# Genetic Profiling Variation Caused by Tissue Type, Error, and Damage

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Declaration:

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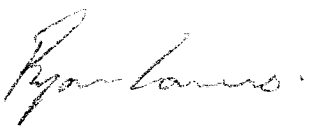

Ryan Lamers

## I. Acknowledgements

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## V. List of Abbreviations

| | | | |
|---|---|---|---|
| **A** | Adenine | **ABI** | Applied Biosystems |
| **aDNA** | ancient DNA | **AP Site** | Apurinic/Apyrimidinic site |
| **APS** | Ammonium Persulfate | **bp** | Base Pair |
| **C** | Cytosine | **CE** | Capillary Electrophoresis |
| **dATP** | deoxyadenine triphosphate | **dCTP** | deoxycytosine triphosphate |
| **EDTA** | Ethylene-Diamine-Tetra-Acedic acid | **EtBr** | Ethidium Bromide |
| | | **EtOH** | Ethanol |
| **G** | Guanine | **GuSCN** | Guanidinium Thiocyanate |
| **HCl** | Hydrochloric Acid | **HV1; HVI** | Hypervariable Region 1 |
| **HV2; HVII** | Hypervariable Region 2 | **Kb** | Kilobase |
| **KCl** | Potassium Chloride | **Mg** | Magnesium |
| **$MgCl_2$** | Magnesium Chloride | **$MgSO_4$** | Magnesium Sulfate |
| **mtDNA** | Mitochondrial DNA | **NaCl** | Sodium Chloride |
| **nDNA** | Nuclear DNA | **PAGE** | Polyacrylamide Gel Electrophoresis |
| **PCR** | Polymerase Chain Reaction | | |
| ***Pfu*** | *Pyrococcus furiosus* | **PK** | Proteinase K |
| **PPi** | Pyrophosphate | **PTB** | N-phenacyl thiazolium Bromide |
| **RCRS** | Revised Cambridge Reference Sequence | **RFLP** | Restriction Fragment Length Polymorphism |
| **RFU** | Relative Fluorescence Units | | |
| **RNA** | Ribonucleic Acid | **ROS** | Reactive Oxygen Species |
| **rRNA** | Ribosomal RNA | **SDS** | Sodium Dodecyl Sulfate |
| **$SiO_2$** | Silica Oxide | **SNP** | Single Nucleotide Polymorphism |
| **STR** | Short Tandem Repeat | | |
| **T** | Thymine | ***Taq*** | *Thermus acquaticus* |
| **TBE** | Tris-Boric acid-EDTA | **tRNA** | Transfer RNA |
| **UV** | Ultraviolet | **VNTR** | Variable Number of Tandem Repeats |

## VI. Abstract

Genetic profiling variations caused by tissue type, error, and damage have been studied only minimally in the past. Even though it is well accepted that damage to the DNA molecule and PCR induced error occur, little focus has been given to their roles in generating variation within genetic profiles. Through the generation and compilation of mitochondrial DNA (mtDNA) sequence data, autosomal short tandem repeat (STR) data, and Y-chromosome STR (Y-STR) data, the incidence of damage, error and mutation was assessed during this study. Mitochondrial DNA sequence data was analyzed among 255 replicates from 42 aDNA samples in 9 populations. Intra-individual genetic profiling variations were observed in 28 replicates from 18 individuals, the majority of which can be explained by damage to the DNA molecule (hydrolytic and oxidative). In 15 replicates from 1 modern individual, no Y-STR profiling variations were observed. Conversely, ancient Y-STR data yielded multiple variations, all of which are attributable to PCR error. In 64 replicates from 6 individuals, only 4 alleles in total were observed to consistently amplify. The remaining loci exhibited variation among multiple replicates from each individual. To overcome such profiling problems, 8 hemi-nested Y-STR singleplexes were designed and optimized. For autosomal STRs, booster STR amplification was tested for its potential use, however, the results were unreliable. The data obtained throughout this study are consistent with previous reports pertaining to DNA damage. In addition, this research has shown that specific damage is acting on particular populations presumably due to their depositional environments. This research leads into future studies concerning DNA damage detection and repair for the recovery of low copy number DNA templates.

## VII. Introduction

Since the discovery of its structure over 50 years ago, significant advancements have been made to our knowledge and understanding of deoxyribonucleic acid (DNA). During the last 25 years, extensive research has focused on the mechanisms of various cancers and diseases, as well as the individualizing characteristics found within this intricate biomolecule. These individualizing characteristics exist in regions of DNA that are specific to each individual. To better study these regions, various techniques such as mitochondrial DNA (mtDNA) profiling, short tandem repeat (STR) profiling and single nucleotide polymorphism (SNP) profiling have been developed. These profiling techniques enable researchers to identify genetic differences between individuals. Researchers use the variation between individuals for a variety of purposes and in the case of individual identification they use this variation to their advantage. However, the presence of variation within one individual is not well studied or understood. Intra-individual DNA variation can be caused by a multitude of factors such as damage to the DNA molecule, mutation, analytical errors and features specific to different types of DNA like mitochondrial DNA heteroplasmy. The causes of this observed variation can occur at various stages, such as during one's lifetime, perimortem, postmortem, or at any step during the analysis of a sample, but one thing remains the same; all will complicate the interpretation of data obtained.

### i. Structure and Types of DNA

Within every living organism resides a highly complex macromolecule called deoxyribonucleic acid (DNA). This extremely large organic polynucleotide has been referred to as the genetic "blueprint" of life because it stores all the necessary hereditary

information required for a cell to survive. DNA is passed on from one generation to the next and exists in a variety of different types. The most common types of DNA within a cell can be found both in the nucleus, and the mitochondria. Other types of DNA, such as extrachromosomal and minicircles also exist, however, nuclear and mitochondrial DNA are typically the only types used for profiling. All of the DNA residing within a cell can be referred to collectively as its genome (Alberts, 2002; Butler, 2005). However this term "genome" can be used to describe the different types of DNA, depending on where that DNA is found, such as mitochondrial genome or nuclear genome. In humans the entire nuclear genome is made up of 46 chromosomes, structured in 23 pairs and is approximately three billion bases in length (Alberts, 2002). Twenty-three chromosomes come from an individual's mother and the complementary twenty-three come from the father. The mitochondrial genome on the other hand is extra-chromosomal DNA that is inherited strictly through the maternal cell line and is only a fraction of the size of its nuclear counterpart (on average 16,569bp). The function of both mitochondrial and nuclear DNA (nDNA) is to direct the synthesis of all the necessary enzymes, proteins and molecules required for cellular metabolism and survival. In addition these genomes are required to self-replicate, in order for cells to successfully replicate, divide and pass on the genetic "blueprint" to successive generations of cells. There are two copies of each nuclear chromosome per cell except red blood cells that contain no nuclei and therefore, no DNA. On the other hand, mtDNA exists in extremely high copy number within the cell as there are multiple copies of this genome per mitochondrion and multiple mitochondria per cell. Although these two types of DNA (nuclear and mitochondrial) are very different in terms of their genome structure, associated proteins, size, function, copy

number and mode of inheritance, their primary genetic structure is the one thing that remains the same.

a. Structure of DNA

The structure of DNA was first discovered in 1953 by James Watson and Francis Crick at Cambridge University (Watson and Crick, 1953b). This stemmed from a compilation of numerous other studies in the early 1950's performed by researchers attempting to understand the molecular structure and function of DNA. Previous theories described the structure of DNA as three phosphate backbone subunits intertwined together (Pauling and Corey, 1953), however, it was not until 1953 that Watson and Crick identified the structure as being two helical chains wrapped around one central axis in a right handed conformation (Figure 1) (Watson and Crick, 1953b). These helical chains are comprised of alternating deoxyribose sugar and phosphate groups (Watson and Crick, 1953b; Watson and Crick, 1953a). Attached to the deoxyribose sugar are any one of the four nitrogenous bases; guanine (G), adenine (A), cytosine (C), and thymine (T). Of these, the purines, guanine and adenine pair only with the pyrimidines cytosine and thymine respectively. The double helix is formed when nitrogenous bases are attached to the sugar-phosphate backbone, and the two helices are held together through hydrogen bonding between complementary bases on each strand (Watson and Crick, 1953b; Watson and Crick, 1953a). The phosphate groups within the backbone of the DNA strands are attached at the 5' and 3' carbons of consecutive deoxyribose sugars through the formation of ester bonds, while the nitrogenous bases are attached to the 1' carbon of the same sugar through a N-glycosyl bond. The two helices within the double helix run in the 5' to 3' direction anti-parallel to each other. The sequence of a DNA molecule is

usually referred to as a single stranded sequence, when the sequence of one strand is known (template or sense strand), the sequence of the other strand can be determined by reverse complimenting the known sequence. Due to the variability in the sequence of DNA, any nucleotide combination may be possible. Although the particular sequence of DNA may appear to occur at random, we know that as Watson and Crick hypothesized in the 1950's, the nucleotide arrangement exists in a very precise manner. The actual nucleotide sequence within DNA is responsible for the successful development and functioning of every cell within each individual. All of the necessary information required for the production of essential enzymes and other proteins is encoded within the nucleotide sequence of the DNA molecule (Hartl and Jones, 2005).

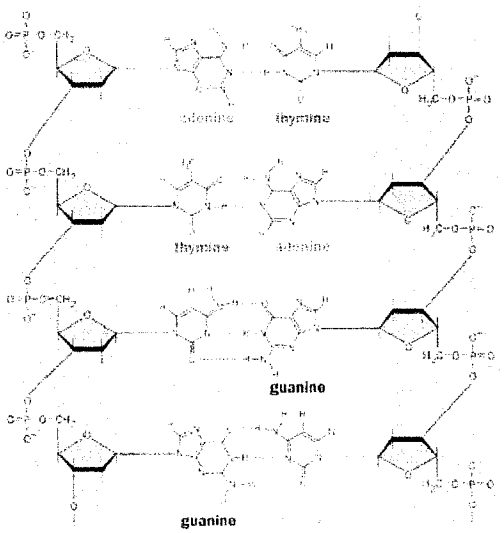



Figure 1: Structure of the DNA double helix (Huxley, 2003).

b. Nuclear DNA

The nuclear genome is highly conserved from one individual to the next and is responsible for coding the vast majority of proteins that are required for the cellular functioning required for an organism to survive. As its name implies, nDNA resides in

the nucleus of every cell where it remains highly compacted (Figure 2) until it is required for transcription or replication. This compaction is necessary for a variety of reasons ranging from protection to packing size.

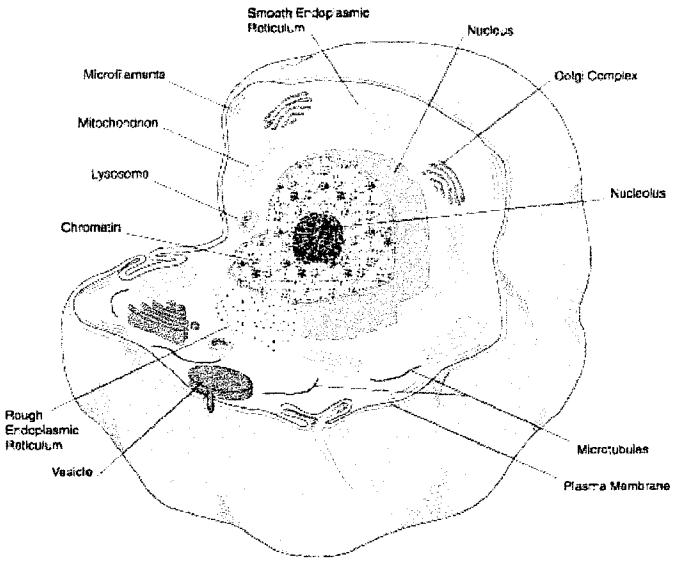



Figure 2: Image of a human cell indicating the location of such organelles as the nucleus, and mitochondria. (Picture taken from http://www.paternityexperts.com/images/animal%20cell.gif).

The proper function of each cell is highly dependent upon the information contained within the DNA molecule. Therefore, it is essential that this information be protected from damage caused by one of the many harmful agents found within the cell. Also, the genome is so large that if it were stretched out end to end, it would be far too long to fit into the nucleus. For example, chromosome 22 alone when stretched out end to end extends approximately 1.5 cm. In reality, when compacted, this chromosome only measures approximately 2 μM in length (Alberts, 2002). The cell achieves this level of compaction by utilizing proteins called histones (Figure 3). The histone-DNA complex forms the most basic level of compaction, the nucleosome (Alberts, 2002). Each nucleosome comprises 146 base pairs of nDNA wrapped around the histone complex made up of eight histones (Alberts, 2002). The adjacent nucleosomes are joined by a

linker region of DNA ranging from a few base pairs up to about 80 base pairs (Alberts, 2002). Further compaction is achieved by nucleosomes stacking on top of one another forming what is known as chromatin and ultimately leading to the structure of the chromosome (Alberts, 2002).

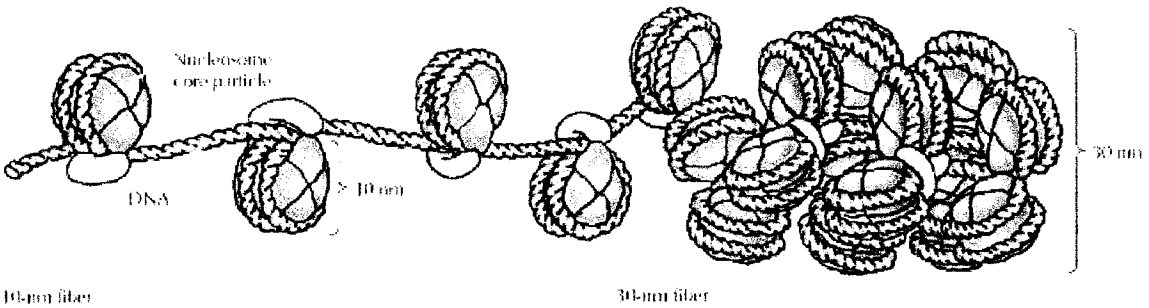



Figure 3: Compaction of DNA forming the nucleosome (histone octamer) and further compacting to form the chromatin fibre. (Picture taken from http://edoc.hu-berlin.de/dissertationen/seitz-stefanie-2004-10-20/HTML/chapter1.html#N100AF).

Nuclear DNA from one individual to the next is highly conserved with approximately 99.9% of the human nuclear genome being shared between different human beings. It is the remaining 0.1% or approximately 3 million bases that differ between individuals (with the exception of identical twins who share 100% of their DNA). It is within this 0.1% of DNA that scientists analyse and study to find characteristics, or polymorphisms that can be profiled to identify individuals.

While the nuclear genome is inherited half from the mother and half from the father, there is one region that is an exception to this and has intricate implications on its mode of inheritance. Normally one chromosome from each chromosome pair in the nuclear genome comes from each parent. These are referred to as the autosomal chromosomes. The sex chromosomes however are different to this inheritance. The sex chromosomes in humans are the X and Y chromosomes. The X chromosome can be inherited very similarly to the autosomes but the Y chromosome is passed on only from

father to son and does not recombine during transmission. This indicates that aside from the occurrence of mutation, all paternally related males will share the same Y-chromosomal profile. In this way the Y chromosome is very similar to the mitochondrial genome being inherited from only one parent and replicating in a clonal manner. Both these genetic regions (mtDNA and the Y chromosome) are useful for genealogy and inheritance studies and can be used very successfully for the identification of missing persons. However, because the Y chromosome exists in only one copy per cell, it is not typically useful for highly degraded samples of DNA. In cases such as this, mitochondrial DNA found in multiple copies becomes an extremely useful tool.

c. Mitochondrial DNA

Mitochondria are organelles that are found within the cytoplasm of a cell (Figure 2). They possess and regulate all of the genes required for their own maintenance and replication. Mitochondria are essentially the "powerhouses" of the cell, generating all of the energy that the cell may require. Each mitochondrion possesses one or more copies of its own DNA, mitochondrial DNA (mtDNA). Since the 1980's, the use of mtDNA as an investigative tool has become increasingly popular for disease studies, population genetics research, or studies when the DNA is suspected of being highly fragmented. Reasons for the analysis of mtDNA in these studies are primarily due to its high copy number in cells, and its maternal mode of inheritance. In addition to this, the genome contains highly variable sequences of DNA where the likelihood of two unrelated individuals sharing the same profile is relatively low.

Mitochondrial DNA exists in extremely high copy number compared to the two copies of nDNA per cell. There are 1-10 copies of the mitochondrial genome per

mitochondrion and potentially 1000's of mitochondria per cell (depending upon the energy demands of the cell) (Budowle *et al.*, 2003). Also, in contrast to nDNA, mtDNA is only inherited through the matriline. This means that barring any mutation, all maternally related individuals should possess the exact same mitochondrial DNA sequence. This feature becomes particularly useful in studies involving population migration patterns, or cases involving missing persons (among others) where a maternal relative can help determine the mtDNA profile of the missing individual.

The mitochondrial genome is a circular, super-coiled, double stranded DNA molecule approximately 16,569 bases in length (Figure 4). The entire mitochondrial genome was sequenced in 1981 by Anderson *et al.*, and since then this sequence has been used worldwide as a reference for which all other mtDNA sequences are compared. Resulting from the fact that mtDNA molecules are not wrapped around histones, they are more susceptible to mutation and damage than nDNA, and thus, individuals varying from the typical length or sequence of the genome are not uncommon.

The mitochondrial genome is extremely compact and codes for 13 polypeptides and approximately 80 protein subunits involved in carrying out the functions of oxidative phosphorylation. The genetic instructions required to create 2 ribosomal ribonucleic acids (rRNA) and 22 transfer RNA's (tRNA) are also encoded within this genome (Budowle *et al.*, 2003). Given that all of the essential genes for its own maintenance and replication reside within this genome, it is capable of self-replicating and functioning without the need for genes expressed by the nuclear genome. However extensive studies are currently focusing on the interaction if any, between the nuclear and mitochondrial genomes.

There is one region of this genome that does not code for any proteins and is referred to as the non-coding region, control region, or D-loop. It is within this region that most non-maternally related individuals show sequence variability in the order of approximately 3% (Stoneking, 2000). Due to this, scientists can successfully distinguish between two non-maternally related individuals in both forensic and non-forensic contexts (Howell *et al.*, 1996; Butler and Levin, 1998). The D-loop is approximately 1,100 bases long and contains two hypervariable regions (some identify three hypervariable regions). These regions acquired their names due to their elevated mutation rates, resulting in a high degree of variation between most unrelated individuals. Hypervariable region 1 (HV1) encompasses the region from nucleotide position 16,024 through to nucleotide position 16,365, and hypervariable region 2 (HV2) spans nucleotide position 73 to nucleotide position 340 (Budowle *et al.*, 2003). The differences observed within these two regions do not appear to be distributed uniformly, but seem to occur in close proximity to clusters of "hot spots" (Hummel, 2003). Hotspots are regions of DNA that tend to mutate more rapidly than others.

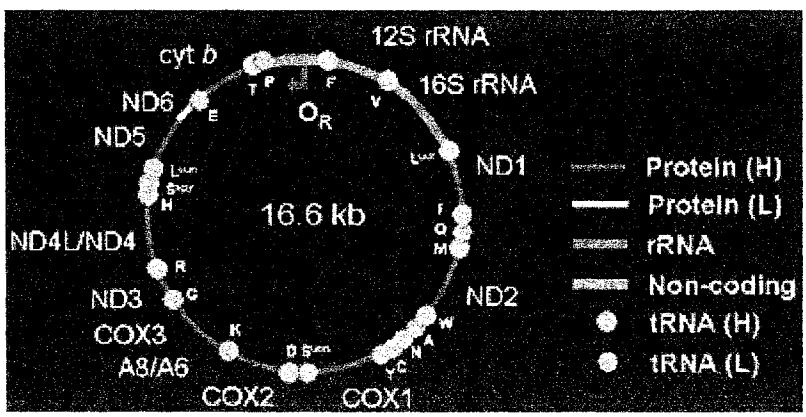



Figure 4: Structural makeup of the mtDNA genome. (Picture taken from http://www.mrc-dunn.cam.ac.uk/research/mtdna.html).

## ii. Introduction to DNA Profiling:

DNA typing has been used for decades as a tool to study a variety of applications. DNA profiling has been used in the past, and will continue in the future to be utilized as a means of studying evolutionary biology, forensic science, and population genetics, among many other areas of research and applications. Within the realm of DNA profiling there are currently three main techniques that are routinely employed in forensic and research facilities. These three techniques are mitochondrial DNA profiling, short tandem repeat (STR) profiling, and single nucleotide polymorphism (SNP) profiling. These are the profiling techniques that will be examined in this research.

### a. Mitochondrial DNA Profiling

Mitochondrial DNA profiling is frequently used in evolutionary studies, mtDNA disease detection, as well as cases in forensic science. Mitochondrial DNA is often analyzed when DNA samples are highly degraded or a maternal comparison is required. The most commonly used method to type mtDNA is through the use of sequencing (Sanger *et al.,* 1977). DNA sequencing is frequently used for a variety of biochemical applications and results in the nucleotide sequence of either nuclear or mitochondrial DNA being produced. Using mtDNA sequences, researchers are capable of making reasonable assumptions about past population structures, as well as the migration patterns of various populations. Mitochondrial DNA sequences can also be utilized to identify the remains of deceased individuals, such as unidentified war victims (Holland *et al.,* 1993), or persons considered as being of historical significance (Ivanov *et al.,* 1996; Dissing *et al.,* 2006). When analyzing mtDNA profiles, the analyst is typically looking for highly informative polymorphisms at a variety of different nucleotide positions in attempt to

determine the individual's haplotype (Bendall *et al.,* 1997; Green *et al.,* 2000; Kline *et al.,* 2005). An individual's haplotype describes the set of polymorphisms they possess, and are typically inherited as an entire unit. Each individual possesses their own haplotype, however, when multiple individuals possess the similar haplotypes, they form what is referred to as a haplogroup. Although all sequence variations, both nuclear and mitochondrial, are single nucleotide polymorphisms (SNP), for the purpose of this paper, we will refer only to SNPs in relation to nuclear DNA. All variations within the mitochondrial genome will be referred to as: polymorphisms, point mutations, substitutions, or sequence variations. Mitochondrial DNA typing is extremely informative when studying past populations or for the detection of disease, however, due to this genome's mode of inheritance; it has a low discriminatory power when comparing one individual to another. For this reason, short tandem repeats (STR) are the preferred method of analysis in forensic science.

b. STR Profiling

The first polymorphic DNA marker in humans was described by Wyman and White in 1980. Since then, there have been tremendous advancements in DNA profiling, in particular, the profiling that occurs at STR loci. STR profiling has been used for many years and is a very important part of everyday forensic casework. Sir Alec Jeffreys first introduced DNA typing to the scientific community in 1985 for use in an immigration case in England (Jeffreys *et al.,* 1985a). Jeffreys developed this technique the year prior, when he observed that sequence similarities among repeat units at different loci (minisatellite core repeat) (Jeffreys *et al.,* 1985b), made possible the development of hybridization probes to produce a Southern Blot profile. Due to the extreme variability

of these sequences between individuals, he termed them 'DNA fingerprints' (Jeffreys *et al.,* 1985c). Since that time, DNA fingerprinting, or profiling has become widely accepted as a means of conducting various scientific and medico legal applications. Of these, paternity and maternity testing, criminal investigations, and other applications of forensic science are the most widely utilized use of this procedure. DNA profiling is favourable due to its high sensitivity to minute traces of DNA, as well as its incredible ability to discriminate between different individuals, often with the random match probabilities being in the order of one in billions.

The methods first employed by Dr. Alec Jeffreys and his colleagues at Leicester University in 1985 were used to solve an immigration dispute (Jeffreys *et al.,* 1985a). One year later, in 1986, these same methods were again used to help solve two homicides in Enderby, England resulting in the exoneration of a convicted individual (Wong *et al.,* 1987). These methods were performed to analyze variable numbers of tandem repeats (VNTR) within specific sequences of DNA. This profiling technique was based on restriction fragment length polymorphisms (RFLPs) (Carey and Mitnik, 2002). Since 1985 however, other methods of DNA profiling have been developed (Carey and Mitnik, 2002) including the use of autosomal short tandem repeats (STR), Y-chromosome STRs, mitochondrial DNA and SNPs. These different methods have been made possible due to the discovery of the polymerase chain reaction (PCR) in 1983 (Mullis *et al.,* 1986). This technique now allows scientists to amplify minute quantities of DNA, thus, increasing the sensitivity to trace samples. DNA profiling has been used for many years as the primary method for the individualization of a variety of biological samples. Of the various methods available to the DNA analyst, autosomal STR profiling is the most highly

utilized due to the extreme level of discrimination that can be achieved. This level of discrimination is ever increasing through the use of multiplexing, which is capable of analyzing dozens of loci simultaneously. The extent to which STR profiling is used for individualization purposes in the future may change given the development and strong insurgence of single nucleotide polymorphism analysis.

The use of PCR has greatly increased the number of loci that can be analyzed simultaneously; however, this also presents a challenging DNA separation and detection problem. Within a multiplex, it is possible to have as many as 20 or 30 fragments of DNA present that must be separated from one another. Adding to the difficulty is the fact that these fragments may be equal in size or differ by as little as one base pair. Presently, capillary electrophoresis is the most frequently used separation method, however in the past electrophoresis using polyacrylamide, agarose and slab gels was routinely used (Buel *et al.*, 1998; Butler *et al.*, 2004; Butler, 2005). The advantages of using capillary electrophoresis over other electrophoretic techniques primarily stem from the ability to fully automate the injection, separation, and detection steps allowing multiple samples to be run unattended (Butler, 2005). Other advantages are the relatively short periods of time required to analyze samples, as well only minute quantities of product are required for analysis (Butler *et al.*, 2001; Butler, 2005).

Once the STR fragments have been separated it is necessary for the size of each to be detected. Although many detection methods are available, fluorescence detection is the most commonly used for the analysis of STR (Butler, 2005). For STR fragment detection, fluorescently labeled primers are typically used where a fluorophore is bound to the 5' end of the forward primer or the 3' end of the reverse primer. Using a variety of

different fluorophores in a multiplex eliminates problems associated with different fragments of equal size. The different fluorophores attached to fragments of equal size will emit light at different wavelengths, thus allowing for the detection of each fragment individually (Butler *et al.*, 2001; Moretti *et al.*, 2001; Butler *et al.*, 2004; Butler, 2005). The use of fluorescently labeled primers has been one of the greatest advancements in STR fragment detection, allowing for increased sensitivity as well as the ability to multiplex similar sized fragments of DNA.

c. SNP Profiling

The third and final DNA typing method that will be discussed is SNP profiling. SNP profiling is not routinely used in forensic casework; however, it is frequently used in disease studies where the genes that underlie complex diseases are now being identified (Syvanen, 2001). It was not until recently that SNP profiling became a technique of serious interest, due to its ability to identify genes that predispose individuals to common multifactorial disorders (Syvanen, 2001). One primary technique used to achieve these discoveries, involves the use of linkage disequilibrium mapping (Risch and Merikangas, 1996; Schork *et al.*, 1998).

The first use of SNP profiling came in 1979 when allele specific oligonucleotide probes (ASOP) were hybridized to DNA in attempt to detect a single base polymorphism (Wallace *et al.*, 1979). This technique was again used in 1983 to detect the sickle cell mutation with Southern Blotting (Conner *et al.*, 1983). With the use of the polymerase chain reaction, many different methods of SNP genotyping are currently being employed. Some of the methods commonly utilized currently are, microarrays, TaqMan™ and molecular beacon assays, dot blot or reverse dot blot methods, and DNA polymerase

assisted SNP genotyping such as single base primer extension or single nucleotide extension and pyrosequencing (Syvanen, 2001). Although early methods only allowed researchers to analyze one locus at a time, presently it is common to see multiplexes that incorporate tens to hundreds of SNPs, or microarrays analyzing hundreds of thousands or even millions of SNPs at one time.

Recently, even forensic scientists have begun taking advantage of the immense information single nucleotide polymorphisms have to offer. Although SNP profiling has not been accepted into court as yet, it has begun to be utilized as an investigative aid to law enforcement agencies. One company in Florida has recently introduced a test called DNAWitness™ to the forensic community which is a service provided to law enforcement agencies to narrow down the list of possible suspects of a crime. This is done by analyzing 176 SNPs from crime scene samples where no suspect has been identified. Based on the profile generated, a software program compares this profile to a database of thousands of other profiles in order to determine the most probable ethnic group that the crime scene sample came from. With over 3 million SNPs in the human genome, this method of DNA typing would be capable of providing individualization of a given biological sample. However, the lack of population frequency data and the small size of the current databases prevents this type of profiling from being applied to current investigations.

### iii. Causes of Genetic Profiling Variation

a. DNA Damage

Postmortem degradation of a cell or organism begins immediately following death of the cells. This is due to the activity of many chemicals and enzymes, in particular, the

uncontrolled activity of lysosomal nucleases (Paabo *et al.,* 2004). In addition to this, a variety of organisms such as bacteria, fungi and insects feed on the exposed macromolecules aiding in their destruction (Eglinton and Logan, 1991). During the life of the cell, destructive enzymes remain compartmentalized and thus, do not degrade the DNA. Also present within the living cell, are repair mechanisms that are in place to correct any errors occurring within the DNA sequence (Lindahl, 1993). There are a variety of different types of damage that may occur to DNA postmortem such as single or double stranded breaks (Paabo *et al.,* 2004), oxidative (Lindahl, 1993; Paabo *et al.*, 2004) and hydrolytic (Lindahl, 1993; Paabo *et al.*, 2004) lesions, and cross linking of DNA to other biomolecules such as DNA, RNA, and proteins (Figure 5) (Paabo *et al.,* 2004). Past studies have shown that damage of various forms to the DNA molecule does occur and frequently results in the observance of sequence variation within multiple replicates from the same individual (Hofreiter *et al.,* 2001a; Hofreiter *et al.,* 2001b; Hofreiter *et al.,* 2002; Paabo *et al.,* 2004; Mitchell *et al.,* 2005).

Deoxyribonucleic acids are much more stable than ribonucleic acids (RNA). As DNA is much less susceptible to hydrolytic cleavage, attacking the phosphodiester bonds compared to its counterpart RNA. This is due to the removal of the hydroxyl group on the 2' carbon of the ribose sugar. Although DNA is less susceptible than RNA to this type of damage, it can and does still occur. When the hydrolytic attack takes place, it typically acts on the phosphodiester backbone resulting in fragmentation of the DNA molecule. This is the most obvious form of degradation to DNA and occurs postmortem resulting in a reduction in sequence length to typically 100 to 500 base pairs (Paabo, 1989; Hofreiter *et al.*, 2001b). The cause for this massive reduction in size is due to both

enzymatic processes, as well as hydrolytic damage to the phosphodiester backbone resulting in single or double strand nicks (Shapiro, 1981; Lindahl, 1993). These nicks within the DNA may not become apparent until PCR when the two strands are denatured and the many small fragments dissociate from one another.

Due to the absence of the 2' hydroxyl group on the ribose sugar in DNA, hydrolytic attack occurs less to the backbone, but more frequently to the N-glycosyl bond between the deoxyribose molecule and the nitrogenous base. This attack occurs more frequently to purines compared to pyrimidines and often leaves what are referred to as apurinic or abasic sites (AP) (Lindahl and Nyberg, 1972; Lindahl and Karlstrom, 1973; Schaaper *et al.*, 1983; Lindahl, 1993). Once depurination or depyrimidination occurs *in vivo*, the DNA molecule remains present with the AP site for a few days in the cell until it either undergoes cleavage by an elimination process or is repaired (Lindahl and Andersson, 1972; Doetsch and Cunningham, 1990). *In vivo* enzymes (AP endonucleases) exist to rapidly repair these damaged sites prior to replication, otherwise if left unrepaired would result in mutation when DNA polymerase inserts an incorrect nucleotide as it reads through the AP site (Neto *et al.,* 1992). AP sites can also induce strand breakage, (Friedberg *et al.,* 1995) or block DNA polymerase activity during both *in vitro* and *in vivo* amplification and replication (Lindahl, 1993). AP endonucleases repair the damaged nucleotide by cleaving the 5' phosphodiester bond, leaving the AP site to be removed by another phosphodiesterase. The gap is then filled by DNA polymerase and the nick sealed by ligase (Doetsch and Cunningham, 1990; Dianov *et al.*, 1992). Due to the fact that postmortem cells have lost the functional excision-repair mechanisms, AP sites become a problem for the amplification and interpretation of DNA sequence data. The

levels of depurination or depyrimidination within any given sample are dependent upon many factors. Time since death and the taphonomy of the environment in which the sample is stored are two major factors affecting its preservation. During PCR at an AP site, there is no nucleotide present for the DNA polymerase to read from while extending the daughter strand. It is during this period when misincorporations may occur due to the random insertion of a nucleotide into the DNA. The new strand will then go on to be replicated millions of times throughout the cycling process and result in the sample appearing to be either heteroplasmic for two or more nucleotides or homoplasmic for a nucleotide that does not truly exist. The level of heteroplasmy or homoplasmy would be dependent upon the number of damaged molecules present in the sample prior to amplification.

Aside from depurination or depyrimidination, hydrolytic damage also occurs within the nitrogenous base itself causing base modification or deamination. Of the nucleotides composing DNA, cytosine and 5'methylcytosine are the primary targets for this type of hydrolytic damage, which results in their conversion to uracil or thymine respectively (Shapiro, 1981). These deamination products would then result in a C→T transition upon further replication both *in vivo* and *in vitro*. Once again, *in vitro* repair mechanisms are nonexistent and therefore most, if not all, deamination products will result in a mutation being observed. *In vivo* repair mechanisms exist to correct these mutations prior to transcription or replication. 5-methylcytosine deamination occurs three to four times more frequently than cytosine deamination (Lindahl and Nyberg, 1974; Ehrlich *et al.*, 1990) and are repaired less often (Lindahl, 1993).

Cytosine deamination products are quickly repaired through the excision of the uracil moiety by uracil-DNA glycosylase followed by removal of the AP site by AP endonuclease and the rest of the base excision-repair pathway. Due to the deamination product of 5-methylcytosine being thymine, it is not corrected by this same repair pathway, but is instead corrected by much less efficient mismatch repair mechanisms. Therefore, a higher degree of mutation will develop and remain as a result of the deamination of 5-methylcytosine compared to cytosine (Lindahl, 1993). Given that 5-methylcytosine residues function as endogenous mutagens and carcinogens in humans (Rideout *et al.,* 1990), approximately one third of single-site mutations observed in inherited human diseases arise from deamination at methylated CpG islands (Cooper and Youssoufian, 1988).

Deamination can also occur at purine nucleotides, however, this is observed at a much lower rate. When deamination occurs at adenine residues, hypoxanthine is created which then preferentially base pairs with cytosine causing a transition from an A/T bond to a G/C bond. When guanine is deaminated, xanthine is liberated, which preferentially base pairs with cytosine (Lindahl, 1993). In this instance, no noticeable mutation occurs because conventional base pairing will resume in the next phase of replication. The repair mechanisms for these deamination products are similar to the one employed for cytosine deamination products, however different DNA glycosylases are utilized (Dianov and Lindahl, 1991).

Oxidative damage can also have mutagenic effects on the DNA molecule, aiding in the fragmentation process by causing lesions. The major causes of oxidative damage are hydroxy radicals or peroxide radicals and hydrogen peroxide (Paabo *et al.,* 2004).

The process of DNA oxidation most commonly results in the production of 8-hydroxyguanine from guanine (Kasai and Nishimura, 1984; Shibutani *et al.*, 1991), although the oxidative attack occurs at the double bonds of both purines, and pyrimidines which then leads to ring fragmentation. This attack can also take place within the deoxyribose molecule resulting in fragmentation of the sugar ring (Lindahl, 1993; Friedberg *et al.*, 1995). Once the 8-hydroxyguanine molecule is formed, it preferentially base pairs with adenine rather than cytosine inducing the transversion from a G/C bond to a T/A bond during the following replication or amplification (Kasai and Nishimura, 1984; Shibutani *et al.*, 1991). *In vivo* this mutation is again repaired by a specific DNA glycosylase, and thus, typically does not exist long enough to have profound effects (Lindahl, 1993). The activity of this DNA glycosylase is so efficient that when the gene producing this enzyme is inactivated in *E. coli*, there is approximately a 10-fold increase in the spontaneous mutation frequency (Michaels *et al.,* 1992). When this damage occurs in postmortem tissues, no repair mechanism is available and a mutation will be observed. When fragmentation of either the sugar or base rings occurs postmortem, amplification will not proceed beyond these sites due to blockage of the DNA polymerase (Paabo *et al.,* 2004).

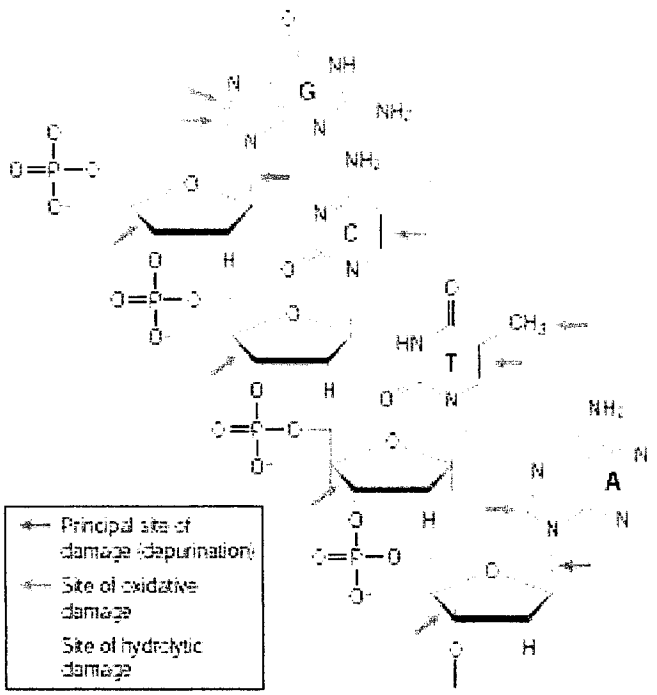



Figure 5: Primary types and sites of DNA damage (Hofreiter *et al.*, 2001b).

Low to nonexistent oxygen metabolism within the nucleus prevents much of the oxidation of nDNA residing within this organelle (Joenje, 1989). It is believed that one major reason separate nuclei evolved in eukaryotes was to protect the nDNA from the reactive oxygen species (ROS) that are present within the mitochondria (Lindahl, 1993). This then leads us to believe that due to the close proximity of mtDNA to ROS, these molecules would be more prone to oxidative damage (Figure 6). Compound the close proximity with the fact that mtDNA receive very little protection from damage since they are not wrapped around histones (Lindahl, 1993). Nucleoids are one feature that may provide some protection to the mtDNA molecule, however, the occurrence of vertebrate mtDNA nucleoids has only recently been studied, leaving their nature, number, and dynamics largely unknown (Legros *et al.*, 2004). The nucleoid provides a level of compactness in addition to mtDNA supercoiling where proteins bind the DNA molecule

that wrap, bend and compact the molecule (Hartl and Jones, 2005). Whether the occurrence of the nucleoid within human mtDNA confers protection is yet to be determined. As is expected, mtDNA contain higher levels of 8-hydroxyguanine compared to nDNA within normal cells and these levels become elevated even higher in cells where the mitochondria are exposed to oxidative stresses (Richter *et al.,* 1988). Due to this, it is possible that the elevated mutation rate, and rapid evolution of the mitochondrial genome is largely affected by oxidative damage (Lindahl, 1993). It is also possible that oxidative damage is responsible for many maternally inherited diseases such as Leber's hereditary optic neuropathy (Wallace *et al.,* 1988; Linnane *et al.,* 1989).

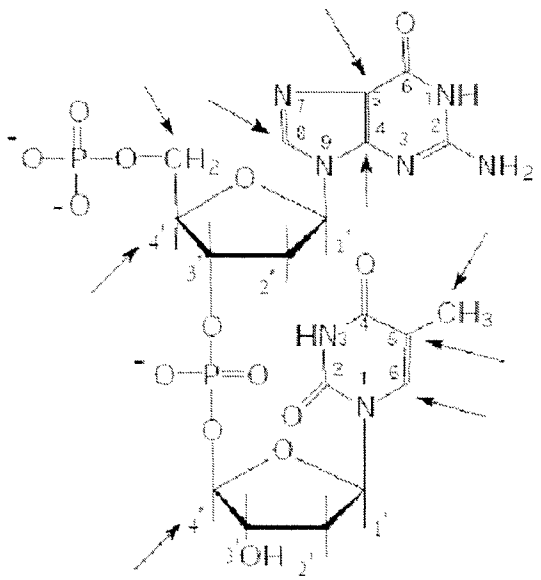


Figure 6: Schematic indicating the primary locations of free radical attack. (Image taken from http://www.medicine.uiowa.edu/frrb/SRFRS/sunrisefreradschool97schafer.pdf).

DNA damage due to crosslinks (e.g. Maillard products) are also problematic because they too distort the DNA molecule and block DNA polymerases (Figure 7) (Paabo, 1989; Snustad and Simmons, 2000). Crosslinking results from the condensation reactions between sugar molecules and primary amino groups in proteins (Paabo *et al.,* 2004). Damage of this type can be effectively repaired *in vivo* by nucleotide excision

repair pathways before detrimental effects occur (Snustad and Simmons, 2000). *In vitro*, DNA crosslinks have been shown to be repairable with the addition of N-phenacylthiazolium bromide (PTB) (Vasan *et al.*, 1996), which breaks these products and allows amplification to proceed.

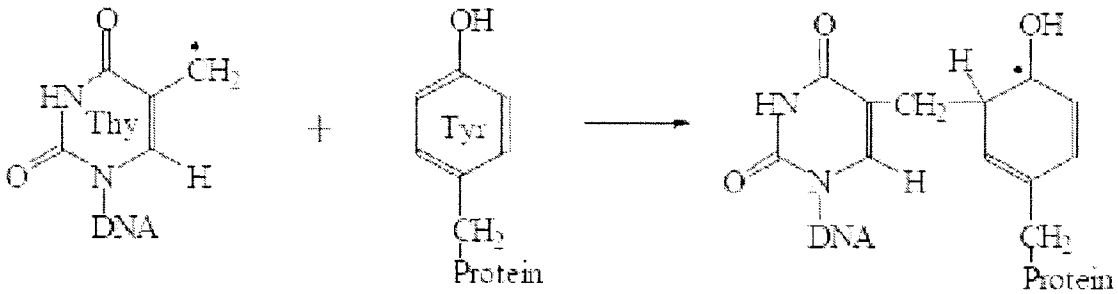



Figure 7: Image showing the formation of a DNA/Protein crosslink. (Image taken from http://www.medicine.uiowa.edu/frrb/SRFRS/sunrisefreradschool97schafer.pdf).

Over the last few years the repair of postmortem damage to the DNA molecule has become of interest particularly to the aDNA community in an attempt to recover DNA molecules from highly degraded samples. This is of particular interest in aDNA studies where extensive damage resulting from strand breaks and base modifications exist at extreme levels. To date no detection method has been developed in an attempt to determine the extent and type of damage that is present within any given sample. Thus far, speculation has been made as to the types and extent of damage that is present, and the addition of various enzymes (glycosylases) and chemicals (PTB) have been added in hopes that they liberate heightened levels of viable DNA templates. In the near future detection systems will be in place to aid the scientist in determining the types of damage present in a sample which will then aid in its recovery, yielding higher copy number DNA templates.

b. Mutation

Mutations within DNA can occur in either the germline or somatic cell line of an individual, and can be either spontaneous or induced. Mutations that occur in the somatic cell line affect only the individual who possesses them whereas mutations that occur in the germline are potentially inherited by the subsequent offspring of that individual. Spontaneous mutations develop through uncontrollable events that typically occur during replication. Induced mutations on the other hand result from exposure to a variety of external agents either, physical, biological or chemical, that react with the DNA molecule facilitating a variety of mutational processes. Mutation rates, whether spontaneous or induced, have also been seen to vary between the sexes, as well as between both the nuclear and mitochondrial genomes.

*De novo* germline mutations which are passed on from one generation to the next are in fact the single greatest source of genetic variability resulting in sequence alterations that take the form of either simple base substitutions, insertions, deletions, duplications, inversions, transposition or even turnover at tandem repeat loci (Jeffreys, 1997; Ellegren, 2000a). Germline mutations are the most easily detected mutations, and predominantly lead to problems involving paternity tests. The first examples of germline instability came in 1985 where the appearance of new alleles in DNA fingerprints could not be attributed to either parent during paternity testing (Jeffreys, 1997). Due to the large number of cellular divisions in germline cells, there is an increased chance for mutations to occur and subsequently, be passed on to the next generation. The traditional method for determining germline mutations is the analysis of pedigrees or familial studies (Jeffreys *et al.*, 1988; Jeffreys, 1997; Ellegren, 2000b). Some researchers feel that this

method is inefficient, yielding only average estimates of mutation rates as opposed to reliable information on the mutational behaviour of specific alleles, particularly for loci that are relatively stable (Jeffreys *et al.,* 1997). Other scholars feel that this method is straightforward and conclusive, offering detailed information about the mutation process (Ellegren, 2000b). In saying this, these latter authors also feel that this process is extremely tedious, requiring large pedigrees and extensive genotyping (Ellegren, 2000b). Germline mutations have been found to be much more prevalent in males compared to those in females for most unstable loci (Jeffreys, 1997; Jeffreys *et al.*, 1997; Bois and Jeffreys, 1999; Ellegren, 2000a), however; there are exceptions in stable loci where the levels of instability are similar in both sperm and oocytes (Jeffreys *et al.,* 1988; Dubrova *et al.,* 1997). The reason behind the fact that males have elevated rates of mutation compared to females is theorized to be the result of errors incurred during extensive replications in the male germline.

There are a variety of different theories of how spontaneous mutations occur, but one such theory is generally more accepted as being the primary model for mutation. This model is referred to as the replication slippage model and was first described by Levinson and Gutman in 1987 (Levinson and Gutman, 1987; Brinkmann *et al.*, 1998; Ellegren, 2000b). Replication slippage or slipped-strand mispairing as it is more commonly known, occurs *in vivo* at either homopolymeric or tamdemly repetitive sequences of DNA when either the template or daughter strand becomes un-annealed during replication and re-anneals one or more (typically only one) bases or repeat units upstream (Figure 8). This results in a tandemly repetitive sequence becoming either one or more repeat units shorter or longer depending on which strand is responsible for the

mutation. This also results in homopolymeric stretches of DNA becoming one or more nucleotides longer or shorter, again, depending on which strand is responsible for initiating the mutation. Given that microsatellite sequences have mutation rates considerably higher than unique sequences (Ellegren, 2000a), and due to that fact that single nucleotide polymorphisms (SNPs) are very difficult to detect outside of sequencing, mutations at these loci are typically the only ones frequently encountered and extensively studied. Somatic microsatellites have been shown to be stable in the past from human hair roots, blood, vaginal swabs, semen, and saliva secretions (Lygo *et al.*, 1994; Sparkes *et al.*, 1996). Although observing any intra-individual somatic variation seems unlikely, it would be of extreme importance when analyzing DNA profiles involving both forensic and other contexts (Hoff-Olsen *et al.*, 2001).

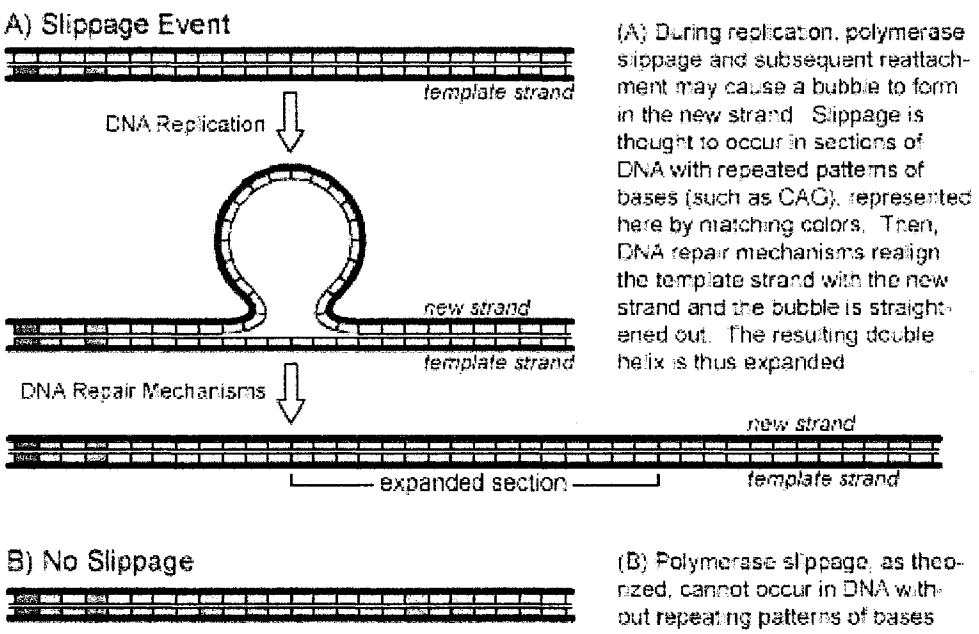



Figure 8: Schematic showing the process of replication slippage. The slippage event shown in A) can also be reversed where the template strand loops out causing the new strand to be shorter in length. (Picture taken from http://www.stanford.edu/group/hopes/causes/mutation/f_q05slippage.gif).

Within microsatellite mutations it is typically assumed that mutant alleles arise from their size closest counterparts (Ellegren, 2000b). Brinkmann *et al.* (1998), when reviewing data from previous studies (Valdes *et al.*, 1993; Weber and Wong, 1993; Di Rienzo *et al.*, 1994), as well as their own, concluded that approximately 90% of all STR mutation events arise from a single-step mutation process. Variant alleles arising from double-step mutation events also occur albeit at much lower frequencies than single-step mutations, and multi-step mutation events are even more rare (Brinkmann *et al.,* 1998). The frequency and direction of the mutation process is influenced by both the size of the repeat unit, as well as the allele length. Previous studies indicate that the mutation process at microsatellite loci is more active on longer alleles and favours the expansion of allele lengths as opposed to contraction (Primmer *et al.,* 1996; Brinkmann *et al.,* 1998; Twerdi *et al.,* 1999). These studies have been regarded only as preliminary and the generalization of this phenomenon has been delayed due to the relatively small sample sizes used in these studies (Xu *et al.,* 2000). In response to this, Xu *et al.* (2000) have conducted similar studies using significantly larger sample sizes. Here it was determined that the rate of contraction mutations increases with allele length whereas the rate of expansion remains constant over all loci tested (Xu *et al.,* 2000). In contrast to previous reports (Rubinsztein *et al.,* 1995; Amos *et al.,* 1996), there seems to be no affect on relative mutation rates within loci based on size differences between the two alleles an individual possesses (known as the allelic span). This is based on the observation that individuals with a high degree of variation between their two allele sizes show no significant increase in the rates of mutation (Ellegren, 2000a). The length of the repeat motif is another factor that influences the mutability of a locus. Instability in

dinucleotide repeats is significantly higher (1.5 – 2.0 times) than the instability of tetranucleotide repeats. Similarly, trinucleotide repeats have a mutation rate that falls between those observed in dinucleotide and tetranucleotide repeats (Chakraborty *et al.*, 1997; Ellegren, 2000a; Sturzeneker *et al.*, 2000). Another factor that has been shown to affect the mutational process at microsatellite loci is the actual repeat sequence. Imperfect repeat sequences have a lower incidence of mutation compared to perfect repeat sequences (Sturzeneker *et al.,* 2000). This is likely due to the fact that slipped-strand mispairing is less apt to occur when the core repeat unit is divided by one or more nucleotides. The occurrence of slipped-strand mispairing is also seen to occur *in vitro* and will be discussed further in an upcoming section.

Along with DNA slippage, DNA replication repair (such as mismatch repair genes) failure can occur in situations where DNA strands do not anneal correctly (Hoff-Olsen *et al.*, 1998; Butler, 2005). This occurs in many types of cancers where cells divide uncontrollably forming a tumor. Not only do the high levels of replication facilitate slippage, but also in cancers there is a high incidence of defects in mismatch repair genes, which aid in the mutational mechanism (Hoff-Olsen *et al.,* 1998). Even in tissues where mismatch repair genes are fully functional, due to the high rates of turnover there is insufficient time for all errors to be recognized and corrected by the repair mechanism.

Aside from strand slippage and replication repair failure, spontaneous mutation can also arise via tautomeric shifts occurring within the nucleotide bases that comprise DNA (Figure 9). When the common keto or amino forms of bases are present in the DNA strand, conventional Waston and Crick base pairing occurs (A with T and C with

G). Occasionally however, the rare form of the nucleotide bases, the imino or enol form will be present in the DNA strand. When this occurs, the base pairing properties are altered which leads to mismatches in the DNA molecule. When the rare imino form of cytosine is inserted, it will then preferentially mispair with adenine in the next round of replication (Suzuki and Suzuki, 1986; Snustad and Simmons, 2000). Similarly, the rare enol form of thymine mispairs with guanine; the rare imino form of adenine mispairs with cytosine and the rare enol form of guanine mispairs with thymine (Suzuki and Suzuki, 1986; Snustad and Simmons, 2000). All of these errors result in base substitutions that take the form of transitions (Suzuki and Suzuki, 1986).

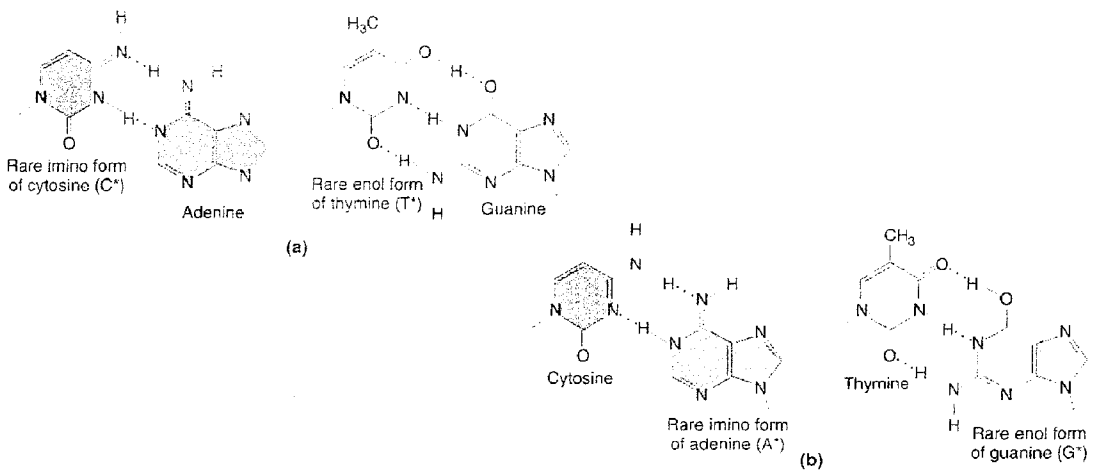



Figure 9: Image showing the incorrect base pairing of (a) rare tautomeric forms of the pyrimidines and; (b) rare tautomeric forms of purines (Griffiths, 2000).

The differences in mutational frequencies seen between male and female individuals has also been shown to be the result of the increased levels of replication occurring in males that are not present in females (Brinkmann *et al.,* 1998). Recent studies have shown that mutations are about five times more likely to occur in males rather than females (Weber and Wong, 1993; Brinkmann *et al.*, 1998; Ellegren, 2000a; Xu *et al.*, 2000), which is due to the larger number of cellular divisions in

spermatogenesis versus oogenesis (Ellegren, 2000b). Estimates have been made that indicate for a 25 year old male, sperm have undergone approximately ten times the number of replications compared to that of an egg belonging to a female of the same age (Vogel and Motulsky, 1997). Overall, the average estimated microsatellite mutation rate within the germline is in the order of $10^{-3}$ to $10^{-4}$ mutations per generation. No reliable mutation rates have currently been proposed for somatic cells due to the difficulty of identifying variations.

Similar mechanisms are responsible for the occurrence of mutations in somatic cell lines as have been discovered in the germline, however, they are much more difficult to study. Due to the rarity of somatic cell mutations, most studies have focused on tumor tissues where mutations are more easily observed. Mutations that occur in the soma affect only the individual who possesses them; however, mutations within this cell type would have great implications for inheritance or hereditary studies, paternity or maternity testing, missing person identification and other applications of forensic science.

It appears as though most mutations occurring within the somatic cell line occur as a result of tumor formation. It is because of this fact that somatic microsatellite instability is more easily studied in tumor tissues (Sturzeneker *et al.,* 2000), however; cancerous tissues are not the only tissues that have been shown to undergo somatic mutation. Studies by Dubrova *et al.* (1996; 1998; 2002) involving victims from the Chernobyl area have indicated that these people have an increased level of mutation due to the increased exposure to radiation. Exposure to radiation and other mutating agents can cause what is referred to as induced mutations.

Induced mutations are the result of an external agent acting on the DNA molecule. External agents that induce mutations are collectively known as mutagens. A mutagen is classified as any agent that raises the rate of mutation above the spontaneous background (Hartl and Jones, 2005). Herman Muller, in 1927, was the first person to show that external agents were capable of initiating mutations. A mutagen could be as simple as water through to alkylating or intercalating agents, base analogs, and ionizing and ultraviolet radiation. The chemical mechanisms involving mutagens include depuriniation, deamination, and single or double stranded breaks.

Depurination is one common outcome resulting from exposure to a mutagen. Depurination often results from hydrolytic damage caused by water, and is the occurrence of a purine nucleotide having its base removed from the deoxyribose sugar (Figure 10). When depurination occurs, the anomaly can be repaired by the apurinic repair system, in which case no mutation ensues, however; if the AP site is not repaired, an adenine is usually inserted opposite the now present hydroxyl group during amplification. This will ultimately result in a mutation to a T/A base pair from the original G/C or A/T base pair (Hartl and Jones, 2005). The reason for this is because various DNA polymerases (e.g. *Taq*) preferentially insert adenine residues when no template is present. Non-template addition typically occurs at the 3' end of an extended DNA fragment, however, AP sites would also be expected to undergo non-template adenine addition at higher frequencies than any of the other bases.

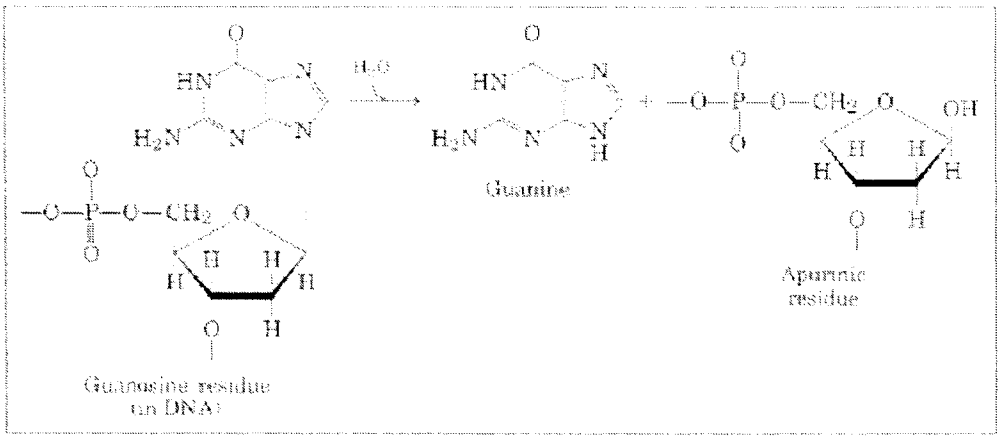



Figure 10: Image illustrating the deamination of guanine yielding an apurinic site (hydroxyl group) on the deoxyribose sugar. (Image taken from http://opbs.okstate.edu/~petracek/Chapter%2025%20figures/Fig%2010-33b.JPG)

Deamination is another mechanism of mutation and is caused by the exposure of DNA to oxidizing or hydrolyzing agents (Figure 11). Nitrous acid is one such oxidizing agent that functions by altering the hydrogen bonding properties and ultimately causes the loss of an amino group in adenine, cytosine, 5-methylcytosine, or guanine. Deamination of cytosine results in uracil (C/G→T/A transition); deamination of 5-methylcytosine results in thymine (C/G→T/A transition); deamination of adenine results in hypoxanthine which then pairs with cytosine rather than thymine (A/T→G/C transition); and the deamination of guanine to xanthine which then pairs with cytosine (no mutagenic effect) (Hartl and Jones, 2005).

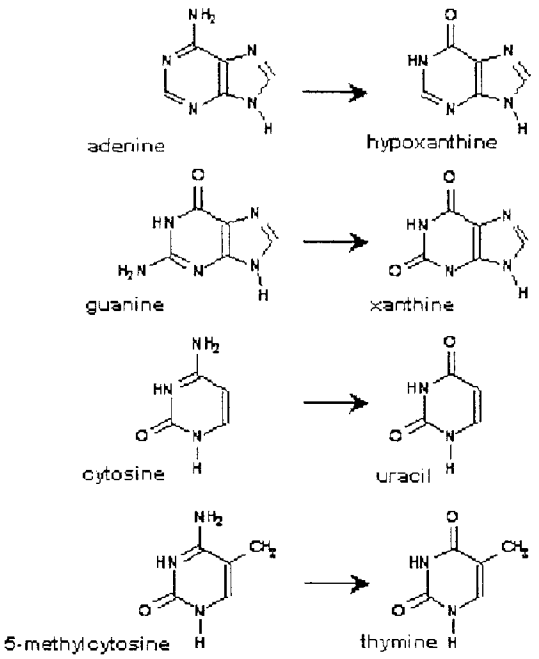
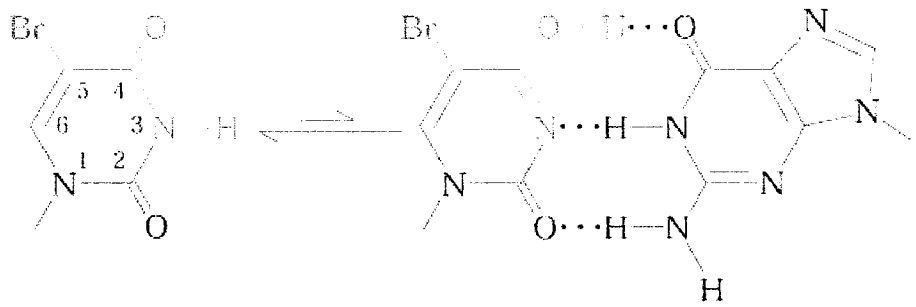



Figure 11: Schematic showing the deamination products of the nitrogenous bases composing DNA. (Image taken from http://web.mit.edu/noppg/deamination.gif).

Base analogs are other agents that induce mutations. These are molecules that closely resemble the actual bases normally incorporated into the growing DNA strand (Snustad and Simmons, 2000; Hartl and Jones, 2005). They cause mutations by becoming incorporated into the growing DNA strand and mispair with another nucleotide. This is seen with 5-bromouracil which is an analog of thymine (Figure 12). When 5-bromouracil is in its common keto form it will pair with adenine, however, it occasionally shifts to its rare enol form causing it to pair with guanine. When the enol form is incorporated, this will induce a transition from a G/C bond to an A/T bond, however; when the common keto form is incorporated, the result will be a transition from an A/T bond to a G/C bond (Snustad and Simmons, 2000; Hartl and Jones, 2005).



**5-Bromouracil (5BU) (keto tautomer)** **5BU (enol tautomer)** **Guanine**

Figure 12: Image showing the tautomeric forms of 5-bromouracil. When 5-bromouracil is incorporated into the DNA it preferentially base pairs with adenine while in its common keto form, however, it will base pair with guanine when incorporated in its enol form. (Image taken from http://www.mun.ca/biochem/courses/3107/images/VVP/Ch24/24-24.%205-Bromouracil.jpg)

Some chemical mutagens such as nitrogen mustards or ethyl methylsulfonate (EMS) are referred to as alkylating agents (Snustad and Simmons, 2000; Hartl and Jones, 2005). These mutagens act by adding bulky side groups to DNA bases that cause either base mispairing or distort the DNA molecule. When either guanine or thymine undergo alkylation, mispairing will occur resulting in a mutation from an A/T bond to a G/C bond or vise versa (Hartl and Jones, 2005).

Other types of chemical mutagens are the intercalating agents. Of these acridine dyes are most common. Originally it was thought that because acridines have very similar dimensions to the bases within DNA, they were capable of intercalating themselves between the base pairs, thus, causing distortion of the DNA molecule (Snustad and Simmons, 2000; Hartl and Jones, 2005). This distortion was suspected to cause insertions or deletions within the replicating DNA; however, it is now thought that the actual mutational mechanism occurs as these dyes interfere with topoisomerase II. Topoisomerase functions to relieve torsion strain by nicking the DNA molecule on one strand, unwinding the DNA, relieving the stress and then seals the nick. Acridines

interfere with this function causing the activity of topoisomerase to take longer than ideal, thus, resulting in the addition or deletion of one or more bases in the growing DNA strand (Hartl and Jones, 2005).

Various forms of radiation are also highly capable of inducing mutations within the DNA molecule. Ultraviolet (UV) radiation is the most common type of radiation and is typically encountered on a daily basis. The mutation is the result of a photoreaction that occurs as a consequence of the absorption of light energy. The most common products are pyrimidine dimers that form primarily between thymine residues adjacent to one another in the same DNA molecule (Figure 13) (Suzuki and Suzuki, 1986; Snustad and Simmons, 2000; Hartl and Jones, 2005). Dimers can also occur between non-adjacent nucleotides within the same molecule or possibly between two pyrimidines on separate molecules that are in close proximity. When dimerization occurs, torsion within the DNA molecule(s) inhibits both transcription and replication from occurring (Hartl and Jones, 2005).

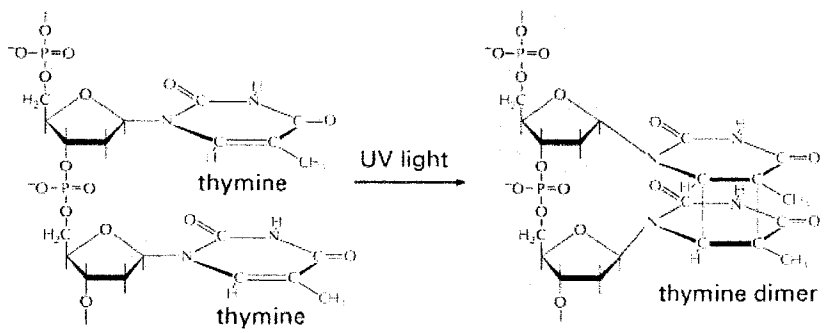



Figure 13: Image showing the formation of a pyrimidine (thymine) dimer caused by UV light (Alberts *et al.*, 2004).

Ionizing radiation is another agent that can cause mutation; however, it is encountered only minimally in our daily lives. Ionizing radiation reacts with water or living tissues, and subsequently results in the formation of oxygen free radicals (Snustad

and Simmons, 2000; Hartl and Jones, 2005). These free radicals then react with other molecules, including DNA, that result in mutations. Ionizing radiation can affect DNA in a variety of ways. It can cause single strand breaks, double strand breaks, nucleotide base alterations and also chromosomal breaks (Hartl and Jones, 2005).

In past studies, mutation rates have been found to significantly differ between different types of DNA. Although much of this discussion has been concerned with mutations that occur within nDNA, the mechanisms and mutations do occur within the mtDNA and are usually found at much higher frequencies. Estimates show that mutations in mtDNA are 6-17 times more frequent than nDNA mutations (Bar *et al.*, 2000), occurring at approximately 0.32 mutations per nucleotide per million years (Sigurgardottir *et al.*, 2000). The difference in mutation rates between nDNA and mtDNA is suspected as being the result of their level of protection, as well as their associated repair mechanisms.

Mutations occurring within an individual will result in the presence of two or more different populations of DNA being represented. The extent of the variations between different tissues is dependent upon when in an individual's lifetime the mutation arises. The presence of multiple DNA profiles within one individual is referred to as either chimerism or mosaicism depending on how the variation arose. The difference between the two is that chimeras possess more than one genetically different cellular population that arises from more than one zygote. Mosaics also have more than one genetically different cell type, however, these arise from only one zygote (Pearson, 2002). Of these two anomalies, mosaicism is more common (although frequencies for both are unknown) and more extensively studied. Mosaicism results from mutations or

chromosomal abnormalities occurring early on in embryological development. As the outcome of this, the individual typically possesses "patches" of genotypically different tissues. If an error in cellular division occurs early enough in development, certain cells may obtain an abnormal number of chromosomes and subsequently, many of the new cells will inherit the defect (Pearson, 2002). There are, however, different levels of mosaicism depending on whether it exists for each chromosome or if it is present within only one chromosome in the individual. To a certain extent, all female individuals exhibit mosaicism as a result of X inactivation. This is the most basic and common form of mosaicism, however, it can also take the form of a duplication of a locus, trisomy of one chromosome, and even be as severe as an individual possessing an additional copy of all chromosomes in one or more groups of cells.

Chimerism is very rare and has only been observed in a few cases worldwide. Of these, most are blood chimeras that developed two DNA profiles while sharing blood supplies with a non-identical twin while in the womb. Studies have begun to show that as much as 8% of non-identical twins result in blood chimerism. It is also speculated that because most multiple conceptions resulting in live births involve one of the twins being lost early in development, it is possible many single births result in some level of blood chimerism (Pearson, 2002). It is difficult to detect chimerism, as extensive genetic testing is required. It is also difficult to detect unless a female is carrying a population of male cells or vice versa (Pearson, 2002).

With the advancement of microsurgical techniques, antibiotics, and immunosuppressive agents, organ transplants have become much more routine in many clinical settings (Brouha and Ildstad, 2001). With this advancement, the problem of

increased levels of false chimerism within the population may arise. Through bone marrow transplants, organ transplants or the mere donation of blood, an individual is capable of obtaining an additional population of DNA that could ultimately be observed during profiling. The most common form of false chimerism is expected to be in bone marrow recipients, when the donor's DNA profile is found circulating within the blood of the recipient. Organ recipients also have the potential of exhibiting an additional DNA profile particularly when tissue from that organ is being tested. In clinical settings problems from this type of chimerism are not likely to occur due to documentation of the complete medical history of an individual, however, in forensic settings, unknown biological materials are often obtained from various different contexts, which may complicate matters.

c. Mitochondrial DNA Heteroplasmy

Mitochondrial DNA heteroplasmy is the occurrence of more than one mitochondrial DNA genome residing within one individual (Huhne *et al.*, 1999; Melton, 2004; Sekiguchi *et al.*, 2004). Heteroplasmy is becoming a more commonly observed phenomenon due to the increased sensitivity of the DNA sequencing chemistries that are available. Although homoplasmy is the baseline state for nearly all individuals, it is becoming generally accepted that some level of heteroplasmy exists in everyone. Given that mtDNA is inherited only maternally, and does not recombine, it remains unchanged from one generation to the next aside from mutations. Due to this feature, mtDNA typing cannot provide an individual profile in the same way that nDNA is capable (Hageman *et al.*, 2002). In saying this, the mitochondrial genome has been shown to have a mutation rate 6-17 times that of nDNA, making it possible to find individuals with differing

mtDNA profiles compared to their maternal relatives' (Bar *et al.,* 2000). When mutations do occur, heteroplasmy may be observed in one or more tissues within the same individual. Past studies of heteroplasmy have been conducted to determine the origin of diseases (Melton, 2004), and not until rélatively recently (Ivanov *et al.,* 1996) has mtDNA analysis and subsequently, heteroplasmy been utilized in forensic contexts. Heteroplasmy observed in forensic cases does not render the profile invalid (Bar *et al.,* 2000), but rather indicates with a much higher degree of certainty that a sample is a match to a suspect. Heteroplasmy can be seen in one of three ways within an individual; more than one mtDNA profile can exist in all tissues; a single profile may be shown in one tissue and a different profile in another tissue; and lastly, heteroplasmy in one tissue and homoplasmy in another (Bar *et al.,* 2000). Most reported cases of heteroplasmy involves a single mutation, however; the extent of intra-individual variation remains largely unknown (Jazin *et al.,* 1996). The degree of heteroplasmy as well as its detectability then becomes a major concern for researchers analyzing mtDNA sequence data (Melton, 2004).

New variations within the mitochondrial genome are primarily generated via mutation. Mutations are abundant in this genome due to the high degree of damage that the mtDNA is exposed to and the fact that it is not protected by being wrapped around histones, rendering mismatch errors much less likely to be detected and repaired (Bogenhagen, 1999). Damage is generated because of its location near oxidative phosphorylation and the byproducts of this metabolism, oxygen free radicals (Chinnery *et al.*, 2000; Melton, 2004). Whether heteroplasmy persists in a maternal lineage or is lost within a few generations seems to be a process of natural selection or random drift acting

on the molecules of mtDNA within the individuals germline (Chinnery *et al.*, 2000; Howell and Smejkal, 2000; Melton, 2004). Heteroplasmy is generally thought to be a transition state between two states of homoplasmy (Howell *et al.*, 1992; Chinnery *et al.*, 2000; Howell and Smejkal, 2000; Melton, 2004). In a study of Holstein cattle, the founder female had homoplasmy in the blood, however, many of the offspring showed heteroplasmy in the same sample type. The heteroplasmy in the offspring was quickly seen to revert back to homoplasmy within relatively few generations. In one case within this study, heteroplasmy was seen to exist and revert back to homoplasmy within only one transmission (Koehler *et al.,* 1991). Occurrences such as this have also been seen to exist in human pedigrees where novel mutations become fixed within one or a few generations (Blok *et al.,* 1997; White *et al.,* 1999). The main theory for the rapid elimination of heteroplasmy resulting in the restoration of homoplasmy has been attributed to the process of a genetic bottleneck.

The genetic bottleneck functions by a single germline mutation occurring in a founder female. This mutation is subsequently passed on to the offspring who would then exhibit some level of heteroplasmy. During the next generational transmission one of three events may occur; (1) the heteroplasmy may now persist in subsequent offspring (albeit at varying degrees); (2) the mutant genome may be negatively selected for and eliminated, resulting in the original genotype being restored; or (3) the bottleneck may select for the newly formed allele and segregate this into subsequent offspring, thus; fixing the new mtDNA genome into the population. The mechanism of this random selection is not entirely understood, but because the number of mtDNA molecules in the mature oocyte is approximately 100,000 (Chen *et al.,* 1995), it is necessary that the

number of segregating molecules be reduced to a very small quantity for the variant to be passed on to the offspring at a detectable level (Melton, 2004). Estimates based on both human and mouse studies using the Wright/Solignac model of genetic drift (Wright, 1968) have suggested that anywhere from 20 to 200 mtDNA genomes are segregated for at the bottleneck. Depending on the mitochondrial genomes selected for at the bottleneck, each offspring would yield differing levels of heteroplasmy and continuous selection in each successive generation would be required to completely fix the new variant into the population. Negative selection of the variant genome at any stage would result in the variant being completely lost and the wild-type restored. The genetic bottleneck would undoubtedly reduce the number of overall mutations that become homoplasmic, due to the sheer probability of selecting a few mutant molecules from extreme numbers of wild-type molecules, however, the bottleneck would function to increase the speed at which new variants do become fixed in the population (Howell *et al.,* 1996).

Although the genetic bottleneck theory is widely accepted as the reason for maintaining a state of homoplasmy, this theory does not explain the occurrence of persistent heteroplasmy. Theories such as the bottleneck and genetic drift only take into account rapid transitions originating at, and returning to, a state of homoplasmy while passing through a short period of heteroplasmy. Persistent heteroplasmy occurs quite frequently in families possessing mitochondrial DNA diseases (Chinnery *et al.,* 1999; Chinnery *et al.,* 2000) and does not appear to act under small bottlenecks or genetic drift (Howell *et al.,* 1992). There are a variety of reasons this may occur, beginning with the possibility that clusters of membrane bound mtDNA molecules are segregated as an

entire organelle unit rather than an independently segregating panmictic population (Howell *et al.,* 1992). Another reason these mutant genomes can remain within the matriline for extended periods of time might be because there are enough wild-type molecules to maintain adequate functioning of the cell even with the presence of the mutants (Taanman, 1999). This would suggest that forces of natural selection are acting for or against the mutant mtDNA molecule at the tissue level. It still remains to be seen whether or not this natural selection occurs during the developmental stages of the oocyte. This is due to the fact that the earlier studies of disease and its testing has only been conducted on one tissue type, usually blood samples of affected individuals and their matrilineal relatives (Melton, 2004). More testing on a greater number of tissues is required to gain a better understanding of the phenomenon.

Although the mitochondrial genome is considered to undergo only maternal inheritance, few reports have described the occurrence of paternal inheritance. Paternally inherited mtDNA molecules typically undergo ubiquitination and their subsequent destruction shortly after fertilization (Sutovsky *et al.,* 1999), however, paternal inheritance was observed to occur in one case (Schwartz and Vissing, 2002) where a man exhibited severe, lifelong exercise intolerance. The patient had a 2-bp deletion within the mtDNA from only skeletal muscle. Based on mtDNA sequencing of the patient's healthy parents, it was determined that the mtDNA genome from skeletal muscle was inherited from the father, without the 2-bp deletion, whereas the mtDNA genome in his blood was inherited from the patient's mother. Given that the patient's healthy father had the same profile in blood as the patient did in skeletal muscle without the 2-bp deletion indicated

that the mutation must have arisen in either early embryogenesis or in the father's germline (Schwartz and Vissing, 2002).

Mitochondrial DNA heteroplasmy can also arise spontaneously within an individual's soma and thus, is not the result of germline inheritance. Two possibilities exist as to when somatic mutation may occur, resulting in heteroplasmy. The first is heteroplasmy that has been present since birth and possibly arose due to mutation during early embryogenesis; and the second is heteroplasmy that arose due to somatic mutation at some stage throughout the individual's life and may or may not be tissue specific (Melton, 2004). Should heteroplasmy arise early during embryogenesis, this would impact studies of mutation rates relating to human evolution and matrilineal pedigrees, however, if a spontaneous mutation arises later in life and is tissue specific, it would greatly impact studies of intra-individual forensic comparisons where a biological sample obtained from a crime scene would exhibit a different profile when compared to a different tissue type from a suspect (Melton, 2004).

Variations within the mitochondrial genome have been shown to occur at different levels in the past between different tissues within the same individual. These variations have also been shown to increase with the age of the individual (Calloway *et al.,* 2000). In one study by Calloway *et al.* (2000), various tissue samples (blood, brain, muscle and heart) were obtained at autopsy from 43 individuals ranging in age from 11 to 85 years old. It was determined that the overall frequency of heteroplasmy was 11.6% and differed significantly across tissue type and with age. The greatest levels of heteroplasmy were in skeletal muscle samples from individuals over 60 years old, followed by brain tissue, then heart and blood. One proposed reason for this increase

with age is due to the increased exposure to oxygen free radicals throughout the course of one's lifetime. As well, exposure is more severe in tissues with high energy demands such as muscle, brain and heart. Similar results were obtained in another study by Jazin *et al.* (1996) where the levels of heteroplasmy were significantly higher in human brain tissues when compared to blood from the same individuals. There was also a 7.7-fold increase in insertions/deletions and a 2.2-fold increase in the overall level of heteroplasmy in individuals over 90 years of age compared to individuals in their late 20's.

Hair is another tissue that shows extensive variation in both the shaft and root when compared to different hairs and tissues from the same individual (Bendall *et al.*, 1997; Melton, 2004; Sekiguchi *et al.*, 2004; Melton *et al.*, 2005). In a review of 691 case studies, Melton *et al.* (2005) found that individual hair shafts exhibited heteroplasmy 11.4% of the time. In another study by Sekiguchi *et al.* (2004), when 160 hairs from each of three individuals were compared to blood, the hair was found to exhibit 4.4% to 5.8% (depending on the method of detection) heteroplasmy even when the blood samples from the same individuals showed only homoplasmy. There have been however, studies (Huhne *et al.*, 1999; Pfeiffer *et al.*, 1999) that show no difference in the profile generated in hairs compared to any other tissue in the body. These studies appear to be somewhat questionable due to small sample sizes, compounded by relatively low success rates.

The mutation rate of the mitochondrial control region has in the past been broadly reported. Mutation rates have been assessed in the range of 0.025 – 0.26/site/1 million years (Myr), and have been as extreme as 2.5site/Myr (Parsons *et al.*, 1997). One recent study (Sigurgardottir *et al.*, 2000) analyzed 272 individuals who were related by 705

mtDNA transmission events and came up with an intermediate mutation rate of 0.32site/Myr. To date, this is the largest study taking into account mtDNA transmission events and is therefore, considered to be a reliable estimate of the actual mutation rate.

Various nucleotide positions have been shown to undergo mutations at much higher rates than the vast majority of sites in the mitochondrial genome. These "hotspots," as they are more commonly referred to, are typically associated with neighboring homopolymeric tracts that are suspected of aiding in the mutation rate during replication at these positions (Melton, 2004). Some of the more commonly observed hotspots are at positions 16,093 and 16,229 in HVI (Tully *et al.*, 2000; Melton and Nelson, 2001) and positions 73, 152, and 189 in HVII (Calloway *et al.*, 2000). Polymorphic hotspots have great implications in forensic science as they are suspected to preferentially undergo postmortem damage more rapidly than other sites in the control region (Gilbert *et al.*, 2003a; Gilbert *et al.*, 2003b). If this were to occur, unsuspected mutations could potentially arise due to error early in the PCR process, yielding a high proportion of the mutant sequence being observed during the sequencing process (Melton, 2004).

Length heteroplasmy is the most common form of heteroplasmy in mtDNA. It is the occurrence of two or more mitochondrial genomes of varying length existing within one individual. This type of heteroplasmy typically occurs at homopolymeric C stretches, where the fidelity of the polymerase is decreased due to the increase in C residues. This is a result of strand slippage during replication or the repetitive incorporation of identical nucleotides causing an error by DNA polymerase (Melton, 2004). Length heteroplasmy may render a mitochondrial sequence unreadable due to

errors reading through the frameshift or due to difficulties reading the data downstream of the mutation (Melton, 2004). It would also be expected that length heteroplasmy be observed at higher rates in tissues with high energy demands. The increased replication of the mtDNA would facilitate the occurrence of strand slippage or errors, thus, generating a higher degree of variant genomes. It is currently thought that length heteroplasmy may be found in everyone at varying levels.

d. Error

During the generation of a DNA profile, errors can occur at any stage. For the purposes of this paper spontaneous mutations occurring *in vivo* will not be considered forms of error. For this study error will imply variations that occur *in vitro* throughout the process of generating and interpreting a profile. Errors can occur at many different stages throughout the analytical process and can result in the observation of many different artifacts within the DNA profile. Errors that occur during the analysis of DNA causing variations to be observed within the profile can be due to misincorporations by DNA polymerase, contamination, or PCR artifacts generated during the amplification of STR loci. PCR artifacts that occur during STR amplification are stutter products, non-template addition, microvariants, three-banded allele patterns, and allelic dropout.

Many of the errors generated can be attributed to the fidelity and processivity of the DNA polymerase employed during the analysis. Numerous studies have been conducted in attempt to determine the misincorporation or error rate of commonly used DNA polymerases. In one such study by Cline *et al.* (1996), six types of DNA polymerases were tested and it was determined that *Pfu* polymerase had the lowest error rate at $1.3X10^{-6}$ mutations/bp/PCR cycle followed by Deep Vent Polymerase ($2.7X10^{-6}$);

Vent polymerase ($2.8X10^{-6}$); *Taq* polymerase ($8.0X10^{-6}$); exo- *Pfu* and UlTma ($5.0X10^{-5}$). The 3'-5' proofreading capabilities of many DNA polymerases adds immensely to their fidelity. *Pfu*, Deep Vent, Vent, and UlTma are all polymerases that have this proofreading capability and thus, it is not surprising that all but UlTma have lower error rates than *Taq* and exo- *Pfu*. Due to the fact that the first thermostable DNA polymerase discovered was *Taq*, it is routinely used in many molecular facilities. It is necessary that the data obtained be analyzed carefully due to the fact that this polymerase has somewhat lower fidelity and no proofreading capabilities compared to the other polymerases. Since the fidelity of DNA polymerases is not 100%, it is likely that scientists will occasionally encounter anomalies like unsuspected polymorphisms. In instances such as this is important to replicate the results prior to reaching any conclusions about the sample to ensure the authenticity of the data. The fidelity of DNA polymerases is also greatly affected by the PCR reagent concentrations and cycling parameters, therefore, optimization of the PCR may be required if copious levels of error are frequently being observed (Eckert and Kunkel, 1990).

It has been suggested by aDNA analysts that cloning be carried out, along with conventional sequencing, for the analysis of highly degraded samples to add to the validity of the data obtained. In addition to cloning, a variety of other suggestions have been made by these researchers to ensure the validity and authenticity of the data (Hofreiter *et al.,* 2001b; Paabo *et al.,* 2004). These have been recommended since modern DNA can very easily contaminate highly degraded samples; as well more errors are expected to occur due to the high degree of damage to the DNA.

Aside from errors initiated by standard PCR and sequencing, artifacts can and do occur during the analysis of STR profiles. These are such things as stutter products, non-template addition, microvariants, three-banded allele patterns, and allelic dropout. Stutter products are allele lengths that are either one repeat unit smaller or larger (most often smaller) than the main allele length. The occurrence of stutter products have been reported since microsatellites were first described (Butler, 2005). These arise *in vitro* in much the same way spontaneous mutations occur *in vivo*, due to strand slippage (Figure 14) (Hauge and Litt, 1993). When analyzing electropherograms of STR profiles in forensic contexts, minor allele lengths that make up less than 15% of the major allele lengths are considered insignificant. Should the stutter product be greater than 15% of the major allele length, contamination, multiple donors or trisomy must be considered. The levels of stutter vary greatly depending on the locus tested and typically, loci with shorter repeat lengths exhibit greater levels of stutter (Butler, 2005). Similarly, the degree of stutter increases with allele length or repeat number and decreases if the sequence of the repeat is imperfect (Walsh *et al.,* 1996). As with standard PCRs and sequencing, the fidelity and processivity of the DNA polymerase employed has a great effect on the level of stutter during the amplification of STR. Slower processivity has shown in the past to increase the amount of stutter (Walsh *et al.,* 1996). This is likely due to the increased time allowed for the two strands of DNA to become dissociated and then re-anneal incorrectly prior to completing the amplification of the allele (Butler, 2005).

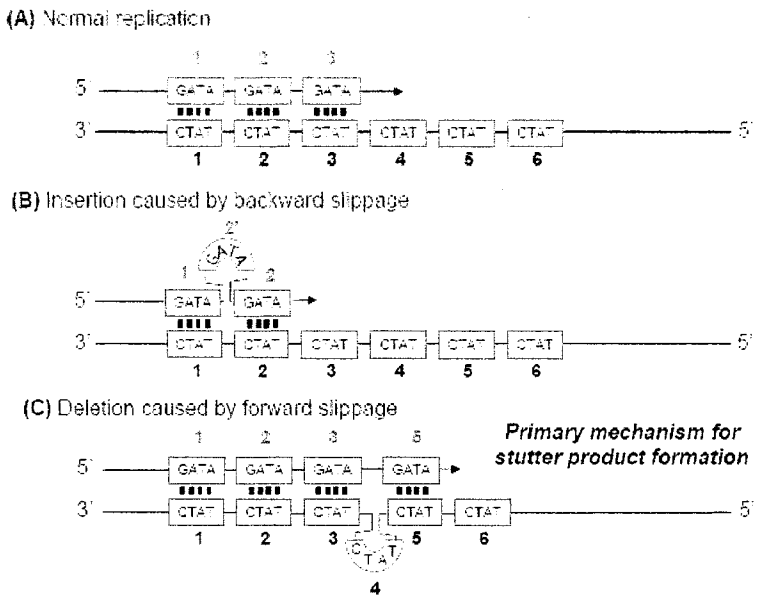



Figure 14: Illustration of slipped-strand mispairing process during PCR (Butler, 2005).

Adenylation is also an artifact of the PCR, however, it does not usually cause as much of a problem as most of the other artifacts when dealing with STR profiles. It occurs when DNA polymerase adds an additional adenine residue to the 3' end of the target sequence (Clark, 1988; Magnuson *et al.*, 1996). This is an artifact most commonly seen when using *Taq* DNA polymerase and does not occur 100% of the time. When this occurs during STR profiling the result is that some of the loci will show alleles that are one nucleotide longer than the target sequence, thus giving the appearance on the electropherogram that the main allele peak has a shoulder peak attached to it (Figure 15). The extent of the non-template addition, or adenylation, is highly dependent upon the template sequence. Due to the fact that the 5' end of the reverse primer determines the most 3' nucleotide on the labeled fragment of DNA (given that the forward primer is labeled), this position greatly affects whether adenylation will occur or not (Magnuson *et al.*, 1996). It has been shown that if the 5' end of the reverse primer is a guanine, the frequency of adenylation is approximately 100% (Brownstein *et al.*, 1996). Ensuring that

all copies of an allele are adenylated during STR amplification is important because at certain loci where both the fully adenylated allele and non-adenylated alleles are recognized as common allele lengths, it may appear as though the individual is one repeat unit shorter than they truly are. This is true for the TH01 locus where the difference between allele lengths 10 and 9.3 is one nucleotide. A non-adenylated 10 allele would appear the same as a fully adenylated 9.3 allele (Butler, 2005). There are a variety of ways to overcome partial adenylation during PCR; however, the most commonly used method is to add a final extension at the end of the PCR. This gives the DNA polymerase extra time to fully adenylate all copies of DNA. Fully adenylating the DNA fragment can also be achieved by ensuring that the non-fluorescently labeled primer in the reaction begins with a 5' guanine.

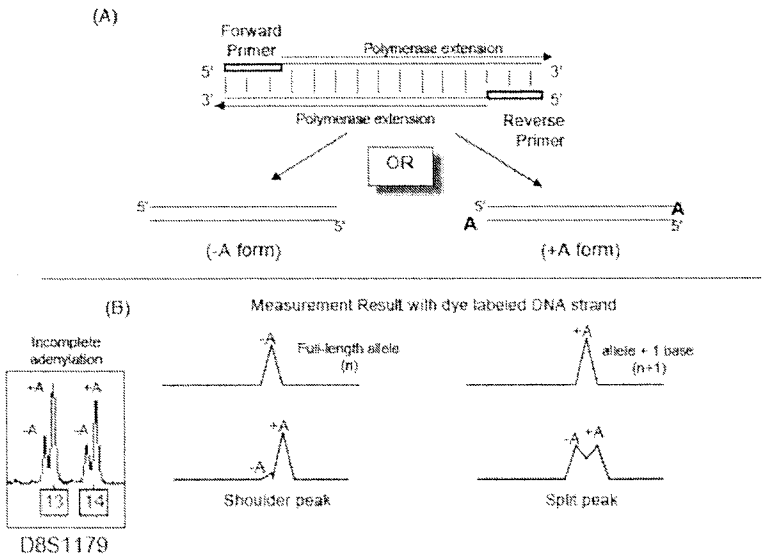



Figure 15: Schematic of non-template addition shown (a) with illustrated measurement result. (b) as the product appears on the electropherogram (Butler, 2005).

Microvariants or, off-ladder alleles, as they are more commonly referred to, are occasionally observed during STR typing. An off-ladder allele is an allele length that is not the same as any allele length provided within the STR kit. These occur when there is

an insertion or deletion within the individual's DNA that is not commonly observed within the general population. There are a number of ways that microvariants have been recorded and reported. The analyst could report the microvariant as the allele length followed by a '.x'(for example 9.x) (Crouse *et al.,* 1999). If the microvariant lays outside of the allelic ladder, it could be recorded as being either '>' or '<' the nearest allele length (Crouse *et al.,* 1999). The most common way to record the off-ladder allele is to label it as its calculated repeat length (Butler, 2005). For example, at the TH01 locus, the repeat motif is a tetranucleotide. For allele lengths of 10, there are 10 full repeats of AATG. The allele length 9.3 has 9 full repeats of AATG but in the seventh repeat there is a deleted A (Puers *et al.,* 1993). Due to this deletion there are only 3 of the 4 nucleotides present at this position, which explains where the .3 in 9.3 originates. As more research is conducted, more of these uncommon alleles are being added to the ladders in the STR kits, alleviating this problem. In forensic cases where crime scene samples are compared to suspect samples, the presence of a microvariant at the same position within two samples would increase the power of discrimination when identifying the donor of the sample.

Three-banded allele patterns are another artifact occasionally observed in STR profiles. The occurrence of three-banded allele patterns could indicate contamination of the sample, trisomy, duplication of the locus, or strand slippage (Figure 16). Contamination could be ruled out relatively quickly by analyzing the rest of the STR profile. If contamination were the case, three-banded allele patterns would be expected to occur at multiple loci throughout the profile. Similarly, strand slippage should also be ruled out relatively easily because two of the three alleles should only be one repeat unit

apart. If this is the case, stutter could be assumed. Should only one locus in the STR profile exhibit a three-banded pattern, and the alleles are approximately equal intensities, trisomy or duplication of the locus must be seriously considered. In any case, the data should be replicated prior to any conclusions being drawn.

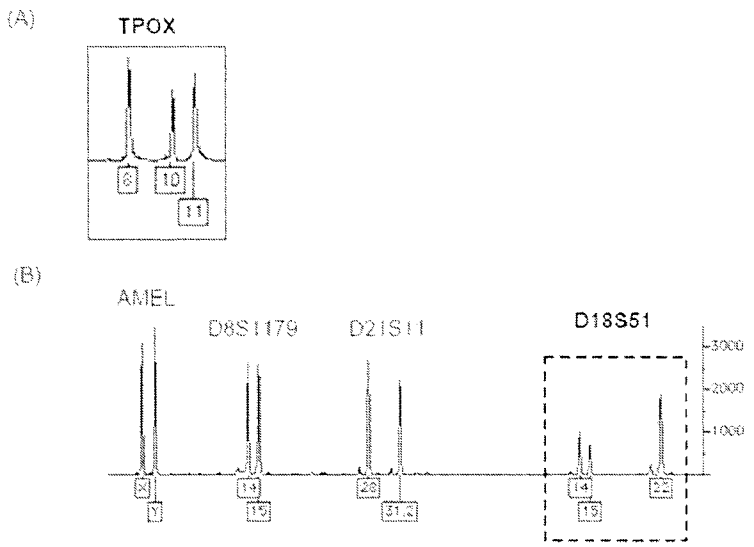



Figure 16: Three-banded allele patterns at (a) TPOX and (b) D18S51 loci (Butler, 2005).

The final artifacts resulting from PCR errors that will be discussed are allelic dropout, and null alleles. Allelic dropout occurs as a result of mutations taking place within the primer binding region of a microsatellite locus (Figure 17 and Figure **18**) (Butler, 2005). Mutations can also occur in the STR itself causing incomplete extension of the target region and the DNA polymerase to fall off the template (Figure 17). Alternatively mutations in the region between the primer binding site and the repeated elements can also occur having the same affect of the DNA polymerase falling off (Figure 17). Mutations such as these can result in the observance of a null allele, which could potentially lead to intra-individual differences within the DNA profile. This would have severe consequences since a false exclusion or false inclusion could potentially

occur in forensic cases. Allelic dropouts occurring in a genotypically normal person would give the appearance that a heterozygote is homozygotic. This phenomenon may also occur in cases of trisomy, thus, generating a "normal" DNA profile for a person who is trisomic at a given locus.

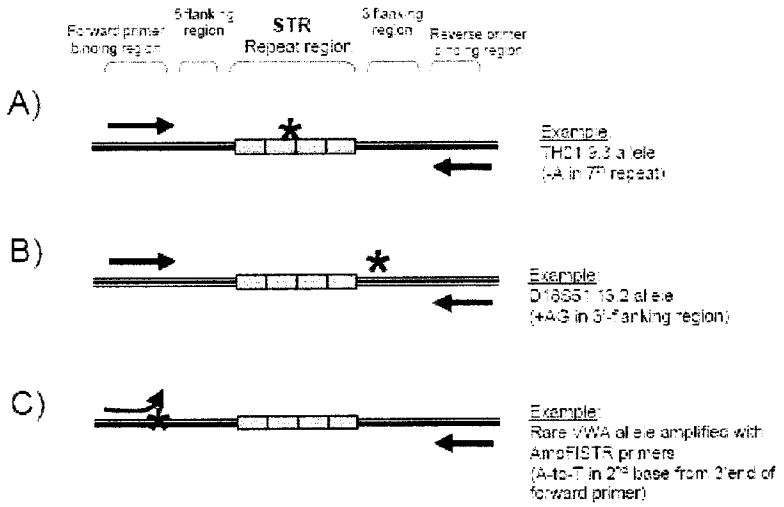



Figure 17: Image showing mutations in or around STR loci causing (a) different allele length, but no effect on primer binding; (b) differing product length, but will not prevent amplification; (c) allelic dropout due to the mutation occurring in the primer-binding region (Butler, 2005).

Addressing null alleles may be problematic; however, there are solutions to this problem. When analyzing crime scene evidence and comparing it to a suspects' DNA, whichever sample type is collected from the crime scene should also be obtained from the suspect (i.e. blood, hair, etc.). This way it can be ensured that the same profile (with or without dropout) would still be generated. It is also necessary that all known and unknown samples be processed using the same protocols and procedures. This ensures that any differences in the profiles are actual differences in the DNA and not a result of differences in the profiling kit. Another way of avoiding allelic dropout is to lower the annealing temperature for specific primers. This way even if there is a mutation within the primer-binding region, the primer may still bind, however this may also cause non-

specific amplification to occur as well (Figure 18). If the mutation is known to occur in a primer-binding region, new primers incorporating this mutation may be used to successfully amplify the locus of interest (Butler, 2005).

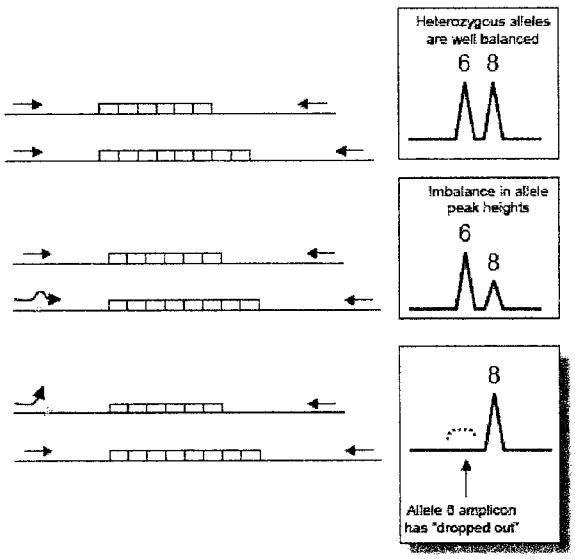



Figure 18: Schematic showing the impact of sequence polymorphism in the primer binding site illustrated with a hypothetical heterozygous individual. Heterozygous peaks may be well-balanced, imbalanced, or exhibit allelic dropout (Butler, 2005).

## VIII. Methodological Background

### i. Sample Preparation

a. Sample Pretreatment

In general, when a DNA testing laboratory receives a biological sample, it will have to undergo some form of preparation prior to the extraction of DNA. There are a variety of sample preparation procedures that may be used; however, the most ideal depends upon the sample type being analyzed. In the majority of cases this preparation step is necessary to remove potential surface contaminants, as well as render the sample manageable in terms of size. As is the case in most aDNA studies, the analysis is typically conducted on bone, teeth, tissue, hair or some other well preserved biological material. In these cases, all surface contamination must be removed prior to extraction. This is due to the fact that modern contaminating DNA from handlers will undoubtedly out compete the aDNA during the analysis. Surface decontamination typically involves a series of washes with detergents, bleach and ethanol followed by surface irradiation with UV light. Further preparation of samples includes cutting, crushing, tearing, or drilling to increase the surface area for extraction purposes. The majority of the time modern samples require very little preparation prior to extraction due to the high molecular weight and high copy number of DNA existing within these samples. Careless handlers still contaminate modern biological samples; however, the DNA within the modern sample often out competes the contaminating DNA during subsequent amplifications. Typically with non-fluid modern samples, a brief bleach and ethanol wash is all that is required in preparation for a sample to be extracted.

b. DNA Extraction

The liberation of nucleic acids from a biological sample is perhaps the most critical step of its analysis. Selecting the most appropriate method of extraction is crucial for maximizing the yield of DNA and often depends on the sample type. In the early stages of an investigation, the wrong decision of what extraction method to use could potentially destroy all information available from the sample (Hummel, 2003). Although the importance of this step cannot be stressed enough, there seems to be a consensus as to which methodologies are most appropriate (Hummel, 2003). For forensic cases, the proteinase K (PK) extraction method (Hansen, 1974) appears to be the standard while aDNA research laboratories most commonly use the guanidinium thiocyanate (GuSCN) extraction method (Boom *et al.,* 1990). These are not the only two extraction methods available to the scientist as there are many other techniques that are available. It is important that the DNA analyst have a strong understanding of the biochemical processes occurring within the reaction in order to determine the best procedure to follow (or modify).

The extraction buffers from various methodologies contain a few common reagents. Tris-HCl, and ethylenediaminetetraacetic acid (EDTA) are used to chelate the cell walls and other molecules present within the sample and stabilize the DNA; sodium or potassium salts are utilized to create an isotonic solution to stabilize nucleic acids; and proteinase K is used to destroy proteins present within the solution (Herrmann and Hummel, 1994), and lyse the cell through the degradation of transmembrane proteins. Often detergents are incorporated into the extraction buffer to disrupt the phospholipid bilayer and gain access to the intracellular contents. For soft tissues, this extraction step

will only take a few hours while hard tissues require much longer incubation times with higher concentrations of EDTA to chelate the hydroxyapatite mineral content (Herrmann and Hummel, 1994).

c. Separation/Purification/Concentration

After the extraction of DNA from the sample of interest, some form of separation, purification or concentration is nearly always required. The choice of purification procedure is at the discretion of the analyst; however, it is essential that it be chemically compatible with the extraction protocol used. During the separation step the sample is usually centrifuged for a period of time in order to physically isolate the DNA from the rest of the cellular debris. This separation step can also occur chemically where reagents of differing densities or polarities are mixed together resulting again in the DNA fraction being separated from other unwanted cellular debris. During the final purification step, the DNA is washed using one of a variety of reagents, which are usually ethanol based, to remove any remaining molecules or substances that could potentially interfere with subsequent methodologies. This purification step typically results in the purified DNA sample being dried in a sterile microcentrifuge tube or suspended in either a buffered solution or sterile water.

Particularly in aDNA studies, further purification is required to remove inhibitors present in the sample. Such inhibitors can include tannins, humic acids, metals, and other molecules such as divalent cations that can potentially be co-purified, and interact negatively with the DNA. Such inhibition can be removed by further washing the sample of DNA, selective binding of the DNA to a resin, or filtering the sample through the use of a size exclusion or bind-elute column (Herrmann and Hummel, 1994).

Many of the same methods employed for extract purification are used for both post-PCR clean-up, as well as post-sequencing purification. Post-PCR clean-up is used prior to sequencing reactions, or further amplifications such as nested, hemi-nested, or booster PCRs. This is necessary to remove any unincorporated deoxynucleotide triphosphates (dNTPs), $Mg^{2+}$ ions, or primers that may potentially interfere with these subsequent reactions. Typically, this purification is carried out using what are known as bind-elute spin columns, which utilize the silica binding properties of DNA in order to first bind the molecules to a silica membrane. Following this, a series of washes are applied to the column, before finally eluting the DNA using an elution buffer (EB) or water.

After the sequencing reaction is complete it is necessary to again purify the sample prior to detecting the sequence using capillary electrophoresis. This is done in order to remove any unincorporated fluorescently labeled dideoxynucleotide triphosphates (ddNTPs) that can interfere with the read of the sequence. This is accomplished using a variety of procedures including size exclusion spin columns or ethanol precipitation. Size exclusion columns separate DNA fragments from other materials present during the sequencing reaction on the basis of size. There are a variety of size exclusion columns commercially available; however, dye terminator removal (DTR) columns are usually always used for filtering the sequencing fragments from the unincorporated fluorescently labeled dye terminators. DTR columns should not be confused with other size exclusion columns that are used, for instance, to remove inhibitors or purify DNA extracts that are capable of filtering higher volumes of sample without reduced efficiency. DTR columns are typically only capable of efficiently

purifying small sample volumes. Various size exclusion columns also filter different sized fragments and should be selected based on the size of the material that is to be purified.

### ii. Polymerase Chain Reaction (PCR)

Kary Mullis developed the polymerase chain reaction (PCR) in 1985 for which he was awarded the Nobel Prize in 1993. This first method of enzymatic amplification *in vitro* was published in 1986 (Mullis *et al.*, 1986) and gave way to significant changes in the way scientists would conduct research. PCR reduced the need to dismiss a forensic sample, or other type of biological specimen because the sample size was considered too small for analysis. "Molecular Xeroxing," as it became referred to, was capable of taking minute quantities of starting template DNA and exponentially amplifying the particular region of interest millions of times. The amplification works by initially denaturing double stranded DNA by heating, resulting in single stranded DNA in solution. An annealing step follows the denaturation where the temperature is lowered and synthetic olgionucleotide primers, specific and complementary to the region flanking the locus of interest, anneal. Once again, the temperature is raised and the enzymatic activity of the DNA polymerase synthesizes the new strand of DNA beginning at the 3' end of the primer.

The initial method developed by Mullis did have some drawbacks such as the problem of denaturing the enzyme (DNA polymerase) which was responsible for synthesizing the new DNA strand every time the sample was heated to 95°C. Heating to this temperature is a necessity as it is required to denature the two strands of DNA prior to the next round of amplification. This required a new DNA polymerase be added prior

to each phase of extension. Due to the fact that the researcher needed to be present during each PCR cycle in order to add extra polymerase, this method became extremely time consuming and would not allow for automation. This was the case until 1988 when Saiki *et al.* discovered a heat stable DNA polymerase from the bacterium *Thermus aquaticus* (*Taq*). *Taq* DNA polymerase, as it is called, is capable of withstanding the high temperatures during denaturation and remains functional during subsequent periods of extension. This discovery allows the amplification process to occur in a shorter period of time, given that there is no need to stop the reaction after each cycle.

Currently, there are a wide variety of different PCR techniques that can be carried out depending on the type of amplification that is to be achieved. Some of the more commonly used amplification mechanisms in forensics and aDNA studies are termed standard PCR, Hotstart PCR, Short Tandem Repeat (STR) amplification, nested or hemi-nested PCR and DNA sequencing PCR.

a. Standard PCR

A standard PCR can also be referred to as a species specific PCR. It typically has three phases to each cycle as was mentioned previously. The denaturing step is typically carried out at approximately 94°C for about 60 seconds (Hummel, 2003). The primer annealing step typically occurs for 60 seconds between temperatures in the range of 50°C to 60°C (Hummel, 2003). These annealing temperatures can vary greatly and are ultimately determined by the melting temperature of the primers along with the desired degree of specificity the analyst requires. The extension phase typically occurs at 72°C and lasts for approximately 60 seconds (Hummel, 2003). This time can also vary depending on the size of the amplicon and the processivity of the polymerase used.

These three phases of PCR constitute one cycle. Depending on the amount of starting template in the PCR the number of cycles will also vary. Typically for modern DNA extracts an excess of 35 cycles is not necessary while for ancient DNA extracts 45 cycles of amplification is not uncommon. In theory, each cycle of PCR results in 100% duplication of the DNA template present within the reaction. Therefore, the number of cycles in a PCR should depend on the quantity of starting template given that amplification occurs exponentially.

The efficiency of the PCR greatly depends on the concentrations of the various reagents present in the reaction mixture. Although the reagents used in the PCR can be purchased from a supplier in the form of a kit, the analyst can make some of the components and then purchase the remainder. All PCRs require the presence of a buffer, which typically contains a variety of the following chemicals: 10 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$ or $MgSO_4$. Frequently the magnesium is omitted from the PCR buffer and is added separately to the reaction mixture. Tris-HCl has been considered the most important component of this buffer because it is a dipolar ionic buffer that maintains a pH between 6.8 and 8.0 at the varying temperatures throughout the reaction (McPherson and Moller, 2000). KCl plays a key role in the primer/template interactions however, at increased concentrations it can result in non-specific amplification (McPherson and Moller, 2000). The concentration of magnesium is significant in determining the specificity of the DNA polymerase, the interaction of the free nucleotides in solution, the binding properties of the primer and the denaturation of the template DNA. While magnesium concentrations too high will result in reduced fidelity of the DNA polymerase, concentrations too low may result again in non-specific amplification

(Erlich, 1989; Henegariu *et al.*, 1997; McPherson and Moller, 2000). It is for these reasons that it may be beneficial to add the magnesium separately to the reaction mixture as opposed to adding it directly to the buffer.

In addition to the reaction buffer, deoxynucleotide triphosphates (dNTPs) must be present for the polymerase to synthesize the new DNA strand. Concentrations ranging from 50 to 200 μM of each dATP, dGTP, dCTP and dTTP should be added to the reaction while care must be taken because too high of concentration will result in errors by the polymerase and concentrations too low will result in the reaction being depleted, ceasing amplification (Erlich, 1989; McPherson and Moller, 2000). Primers are also necessary for amplification to occur as the newly synthesized DNA strand is built upon their 3' ends.

The DNA polymerase enzyme is added in concentrations of approximately 0.5 to 5 units per reaction. The concentration of polymerase added to the reaction mixture is dependent upon the amount of starting template and the number of cycles in the PCR. For longer PCRs, more polymerase would be required as the fluctuations in temperature over many cycles degrades the enzyme and reduces efficiency. Some DNA polymerases, however, can withstand higher temperatures for longer periods of time such as Deep Vent® DNA polymerase. This is the most thermostable DNA polymerase that has a half-life of 23 hours at 95°C or 8 hours at 100°C (NEW England Biolabs; www.neb.com). As well, if the reaction is starting with relatively high template concentrations, it is necessary to add more polymerase to ensure exponential amplification. In most standard PCRs the thermostable *Taq* polymerase is used. This polymerase is essential to the reaction as it is capable of maintaining its functionality after repeated fluctuations in temperatures from

as low as 35°C to as high as 97°C. This enzyme is responsible for synthesizing the new DNA strands extending from the 3' end of each primer. Should polymerase be added in too low of concentration, little to no amplification will occur within the PCR.

The last two elements added to the standard PCR are sterile $H_2O$ and the DNA template itself. The concentrations of these two components are entirely dependent upon one another. Standard PCRs can be anywhere from 25 μL to 100 μL in total volume. Given that in a 25 or 50 μL reaction, all the other components in the reaction make up approximately 5.2 or 10.4 μL of the total volume respectively; the remainder of the volume is to be made up using a combination of template and sterile $H_2O$. For reactions amplifying modern DNA extracts 1 to 2 μL of template is added (whether it is diluted or not is up to the analyst) meaning that the rest of the reaction volume is made up with sterile $H_2O$. The addition of sterile $H_2O$ is necessary as it allows the volumes of the other reagents to be at their optimal concentrations while at the same time creating enough volume for the reaction to take place. For aDNA extracts 10 μL of template is typically added into a 25 μL reaction and the remaining volume consists of sterile $H_2O$. The reason such high volumes of aDNA extracts are added to the reaction is due to the fact that the DNA is highly degraded and that the DNA is present in extremely low copy numbers. This is not a problem for modern DNA and thus, we see the difference in the volume of template added to the reaction. Typically, a desirable starting template concentration will be in the order of $3.0 \times 10^5$ molecules of DNA (McPherson and Moller, 2000).

A detection PCR is a form of standard PCR and is usually carried out for a variety of reasons. It is typically used to determine the success of the extraction procedure, or in

preparation of further analyses such as sequencing. Detection PCRs are typically used to simply determine the presence or absence of DNA after extraction. This technique is used in aDNA to identify if there is enough DNA in the extract and can be used to identify if there is any inhibition present.

b. Primer Selection and Primer Design

One of the most important aspects of the PCR is the proper selection of primers. Two primers are required that are complementary to the DNA sequence flanking the region of interest for the vast majority of PCRs. If the sequence of interest is known, then it is relatively easy to develop primers for the amplification of a given locus (McPherson and Moller, 2000). Aside from the location of the primers, there are a few important rules that should be followed when designing a primer. The primer should be approximately 18 to 30 nucleotides long with a G/C content of 40 to 60%. The annealing temperature can be quite variable, but should be close to that of the other primer used during the reaction. Primers should be designed to avoid repetitive sequences or stretches repeating the same nucleotide in tandem as this facilitates slippage during the annealing phase (McPherson and Moller, 2000). Although it is important to have a C or G at the 3' end of a primer, it is not beneficial to have more than about 3 or 4 as this leads to mispairing at GC-rich regions of DNA (McPherson and Moller, 2000). It is also important to avoid primer sequences that allow the formation of secondary structure due to internal complementarity, or sequences at the 3' end that will facilitate base pairing with itself or the other primer in the pair (referred to as the formation of primer-dimers) (McPherson and Moller, 2000). Primer-dimers are formed by the annealing of one or both of the primers in the reaction, followed by its extension by DNA polymerase. The

newly formed primer-dimer will then become the template in further cycles, consuming much of the primer required for actual amplification of the target sequence. Since smaller target sequences are preferentially amplified, primer-dimers often dominate the PCR, again taking away from amplification of the desired target (McPherson and Moller, 2000). Fortunately, there are a variety of computer programs, both free and available for purchase that analyze one or more primer sequences for the occurrence of secondary structure or complementarity within or between primers. Often, especially in aDNA studies, primer-dimer formation is beneficial as it indicates to the analyst whether the PCR has been inhibited, or if there is not enough template added to the reaction.

The 3' end of the primer should end in either a cytosine or guanine residue. This is to ensure that strong binding occurs at this end because it is here that extension by the DNA polymerase occurs (McPherson and Moller, 2000). This is not the case for the 5' end of the primer where it is possible to add a variety of sequences or labels. It is not uncommon to add a restriction site at the 5' end of a primer to make the PCR product more desirable for incorporation into a vector afterwards for cloning (McPherson and Moller, 2000). Fluorescent labels are also commonly added to the 5' end for easy detection of fragments after amplification. This is generally done for STR reactions or other instances where fragment size detection is desired.

c. Nested or Hemi-nested PCR

A nested or hemi-nested PCR is merely an extension of the standard PCR. A nested PCR is done subsequent to the standard PCR with a new set of primers amplifying a target sequence internal to that of the original amplification. A hemi-nested PCR is similar to the nested PCR, however, only one of the two primers are internal of the

original and the other primer is the same as was used during the original PCR. Both of these techniques are commonly used when amplifying samples of extremely low copy number template. Prior to the nested or hemi-nested PCR it is necessary to purify the products of the standard PCR to ensure that there will be no interference of the previous reagents. Ideally, after the standard PCR, there will be a much higher starting copy number for the nested or hemi-nested PCR to build from. It is estimated that nested PCR leads to a $10^4$-fold increase in sensitivity of the detection of the desired product (McPherson and Moller, 2000). Even if the initial PCR product is poorly represented within a background of non-specific products, it will amplify preferentially due to the unlikelihood that the non-specific products contain the same nucleotide sequence as the new nested primers (McPherson and Moller, 2000).

d. Short Tandem Repeat (STR) PCR

Short tandem repeat PCRs can be either multiplex PCRs, where multiple loci are amplified simultaneously, or can be singleplex PCRs where only one locus is amplified in each reaction. Typically, STR amplifications are multiplex reactions using one of the many kits available for purchase. Multiplex PCR was developed in 1988 (Chamberlain *et al.,* 1988) and since then has been used for a variety of applications, none more frequent then STR analysis (particularly in forensic settings). For aDNA studies the analysis of STRs adds to the individualization of the sample and renders results less susceptible to criticisms of contamination. The reason for this being that if many loci are analyzed at one time, the less likely the data obtained is due to random contamination (Hummel, 2003). Much of the time with aDNA studies it is easier to conduct singleplex STR amplifications given that there is no competition between multiple primer sets for the

very small number of starting DNA molecules. Also, for aDNA studies, it is possible to apply a nested or hemi-nested PCR for amplification of a STR locus in order to maximize the amount of information obtained from a sample.

All STR primer sets that are purchased in a kit come fluorescently labeled for easy and rapid detection using capillary electrophoresis. Within these kits there are three reagents. A reaction buffer, primer set mixture and DNA polymerase. Because these kits are patented, all information on the contents of the buffer or primer sequences is kept confidential. Therefore, to create the reaction mixture, the researcher merely mixes the indicated concentrations of each reagent and has little control in modifying the reaction.

A variety of polymerases can be used for STR reactions; however, most kits come with a hotstart DNA polymerase, such as AmpliTaq Gold. A hotstart polymerase is an enzyme that has an antibody or some other molecule attached to it or is modified in some capacity to require incubation at a specific temperature (typically 95°C), for an extended period of time (usually 10-11 minutes) prior to becoming activated. Hotstart polymerases are extremely beneficial because the PCR mixture can be created at room temperature without the danger of any reaction occurring prior to the activation of the polymerase. Non-hotstart polymerases have the potential to begin the amplification process at room temperature before the samples are placed in the thermocycler. This has the potential to generate many nonspecific PCR products prior to the start of the reaction. Also with hotstart PCR, the extended incubation before amplification begins, ensures that all primer/template interactions are melted prior to the start of amplification. This eliminates any nonspecific products generated during the first round of amplification.

The cycling conditions of the STR PCR are very much the same as for standard PCRs where all primers within the multiplex are designed to anneal at one particular temperature. Should some of the primer pairs within the reaction anneal at differing temperatures it is not uncommon to have two sets of cycling parameters within one reaction where the first few cycles are carried out at a relatively high annealing temperature and the remainder of the reaction is carried out at a lower annealing temperature. This method of cycling is used for the PowerPlex® Y-STR kit (Promega Corporation) where the first 10 cycles have an annealing temperature of 60°C and the remaining 22 cycles use 58°C as the annealing temperature.

An additional step at the end of the STR amplification process, which is common in any PCR where size detection is required, is a final extension. The final extension is usually carried out at approximately 60°C and lasts anywhere from 30 to 60 minutes. This is necessary due to the natural tendency of *Taq* polymerase to add an additional adenine residue at the end of the newly synthesized strand of DNA. To ensure that all synthesized DNA strands are fully adenylated, a final extension is incorporated into the cycling parameters. If this step were avoided, a mixture would be observed where both the fully adenylated and unadenylated forms of any allele would differ by one base pair.

Detection of any fluorescently labeled strand of DNA is typically carried out using capillary electrophoresis. Capillary electrophoresis is a benefit for multiplex reactions where the primers are fluorescently labeled, because it has the capacity to detect multiple colours at the same time. This is not much of a benefit for singleplex reactions however where there is only one fluorescently labeled product to detect at any given time. With the increasing number of loci being analyzed in multiplexes currently, it is

important that multiple coloured tags can be detected simultaneously since many loci are similar or overlap in size. When two loci of interest are similar in size, one locus can be labeled with one colour while the other locus is detected using a different colour. This way the analyst can analyze many different loci in one reaction without concerns of how each will be detected.

e. DNA Sequencing

DNA sequencing is an important application of many fields such as archaeology, anthropology, genetics, biotechnology, molecular biology and forensic science (among others) (Franca *et al.,* 2002). To date there have been numerous methods of DNA sequencing developed with the first coming in the mid 1970's. These methods include the Sanger method; the Maxam & Gilbert method, pyrosequencing, and single molecule sequencing with exonuclease. The first method of DNA sequencing was developed in 1975 by Sanger and Coulson and was referred to as the 'plus and minus' sequencing method (Sanger and Coulson, 1975). This method utilized *Escherichia coli* (*E. coli*) DNA polymerase I and polymerase from bacteriophage T4 (Englund, 1971; Englund, 1972) with differing nucleotide triphosphates (Franca *et al.,* 2002). Two years later, in 1977, Sanger *et al.* developed a more efficient sequencing method that utilized enzymatic polymerization. This method became known as the Sanger dideoxynucleotide method, or the chain terminating method. A modified version of this method is currently the most routinely used method for sequence analysis in molecular biology and can be automated. This method consists of an enzymatic reaction that polymerizes the DNA strands complementary to the template and begins its synthesis from the 3' end of a $^{32}$P-labeled oligonucleotide primer (Sanger *et al.,* 1977). The new DNA strand was synthesized by

incorporating dNTPs until a terminating nucleotide or dideoxynucleotide triphosphate (ddNTP) was incorporated into the strand. This modified nucleotide immediately terminates the extension of the growing DNA strand due to the absence of a hydroxyl group on the 3' carbon of the dideoxyribose sugar (Sanger *et al.,* 1977). The method consisted of four reactions occurring in tandem where each of the reactions contained one of the four ddNTPs. Therefore, all fragments had the same 5' end, however, the residue on their 3' end was dependent upon the ddNTP used in the reaction (Franca *et al.,* 2002). The random incorporation of these ddNTPs generated fragments of every possible length, which were then detected using denaturing polyacrylamide gel electrophoresis where the products from each of the four reactions were run in parallel (Franca *et al.,* 2002). The pattern of bands visualized following autoradiography of the gel indicated the precise sequence of the DNA. An alternative and more commonly used variation to this method was introduced in 1986 where fluorescently labeled ddNTPs were used in the reaction as opposed to the labeled primer (Smith *et al.,* 1986). This allowed for the entire sequencing reaction to occur in only one tube.

There are two major approaches to the Sanger method that are commonly used. The first is shotgun sequencing and the second is primer walking (Griffin and Griffin, 1993). Shotgun sequencing is a random process where the analyst has no control over where sequencing takes place (Franca *et al.,* 2002). This method begins with extensive fragmentation of the DNA followed by cloning. Successfully cloned products are then sequenced extensively with many overlapping sequences being generated (Adams *et al.,* 1996). Due to the extreme redundancy in sequences, it is possible to piece together or 'assemble' the complete unknown sequence. Caveats to this method are the cost of

replicating all sequences multiple times and the large amounts of time required for sequence assembly (Franca *et al.,* 2002). In 1996, a variation of this method was introduced by Venter *et al.*, which made possible shotgunning entire genomes at once. This novel method ultimately led to the rapid sequencing of the entire Influenza virus and human genomes (Venter *et al.,* 2001).

The second approach to the Sanger method is primer walking, which is a direct approach to sequencing an unknown fragment of DNA (Franca *et al.,* 2002). For this method, an unknown sequence of DNA is amplified in a vector. The primers used for the sequencing reaction are specific to the vector, flanking the region of unknown DNA. Once this sequence is generated, it is then possible to create a primer downstream within the region of interest and conduct another sequencing reaction. This is the process of primer walking (Studier, 1989; Martin-Gallardo *et al.*, 1992), which reduces the cost and redundancy of sequence information (Voss *et al.,* 1993) when comparing it to shotgun sequencing.

Another sequencing method introduced to the scientific community was based on chemical degradation and created by Maxam and Gilbert in 1977. This method uses end labeled DNA fragments that are subjected to random cleavage of any of the nucleotide positions using specific chemical agents (Franca *et al.,* 2002). The chemical attack is based on three mechanisms: base modification, excision of the modified base from the deoxyribose sugar, and DNA strand breaking at the sugar position (Maxam and Gilbert, 1977). These reaction products are then separated using polyacrylamide gel electrophoresis (PAGE) where the sequence can be read from four parallel lanes in the gel (Franca *et al.,* 2002). This sequencing technique can be conducted on either single or

double-stranded DNA templates and can begin with either a restriction enzyme digestion (Maxam and Gilbert, 1980), or an inserted or rearranged template of DNA (Maxam, 1980). Next, the DNA strands are end-labeled on one of the strands using either $^{32}$P-phosphate or with a nucleotide linked to the $^{32}$P and incorporated into the end fragment enzymatically (Maxam and Gilbert, 1977). An alternative to this labeling method was developed whereby the restriction fragments through $^{35}$S-dideoxyadenosine 5'-(α-thio) triphosphate and terminal deoxynucleotidyltransferase were used instead (Ornstein and Kashdan, 1985). This labeling method was used because of the following advantages: longer lifetime, low-emission energy, increase in the autoradiograph resolution, and higher stability after labeling (Franca *et al.,* 2002). Due to the hazardous nature of radioactive labels, a variety of fluorescent based labeling methods became widely used. The first of which used a 21-mer fluorescein labeled sequencing primer where the fluorescent dye was bound via a mercaptopropyl or aminopropyl linker arm to the 5'-phosphate of the primer (Rosenthal *et al.,* 1990). The next non-radioactive labeling technique included the use of a biotin marker that was either enzymatically or chemically attached to the oligonucleotide sequencing primer. This marker can then be visualized after electrophoresis by using a streptavidin-bridged enzymatic colour reaction (Richterich, 1989). Another labeling method is one that uses oligonucleotides labeled with alkaline phosphatase, or biotin hybridization to cleave DNA fragments previously transferred to a nylon membrane. These are then visualized by incubating with avidin-alkaline phosphatase, soaking in the chemiluminescent substrate (AMPPD) and then exposed to radiographic film (Tizard *et al.,* 1990).

In general, there are a few main advantages that the Maxam-Gilbert, and other chemical sequencing methods have over the Sanger (dideoxy) method. (1) With chemical methods, a fragment of DNA can be sequenced from the original template and requires no enzymatic amplification beforehand (Franca *et al.,* 2002). (2) No subcloning or PCR is required. As a consequence, for the location of rare bases, the chemical cleavage method cannot be replaced by Sanger's dideoxy method since it analyzes DNA via its complementary sequence and only gives sequence information based on the four canonical bases (Franca *et al.,* 2002). (3) Chemical methods are less susceptible to mistakes with regards to the sequencing of secondary structures or errors induced by polymerase (Boland *et al.,* 1994). (4) Chemical methods have been considered simple, easy to control, and the chemical distinctions between different bases are clear (Negri *et al.,* 1991).

There are also disadvantages to the Maxam-Gilbert method such as: chemical methods are slow and special care is required with use of hazardous materials (Franca *et al.,* 2002). Another problem, and possibly the most severe, is the occurrence of incomplete reactions that result in shorter than desired read lengths (Franca *et al.,* 2002). The reasons for such failures is due to incomplete reactions introducing electrophoretic mobility polidispersion which in turn enlarges the bandwidths causing reduced resolution (separation) between bands of similar lengths (Franca *et al.,* 2002).

Pyrosequencing is another DNA sequencing method and is a real-time technique based on the detection of inorganic pyrophosphate (PPi) during the polymerization process (Nyren and Lundin, 1985; Hyman, 1988; Ronaghi *et al.*, 1996). During this method, each dNTP is tried separately during the nucleic acid polymerization process

where PPi is released when any of the nucleotides are incorporated into the growing strand. The PPi is then converted to ATP and light is emitted via the firefly luciferase enzyme that catalyses luciferin into oxyluciferin (Franca *et al.*, 2002). The number of emitted photons can then be related to the number of nucleotides incorporated into the DNA strand. The sequence of DNA can then be determined by noting if incorporations occur and by counting the number of incorporations in a given attempt (Franca *et al.*, 2002).

There are currently two different pyrosequencing approaches: solid-phase (Ronaghi *et al.*, 1996) and liquid-phase sequencing (Ronaghi *et al.*, 1998b). Solid phase requires a template-washing step between nucleotide additions in order to remove any non-incorporated nucleotides or ATP to avoid false positive results. In order to maintain signal strength, this method must be conducted on a solid support such as a magnetic bead (Franca *et al.*, 2002). For liquid-phase sequencing a wash step is not necessary, however, a nucleotide-degrading enzyme (apyrase) is used in its place to eliminate excess nucleotides and ATP (Franca *et al.*, 2002).

As with all methods, pyrosequencing has several advantages and disadvantages over the other sequencing methods. (1) There is no need for labeled primers, nucleotides or gel electrophoresis with this method (Franca *et al.*, 2002). (2) The reaction is carried out very rapidly (~2 minutes per cycle) and is observed in real-time (Franca *et al.*, 2002). (3) These reactions occur at room temperature and physiological pH (Franca *et al.*, 2002). (4) It is a cost-effective method in comparison to the others mentioned (Ronaghi, 2001). (5) This method can easily be adapted for multiplex reactions (Franca *et al.*, 2002). (6)

Short fragments can easily be sequenced with relatively high signal to noise ratios being observed even after 40 cycles (Ronaghi *et al.,* 1998b).

The disadvantages with this method are: (1) With the repeated washes during the solid-phase method, signal intensity is difficult to maintain due to the loss of template (Ronaghi *et al.,* 1996). (2) The apyrase activity is reduced during the latter cycles of liquid-phase sequencing due to the accumulation of intermediate products (Ronaghi *et al.,* 1998b), as well non-specific interactions between apyrase and DNA is occasionally observed resulting in the loss of the enzyme's nucleotide-degrading activity (Ronaghi, 2000). (3) Difficulty occurs when analyzing homopolymeric stretches of DNA due to the non-linear light response following incorporation of more than five identical adjacent nucleotides (or 10 when using SSB) (Ronaghi *et al.*, 1999; Ronaghi, 2000). (4) Contamination of PPi increases the background noise significantly, reducing the signal to noise ratio (Ronaghi *et al.,* 1998b). (5) The fidelity of the DNA polymerase is not high enough due to the use of an exonuclease-deficient DNA polymerase. A polymerase with a high strand-displacement activity is required to achieve rapid incorporation of nucleotides during the limited reaction time (Ronaghi *et al.,* 1999). (6) For long sequences, the cost per base must be improved (especially for G/C rich sequences) (Franca *et al.,* 2002). (7) Intensity is significantly decreased due to mispriming occurring from either fragmentation or enzymatic degradation (Ronaghi *et al.,* 1998a).

The fourth and final method to be discussed is single-molecule sequencing with exonuclease. This is a laser-based technique that allows rapid sequencing of DNA fragments 40 kb or more in length (Jett *et al.,* 1989). The technique is based on the detection of fluorescent nucleotides in a flowing sample stream (Harding and Keller,

1992). The phases of this method are as follows: the fluorescent labeling of bases in a DNA fragment followed by the attachment of this fragment onto a microsphere; movement of this complex into a flowing buffer stream, followed by the digestion of the labeled DNA with an exonuclease that sequentially cleaves the 3'-end nucleotides. Detection and identification of the fluorescently labeled bases occurs as they pass a focused laser beam (Davis *et al.,* 1991).

Although this method of sequencing is significantly faster than other techniques (Davis *et al.,* 1991; Stephan *et al.,* 2001), the quality of the buffer must be improved and a selection step must be incorporated into the process (Franca *et al.,* 2002). The use of new polymerases, and exonucleases must occur for more rapid and efficient sequencing to occur (Stephan *et al.,* 2001).

f. PCR Inhibition

Inhibition of DNA can occur in a variety of ways and is commonly observed in the processing of aDNA samples, and to a lesser extent in modern DNA samples. Inhibition can act at one or more of the steps within the analytical process and can take the form of either enzymatic inhibition or DNA template inhibition. During the extraction process, inhibitors can interfere with the enzymatic activity of specific components that are required for cell lysis, or nucleic acid degradation and capture. During amplification, inhibitors can interfere with the DNA polymerase activity, through either directly inhibiting the enzyme or disallowing it to gain sufficient access to the template. Both of these occurrences will eliminate amplification of the DNA target (Wilson, 1997).

The first concrete indication of inhibition typically becomes evident after amplification of the DNA sample. PCR inhibition is easily detected by the lack of primer-dimers, smears, or non-specific amplification products. If any of these artifacts are present, then the presence of inhibition must be eliminated (Hummel, 2003). Inhibiting substances can include humic acids, fulvic acids, tannins, maillard products and metal ions; and commonly come from the soil in which the sample is deposited. All of these agents act by inhibiting either the enzymatic activity of the DNA polymerase or interact with the template such that amplification cannot proceed during the PCR. Other potential inhibitors include hemoglobin, urea, heparin, organic and phenolic compounds, glycogen, and fats (Wilson, 1997; Hummel, 2003). In aDNA studies, the yellow to dark brown staining of the sample is an indication that it contains inhibitors, even after purification (Hummel, 2003). In many instances (particularly forensics) inhibition is removed by merely diluting the sample prior to PCR. Should this not be adequate in successfully removing the inhibition, further purification of the sample using an appropriate size exclusion column would be necessary and generally sufficient.

Another cause of inhibition, commonly observed when amplifying modern extracts, is the addition of too much DNA template to the PCR. Not typically observed in aDNA studies due to the extremely low copy number templates, it is relatively easy to overload the amplification of modern DNA PCRs. The mechanism of this inhibition is not completely understood, however, it is suspected to be the product of stochastic effects occurring within the reaction mixture. In contrast to chemical inhibition, DNA overload usually results in the observation of smears on the gel (Hummel, 2003), however it can also result in no amplification being observed. If DNA overload is suspected as being the

reason for PCR failure, the reaction should be repeated using a smaller template volume by either adding less template to the reaction mixture or by diluting the sample beforehand.

## IX. Project

### i. Question

How do tissue type, damage, and error affect the data obtained through genetic profiles?

### ii. Purpose

The purpose of this research is to analyze the occurrence of genetic profiling variations that are caused by such factors as tissue type, damage to the DNA molecule, and error induced throughout the process of analysis. This project will focus on the variations that are observed when profiling multiple replicates from various tissues within the same individual. It will also focus on how such profiling variations can be reduced, if not overcome, in the methodology used to analyze a given sample. Since damage to the DNA molecule, and errors induced throughout the analytical process differ between samples and tissue type, methods will be designed to enhance the recovery of highly degraded samples of DNA in attempt to gain as much information as possible. In order to assess the levels of variation, we will be using mtDNA sequencing and both autosomal and Y-chromosome STR amplification. In attempt to recover microsatellite information from highly degraded samples, novel methodologies will be designed and tested for their potential applicability to future studies. The specific mechanisms of DNA damage and degradation have been studied in the past, and will not be analyzed throughout this research. For the purpose of this research, we will only be analyzing the types of variations observed within or between samples, and make methodological modifications in attempt to overcome such variations.

### iii. Methodology

Human sequence variation will be assessed through mtDNA sequencing of the control region. Sequences will then be detected using capillary elecrophoresis and aligned using BioEdit software. Both ancient and modern DNA sequences will be compiled and compared from data generated in this study and data obtained from previous studies. Once the sequences are aligned, multiple replicates will be compared for the occurrence of variations within the same individual. Should variations be observed within multiple replicates from the same individual, attempts will be made to identify the cause of these variations.

The occurrence of damage and error will also be assessed through the amplification of both autosomal and Y-chromosome STRs from both ancient and modern DNA samples. All STR will be amplified using commercially available amplification kits and fragment detection will again be done using capillary electrophoresis with GeneMapper® ID software. Variations within STRs will be observed through the assessment of all the allele lengths present at various microsatellite loci, allelic dropout, preferential amplification, and off-ladder peaks.

In attempt to overcome damage or error that is observed among STR loci, particularly in low copy number templates, novel methods will be designed and tested for their potential use in future studies. For the recovery of Y-STR data, hemi-nested singleplex PCRs will be designed and optimized such that they too can be detected using capillary electrophoresis. For the recovery of autosomal STR data, the usefulness of booster amplification will be tested using a commercially available STR amplification kit.

### iv. Samples

A variety of sample types will be utilized throughout this research. For the ancient DNA sequence data analysis, bone and teeth samples obtained from multiple individuals in 9 different geographical locations (populations) will be extracted using both chemical and enzymatic methods followed by amplification of the mitochondrial D-loop. Depending on the extent of fragmentation, a variety of amplicons for the target of interest will be used to generate successful sequence data.

When generating modern mtDNA sequence data, buccal swabs will be extracted using an enzymatic extraction method only and hair samples will be extracted using a chemical extraction method only. These extracts will then be amplified for the entire D-loop prior to sequencing.

Samples extracted for the analysis of microsatellites will be hair roots, and will again be extracted using only a chemical extraction method. Following this, the samples will be amplified for either autosomal or Y-chromosome STRs using a commercially available STR amplification kit.

All modern samples will be extracted within 24 hours of being obtained from the subject and subsequently amplified within 48 hours. Ancient samples will be prepared from bone or teeth samples by washing with detergent, bleach, and ethanol, followed by exposure to UV light for a minimum of 24 hours. The samples will then be crushed using a mixer mill and placed in sterile microcentrifuge tubes. These samples will then remain in their dry state at room temperature until extraction is desired. Within 24 hours of extraction, amplification will ensue.

## X. Methods and Procedures

### i. Extraction Protocols

As has been mentioned previously, there are a variety of different extraction protocols available, however, which one to use is often determined by the type of sample being analyzed. Typically in a forensic setting, a proteinase K (PK) extraction method is employed, while in research facilities, especially aDNA research facilities, the guanidinium thiocyanate (GuSCN) extraction method is frequently utilized. For this research a combination of both extraction methods have been used.

**Proteinase K Extraction:**

Norman Hansen first introduced proteinase K (PK) as an enzymatic extraction method in 1974 (Hansen, 1974). Since then, numerous variations of this enzymatic method have been developed. The modified PK extraction method utilized in this study is an extraction method that combines a variety of chemical reagents to successfully extract genomic DNA. The extraction combines the cellular denaturing capabilities of both PK and sodium dodecyl sulfate (SDS) to lyse the cell, which is then followed by the denaturation of proteins by the enzymatic activity of PK. In this study, we will be using this extraction method for a variety of biological samples (bone, teeth, and buccal swabs). Once this extraction is complete, the solution will contain denatured proteins, DNA, RNA and other cellular debris. All denatured proteins and other cellular debris will be separated from the DNA in a later step that utilizes phenol and chloroform/isoamyl alcohol mixtures to physically purify the DNA.

Required Reagents:

Proteinase K (20 mg/mL)

TNE (Tris-NaCl-EDTA)

Sodium Dodecyl Sulfate (20%)

Dithiothreitol (DTT) (0.39 M)

dd$H_2O$

Phenol

Chloroform:Isoamyl Alcohol (24:1 v/v)

Extraction Protocol:

1. Prior to processing samples, prepare 1X extraction buffer for (n+1) samples:
   290 µL TNE
   40 µL 20% SDS
   40 µL DTT (0.39M)
   2 µL Proteinase K (20 mg/mL) + 28 µL water
   Total 400 µL
2. Add 400 µL of 1X Extraction Buffer to each sample. Be sure to include an extraction negative to test reagent purity.
3. Incubate at 37 °C in thermomixer with agitation at 1100 rpm (incubate 3 hours to overnight).
4. Create 2 aliquots per sample of 500 µL Phenol and 500 µL chloroform:isoamyl alcohol (24:1 v/v) in sterile 1.5 mL microcentrifuge tubes. All steps from here on are to be done in the fume hood.
5. Create 1 aliquot per sample of 1000 µL chloroform:isoamyl alcohol (24:1 v/v).
6. Add extract to first tube containing 500 µL phenol and 500 µL chloroform:isoamyl alcohol (24:1 v/v). Shake/vortex until milky emulsion is achieved.
7. Centrifuge for 5 minutes at 13000 rpms.

8. Remove the aqueous (top) layer and place into the second previously prepared 1.5 mL tube containing 500 μL phenol and 500 μL chloroform:isoamyl alcohol (24:1 v/v). When transferring supernatant, do not remove the layer of denatured protein at the interface separating the two layers. This new tube now contains the DNA. Store the previous tube at –80°C.
9. Shake/vortex the tube vigorously.
10. Centrifuge for 5 minutes at 13000 rpms.
11. Remove the aqueous (top) layer and place in the previously prepared 1.5 mL tube containing 1000 μL chloroform:isoamyl alcohol (24:1 v/v). Shake/vortex the tube vigorously.
12. Centrifuge for 5 minutes at 13000 rpm.
13. Carefully remove the aqueous (top) layer and place in a newly labeled 1.5 mL microcentrifuge tube. These tubes now contain extracted DNA ready to be further purified using ethanol precipitation.

**Guanidinium Thiocyanate Extraction:**

Boom *et al.* (1990) first introduced the guanidinium thiocyanate (GuSCN) extraction method in 1990 for the extraction of nucleic acids. This method is frequently used in research facilities and is what is referred to as a chemical extraction. The extraction buffer contains the chaotropic agent GuSCN that dehydrates exposed DNA allowing purification using a silica bead based method (described next). In this study, we will be using this extraction method on many of the same samples extracted with PK; however this method will be used for the extraction of hair roots as well.

Required Reagents:

Guanidinium Thiocyanate (GuSCN) Extraction Buffer

Extraction Protocol:

1. Add 500 μL of GuSCN extraction buffer (or enough to cover sample) to the desired sample in a 1.5 mL tube.
2. Incubate overnight at 56°C with gentile agitation.
3. Purify extract using silica bead purification protocol.

### ii. Purification Protocols

After the extraction of DNA from the sample, it is necessary to remove all possible inhibitors, denatured proteins and other unwanted cellular debris. Ideally, the purification step will leave the researcher with only the pure DNA molecules for future analyses. As will be seen, most routinely employed purification procedures are run in conjunction with a specific extraction procedure. This is because the chemistry of the purification procedure is typically most effective when used with similar extraction chemistries.

**Ethanol Precipitation Purification:**

Ethanol (EtOH) precipitation purification or concentration typically follows the PK extraction. During this extended purification, a chaotropic agent (in this case sodium acetate) is utilized to dehydrate the DNA followed by its exposure to ice cold EtOH of varying concentrations. The exposure to EtOH causes the DNA to precipitate out of solution and is then physically separated from the rest of the solution via centrifugation.

Required Reagents:

3 M Sodium Acetate

100% Ethanol (EtOH)

95% Ethanol (EtOH)

$ddH_2O$

Purification Protocol:

1. Add a 10% volume (approximately 40-50 μL) of 3M sodium acetate to the entire volume (approximately 400-500 μL) of extract to be purified.
2. Briefly shake/vortex.
3. Add 2.5 times the volume (approximately 1000-1250 μL) of ice cold 100% ethanol to the tube containing the extract and sodium acetate.
4. Shake/vortex and place on ice for 30 minutes.
5. Centrifuge tube for 5 minutes at 13000 rpm.
6. Discard supernatant.
7. Wash the extract by adding 500 μL of ice cold 95% ethanol.
8. Shake/vortex
9. Centrifuge tube for 10 minutes at 13000 rpm.
10. Discard supernatant and allow sample to air dry.
11. Prior to PCR, resuspend sample in 100-150 μL of $ddH_2O$ at 37°C for 15 minutes.

**Silica Bead Purification:**

As was mentioned, Boom *et al.* (1990) introduced the silica bead purification in 1990 as an effective way to purify nucleic acids extracted using the GuSCN method. Three years later, Hoss and Paabo (1993) applied this purification method to aDNA extracts with considerable success. Silica based purification is now a method which is routinely used in aDNA laboratories, as well as other research facilities. The chaotropic

agent GuSCN dehydrates the DNA during the extraction, and additionally in the beginning of the purification step, exposing the DNA to the silica beads. Due to the negative charge on the DNA and the positive charge of silica a tight bond is created when the two are mixed and placed on ice. The silica-DNA complex is then washed a series of times using working wash buffer and EtOH before being resuspended in water. The final step in this purification is the addition of $ddH_2O$ and incubation at 56°C for 1 hour. This step is essential for the DNA to disassociate from the silica and exist in solution.

Required Reagents:

Guanidinium Thiocyanate (GuSCN) Extraction Buffer

Acidified Silica Oxide Beads ($SiO_2$)

Working Wash Buffer

100% Ethanol (EtOH)

$ddH_2O$

Purification Protocol:

1. Add 900 GuSCN extraction buffer and incubate at 94°C for 10 minutes.
2. Centrifuge tube at 13000 rpm for 1 minute.
3. Remove the supernatant and place in sterile 1.5 mL tube.
4. Add 900 μL of GuSCN solution (or Sodium Iodide solution) and 20 μL of silica to the sample.
5. Briefly shake/vortex.
6. Place tubes on ice for 60 minutes. Invert to resuspend silica every 15 minutes.
7. Wipe tubes and zip spin.
8. Remove and discard supernatant.

9. Add 500 μL of working wash buffer.
10. Shake/vortex to resuspend silica.
11. Zip spin the tube.
12. Remove and discard supernatant.
13. Repeat wash steps 9-12 if required.
14. Add 150 μL of 100% ethanol.
15. Shake/vortex to resuspend silica.
16. Centrifuge tubes at 13000 rpm for 1 minute.
17. Remove and discard supernatant.
18. Speed-vac silica pellet for 5-10 minutes or air dry for 30 minutes.
19. Resuspend silica in 100 μL $ddH_2O$ for 1 hour at 56°C.
20. Centrifuge samples at 13000 rpm for 1 minute prior to each PCR.

**Size Exclusion Purification:**

Size exclusion purification columns are typically utilized to quickly and effectively purify nucleic acids from other macromolecules or chemical elements. Size exclusion purification is also routinely used to purify PCR products by separating the amplified fragments of DNA from other reagents that may interfere in subsequent analyses such as primers, magnesium ions or fluorescently labeled ddNTPs. There are a variety of size exclusion columns available, and the choice of which to use is entirely dependent upon what is to be removed from the sample. For this study, we will be utilizing size exclusion columns in order to remove potential inhibiters from our extracted DNA, or as sequencing PCR clean-up.

**Bio-Rad Micro Bio-Spin P30 Spin Column:**

P30 size exclusion columns are often used to separate macromolecules based on molecular weight. The column is composed of a matrix of porous polymeric beads. As the solution is washed through this matrix, molecules too large to become trapped inside the porous beads will elute quickly while the matrix will retard smaller molecules, thus purifying the sample. These columns will be used throughout this research to remove potential PCR inhibitors after extraction and the initial purification procedures.

Required Materials/Reagents:

P30 spin column and waste collection tube (one of each for each sample to be purified)

Purification Protocol:

1. Snap off tip of column and place in 2.0 mL collection tube provided.
2. Loosen the lid on the column and centrifuge at 3400 rpm for 2 minutes.
3. Place column in sterile 1.5 mL tube.
4. Add sample to be purified to the center of the column filter.
5. Centrifuge at 3400 rpm for 4 minutes.
6. Purified DNA is now in the 1.5 mL tube.

**Dyex Purification Columns:**

Dyex purification columns will be used during this study for PCR cleanup after the sequencing reaction. These columns quickly remove unincorporated dye terminators, primers, dNTPs and other low molecular weight compounds left after PCR. These columns purify the PCR product making it ready for sequencing by capillary electrophoresis.

Required Materials/Reagents:

Dyex spin column and waste collection tube (one for each product to be purified).

Purification Protocol:

1. Shake/vortex column to resuspend resin.
2. Loosen lid of the Dyex spin column and centrifuge it in its provided collection tube for 2 minutes at 3400 rpm.
3. Transfer spin column to sterile 1.5 mL tube.
4. Add product to be purified to the center of the column's membrane.
5. Centrifuge at 3400 rpm for 2 minutes.
6. Purified product is now in the 1.5 mL tube.

**E.Z.N.A. Dye Terminator Removal (DTR) Columns:**

These DTR columns utilize similar chemistries to, and will be used for the same purposes as Dyex purification columns.

Required Materials/Reagents:

DTR spin column and waste collection tube (one for each sample to be purified).

Purification Protocol:

1. Open the column cap a half turn, snap off the bottom closure.
2. Close the column cap and vortex to resuspend the resin.
3. Place column in the collection tube provided.
4. Centrifuge the column for 2-3 minutes at 3400 rpm.
5. Transfer the spin column to a clean 1.5 mL microcentrifuge tube.
6. Apply the total volume of sequencing PCR product to the center of the spin column.

7. Centrifuge the column for 2-3 minutes at 3400 rpm.
8. Purified sequencing PCR product is now in the 1.5 mL tube.

**Bind/Elute Column Purification:**

Bind-elute columns are typically used for PCR cleanup prior to the sequencing reaction. They can also be used to purify DNA extracts from modern or uninhibited samples. First the extract or PCR product is mixed with a high-salt binding buffer and spun through the purification column. This enables the DNA to bind tightly to the silica membrane. The samples are then washed to elute primers, nucleotides, enzymes and other impurities from the DNA. The pure DNA is then eluted with the addition of a low-salt elution buffer or $ddH_2O$. The PCR purification columns that will be utilized are capable of purifying DNA ranging in size from 100 bp to 10 Kb.

**QIAquick PCR Purification Columns:**

These columns will be used for the above-discussed procedures, pre-sequencing PCR cleanup, or purification of modern DNA extracts.

Required Materials/Reagents:

QIAquick PCR purification column and waste collection tube (one for each sample to be purified).

PB Buffer (Provided)

PE Buffer (Provided)

$ddH_2O$

Purification Protocol:

1. Add 5 volumes of PB Buffer to 1 volume of PCR product or extract.
2. Place QIAquick spin column in 2 mL collection tube provided.

3. Add entire volume of PB Buffer/PCR product (or extract) solution to the center of the column. Note that the column only holds 750 μL; therefore, steps 3-5 may need to be repeated depending on sample volume.
4. Centrifuge the column for 1 minute at 13000 rpm.
5. Discard the flow-through, and place the spin column back into the collection tube.
6. Add 750 μL of PE Buffer to the center of the spin column.
7. Centrifuge the column for 1 minute at 13000 rpm.
8. Discard the flow-through, and place the spin column back into the collection tube.
9. Centrifuge the empty column for 1 minute at 13000 rpm.
10. Place the spin column in a sterile 1.5 mL microcentrifuge tube.
11. Add 30-50 μL of $ddH_2O$ to the center of the column to elute purified PCR products and 100-150 μL of $ddH_2O$ for purified extracts.
12. Centrifuge spin column for 1 minute at 13000 rpm.
13. Purified PCR product or extract is now in the 1.5 mL tube.

**E.Z.N.A. Cycle Pure Columns:**

These purification columns will only be used for pre-sequencing PCR cleanup, and not the purification of DNA extracts.

Required Materials/Reagents:

Cycle-Pure column and waste collection tube (one for each product to be purified).

Buffer XP1 (provided).

SPW Buffer (provided).

$ddH_2O$.

Purification Protocol:

1. To PCR product, add equal volumes of Buffer XP1
2. Apply PCR product and Buffer XP1 mixture to the center of the Cycle-Pure purification column assembled in a 2 mL collection tube.
3. Centrifuge at 13000 rpm for 1 minute.
4. Discard the eluate from the collection tube.
5. Wash the column by adding 700 μL of SPW Buffer (diluted with absolute ethanol) to the column.
6. Centrifuge at 13000 rpm for 1 minute.
7. Discard the eluate from the collection tube and repeat steps 5-6 for an additional wash.
8. Discard the eluate from the collection tube and centrifuge empty column at 13000 rpm for 1 minute.
9. Place column in a sterile 1.5 mL tube.
10. Add 30-50 μL of $ddH_2O$ to the center of the column's membrane.
11. Centrifuge at 13000 rpm for 1 minute.
12. Discard the column.
13. Purified PCR product is now in the 1.5 mL tube.

### iii. Amplification Protocols

**Primer Selection:**

When amplifying DNA there are a variety of primers, both mitochondrial and nuclear, that can be used depending on the target of interest. For this research, aside from STR and Y-STR amplification, all primers utilized will target regions of the

mitochondrial D-loop and have previously been designed and optimized. The annealing temperatures for mitochondrial primers have previously been determined; therefore, further optimization is not necessary. Below is the list of all mitochondrial primers that will be used, for this research.

| Primer name and sequence | Primer length (bp) | Tm (°C) | G/C content (%) |
|---|---|---|---|
| mt15971F TTAACTCCACCATTAGCACC | 20 | 58.53 | 45.0 |
| mt16210F CCCATGCCTACAAGCAAGTA | 20 | 56.0 | 45.0 |
| mt16322R TGGCTTTATGTACTATGTACTG | 22 | 57.8 | 36.4 |
| mt16420R TGATTTCACGGAGGATGGTG | 20 | 60.4 | 50.0 |
| mt1F GATCACAGGTCTATCACCC | 19 | 60.5 | 52.6 |
| mt15F CACCCTATTAACCACTCACG | 20 | 60.4 | 50.0 |
| mt279R GATGTCTGTGTGGAAAGTGG | 20 | 60.4 | 50.0 |
| mt155F TATTTATCGCACCTACGTTC | 20 | 46.3 | 40.0 |
| mt389R CTGGTTAGGCTGGTGTTAGG | 20 | 62.5 | 55.0 |
| mt408R CTGTTAAAAGTGCATACCGCCA | 22 | 61.5 | 45.5 |

Primers were designed specifically for this research, that target the Y-chromosome for the amplification of highly degraded samples. These primers will be listed in the Singleplex STR Amplification section to follow.

**Standard Amplification:**

After the extraction of DNA from a sample is completed, a standard, or detection PCR is performed using the purified template. This PCR will serve three main purposes. It will determine the presence of any viable DNA in the extracted sample. As well, the level of fragmentation can be observed, and finally, target regions of interest for sequencing will be amplified.

All standard PCRs in this study will contain the following mixture with corresponding concentrations of each reagent.

| Reagents | Final Concentration |
|---|---|
| 10X Buffer, minus Mg | 1X |
| 10mM dNTP mixture | 200 μM |
| 10 μM Forward Primer | 0.2 μM |
| 10 μM Reverse Primer | 0.2 μM |
| 50 mM $MgCl_2$ | 2 mM |
| Platinum *Taq* DNA Polymerase (5U/μL) | 1.0 U |
| $ddH_20$ | N/A |
| Template | N/A |

* The volume of $ddH_20$ added to the reaction depends on the total volume of the reaction and the amount of DNA template added.

All standard amplifications will be conducted using the following PCR cycling parameters.

| Temperature (°C) | Time | Cycle Number |
|---|---|---|
| 94.0 | 2:00 | 1 |
| 94.0 | 1:00 | *35-45 |
| **60.0 | 1:00 | |
| 72.0 | 2:00 | |
| 4.0 | Hold | 1 |

* The cycle number will vary depending on the sample type. 35 cycles will be used for modern samples while 45 will be used for ancient or highly degraded samples.

** This annealing temperature varies depending on the sequence of the primer; however, for all mitochondrial primers used in this study the annealing temperature is 60°C.

**Multiplex Short Tandem Repeat (STR) Amplification:**

Amplification of both autosomal and Y-chromosome STR are carried out using commercially available kits. Since these kits are proprietary, it is unknown to the user exactly what is contained within them (i.e. primers, reagent concentrations, etc.). The kits utilized for this research were supplied by Applied Biosystems (ABI) and Promega

Corporation. The use of these kits was carried out in accordance with the manufacturer's protocols.

**AMPF*l* STR® Identifiler® PCR Amplification Kit:**

This STR amplification kit is manufactured by ABI and co-amplifies 15 loci on the autosomal chromosomes plus the amelogenin locus on the sex chromosomes in one reaction. This kit utilizes 5-dye chemistry and is run on the ABI PRISM® 3100 Genetic Analyzer after the reaction is complete.

Amplification Protocol:

1. For all AMPF*l* STR® Identifiler® PCR amplifications, combine the following reagents in a 0.2 mL tube.

| Reagents | Volume per reaction (μL) |
|---|---|
| AmpF*l* STR Identifiler Mastermix | 10.5 |
| AmpF*l* STR Identifiler Primer Mix | 5.5 |
| AmpliTaq Gold Polymerase (5U/μL) | 0.5 |
| $ddH_2O$ | N/A |
| Template | N/A |

* The total reaction volume is 25 μL. Only 15 μL of mastermix, primer and AmpliTaq will be added to the reaction. The extra volume is for pipetting error purposes only. The volume of $ddH_2O$ varies depending on the volume of template added to the reaction.

2. Amplify all AMPF*l* STR® Identifiler® STR reactions using the following PCR cycling parameters.

| Temperature (°C) | Time | Cycle Number |
|---|---|---|
| 95.0 | 11:00 | 1 |
| 94.0 | 1:00 | 28 |
| 59.0 | 1:00 | |
| 72.0 | 1:00 | |
| 60.0 | 1:00:00 | 1 |
| 4.0 | Hold | 1 |

3. Remove products from the thermocycler and wrap in aluminum foil.

4. Store at 4°C until fragment detection using the ABI PRISM® 3100 Genetic Analyzer.

**PowerPlex® Y System:**

This amplification kit is manufactured by Promega Corporation and co-amplifies 12 loci on the Y-chromosome in one reaction. This kit uses a three-colour detection system and is run on the ABI PRISM® 3100 Genetic Analyzer after the reaction is complete.

Amplification Protocol:

1. For all PowerPlex® Y STR amplifications, combine the following reagents in a 0.2 mL tube.

| Reagents | Volume per reaction (μL) |
|---|---|
| Gold ST*R Buffer | 2.5 |
| PowerPlex Y 10X Primer Mix | 2.5 |
| AmpliTaq Gold Polymerase (5U/μL) | 0.55 |
| $ddH_2O$ | N/A |
| Template | N/A |

* These reaction volumes do not account for error and are intended for a 25 μL reaction.

2. Amplify all PowerPlex® Y STR reactions using the following PCR cycling parameters.

| Temperature (°C) | Time | Cycle Number |
|---|---|---|
| 95.0 | 11:00 | 1 |
| 96.0 | 2:00 | 1 |
| 94.0 | 1:00 | 10 |
| 60.0 | 1:00 | |
| 70.0 | 1:30 | |
| 90.0 | 1:00 | 22 |
| 58.0 | 1:00 | |
| 70.0 | 1:30 | |
| 60.0 | 30:00 | 1 |
| 4.0 | Hold | 1 |

3. Remove products from the thermocycler and wrap in aluminum foil.

4. Store at 4°C until fragment detection using the ABI PRISM® 3100 Genetic Analyzer.

**Singleplex Short Tandem Repeat (STR) Amplification:**

For highly degraded samples, it is sometimes necessary to amplify one STR target at a time in order to generate a profile. For this research, previously published Y-STR singleplex primers were used to amplify various loci on the Y-chromosome. These primers are listed in the table below.

These previously published primers contain various fluorescent labels that are detected through running on the ABI PRISM® 3100 Genetic Analyzer after the reaction has completed.

| Locus | | Sequence (5'-3') | $T_m$ (°C) | Reference |
|---|---|---|---|---|
| DYS385a/b | F | (6 FAM)AGCATGGGTGACAGAGCTA | 56.9 | (Butler *et al.*, 2002) |
| | R | CCAATTACATAGTCCTCCTTTC | 59.7 | (Butler *et al.*, 2002) |
| DYS389I/II | F | (VIC)CCAACTCTCATCTGTATTATCTATG | 54.2 | (Butler *et al.*, 2002) |
| | R | GTTATCCCTGAGTAGTAGAAGAATG | 59.0 | (Butler *et al.*, 2002) |
| DYS390 | F | TATATTTTACACATTTTTGGGCC | 57.2 | (Butler *et al.*, 2002) |
| | R | (6 FAM)GTGACAGTAAAATGAAAACATTGC | 57.0 | (Butler *et al.*, 2002) |
| DYS391 | F | CTATTCATTCAATCATACACCCA | 58.2 | (Ruitberg and Butler, 2000) |
| | R | (6 FAM)GATAGAGGGATAGGTAGGCAGGC | 60.0 | (Butler *et al.*, 2002) |
| DYS392 | F | AAAAGCCAAGAAGGAAAACAAA | 56.0 | (Ruitberg and Butler, 2000) |
| | R | (PET)AAACCTACCAATCCCATTCCTT | 59.7 | (Ruitberg and Butler, 2000) |
| DYS393 | F | AACTCAAGTCCAAAAAATGAGG | 57.8 | (Kayser *et al.*, 1997) |
| | R | (VIC)GTGGTCTTCTACTTGTGTCAATAC | 61.7 | (Kayser *et al.*, 1997) |
| DYS438 | F | TGGGGAATAGTTGAACGGTAA | 59.5 | (Ruitberg and Butler, 2000) |
| | R | (6FAM)GGAGGTTGTGGTGAGTCGAG | 64.5 | (Ruitberg and Butler, 2000) |
| DYS439 | F | ACATAGGTGGAGACAGATAGATGAT | 59.7 | (Ruitberg and Butler, 2000) |
| | R | (NED)GCCTGGCTTGGAATTCTTTT | 58.4 | (Ruitberg and Butler, 2000) |

Amplification Protocol:

1. For all singleplex amplifications, combine the following reagents in a 0.2 mL tube.

| Reagents | Final Concentration |
|---|---|
| 10X Buffer, minus Mg | 1X |
| 10mM dNTP mixture | 200 μM |
| 10 μM Forward Primer | 0.2 μM |
| 10 μM Reverse Primer | 0.2 μM |
| 50 mM $MgCl_2$ | 2 mM |
| Platinum *Taq* DNA Polymerase (5U/μL) | 1.0 U |
| $ddH_20$ | N/A |
| Template | N/A |

2. All singleplex reactions will be amplified using the following PCR cycling parameters. Changes will be made to the annealing temperatures depending on the primers used. The annealing temperatures for primers from each locus are listed in the table of primers above.

| Temperature (°C) | Time | Cycle Number |
|---|---|---|
| 94.0 | 2:00 | 1 |
| 94.0 | 0:30 | 45 |
| Varies with primers | 1:00 | |
| 72.0 | 2:00 | |
| 4.0 | Hold | 1 |

3. Remove products from the thermocycler and wrap in aluminum foil.
4. Determine if amplification was successful by running samples on a polyacrylamide gel. If successful, proceed to step 5.
5. Store at 4°C until fragment detection using the ABI PRISM® 3100 Genetic Analyzer.

**DNA Sequencing Reaction:**

The DNA sequencing reaction for this research will utilize chain terminating chemistries. This research will use the BigDye® Terminator v3.1 Cycle Sequencing Kit provided by ABI and is run on the ABI PRISM® 3100 Genetic Analyzer after the reaction

is complete. The reagent concentrations have been modified from the manufacturer's instructions and are as follows:

DNA Sequencing Protocol:

1. For all DNA sequencing reactions, combine the following reagents in a 0.2 mL tube.

| Reagents | Volume per reaction (μL) |
|---|---|
| BigDye® Terminator v3.1 | 3.0 |
| Either forward or reverse primer from PCR | 0.3 |
| Purified PCR product | 7.0 |

* These volumes are intended for 10 μL sequencing reactions.

2. All DNA sequencing reactions will be amplified using the following PCR cycling parameters.

| Temperature (°C) | Time | Cycle Number |
|---|---|---|
| 94.0 | 0:30 | 35 |
| 50.0 | 0:15 | |
| 72.0 | 4:00 | |
| 4.0 | Hold | 1 |

3. Purify PCR product using either Dyex or E.Z.N.A. size exclusion DTR columns.
4. Desiccate the purified sequencing product.
5. Store at 4°C until sequence detection using the ABI PRISM® 3100 Genetic Analyzer.

**iv. Detection Protocols**

There are many different detection systems available to determine the success of the amplification or sequencing of a given sample. For this research we will be using Polyacrylamide Gel Electrophoresis (PAGE), Agarose Gel Electrophoresis, and Capillary Electrophoresis.

**Polyacrylamide Gel Electrophoresis:**

Polyacrylamide Gels will be used in this research for the detection of amplified DNA products. This method of detection is beneficial due to its sensitivity to small quantities of PCR product and its ability to resolve fragments of DNA differing in as little as one base pair. Polyacrylamide gels will be used for this research when the size of the PCR product is of interest.

Required Reagents:

| | |
|---|---|
| 5X TBE Buffer | 20 mL |
| Acrylamide | 12.5 mL |
| $ddH_2O$ | 66.5 mL |
| TEMED | 90.0 μL |
| 10% APS | 900 μL |

Gel Making Protocol:

*This procedure will make 12 – 6% Polyacrylamide gels:

1. Prepare 10% APS solution by mixing 0.1 g APS to 1.0 mL $ddH_2O$.
2. Place a 125 mL Pyrex flask on ice and allow to cool.
3. Set up gel cassettes and combs ahead of time.
4. Add all reagents in same order as listed above.
5. Swirl mixture every 2 minutes and keep on ice at all times.
6. Fill gel cassettes from one corner using a transfer pipette ensuring that no air bubbles are present.
7. Place gel comb into the filled cassette.
8. Allow gels to set for 1 hour at room temperature.

9. Store at 4°C until use.

Gel Apparatus Preparation:

1. Obtain gel cartridge and remove the strip of tape and comb.
2. Place the gel cartridge into the gel apparatus. Use an empty cartridge or spacer block if running only one gel.
3. Fill the gel apparatus with 1X TBE running buffer.
4. Purge wells with a Pasteur pipette to remove any air bubbles.

Loading and Running the Polyacrylamide Gel:

1. Mix 7 μL of PCR product with 3 μL of 6X loading dye.
2. Load 3 μL of size standard and all samples into the wells of the gel.
3. Set the voltage to 120 and the time to 40 minutes on the electrophoretic power pack.
4. After gel has run, stain for 15 minutes with ethidium bromide (EtBr).
5. Rinse gel with $ddH_2O$, view it on the transilluminator (wavelength UV B) and photograph.

**Agarose Gel Electrophoresis:**

Agarose gels will also be used to detect the presence of DNA after the standard PCR. This method is beneficial when there is only one DNA fragment suspected of being present after PCR. Due to the low resolution of these gels, they will only be used to determine presence or absence of DNA after PCR, prior to sequencing.

Required Reagents:

| | |
|---|---|
| 1X TBE Buffer | 25 mL |
| Agarose | 0.375 g |

10 mg/mL ethidium bromide (EtBr) 2.0 μL

Gel Making Protocol:

* This procedure will make 1 – 1.5% Agarose gel.

1. Measure out 25 mL of 1X TBE buffer and add it to a 125 mL Pyrex flask.
2. Weigh out 0.375 g of agarose and slowly add it to the 1X TBE while continuously swirling the solution.
3. Cover the top of the flask with aluminum foil.
4. Heat the solution while swirling every 1-2 minutes until agarose is completely dissolved.
5. Allow the mixture to cool for 5 minutes.
6. Add 2.0 μL of 10 mg/mL EtBr stain.
7. Pour gel into the agarose plate and insert the gel comb.
8. Allow the gel to set for 30 minutes at room temperature. Place the gel in the fridge to speed up this process.

Loading and Running the Agarose Gel:

1. Remove the comb from the gel.
2. Place the gel into the gel apparatus.
3. Fill the gel apparatus with 1X TBE until the gel is completely submerged.
4. Purge the wells with a Pasteur pipette to remove any air bubbles.
5. Mix 6 μL of PCR product with 2.5 μL of 6X loading dye.
6. Load 3 μL of size standard and all samples into the wells of the gel.
6. Set the voltage to 110 and the time to 25 minutes on the electrophoretic power pack.

7. After the gel has run, rinse with $ddH_2O$, view it on the transilluminator (wavelength UV B) and photograph

**Capillary Electrophoresis (CE):**

The ABI PRISM® 3100 Genetic Analyzer will be utilized as the detection system for all multiplex STR, multiplex Y-STR, singleplex Y-STR, and sequencing reactions. This genetic analyzer is extremely beneficial to the researcher due to its ability to resolve fragments of DNA differing by as little as one base pair. This instrument is also beneficial because multiple fragments of DNA within the same size range can also be deciphered by the incorporation of fluorescently labeled primers during amplification. It is this feature that allows multiplexes as large as the ones used in this study to be successfully analyzed.

**DNA Sequence Detection:**

Required Reagents:

Hi-Di Formamide

Sample Preparation and Loading:

1. Resuspend each sample to be sequenced in 15 μL of Hi-Di Formamide.
2. Vortex the sample for 1 minute and briefly zip spin the tube.
3. Heat the sample to 95°C for 3 minutes, immediately followed by placing on ice for 2 minutes.
4. Vortex the sample and briefly zip spin the tube.
5. Place on ice until ready to load on the CE.
6. Pipette all 15 μL of sample product into the appropriate wells of a 96- or 384-well ABI Plate.

7. Ensure that all 16 wells selected for the detection contain either 15 μL of sample or 15 μL of Hi-Di Formamide.

**ABI AMPF/ STR® Identifiler/ ABI Y-Filer Fragment Detection:**

Required Reagents:

Hi-Di Formamide

500-LIZ Size Standard

Sample Preparation and Loading:

1. Prepare a clean 0.5 mL tube for each sample to the analyzed being sure to add an extra tube for an Allelic Ladder sample.
2. To each tube add 1 μL of the appropriate sample to be analyzed (or 1 μL of allelic ladder), 0.3 μL of 500-LIZ Size Standard, and 9.0 μL of Hi-Di Formamide.
3. Briefly Vortex and zip spin the samples.
4. Heat the sample to 95°C for 3 minutes, immediately followed by placing on ice for 2 minutes.
5. Vortex the sample and briefly zip spin the tube.
6. Place on ice until ready to load on the CE.
7. Pipette all 10.3 μL of sample product into the appropriate wells of a 96- or 384-well ABI Plate.
8. Ensure that all 16 wells selected for the analysis contain either 10.3 μL of sample or 10.3 μL of Hi-Di Formamide.

**PowerPlex® Y System Fragment Detection:**

Required Reagents:

ILS 600 Size Standard

Hi-Di Formamide

Sample Preparation and Loading:

1. Prepare a clean 0.5 mL tube for each sample to the analyzed being sure to add an extra tube for an Allelic Ladder sample.
2. To each tube add 1 μL of the appropriate sample to be analyzed (or 1 μL of allelic ladder), 0.3 μL of ILS 600 Size Standard, and 9.0 μL of Hi-Di Formamide.
3. Briefly Vortex and zip spin the samples.
4. Heat the sample to 95°C for 3 minutes, immediately followed by placing on ice for 2 minutes.
5. Vortex the sample and briefly zip spin the tube.
6. Place on ice until ready to load on the CE.
7. Pipette all 10.3 μL of sample product into the appropriate wells of a 96- or 384-well ABI Plate.
8. Ensure that all 16 wells selected for the analysis contain either 10.3 μL of sample or 10.3 μL of Hi-Di Formamide.

**Genescan Fragment Detection:**

Required Reagents:

Appropriate Size Standard (Typically 500-LIZ)

Hi-Di Formamide

Sample Preparation and Loading:

1. Prepare a clean 0.5 mL tube for each sample to the analyzed being sure to add an extra tube for an Allelic Ladder sample.
2. To each tube add 1 μL of the appropriate sample to be analyzed (or 1 μL of allelic ladder), 0.3 μL of the selected Size Standard, and 9.0 μL of Hi-Di Formamide.
3. Briefly Vortex and zip spin the samples.
4. Heat the sample to 95°C for 3 minutes, immediately followed by placing on ice for 2 minutes.
5. Vortex the sample and briefly zip spin the tube.
6. Place on ice until ready to load on the CE.
7. Pipette all 10.3 μL of sample product into the appropriate wells of a 96- or 384-well ABI Plate.

Ensure that all 16 wells selected for the analysis contain either 10.3 μL of sample or 10.3 μL of Hi-Di Formamide.

## XI. Results

### i. Sequence Data Analysis

a. Ancient DNA Samples

A total of 255 ancient mitochondrial DNA sequence alignments from 42 individuals within 9 different populations were analyzed for variations within different regions of the human mitochondrial control region. The complete sequence alignments can be seen in Appendix 1A along with the complete table of variations, tissue types analyzed and data references (Appendix 1B). As is seen in Table 1, the most heavily sampled population was population 1 with 9 individuals and a total of 54 mtDNA sequences being generated. Following population 1, Populations 6, 7, and 8 were the next most studied with a total of 6, 7, and 8 individuals being analyzed and 43, 49, and 48 sequences in total being generated. Populations 2, 3, 4, 5, and 9 were the least represented with 4, 1, 1, 3, and 3 individual(s) being analyzed generating 25, 3, 11, 10, and 12 mtDNA sequences respectively.

Table 1: List of samples from each population with corresponding number of replicates.

| Population | # of Individuals Sampled | Combined # of Replicates from all Individuals |
|---|---|---|
| 1 | 9 | 54 |
| 2 | 4 | 25 |
| 3 | 1 | 3 |
| 4 | 1 | 11 |
| 5 | 3 | 10 |
| 6 | 6 | 43 |
| 7 | 7 | 49 |
| 8 | 8 | 48 |
| 9 | 3 | 12 |
| Total | 42 | 255 |

All sequences were aligned and compared to the revised Cambridge reference sequence (RCRS) to observe any polymorphisms present in the samples. Over 650 polymorphisms were observed in all 255 sequences, however; only those that have not previously been observed within the entire human population were regarded as possible areas of damage or error. The selection of these polymorphisms based on this selection principle does not assume these are the only evidence of damage, error or mutations. In order to eliminate all polymorphisms that have been observed previously in the human population, the MITOMAP website (http://www.mitomap.org/) was consulted. There, a list of all known, both published and unpublished, polymorphisms occurring within the human mitochondrial control region were obtained. After screening the sequences in this study and eliminating all previously reported polymorphisms, 46 variations remained in total for all 9 populations. The table of all variations considered to be damage or error in this study can been seen in Appendix 1C. As can be seen in Table 2, there were 15 potential damage or error sites remaining in Population 1 that corresponds to 8.7% of all variations observed within this population. The remaining polymorphisms were all transitions from C→T. Within Population 2, 8 polymorphisms remained making up 21.6% of all originally observed variations. Of these 87.5% were transversion type and the remaining 12.5% transitions. After screening all sequences from Populations 3, and 4, no unique polymorphisms remained. Within the 5$^{th}$ and 6$^{th}$ Populations studied, there were 1 (4.3% of all polymorphisms in this population) and 3 (5.2% of all Population 6 variations) unique polymorphisms remaining respectively, all of which being transversions resulting in the departure from a cytosine residue. Populations 7, and 8 observed 6, and 3 polymorphisms remaining respectively corresponding to 3.8% and

4.8% of all variations initially observed within these populations. Of the remaining polymorphisms within Population 7, 83.3% were transversion type and 16.7% were transitions. Within Population 8, 50% of the remaining 8 polymorphisms were transitions and the rest transversions. Lastly, in Population 9, there were 5 unique polymorphisms remaining, which correspond to 16.7% of the total number of polymorphisms observed within this population. Of these, 4 (80%) are transitions and 1 (20%) is a transversion.

Table 2: Percentage of all variations within each population that are unique along with the percentage of each type of base substitution.

| Population | Number of possible damage/error sites in all replicates | Percentage of all variations that are unique | Percent Transversions/Transitions |
|---|---|---|---|
| 1 | 15 | 8.7% | 100% Transitions |
| 2 | 8 | 21.6% | 87.5% Transversions; 12.5% Transitions |
| 3 | 0 | 0.0% | N/A |
| 4 | 0 | 0.0% | N/A |
| 5 | 1 | 4.3% | 100% Transversion |
| 6 | 3 | 5.2% | 100% Transversions |
| 7 | 6 | 3.8% | 83.3% Transversions; 16.7% Transitions |
| 8 | 8 | 4.8% | 62.5% Transitions; 37.5% Transversions |
| 9 | 5 | 16.7% | 80% Transitions; 20% Transversions |

Of the 46 polymorphisms of interest in this study, the frequency of transition type substitutions compared to transversion type are very similar as can be seen in Table 3. The overall frequency of transitions is 56.5% whereas the frequency of transversions is 43.5%. Of the four potentially modified bases, cytosine undergoes replacement significantly more often than any of the others (69.6% of the time), as is seen in Table 4. Following cytosine substitutions, adenine is replaced next most frequently (13.0% of the

time) followed by the equal replacement of both guanine and thymine, which are substituted for at a frequency of 8.7%.

Table 3: Percentage of each type of base substitution observed in all populations combined.

| Type of Base Substitution | Overall Percentage Observed |
|---|---|
| Transition | 56.5% |
| Transversion | 43.5% |

Table 4: Overall percentage of each modified base.

| Bases Modified | Overall Percentage Observed |
|---|---|
| Cytosine (C) | 69.6% |
| Adenine (A) | 13.0% |
| Guanine (G) | 8.7% |
| Thymine (T) | 8.7% |

Table 5: Overall percentage of each base substitution observed.

| Base Substitution | Overall Percentage Observed |
|---|---|
| C→T | 50.0% |
| C→A | 10.8% |
| C→G | 10.8% |
| A→C | 8.7% |
| G→T | 6.5% |
| T→G | 4.4% |
| A→G | 4.4% |
| G→C | 2.2% |
| T→C | 2.2% |

The most common transitions are from cytosine to thymine, which occur at a frequency of 50.0% (Table 5). Following this cytosine is replaced with both adenine and guanine at a frequency of 10.8% (Table 5). The transversion from adenine to cytosine was observed to occur at the same frequency (8.7%) as transversions from cytosine to guanine or adenine; however, transversions from guanine to thymine were less frequent, only occurring 6.5% of the time (Table 5). Transversions from thymine to guanine and, transitions from adenine to guanine were observed only rarely with frequencies of 4.4%

each (Table 5). Similarly, transversions from guanine to cytosine and transversions from thymine to cytosine occurred infrequently as well at 2.2% (Table 5).

b. Modern DNA Samples

The analysis of mitochondrial DNA sequence data from modern individuals was carried out in a similar manner as was done for the analysis of ancient samples. However, all sites showing variations (not just those unobserved in the MITOMAP database) were considered possible site of damage or error. As can be seen in Table 6, 163 sequences from 52 individuals were compared to the RCRS as can be seen in. A table of all polymorphisms found within all replicates can also be seen in Appendix 1D. Both polymorphisms that have not previously been observed within the human population and those that were not seen in multiple replicates from the same individual were included in this study as potential areas of damage or error.

Table 6: List of the total number of replicates from each modern individual tested.

| Individual | # Of Replicates |
|---|---|
| 1 | 48 |
| 2 | 5 |
| 3 | 6 |
| 4 | 4 |
| 5 | 3 |
| 6-52 | 2 each |
| 53 | 5 |
| Total | 163 |

After analyzing all sequence variations, Individual 1 had the highest number of potential damage or error sites with 8. Of these, 4 were transitions and 4 were transversions as is seen in Table 7. Following this, Individuals 2, 3, 5, 36, and 45 all had one possible site of error or damage. All of these variations took the form of

transversions except for Individual 36 which was a transition. Individuals not shown in Table 7 showed no sites of possible error or damage. A table of all polymorphisms considered to be either damage or error within this study can be seen in Appendix 1E.

Table 7: Percentage of all variations found within an individual that are unique, and the type of base substitution.

| Individual | Number of possible damage/error sites in all replicates | Type of Base Substitution |
|---|---|---|
| 1 | 8 | 50% Transitions; 50% Transversions |
| 2 | 1 | Transversion |
| 3 | 1 | Transversion |
| 5 | 1 | Transversion |
| 36 | 1 | Transition |
| 45 | 1 | Transversion |
| Total | 13 | 61.5% Transversions; 38.5% Transitions |

As Table 8 indicates, cytosine residues were substituted for at a much higher frequency (46.1% of all substitutions) than any of the other three bases. Adenine and thymine were the next most frequently substituted bases at 23.1%, followed by guanine at 7.7%.

Table 8: Overall percentage of each modified base.

| Bases Modified | Overall Percentage Observed |
|---|---|
| Cytosine (C) | 46.1% |
| Adenine (A) | 23.1% |
| Thymine (T) | 23.1% |
| Guanine (G) | 7.7% |

As seen in Table 9, transversions from cytosine to guanine were most frequent, occurring in 30.7% of all unique base substitutions observed. Following this, transitions from adenine to guanine occurred 23.1% of the time, and transversions from thymine to guanine occurred 15.4% of the time. The least common base substitutions were guanine

to cytosine and cytosine to adenine transversions; and cytosine to thymine and thymine to cytosine transitions, all occurring only 7.7% of the time.

Table 9: Overall percentage of each base substitution observed.

| Base Substitution | Overall Percentage Observed |
|---|---|
| C→G | 30.7% |
| A→G | 23.1% |
| T→G | 15.4% |
| G→C | 7.7% |
| C→T | 7.7% |
| C→A | 7.7% |
| T→C | 7.7% |

### ii. STR and Y-STR Data Analysis

a. Ancient Y-STR Data Analysis

64 Y-STR profiles were analyzed from 6 ancient individuals. All individuals were analyzed over 16 Y-STR loci and the results of each profile generated can be seen in Appendix 2A. As seen in Table 10, Individual 1 was replicated 22 times, Individual 2 was replicated 9 times, Individual 3 was replicated 6 times, Individual 4 was replicated 9 times, Individual 5 was replicated 5 times, and Individual 6 was replicated 13 times.

Among all replicates from Individual 1 there were a maximum of 4 different allele lengths observed within each of three loci (DYS392, DYS393 and DYS438). Within this individual no alleles consistently amplified while 15 out of the 16 loci amplified in at least one of the replicates. Within Individual 2, the most polymorphic locus was DYS393 where 3 different allele lengths were generated over all replicates. The DYS391 locus amplified two times for Individual 2 and generated an allele length of 10 both times.

Table 10: List of Y-STR profiles generated from ancient DNA extracts indicating the maximum number of alleles amplified at one locus, the alleles that were consistently amplified, and the loci that exhibited complete dropout.

| Individual | # Of Replicates | Max. # Of alleles at one locus | Alleles consistently amplified | Non-amplified Loci |
|---|---|---|---|---|
| 1 | 22 | 4 (DYS392, 393, 438) | None | DYS389II |
| 2 | 9 | 3 (DYS393) | 10 allele replicated 2 times at DYS391 | DYS385a/b; 389I/II; 439; 448; 456; 458; YGATAH4 |
| 3 | 6 | 3 (DYS392, 438) | None | DYS391; 437; 439; 448; 456; 458; YGATAH4 |
| 4 | 9 | 3 (YGATAH4) | None | DYS19; 391; 437; 439; 448; 456; 458; Amel. |
| 5 | 5 | 1 allele at 5 loci | 10 allele replicated 2 times at DYS385a/b | DYS19; 389I/II; 390; 391; 437; 448; 456; 458; YGATAH4; Amel. |
| 6 | 13 | 4 (DYS458) | 13 allele at DYS456; 17 allele at DYS3845a/b | DYS19; 389II; 390; 448; Amel. |

Eight loci amplified at least once within all 9 replicates however, extensive dropout occurred at the remaining eight loci (DYS385a/b, DYS389I/II, DYS439, DYS448, DYS456, DYS458, and YGATAH4). Individuals 3 and 4 were similar in that no alleles consistently amplified. Individual 3 had a maximum of 3 different alleles amplify at 2 loci (DYS392, and DYS438) and Individual 4 had 3 alleles amplify at only one locus (YGATAH4). Both individuals exhibited extensive dropout in at least 7 loci where no amplification occurred in any of the replicates. Individual 3 had dropout at 7 of 16 loci (DYS391, DYS437, DYS439, DYS448, DYS456, DYS458, and YGATAH4) while Individual 4 had dropout at 8 of the 16 (DYS19, DYS391, DYS437, DYS439, DYS448, DYS456, DYS458, and Amelogenin). Within Individual 5, only 1 allele was present at 5 loci and at the DYS385a/b locus the 10 allele was replicated 2 times without the presence

of another allele of differing size. No amplification was observed at DYS19, DYS389I/II, DYS390, DYS391, DYS437, DYS448, DYS456, DYS458 YGATAH4 or Amelogenin loci. Individual 6 showed the presence of 4 different allele lengths at the DYS458 locus among all replicates. Alleles 13 and 17 were consistently amplified at DYS456 and DYS385a/b respectively. No amplification was observed at DYS19, DYS389II, DYS390, DYS448, or the Amelogenin loci.

b. Modern STR and Y-STR Data

STR and Y-STR profiles were generated from 15 separate hair roots all from one modern male individual using commercially available STR and Y-STR kits. Autosomal STR profiles were generated using the AMPF*l* STR® Identifiler® PCR Amplification Kit, and Y-STR profiles were generated using the PowerPlex® Y System Amplification Kit. No variations were observed among any of the 15 replicates from either the STR or Y-STR profiles generated. Table 11 and Table 12 give both the STR and Y-STR profiles that were generated in 100% of the replicates.

Table 11: STR profile generated in all replicates from one modern individual.

| Locus | D8S1179 | D21S11 | D7S820 | CSF1PO | D3S1358 | TH01 | D13S317 | D16S539 |
|---|---|---|---|---|---|---|---|---|
| Allele | 10, 13 | 29, 32.2 | 8, 9 | 10, 14 | 15, 18 | 6, 9 | 11, 12 | 11, 13 |

Table 11 continued.

| Locus | D2S1338 | D19S433 | vWA | TPOX | D18S51 | AMEL. | D5S818 | FGA |
|---|---|---|---|---|---|---|---|---|
| Allele | 18, 19 | 14, 15 | 17, 18 | 8, 11 | 12, 14 | X, Y | 10, 12 | 21, 21 |

Table 12: Y-STR profile generated in all replicates from one modern individual.

| Locus | DYS391 | DYS389I | DYS439 | DYS389II | DYS438 | DYS437 |
|---|---|---|---|---|---|---|
| Allele | 10 | 14 | 12 | 30 | 10 | 14 |

Table 12 continued.

| Locus | DYS19 | DYS392 | DYS393 | DYS390 | DYS385a/b |
|---|---|---|---|---|---|
| Allele | 15 | 11 | 13 | 23 | 12, 16 |

### iii. Hemi-nested Y-STR Amplification

Hemi-nested PCR primers were developed and optimized for 8 regions of the Y chromosome. These 8 primers were designed in such a way that they could be used in conjunction with previously designed primers amplifying various Y-STR loci. Singleplex primers already existed for the amplification of the following loci: DYS385, DYS389, DYS392, DYS390, DYS391, DYS393, DYS438, and DYS439. Given that one of the two primers at each locus was fluorescently labeled, the new primer was designed to anneal upstream of this primer in order to avoid product from the initial PCR being observed during the size detection of the hemi-nested PCR product. Table 13 gives the details for the primers designed in this study.

Table 13: Details for primers developed in this study.

| Locus | | Sequence (5'-3') | $T_m$ (°C) |
|---|---|---|---|
| DYS385a/b | F | GAACTGAAATGATGGCACTGC | 61.4 |
| DYS389I/II | F | CTGTCTGTCTATCTATCTATCTATC | 59.7 |
| DYS390 | R | GTGGGAGAAATGGATGAC | 58.4 |
| DYS391 | R | ATT GCC ATA GAG GGA TAG G | 58.3 |
| DYS392 | R | AGAAGTCAAAACAGAGGG | 56.1 |
| DYS393 | R | ATGAGAACAGACTAATACATAC | 56.0 |
| DYS438 | R | TGAACCTGGGAAGTGGAG | 60.7 |
| DYS439 | R | CAAAATGTTGGGATTACAGGC | 59.5 |

As was mentioned, and is seen in Table 14, the newly designed primers were designed to anneal upstream of the fluorescently labeled inner primer. They either overlap the inner primer or anneal up to 66 base pairs (bp) upstream. The new outer DYS385 forward primer anneals 5 bp upstream of the nested forward primer. At DYS389, the forward outer primer binds 51 bp upstream, while at DYS390, the reverse outer primer overlaps the binding region of the nested primer by 4 bp. The outer reverse primer at DYS391 overlaps the nested primer binding region by 13 bp, whereas the outer

reverse primer at DYS392 anneals 6 bp upstream. The outer primer at DYS393 anneals 66 bp upstream of the nested primer while at DYS438, there is a 4 bp overlap with the primer binding region of the nested primer. Lastly, at DYS439 the outer primer binds 13 bp upstream of the previously designed nested reverse primer.

Table 14: Distance upstream of the nested primer binding region that each corresponding outer primer anneals.

| Locus | Distance upstream outer primer anneals |
|---|---|
| DYS385 | 5 bp |
| DYS389 | 51 bp |
| DYS390 | 4 bp overlap of inner primer |
| DYS391 | 13 bp overlap of inner primer |
| DYS392 | 6 bp |
| DYS393 | 66 bp |
| DYS438 | 4 bp overlap of inner primer |
| DYS439 | 13 bp |

Table 15 describes the allele ranges for each locus tested along with the size range that each locus will encompass. Using the outer primers designed for this study, the initial amplification process will generate slightly larger fragments (Table 14), however, the second amplification using the inner primers will reduce the size of the locus to those given in Table 15.

Table 15: Allele ranges and PCR product sizes for Y-STR loci used in this study.

| Locus | Allele Range | Size Range (bp) | Reference to Locus |
|---|---|---|---|
| DYS385 | 7-28 | 241-324 | (Schneider *et al.*, 1998) |
| DYS389I | 9-17 | 143-171 | (Kayser *et al.*, 1997) |
| DYS389II | 24-34 | 255-295 | (Kayser *et al.*, 1997) |
| DYS390 | 17-28 | 189-283 | (Kayser *et al.*, 1997) |
| DYS391 | 6-14 | 89-121 | (Kayser *et al.*, 1997) |
| DYS392 | 6-17 | 93-125 | (Kayser *et al.*, 1997) |
| DYS393 | 9-17 | 108-141 | (Kayser *et al.*, 1997) |
| DYS438 | 6-14 | 133-173 | (Ayub *et al.*, 2000) |
| DYS439 | 9-14 | 116-136 | (Ayub *et al.*, 2000) |

### iv. Booster STR Amplification

A feasibility study was conducted to determine the efficacy of applying booster STR amplifications to highly fragmented DNA templates. DNA was extracted from one female individual and amplified using the AMPF*l* STR® Identifiler® PCR Amplification Kit. This was initially done in order to determine the appropriate amount of template required for optimal amplification, as well as to generate a known STR profile for the individual (Table 16).

Table 16: STR profile of the female individual used in the booster STR feasibility study.

| Locus | D8S1179 | D21S11 | D7S820 | CSF1PO | D3S1358 | TH01 | D13S317 | D16S539 |
|---|---|---|---|---|---|---|---|---|
| Allele | 13, 13 | 30, 30 | 8, 10 | 11, 12 | 15, 18 | 6, 7 | 8, 12 | 9, 15 |

Table 16 continued.

| Locus | D2S1338 | D19S433 | vWA | TPOX | D18S51 | AMEL. | D5S818 | FGA |
|---|---|---|---|---|---|---|---|---|
| Allele | 17, 17 | 13.2, 15 | 16, 18 | 8, 8 | 12, 16 | X, X | 11, 11 | 21, 23 |

Amplification using the Identifiler® kit was performed on a DNA template dilution series from the same female individual until no detectable amplification occurred. As is described in Table 17, a full STR profile was obtained from the 1:10 dilution. Nearly a full profile was generated with the 1:100 dilution however, dropout of the 13.2 allele did occur at locus D19S433. For the 1:1 000 dilution, minimal amplification occurred. Four alleles at 4 loci were called for; however dropout of the 15 and 7 alleles occurred at the D3S1358 and TH01 loci while alleles 18 and 6 amplified respectively between 50 and 70 RFU's. Allele 13 amplified at 60 RFU at locus D8S1179. At locus CSF1PO and off-ladder allele as well as a 15 allele was called for that was not initially observed in the original profile. No profile was obtained

whatsoever during the first round of amplification using the 1:10 000 dilution. The electropherograms for these initial reactions can be seen in Appendix 3A.

Table 17: Degree of profile generated for the various dilutions.

| Dilution | Degree of Profile Obtained |
|---|---|
| 1:10 | Full profile obtained |
| 1:100 | 15/16 loci amplified; dropout of 13.2 allele at D19S433 |
| 1:1 000 | Partial profile obtained; 4 alleles amplified in total, 1 was an incorrect allele call (allele 15 at CSF1PO). |
| 1:10 000 | No profile obtained |

All of the above-described reactions consisted of 25 μL total volume and 2.5 μL of template for each dilution series. After the initial reactions were carried out and run on the ABI PRISM® 3100 Genetic Analyzer, the remaining product was purified using P-30 size exclusion columns and used as the template for the booster PCR. The booster STR reactions were again conducted using the Identifiler® STR amplification kit, with 2.5 μL of purified template in a 25 μL reaction.

Table 18 gives the resultant profiles generated for the booster amplification of the dilution series amplified previously. The electropherograms for each booster reaction can be seen in Appendix 3B. No booster PCR was carried out for the 1:10 dilution due to the fact that a full profile was generated during the initial amplification.

As seen in Table 18, the 13.2 allele that dropped out at D19S433 during the initial amplification of the 1:100 dilution but amplified during the booster PCR, however, a high degree of non-specific amplification and error occurred during this second reaction as well resulting in off-ladder or incorrect alleles being generated. Of the 15 loci that amplified, 5 resulted in incorrect allele calls. The same is true for the booster amplification of the 1:1 000 dilution. A variety of alleles that were not amplified in the

initial PCR were recovered during the booster amplification, however, at the same time many loci experienced wide ranges of error. All loci amplified, but 9 resulted in incorrect alleles being called for. For the 1:10 000 dilution, 11 loci remained un-amplified as was seen in the initial amplification. Of the 5 loci that did amplify, 3 alleles were correctly called while dropout of the other alleles and non-specific amplification occurred throughout. In all reactions, varying degree of off-ladder peaks were observed as can be seen in the electropherograms.

Table 18: Profiles generated during the booster STR amplifications using 2.5 µL of the previously amplified dilution series.

| Locus → / Dilution ↓ | D8S1179 | D21S11 | D7S820 | CSF1PO | D3S1358 | TH01 | D13S317 | D16S539 |
|---|---|---|---|---|---|---|---|---|
| 1:100 | 13, 20 | 30, 30 | 8, 10 | 11, 11 | 15, 18 | 5.3, 6, 6.3, 7 | 8, 12 | 9, 15 |
| 1:1 000 | 13, 19 | 30, 30 | 10, 10 | 11, 11 | 18, 18 | 5.3, 6 | 8, 8 | 9, 15 |
| 1:10 000 | ---------- | 30, 30 | --------- | ---------- | 15.2, 15.2 | ------- | ---------- | ---------- |

Table 18 continued.

| Locus → / Dilution ↓ | D2S1338 | D19S433 | vWA | TPOX | D18S51 | AMEL. | D5S818 | FGA |
|---|---|---|---|---|---|---|---|---|
| 1:100 | ---------- | 10, 13.2, 15 | 16, 18 | 8, 8 | 12, 16 | X, X | 11, 11 | 20.2, 21, 23 |
| 1:1 000 | 17, 17 | 10, 13.2 | 16, 16 | 8, 8 | 16, 16 | X, X | 11, 11 | 21, 23 |
| 1:10 000 | ---------- | ---------- | 16, 16 | -------- | --------- | X, X | 11, 11 | ------- |

A second booster PCR was carried out using the same template DNA as used in the above described booster PCR however, 5.0 µL of template was added to the 25 µL Identifiler® reaction mixture as opposed to 2.5 µL used previously. Table 19 summarizes the results of these reactions. The electropherograms can be seen in Appendix 3C.

For the 1:100 dilution the profile showed complete allelic dropout at 5 of the 16 loci including the amelogenin locus. Of the remaining 11 loci that amplified, 2 resulted in erroneous alleles being called for. The 1:1 000 dilution showed amplification at all 16 loci where 7 resulted in incorrect allele calls due to either dropout or error. The 1:10 000 dilution only amplified at 6 loci of which 2 were erroneous in the allele calls. As can be seen in the electropherograms, many off-ladder peaks were present during these amplifications as well.

Table 19: Profiles generated during the booster STR amplifications using 5.0 μL of the previously amplified dilution series.

| Locus → / Dilution ↓ | D8S1179 | D21S11 | D7S820 | CSF1PO | D3S1358 | TH01 | D13S317 | D16S539 |
|---|---|---|---|---|---|---|---|---|
| 1:100 | ---------- | 30, 30 | 8, 10 | 11, 12 | 15, 18 | 5.3, 6, 6.3, 7 | 8, 12 | 9, 15 |
| 1:1 000 | 13, 19 | 30, 30 | 10, 10 | 11, 11 | 18, 18 | 5.3, 6 | 8, 8 | 9, 15 |
| 1:10 000 | ---------- | 30, 30 | --------- | ---------- | 15.2, 15.2 | ------- | ---------- | ---------- |

Table 19 continued.

| Locus → / Dilution ↓ | D2S1338 | D19S433 | vWA | TPOX | D18S51 | AMEL. | D5S818 | FGA |
|---|---|---|---|---|---|---|---|---|
| 1:100 | ---------- | 10, 13.2, 15 | 16, 18 | 8, 8 | 12, 16 | --------- | --------- | ------- |
| 1:1 000 | 17, 17 | 10, 13.2 | 16, 16 | 8, 8 | 16, 16 | X, X | 11, 11 | 21, 23 |
| 1:10 000 | 17, 17 | ---------- | 16, 16 | -------- | --------- | X, X | 11, 11 | ------- |

## XII. Discussion

Various methods have been employed to analyze the occurrence of DNA damage, error and mutation in both ancient and modern DNA samples. To achieve this, sequence data, autosomal STR data, and Y-chromosome STR data have been analyzed. Not only was the occurrence of damage and error observed, but methods were also developed to assist in the recovery of such damaged molecules. These new methods were tested to determine the efficacy for potential future use. The occurrence of mutation, on the other hand, was not observed at any stage throughout this study.

Although the specific mechanism for each type of damage (i.e. oxidative, hydrolytic, enzymatic, etc.) was not studied, nor were the specific causes of error, these results remain highly informative for forensic, anthropological and archaeological studies. They add to the understanding of postmortem degradation, as well as indicate the necessary means required for the successful recovery of both ancient and modern biomolecules.

### i. Sequence Data Analysis

a. Ancient DNA Samples

The analysis of ancient mtDNA sequences was performed in order to assess the levels of damage leading to the generation of an incorrect profile. Multiple replicates from each individual were analyzed as a means of locating such damage sites; as well multiple individuals from specific geographic regions were analyzed as an entire population to aid in estimating the types and mechanisms of damage observed within a specific geographical location. Although it is well accepted that damage to the DNA molecule over time does occur, very few studies have addressed the extent to which it

occurs or have analyzed the different types of damage that occur in various geographical regions (Handt *et al.*, 1996; Kolman and Tuross, 2000; Hansen *et al.*, 2001; Hofreiter *et al.*, 2001a). The significance of analyzing the types of damage present in different geographical regions, based on such things as burial preparation practices or taphonomy, is that in future studies researchers will already have an indication of what type of damage can be expected to occur in collections they are about to analyze.

In this study, single base damage was assessed by multiple sequence alignment of 255 sequences from 42 individuals. Samples varied in their age, tissue type, and geographical location from which they were recovered. All sequences were aligned to the revised Cambridge reference sequence (RCRS), which was used as the standard DNA sequence to which all other sequences were compared. Any variations found within the compiled sequences were then further analyzed as potential damage or error sites. Given that most individuals possess some degree of sequence variation in comparison to the RCRS, nucleotide positions that were found to occur in multiple replicates were considered as belonging to the individual's consensus sequence and thus, discarded as possible sites of damage or error. Also, due to the fact that ancient DNA samples are extremely susceptible to contamination from modern human DNA, only those polymorphisms that have not been observed within the human population were regarded as sites of interest. From analyzing multiple replicates from the same individual it is possible to observe the presence of polymorphisms that periodically occur in some but not all of the replicates. For example, in Individual 15, 3 out of the 7 replicates that amplified nucleotide position 16,217 showed the presence of both a cytosine residue as well as a thymine residue while all other replicates showed only the presence of the

thymine residue. Upon further analysis of the elecropherograms from these 3 sequences, it was determined that the cytosine residue that makes up the minor portion is likely to be the result of a contaminating DNA profile. Similarly, with individual 2, replicate 5; there is both a cytosine residue and a thymine residue at nucleotide positions 16,290 and 16,291 where the thymine residue is not found in any of the other 5 sequences at this position. When analyzing the electropherogram at these positions, it is again found that the variant nucleotide (thymine) makes up only the minor portion and again can be attributed to either the archaeologist or another handler of this sample. The profile of the analyst in this project is known and could be excluded as a source of the contamination. As was already mentioned, due to the relative ease of contamination, only those polymorphisms that have yet to be discovered in the human population were held as sites of interest. Cloning of the PCR product prior to sequencing would provide the separation of both the major and minor contributors into separate colonies. This would then result in the consensus sequence being observed after sequencing of one colony while the contaminating sequence would be observed in another colony of clones.

In order to focus our attention on polymorphisms that have not been previously observed within the human population, the MITOMAP website was consulted where a list of all published and unpublished polymorphisms was obtained. In keeping consistency with other studies similar to this (Gilbert *et al.,* 2003b), insertions and deletions that were observed within any sequences were removed from the analysis. Insertions and deletions are particularly difficult to assess at homopolymeric tracts due to the difficulty in determining the consensus sequence of the individual at these locations.

It is suspected that most of the unique polymorphisms observed within the study of ancient samples are a result of damage to the DNA molecule as opposed to polymerase error, however, the possibility remains that misincorporations by *Taq* polymerase did occur within the early stages of replication. It is generally considered that when the starting template number is more than 1,000 copies, postmortem damage rates are unlikely to affect the results, however, when only a few copies initiate the PCR, the resulting sequences are likely to contain artifacts (Handt *et al.,* 1996; Krings *et al.,* 1997). A wide variety of artifacts have been observed within the ancient mitochondrial sequences analyzed in this study, and have lead to approximately equal frequencies of transitions to transversions (56.5% to 43.5%). This is another indicator that high levels of damage have occurred to the DNA molecules given the fact that the naturally mutating mitochondrial genome observes higher rates of transition type substitutions compared to that of transversion types (Malyarchuk and Rogozin, 2004). In past studies, transition:transversion ratios have been observed to occur anywhere from 12:1 to 37:1 (Horai and Hayasaka, 1990; Hasegawa and Horai, 1991; Vigilant *et al.*, 1991; Pesole *et al.*, 1992; Tamura and Nei, 1993). In one study, transition:transversion ratios of 18.2:1, 12.2:1, and 15.7:1 were observed for HVI, HVII, and both HVI and II respectively based on parsimony analyses (Tamura and Nei, 1993). In a more recent study slightly lower ratios were observed using maximum likelihood ratios of 15.6:1 for HVI and 7.2:1 for HVII (Meyer *et al.,* 1999). Within this same study (and many others) the occurrence of pyrimidine transitions was observed more frequently than purine transitions. It was observed that pyrimidine:purine transitions occur at a ratio of 3.5:1 in HVI and 1.7:1 in HVII (Meyer *et al.,* 1999). Observing the relatively equal occurrence of transitions and

transversions (ratio of 1.3:1) within this study further strengthens the probability that damage or error is occurring within these ancient DNA extracts. This is also coupled with the fact that variations are occurring within replicates from the same individual. Another indication that damage and error is responsible for the variations among single individuals is that these variations have not previously been observed within any human population. This is not to say that they were not present in past human populations, thousands of years ago when some of the individuals were alive, however, it does strengthen the proposal that these variations are a result of damage to the DNA molecule or error via misincorporations by the *Taq* DNA polymerase.

Within the realm of transition substitutions, there are two types of miscoding lesions termed "type 1" and "type 2" (Hansen *et al.*, 2001; Gilbert *et al.*, 2003a), both of which have been observed within the dataset analyzed in this study. Type 1 miscoding lesions are either A→G or T→C transitions and type 2 is either transitions from C→T or G→A. Each type has two different possible phenotypes because either of the complementary DNA strands can be sequenced after amplification. For example, a C→T transition may be observed as such, however, if the complementary strand is sequenced, a G→A transition will be observed. This holds true for type 1 transitions as well where an A→G degradation may be observed as either A→G or T→C transitions (Hansen *et al.*, 2001; Hofreiter *et al.*, 2001a; Gilbert *et al.*, 2003a). By far the most common form of postmortem damage is the type 2, C→T transition (Hansen *et al.*, 2001; Hofreiter *et al.*, 2001a; Gilbert *et al.*, 2003a; Gilbert *et al.*, 2003b). Our findings are highly consistent with these previous observations where 50% of all suspected damage sites resulted in a C→T transition. The most probable cause of this form of miscoding lesion has been

previously described as the deamination of cytosine into uracil, which is an analog of thymine (Paabo, 1989; Lindahl, 1993; Hoss *et al.*, 1996; Hansen *et al.*, 2001). Similarly, had the original cytosine been methylated, deamination would have resulted in the formation of thymine directly, resulting in this same substitution. Although deamination is the most probable cause of this lesion, oxidative damage resulting in the formation of 5-hydroxycytosine and/or 5-hydroxyuracil (both analogs of thymine) (Dizdaroglu *et al.*, 2002) cannot be ignored, nor can tautomeric shifts which would also result in this type of transition (shown in Figure 9 of the introduction). Polymerase induced error is another possible cause for these substitutions, however, it is highly unlikely that this occurred since all individuals who showed type 2 transitions showed them in multiple replicates. This is an indication that hydrolytic damage (the most common form of damage) was acting on the sample as a whole.

Of the type 1 transitions observed within this study, A→G substitutions occurred at 4.4% and T→C transitions occurred at only 2.2%. The most likely cause of this type of miscoding lesion is the deamination of adenine to hypoxanthine which is an analog of guanine (Hansen *et al.*, 2001; Hofreiter *et al.*, 2001a). Oxidative damage, however, cannot be ruled out as the causative agent, which would result in the formation of 8-hydroxyadenine from adenine, an analog of guanine (Dizdaroglu *et al.*, 2002), nor can the occurrence of tautomeric shifts be dismissed as the cause of substitution. Replication error is also a possible reason for these substitutions as *Taq* may have erroneously inserted the incorrect nucleotide during replication, or may have read through an AP site randomly inserting the adenine nucleotide. Some strength is given to the assumption that damage (more specifically, deamination) caused the type 1 transition in Individual 36 as

2 of the 3 transversions of this type occur in two separate replicates from this individual. As well, the other polymorphism observed within this individual is a type 2 transition, which is likely also the result of deamination.

Even though the rate of transversion was only slightly lower than that of transitions within this study, the variety of transversions observed was much greater. In keeping consistency with the nomenclature put forth by Hansen *et al.* (2001) for transitions, we will use the following nomenclature for the various transversions observed within this study: Type 1 (C→G or G→C); type 2 (C→A or G→T); and type 3 (A→C or T→G). Note that a fourth type (type 4) would be T→A or A→T, however, no transversions of this type were observed within this study.

Type 1 transversions accounted for 13.0% of all suspected miscoding lesions observed within this study. Given that these transversions cannot be explained by deamination, it is probable they result from oxidative damage to the DNA molecule. This type of transversion occurs by oxidatively damaging the cytosine residue forming either 5-hydroxycytosine or 5-hydroxyuracil intermediates, which then base pairs with cytosine. Further replications will then replace the original cytosine residue with guanine (Dizdaroglu *et al.*, 2002). Tautomeric shifts cannot explain transversions, as only transitions will result from the presence of the rare enol tautomer in the DNA strand (Figure 9).

Type 2 transversions account for 17.3% of all miscoding lesions found within this study. This type of transversion cannot be caused by hydrolytic deamination however, the intermediate 8-hydroxyguanine is typically formed through oxidative damage of guanine. This intermediate then pairs with adenine causing the transversion, and in fact,

is the single most common form of oxidative damage occurring to the DNA molecule (Kasai and Nishimura, 1984; Shibutani *et al.*, 1991). Another potential cause of this transversion is again, the oxidative attack on guanine forming the intermediate 2, 6-diamino-4-hydroxy-5-formamidopyrimidine which is also an analog of thymine (Dizdaroglu *et al.*, 2002).

While no deamination products are capable of forming type 3 transversions, a number of oxidative damage intermediates can aid in the process. Oxidative damage to adenine can result in the formation of either 8-hydroxyadenine, 2-hydroxyadenine, or 4, 6-diamino-5-formamidopyrimidine, which are all capable of base pairing with guanine (Dizdaroglu *et al.*, 2002). The next round of amplification would then ensure the complete transversion of the original adenine.

Although none were observed in the dataset, type 4 transversions can occur through two different intermediates of oxidative damage. Oxidative damage to the adenine moiety can result in the formation of either 2-hydroxyadenine, or 4, 6-diamino-5-formamidopyrimidine. These intermediates can then incorrectly base pair with adenine resulting in the transversion from A→T.

It is possible that all substitutions found within this study (both transitions and transversions) could have been formed through errors induced by the *Taq* polymerase during amplification; however, this is only a remote possibility. In determining whether damage or error is the causative agent for variations within replicates from the tested individuals, it is necessary to consider the amount of variation attributable to a particular type of damage. Here we will analyze each population individually to assess whether damage, or error are the result of the observed variations (Appendix 1C). It should be

noted that in specific populations it may not be possible to clearly determine the cause of variation based on the lack of information provided by the sample.

Within Population 1, we can be relatively certain that a high degree of damage is present in the samples. This is evident from the fact that hydrolytic deamination of cytosine bases is the most abundant form of damage among ancient DNA templates; coupled with the fact that this is the only type of variation observed within all individuals of this population. This is a strong indication that extensive amounts of hydrolytic damage were present in these samples and that polymerase error is not the cause of the observed variations.

Within Population 2, it appears that a high degree of oxidative damage is acting on the samples. This is evident from the fact that individual 10 has 4 sites involving the products of oxidative damage. One of the 4 sites is a type 1 transition that could be caused by deamination of adenine, however, given that the rest of the damage observed within this Individual, and Population, is oxidative it is likely that this is the cause of the transition also. Within Individual 11 (Population 2), all 3 substitutions are type 3 transversions, which again is caused by oxidative damage. Within Individual 12, there is one site of potential damage; however, there are no other sites within this individual that show any form of damage. This is then a potential site of error; however, the substitution observed is consistent with the oxidative damage observed in other individuals from this population that leads us to believe error still may not be the cause.

In one replicate from Individual 18 (Population 5), one variation was observed. Given that no other damage was observed within this Individual, or even within this Population, it adds to the potential that polymerase error is the cause of this variation.

In Population 6, we can be relatively certain that oxidative damage is acting on the DNA templates as opposed to error. This is due to the fact that the most common form of free radical induced damage, oxidative damage resulting in type 2 transversions, is present in 3 individuals from this Population. This type of damage, results in the guanine being replaced by thymine through the intermediate 8-hydroxyguanine. Seeing as though the same mutation is present within 3 other individuals of this Population, we can attribute this variation to damage.

Two individuals from Population 7 showed extensive damage in 1 to 3 replicates. Individual 29 had 3 sites involving type 1 transversions within one replicate while Individual 31 had 3 sites of variation. Two of these sites were type 2 (G→T) transversions while the other was a type 2 (C→T) transition. All of these substitutions can be attributed to oxidative damage; however, the type 2 transition may be indicative that both hydrolytic deamination as well as oxidative damage was affecting this Population. Again, it is not suspected that PCR error is the cause of the observed variations.

Within Population 8, it is suspected that both hydrolytic and oxidative damage were present based on the mixture of substitution types observed. Within Individual 36, 3 substitutions are present in two replicates that can all be explained by hydrolytic deamination. Individual 38 has a combination of three transversions that can only be caused by oxidative damage and 2 C→T transitions that can be caused by either deamination or oxidative damage. Given the likelihood that C→T transitions are mainly caused by deamination and the fact that oxidative damage is present within the samples, it is suggested that both forms of miscoding lesion are present in this Population.

Only one individual from Population 9 showed any form of potential damage or error among all of the samples that were tested within this population. Four C→T transitions were observed in one replicate while one C→G transversion was observed in a second replicate. This would indicate that hydrolytic deamination is the main source of damage to this sample while the transversion may be the result of PCR error. It is also possible that the majority of damage to this sample is hydrolytic damage and to a much lesser extent oxidative.

Of particular interest in this study are Populations 1 and 6 as they are excellent examples of two Populations with two different types of damage acting on the DNA molecules. As is seen in Population 1 only type 2 (C→T or G→A) transitions were observed while in Population 6, only type 2 (C→A or G→T) transversions were found. As was mentioned previously, the most common form of hydrolytic deamination causes the transition from C→T while the most common form of oxidative damage results in the C→A or G→T transversions. The importance of this observation lies in the fact that these two Populations were recovered from two very different geographical locations (Appendix 1B). The two different types of DNA damage are suspected of being directly related to the taphonomic conditions and the deposition environment from which the individuals were recovered. Similarly, with Populations 2 and 7, assuming the single C→T transition in Individual 31 is the result of oxidative damage as opposed to deamination, these Populations too indicate a particular form of damage (oxidative) that is likely associated with the taphonomy and the deposition environment from which the remains were recovered. Individual 11 from Population 2 is also of particular interest on its own because of the consistency in the type of damage observed. Not only is oxidative

damage observed to occur within the entire population, but also one of two particular types of oxidative damage is present within this individual. The A→C transversion observed can only occur through the formation of either 8- or 2-hydroxyadenine, or 4, 6-diamino-5-formamidopyrimidine. This is an example of the specificity different forms of damage have on the DNA molecule.

Within the dataset analyzed, there does not appear to be any significant increase in potential damage or error sites based on the tissue type analyzed. Of the nine Populations studied, three (Populations 1, 2, and 3) analyzed both bone and teeth from the individuals present (Appendices 1B and 1C). Within Population 1, one individual was analyzed from bone while the others were sampled from teeth. The bone sample studied revealed no indications of damage or error, however, the teeth samples observed unique variations potentially attributable to damage or error to varying degrees. This population may not provide a good indication of the stability of bone compared to teeth as it may be the mere fact that more teeth samples were tested compared to bone, thus increasing the potential for damage or error to be observed. Also, the possibility that microenvironments exist within the general area that the remains were recovered, thus, varying levels of preservation among different samples obtained from the same region may be observed.

Population 2 contained a total of 4 individuals, two of which were analyzed through teeth samples and two were analyzed through bone samples. Within this population there again, appeared to be no increase in damage or error based on the tissue types examined. One tooth sample showed no unique variations whatsoever, while the

other showed three sites of potential damage or error. Of the bone samples, one exhibited four sites of potential damage or error, while the other exhibited one.

Population 3 is the final populations in which bone and teeth were both analyzed. Within this population, no potential damage or error sites were observed among any of the samples analyzed. Based on these data, there is no difference in the level of variation observed between these two tissue types. This does not indicate that these two tissue types exhibit the same level of stability, given that no polymorphisms indicative of damage or error were observed throughout the entire population. This may however, provide some indication of the preservation of the sample as a whole, based on the depositional environment.

Weaknesses in this study of tissue type may come from the fact that very few (2) tissue types were analyzed from all aDNA samples. In addition to this, multiple tissue types were not assessed from one individual, which would be required to undoubtedly determine whether tissue type from aDNA extracts affects the levels of damage or error that occur. Similarly, numerous samples from the same tissue would also be required (i.e. multiple teeth, bone, skin samples, hairs, etc.) to reach a valid conclusion. In saying this, it is often difficult to obtain a variety of tissue types from ancient DNA samples, as their destruction is typically desired to be kept to an absolute minimum.

b. Modern DNA Samples

In addition to ancient mtDNA sequence analysis, mtDNA sequences were also obtained within this study for the analysis of damage or error occurring to modern DNA samples. In theory, variations observed during the analysis of modern DNA samples should arise primarily due to PCR error occurring in the early stages of amplification.

This is resultant of the fact that modern DNA templates exist in relatively high copy number largely undamaged. The possibility does exist (albeit slight) that if the yield of modern DNA after its extraction is poor, low numbers of template may be added to the PCR. If this was to occur and one or more of the templates were damaged, modern extracts could also show variation due to this as opposed, or in addition to, PCR error.

Multiple sequence alignment was conducted in this study where all sequences were compared to the RCRS. For the consideration of this study, polymorphisms that have not previously been observed within the human population were again considered as potential sites of damage or error. In addition to these polymorphisms, all other variations that were observed between replicates from one individual were also considered. This was done for modern DNA samples, but not for ancient ones, given that modern DNA extracts are much more difficult to contaminate than aDNA extracts. If contamination is present within a modern DNA sample, the endogenous DNA often out competed the contaminant DNA templates.

As was also the case with the aDNA sequence data analysis, the levels of transitions compared to transversions were indicative that error or damage was acting on the DNA. For this analysis the transition to transversion ratio was 0.63:1 indicating that the number of transversions far outnumberd those of transitions. This is extremely rare to observe given that in the naturally mutating mitochondrial genome, transitions greatly outnumber transversions.

Of the substitutions observed within this study, type 1 transversions (C→G or G→C) were most frequent, occurring 38.4% of the time. These substitutions can be caused by oxidative damage to the DNA molecule resulting in the formation of 5-

hydroxycytosine or 5-hydroxyuracil, both of which are capable of base pairing with cytosine. This transversion type could also be explained by misincorporations by *Taq* polymerase early during amplification; however, they are not capable of being formed by either the insertion of tautomers or hydrolytic deamination.

Type 2 transversions were observed minimally within this study, occurring only 7.7% of the time. This transversion type is typically the result of oxidative damage to the DNA molecule resulting in the formation of 8-hydroxyguanine or 2, 6-diamino-4-hydroxy-5-formamidopyrimidine. Both of these intermediates are then capable of base pairing with adenine inducing the transversion from G→T. Again, this transversion type cannot occur from deamination or the incorporation of tautomers; however, PCR error remains a possibility.

Type 3 transversions were observed to be the third most frequent base substitution within this dataset occurring 15.4% of the time. As with all transversions, this cannot occur through tautomeric shifts, however, it can occur through oxidative damage resulting in 8-hydroxyadenine, 2-hyrdoxyadenine, or 4, 6-diamino-5-formamidopyrimidine being formed. All of these intermediates are then capable of base pairing with guanine resulting in the observed transversion. Hydrolytic damage will not cause this type of base substitution; however, misincorporations by Taq polymerase can. As with the analysis of aDNA sequences, the occurrence of type 4 transversions was not observed. This type of base subtituton would have resulted in the replacement of thymine with adenine or adenine with thymine.

Type 1 transitions (A→G or T→C) were the second most frequently observed base substitutions within this dataset occurring 30.8% of the time. These substitutions can

arise through both oxidative and hydrolytic damage to the DNA molecule, as well as through tautomeric shifts and PCR error. Hydrolytic deamination results in the formation of hypoxanthine from adenine which then base pairs with cytosine while oxidative damage results in 8-hydroxyadenine being formed which is also capable of base pairing with cytosine to induce this mutation.

Type 2 transitions are typically associated with hydrolytic damage to the DNA molecule. This process occurs through the deamination of cytosine or 5-methylcytosine forming uracil and thymine respectively. Following deamination these intermediates then base pair with adenine inducing the transition from cytosine to thymine. In this study, type 2 treansitions were observed only minimally at 7.7% of all the base substitutions that occurred.

Given the data obtained throughout this study, strength is added to the fact that error is the most likely cause for the variations observed among modern DNA extracts as opposed to damage to the DNA molecule. This is evident by the fact that extremely low occurrences of both type 2 transitions and type 2 transversions were observed. Typically, type 2 transitions are the most frequently observed substitutions resulting from high levels of hydrolytic deamination occurring within the DNA. Similarily, type 2 transversions are the most frequently encountered substitutions resulting from oxidative damage to the DNA molecules. Given that neither of these commonly observed damage products were observed to a great extent (15.4% combined, as opposed to 67.3% in the aDNA sequence analysis), it leads us to believe that error is the primary cause for the variations occurring among the modern DNA extracts. These data are compounded by the fact that modern DNA extracts exist in relatively high copy number and are largely

undamaged. This study supports the theory that the primary cause of variation within modern DNA extracts is PCR error as opposed to damage to the DNA molecule.

Of particular interest in this dataset are the replicates analyzed from Individual 1 and Individual 53. Individual 53 is of interest because 5 replicates from 5 different tissue types (blood, buccal, tooth, skin, and hair root), were analyzed for the occurrence of intra-individual variation resulting from tissue type. There appears to be no effect of tissue type on the mitochondrial sequence profile obtained from Individual 53, given that no variations were observed among all 5 of the tissue types tested. Individual 1 on the other hand, did display variations among multiple replicates. All 48 sequences analyzed from Individual 1 were generated using separate hair roots obtained from the scalp of the individual. Out of the 48 sequences analyzed, 8 showed polymorphisms that were not present in any other replicate from this Individual. As has been seen in the past, heteroplasmy within individual hair shafts, as well as different hair shafts from one individual is not uncommon (Calloway *et al.*, 2000; Melton, 2004; Melton *et al.*, 2005), however, heteroplasmy within hair roots is not frequently observed. The variations observed between hair roots could be the result of either damage to the template, heteroplasmy existing within the sample, or the result of PCR error. Although it is difficult to determine, we feel that the variations observed within this study in hair roots from Individual 1 are the result of heteroplasmy within the hair shaft adjacent to the root. When preparing the hair root samples for extraction, approximately 0.75 to 1.0 cm of the hair shaft was cut and placed into the extraction buffer.

### ii. STR and Y-STR Data Analysis

The analysis of ancient Y-STRs, and both modern STRs and Y-STRs was done in attempt to observe intra-individual variation as a result of either polymerase induced errors or DNA damage in the form of fragmentation. Ancient Y-STR data were analyzed to test the presence of variation primarily due to the effects of DNA fragmentation. Modern STR and Y-STR data were analyzed in this study to observe whether any intra-individual DNA variation exists due to mutations within the DNA molecules or as a result of PCR error. Although damage to the DNA molecule begins very rapidly after death, modern DNA extracts are not likely to show signs of degradation, as high concentrations of non-damaged template will outnumber those with damage. Since modern DNA extracts generally contain greater than 1,000 template molecules at the beginning of amplification, the few potentially damaged templates will not affect the outcome of the PCR (Handt *et al.,* 1996; Hansen *et al.,* 2001).

a. Ancient Y-STR Data Analysis

Genetic analysis of both STRs and Y-STRs from ancient or highly degraded DNA samples is of extreme importance for both forensic and archaeological studies. In this study we have observed that Y-STR loci from ancient remains are highly unstable primarily as the result of DNA degradation. Degradation to the DNA molecule occurs rapidly after death resulting in fragment lengths much shorter than ideal for STR typing. Our results indicate that with as many as 22 replicates in one individual, no undisputable allelic determinations were possible. This contradicts previous statements that 4 replicates are necessary to overcome PCR artifacts such as slippage and/or allelic dropout (Hummel and Herrmann, 1996). We feel that four replicates for aDNA STR profiling is

an acceptable minimum, however, the extent to which samples are replicated for authentication is entirely dependent upon the preservation of the DNA templates themselves.

Within the dataset analyzed for this study, the incidence of PCR error due to slippage occurred quite frequently with anywhere between 1 and 4 alleles being amplified at any one locus. This is seen in Individual 1 where 4 alleles were amplified at 3 different loci over multiple replicates. Even within replicates, varying allele lengths were observed. The same is true for Individual 6, where 4 different allele lengths were amplified at locus DYS458 of which two were observed to amplify in one replicate. Artifacts such as these were observed in all individuals tested and are the result of PCR error. As was mentioned, this form of PCR error is the result of *Taq* polymerase slippage, typically resulting in allele lengths one or two repeat units longer or shorter than the actual length. We have seen in this study when analyzing highly degraded samples, the allele lengths generated can span up to 6 repeat units at one locus. Polymerase slippage artifacts become more evident in aDNA studies (as opposed to studies involving modern DNA) as a result of the small amounts of viable templates in the aDNA extracts. It has been estimated that for single copy gene targets, only 5 intact aDNA templates are present at the onset of amplification (Hummel and Herrmann, 1996). Compound this with the fact that increased cycling during amplification of aDNA STR increases the levels of slippage by-products. This is not to say that *Taq* polymerase slippage is more common in studies involving aDNA compared to those of modern DNA, but given the smaller amount of starting DNA templates in ancient studies, the effects of error are much more profound. STR amplifications suffer from the increased number of cycles

more than any other types of PCR even in modern amplifications, where the result is a high level of background noise (Hummel and Herrmann, 1996).

Aside from polymerase slippage artifacts, allelic dropout was frequently observed throughout this study as well. The number of non-amplified loci found for each individual is evidence of these occurrences. All individuals in this study exhibited allelic dropout to some degree. Individual 1 had the least amount of dropout with only one locus remaining unamplified after 22 replicates. The rest of the individuals tested observed dropout ranging from 5 to 11 loci after as many as 5 to 9 replicates were performed. Allelic dropout can arise for a variety of reasons such as template inhibition, damage to the primer binding region, and/or stochastic effects resulting in multiple primer sets competing for the limited amount of viable template. With Y-STR amplifications, allelic dropout is easily detected as typically only one allele is expected to amplify at each locus. Therefore, if dropout occurs, no amplification whatsoever will be observed at a given locus. This is not the case in autosomal STR where an individual can be heterozygous for any locus. Dropout in these instances could generate the appearance of a homozygous individual from someone who is actually heterozygotic. As some researchers have suggested, this is the most crucial point in DNA typing where the consequences of dropout could be detrimental (Hummel and Herrmann, 1996). To overcome the problems associated with the amplification of ancient Y-STRs we have designed and developed a method involving hemi-nested Y-STR amplification.

b. Modern STR and Y-STR Data

The most common use of autosomal and Y-chromosome STRs is in the application of forensic science. Occasionally in the past it has been shown that intra-

individual variation exists at various microsatellite loci typically as the result of mutation (Roffey *et al.,* 2000; Rolf *et al.,* 2001; Rolf *et al.,* 2002), and occurs particularly within tumor tissues (Schwark *et al.,* 2004). Most variations within human STR loci arise either as the result of polymerase slippage, resulting in allelic variations or variations within the primer binding region which causes a potentially phenotypically normal individual to appear genotypically different.

For this study we analyzed both autosomal and Y-chromosome STRs for the appearance of intra-individual variations. Separate male hair roots were chosen for this study due to the fact human hair is the most commonly recovered sample from crime scenes. This tissue type is also of interest because of its clonal growth (Linch *et al.,* 2001). Panmictic populations would be expected to show less mutation, as any that occur would be diluted out, however, mutations within clonal populations would be easily detected. Also, past studies of mitochondrial DNA profiling, have shown that human hair shafts are highly prone to heteroplasmy more than any other human tissue (Calloway *et al.,* 2000; Melton *et al.,* 2005). Based on this, and for the reasons stated previously, our preliminary study of intra-individual STR variation was conducted on modern hair roots. This tissue type was chosen as opposed to hair shaft due to the extreme difficulty in obtaining high enough levels of viable templates to generate full STR profiles.

For this and other modern DNA studies, it is expected that any variations or anomalies observed within or between profiles generated is the result of contamination or mutation within the samples as opposed to PCR error or damage to the DNA molecules. This is suggested because greater than 1,000 copies of template DNA are expected to be present at the beginning of amplification along with the fact samples are relatively fresh

(being amplified for STR within 48 hours of extraction). Although DNA damage and error can occur, the high concentration of intact DNA template will overwhelm and out compete the variant templates. For forensic cases, minor allele lengths that make up less than 15% of the major allele length at the same locus are considered as being PCR artifacts generated as the result of error or damage and are not considered relevant. These same guidelines have been followed throughout this study as well.

Of all 30 STR profiles generated, both autosomal STR and Y-STR, no variations were observed. The occurrence of "stutter peaks" generated through strand slippage during amplification was observed numerous times, however, the peak height was never observed to be greater than 15% that of the major peak. All stutter products generated during this study are consistent with previously reported indications that the majority of the time they will only be one repeat unit shorter than the actual allele length.

Given that no variation was observed within this preliminary study and the fact that intra-individual STR variation is only seldom reported in the literature, this study was not continued. A study of this magnitude would be better designed as a multi-center study with emphasis on DNA samples that have been previously reported as containing variations. This would greatly increase the number of samples examined while reducing the individual cost of analysis for each testing facility.

### iii. Hemi-nested Y-STR Amplification

The crucial point in aDNA STR typing is the amount of sample required for successful amplification and replication. This becomes a particular problem in cases involving historic persons of cultural interest or other valuable samples where destruction must be kept to a minimum (Hummel *et al.,* 1999). Due to this, it is necessary to have

more sensitive methodologies in order to eliminate the need for large volumes of sample, and to eliminate the issue surrounding the authenticity of STR amplifications in ancient DNA studies. To develop a more sensitive method for the recovery of ancient Y-STRs, we utilized previously existing Y-STR singleplex primer pairs and designed a hemi-nested amplification system to complement these previously published primers. To this date such a technique has never been developed for Y-STR amplification.

The eight primers designed for this study were unlabelled and either overlapped the binding location of the labeled inner primers or were located a few nucleotides upstream of the previously published labeled primers. These primers were designed to anneal upstream of the previously developed fluorescently labeled primers in order to avoid generating labeled products of varying size that would ultimately be detected through capillary electrophoresis. Primer optimization proved to be somewhat tedious as extensive secondary structure remained at high temperatures within the primer binding regions of four of the new outer primers. This issue was easily overcome by utilizing the additive dimethyl sulfoxide (DMSO), which effectively lowers the melting temperature of the template allowing it to exist linearly at lower temperatures. Of the remaining four primers, no secondary structures significantly influenced their binding abilities, and thus, require no additives.

The hemi-nested PCR works by amplifying a larger fragment of DNA during the first amplification that will then be used as the template for subsequent amplifications utilizing fluorescently labeled nested primers. Due to the limited quantity of desirable templates during the initial PCR, little amplification is expected to occur; as well products of what amplification does occur may potentially be non-specific. Since the

product of the first PCR is used as template for the hemi-nested amplification, the starting template concentration will be higher. The non-specific product from the first amplification will not affect the hemi-nested PCR, as the specificity of the nested primer pairs will not amplify this product. Also, after the initial round of PCR involving the outer primer pairs, it is important to purify the PCR product prior to carrying out the hemi-nested PCR. This is required to ensure that no interference with the original primers and other reagents occurs during the second PCR.

The next step in this study is to apply this methodology to multiple aDNA extracts in a validation study. Utilization of this methodology will allow the amplification and replication of Y-STR loci from ancient or highly degraded samples of DNA without the need to consume large amounts of sample in the process. This technique will allow for the reliable amplification of Y-STR alleles without the occurrence of PCR artifacts as was observed during the ancient Y-STR data analysis portion of this research.

Hemi-nested Y-STR amplification is a sensitive technique capable of eliminating the occurrence of allelic dropout, stutter products, or three-banded allele patterns. This is primarily due to the increased sensitivity that occurs through the utilization of the nested primers. Although the initial amplification may not be sensitive or specific, the effects are not seen after the nested amplification due to the increased amount of starting template. In addition to this, the non-specific product generated during the initial PCR, will not be observed because these fragments will not be labeled. Only the nested primer is labeled and will be detected by CE.

### iv. Booster STR Amplification

Booster, or biphasic, PCR was attempted on human DNA over a series of dilutions using the AMPF*l* STR® Identifiler® STR Amplification Kit. This attempt was made to assess the potential for recovering highly fragmented autosomal STR from both aDNA extracts, as well as highly degraded modern DNA samples. The sample utilized in this study was composed of intact female DNA. The STR profile for this sample was generated prior to any dilution series being applied in order to generate a standard profile for which all subsequent profiles were compared. A dilution series of the sample was conducted to determine the sensitivity limitations of the STR amplification kit, as well to determine the level of template required in the first PCR that would allow for further use in the booster PCR. This was necessary because it is undesirable to generate a full profile during the initial PCR when the recovery of alleles through booster PCR is to be assessed.

The initial booster amplification was conducted with 2.5 μL of purified product from the initial STR amplification reactions. Using this amount of template for the booster PCR recovered many of the un-amplified alleles from the initial PCR; however, multiple anomalous alleles were also observed in all the profiles generated. In addition to this PCR artifact, dropout also occurred frequently as did the appearance of off-ladder peaks throughout. Preferential amplification of one allele at multiple loci was also observed to occur quite frequently throughout all profiles generated. This is evidenced by the occurrence of one allele peak being of greater intensity than the other.

An additional booster PCR was then conducted using the same PCR product, however, 5 μL of template was used for this trial. An increased concentration of template

was used in attempt to reduce the levels of off-ladder peaks, allelic dropout, and other PCR artifacts. The theory is that with increased template the erroneous PCR product would be overwhelmed and out competed by the wild-type PCR product, from which all subsequent amplifications will stem. This theory proved to be unsuccessful as the occurrence of PCR error increased substantially, generating artifacts. Due to the extremely low number of templates present in the initial PCR, extensive error is suspected to have occurred, thus, increasing the concentration of PCR product in the booster PCR merely increased the levels of erroneous template from which the boosted amplification was initiated.

Based on these results, the booster amplification of STR loci initially appears to be unreliable in recovering un-amplified alleles from the initial round of amplification. This is evidenced by the fact that the alleles, which dropped out during the initial PCR, were recovered during the booster amplification; however, an extensive amount of PCR error was also generated throughout the process. Rather than helping to determine the individual's genotype, this increases the confusion as to which allele lengths exist within the sample and which are mere PCR artifacts. In order for the booster STR amplification procedure to be successful, attempts need to be made to increase the fidelity of the initial STR amplification where the template DNA is extremely limited. Very few options exist that will aid in this; however, increasing the quality of DNA polymerase used may reduce the amount of slippage that occurs, due to its increased processivity. Should a technique be designed to determine what type, if any damage is present in low copy number samples, a repair system could be employed, which would increase the level of intact templates for the initial amplification to initiate.

## XIII. Conclusion

Postmortem degradation to DNA is one of the biggest hurdles forensic scientists, molecular biologists, molecular anthropologists, molecular archaeologists and other scientists have to face when analyzing a degraded biological sample. The damage that is present within a sample is largely unknown at the outset of an investigation and can only be presumed based on the taphonomy, or environmental conditions, from which the sample was recovered. This is a particular problem when sample size is limited, or sample destruction is undesirable, as is the case in historical and forensic studies.

Throughout this research we have identified a variety of damage types and trends within population groups from ancient DNA sequences. Both the trends and types of damage were found to be consistent with the few previous studies conducted in this area. Based on the results obtained through this research, as well as relevant previous studies, it can be concluded that damage to the DNA molecule is extremely specific for the surrounding environmental conditions, and should be considered on a case-by-case basis.

We have also identified error induced through PCR of modern DNA sequences, as well as ancient Y-chromosome STRs. The lack of viable template available at the onset of amplification is the likely cause of PCR artifacts as any damage within the DNA molecule, or polymerase induced error, is likely to be more pronounced than in studies where large amounts of template are present during amplification. Intra-individual variation of modern mtDNA sequences is likely to occur via misincorporations during the initial cycles of amplification or in later stages of amplification where the amount of starting template is lower than ideal.

In order to overcome the small amount of starting DNA template in highly degraded modern or ancient samples, two methodologies were designed and tested in this research to recover STR profiles. A hemi-nested PCR was designed and optimized for the recovery of Y-STRs, while the efficacy of a booster amplification system was tested for the recovery of autosomal STRs. The design and optimization of the hemi-nested Y-STR amplification was a success, while the booster PCR design for autosomal STRs must be reconsidered. There are a variety of potential tests that can be considered for future applications that will render this a valuable technique for STR amplification of both aDNA and modern DNA studies where templates are degraded.

There are a variety of problems associated with the occurrence of damage to DNA molecules that will result in the loss of information available from a given sample. This research aids in the advancement of our knowledge to this area since there have been only a few studies focused on damage to DNA in the past. This research will also lead into future studies that can rectify these issues, allowing for the maximization of information obtained from all sample types including both ancient and modern samples.

## XIV. Future Considerations

This research has provided insight into the occurrence of DNA damage, as well as proposed mechanisms for the various types of damage observed. Given that trends have been identified within individuals from the same populations, the focus can now be applied to determining the types of damage within a sample prior to its extraction. If this can be done even the most highly degraded ancient DNA samples will be capable of providing as much information as a fully intact template. Future studies can now focus on creating a detection system that can be applied to the first extract of a DNA sample or potentially prior to any extraction whatsoever. Once this detection system has been applied to a sample, an appropriate repair system can then also be applied for the various types of damage observed as being present. These two techniques, when used together, will greatly increase the yield of viable DNA template for further analyses. To further this research, with enough sampling, generalizations can be made to identify the best repair mechanism for samples that are obtained from a variety of taphonomic conditions. In using these techniques, it will be possible to eliminate nearly all intra-individual variations that arise as the result of damage to the DNA molecules. This is of particular importance to samples with low copy number templates, as any damage will result in variations being observed.

Validated detection and repair systems would be the ultimate solution to damaged DNA templates, particularly to aid in the recovery of highly fragmented DNA. Without the availability of such techniques, nested, hemi-nested, and booster PCRs will be beneficial in obtaining the maximum level of information possible from a sample.

The hemi-nested Y-STR amplification system developed during this research can now be applied to a series of ancient DNA samples that have been observed in the past as being too degraded to yield any valuable Y-STR data. Future directions for this part of the project will be directed at the validation of this technique. Following this, a similar system will then be developed for autosomal STRs since the recovery of these microsatellites is much more informative than that obtained from Y-STRs.

Further research is necessary to optimize the booster PCR for autosomal STRs. Recently a mini-STR kit amplifying the same loci as the AMPF*l* STR® Identifiler® PCR kit has been developed by Applied Biosystems and will be available to consumers in the near future. This mini-STR kit can potentially serve as a nested PCR for use after the initial amplification using the original Identifiler® STR kit. By nesting the primers, allelic dropout or the extensive amounts of preferential amplification may be overcome. Additionally, a higher quality DNA polymerase can be used during the initial amplification in attempt to reduce the levels of slippage occurring within the limited amount of template.

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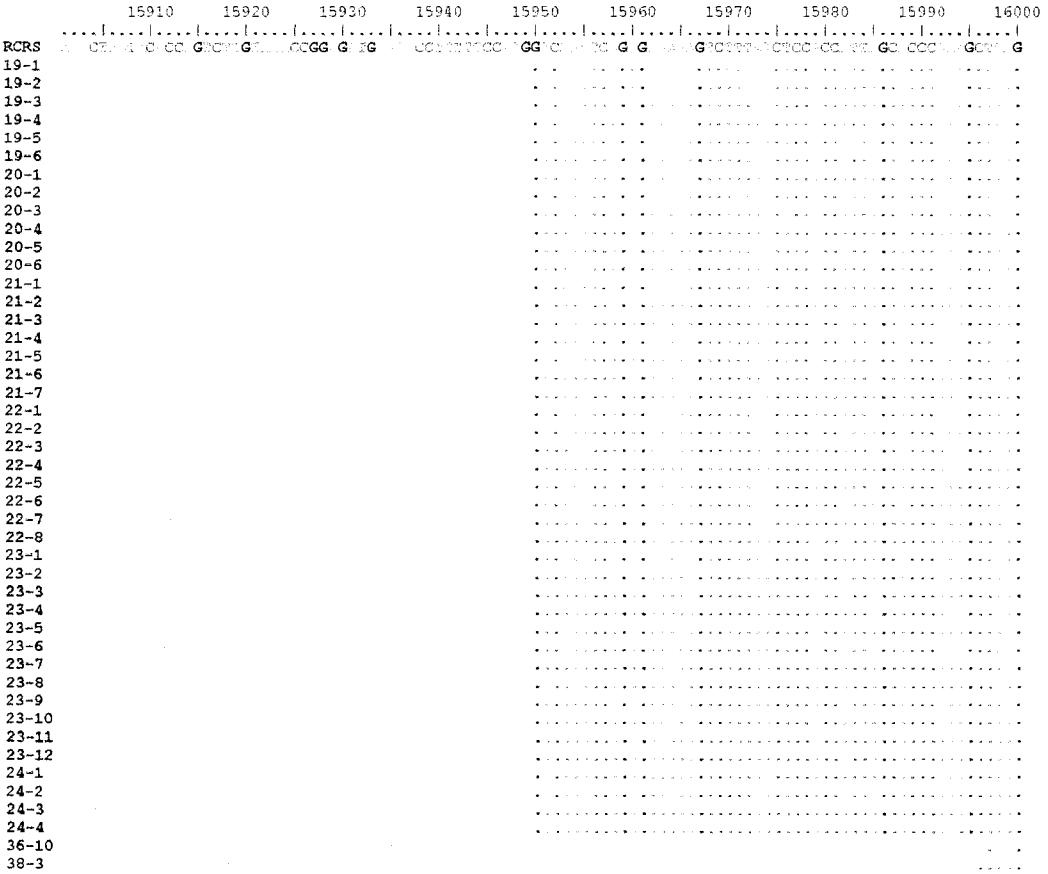
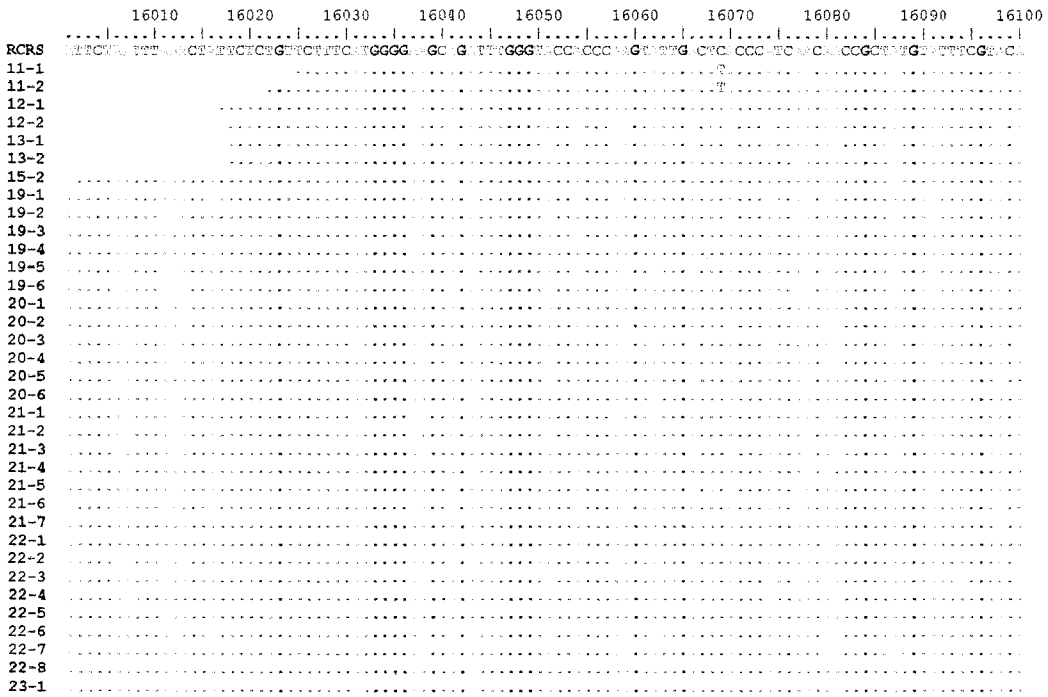
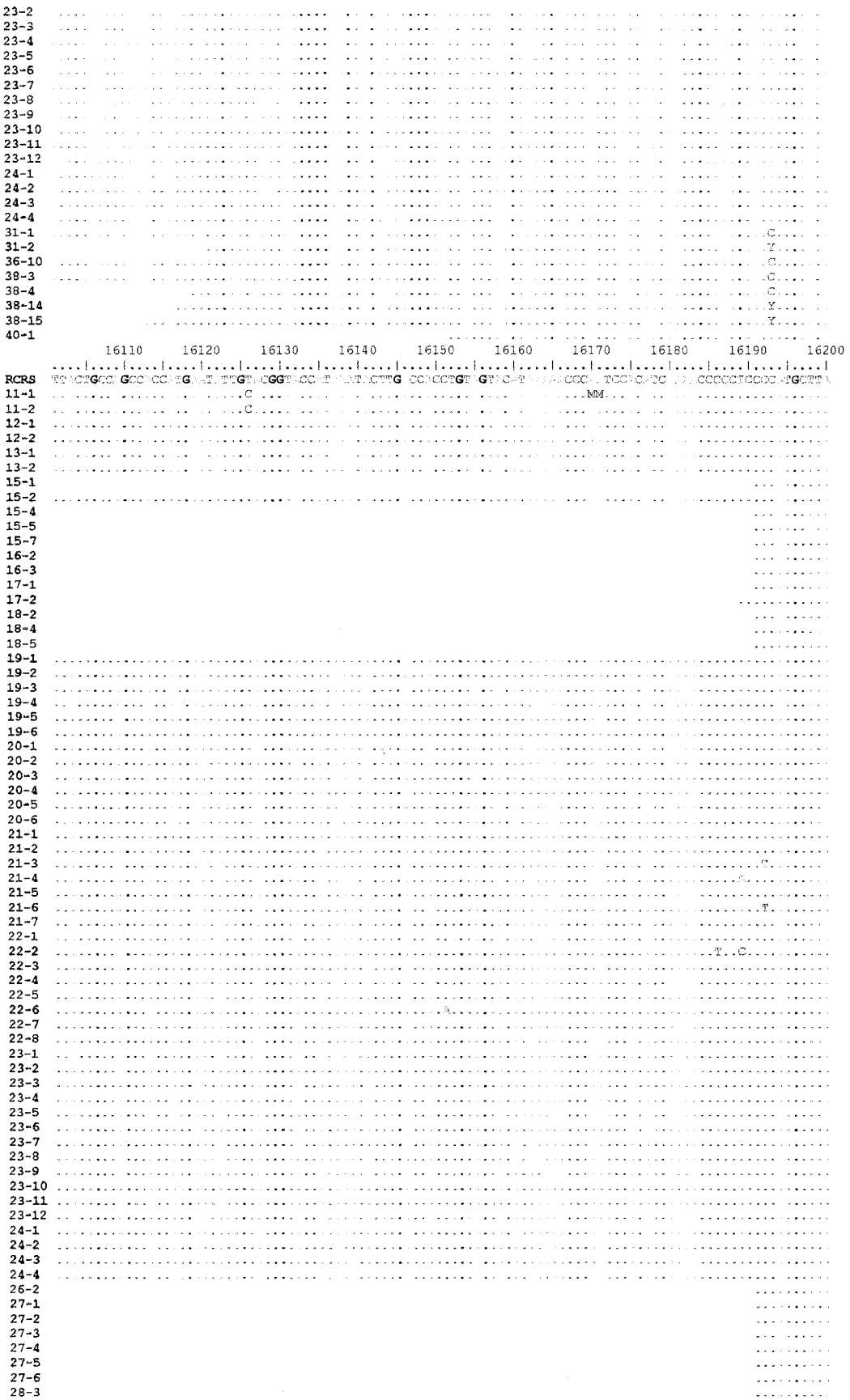
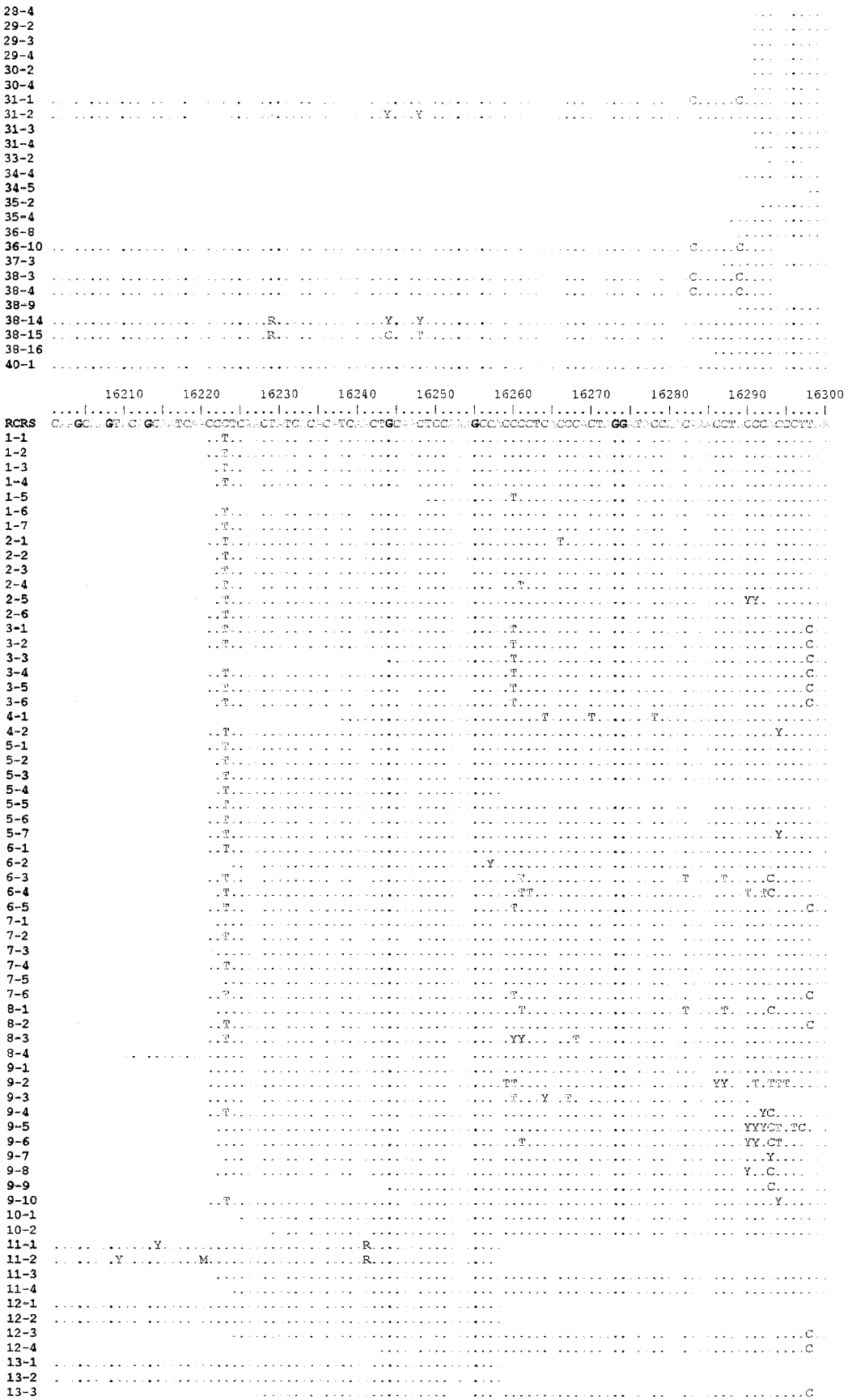
## XVI. Appendix 1A

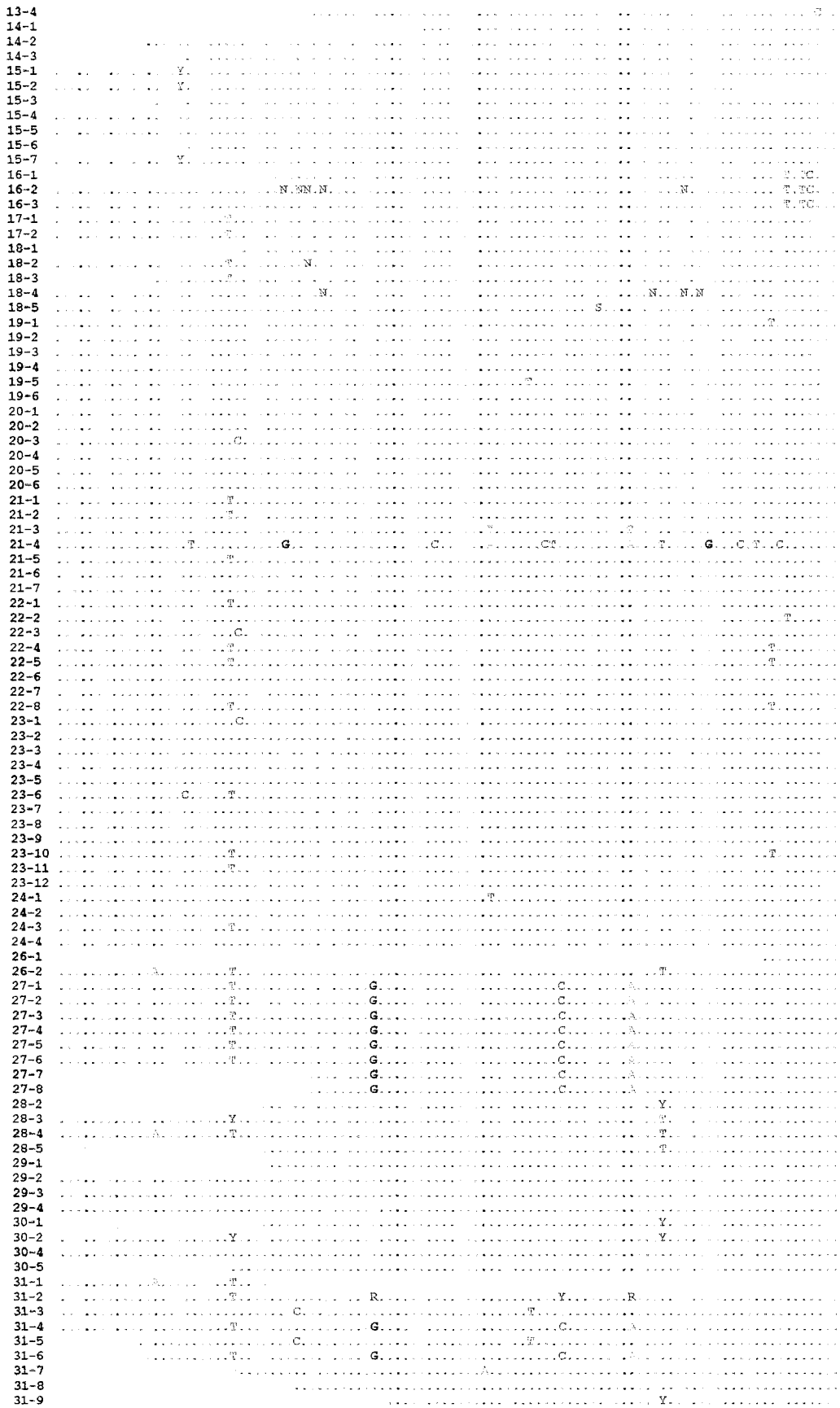
BioEdit multiple sequence alignment for aDNA samples analyzed in this study.

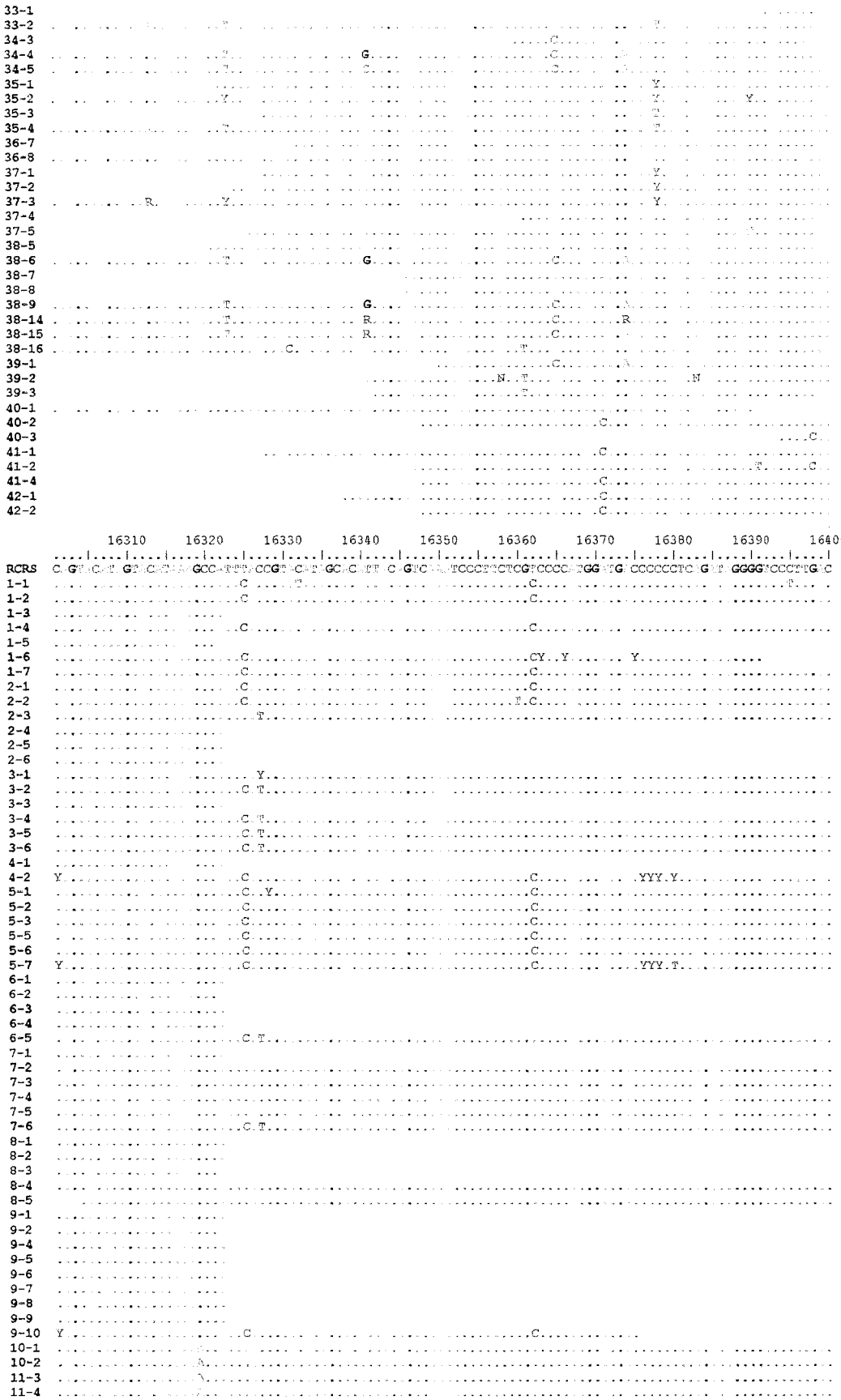


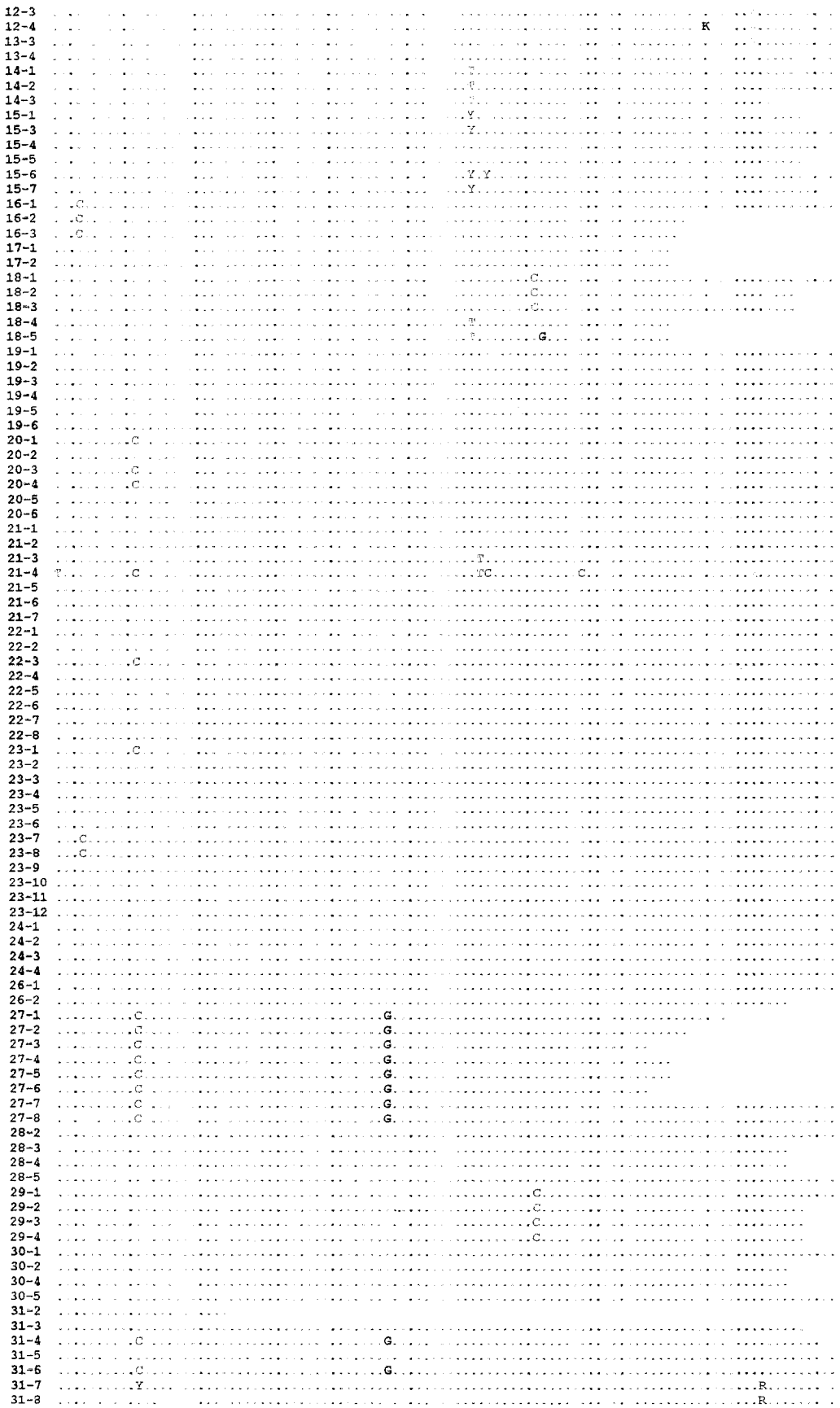



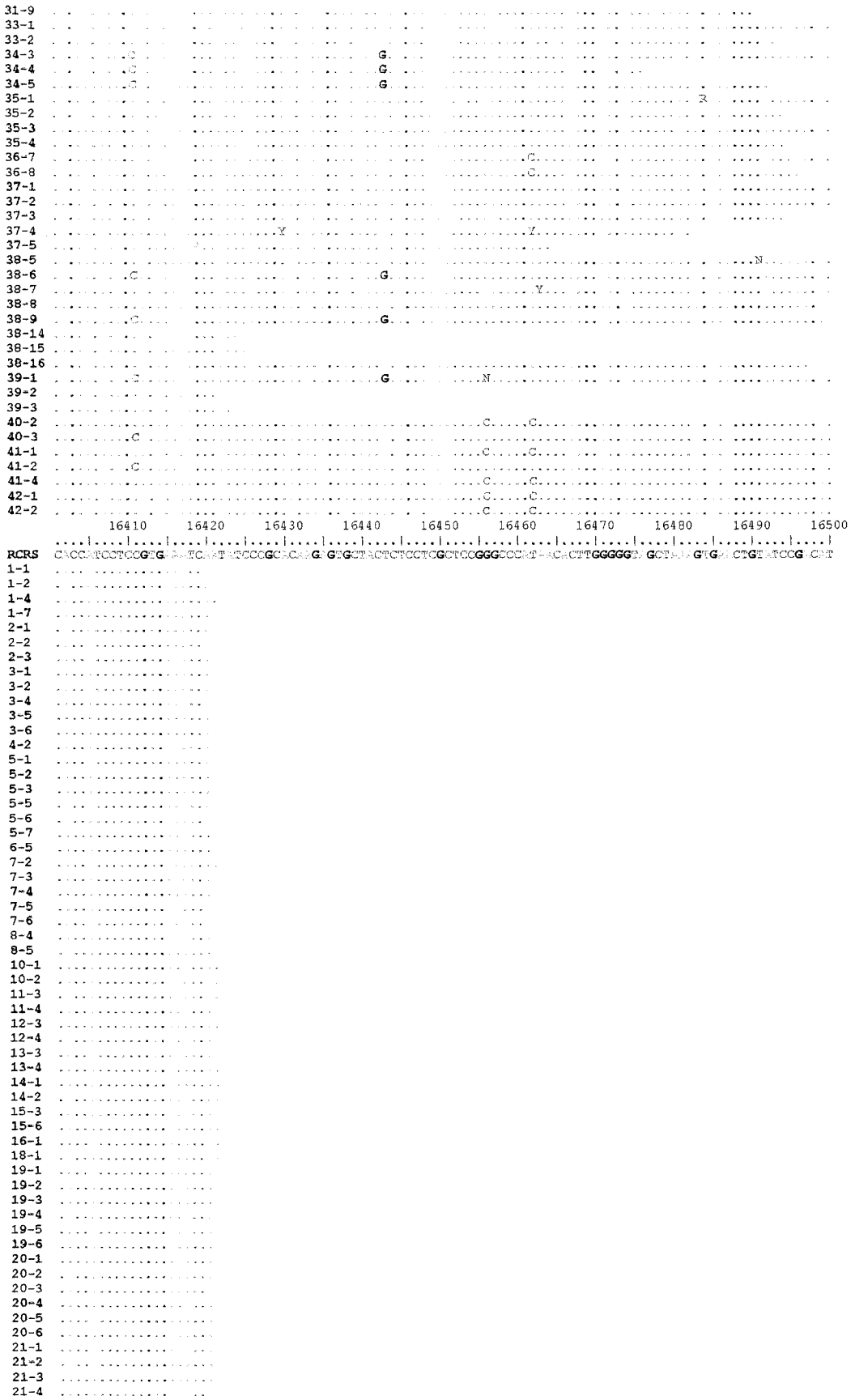


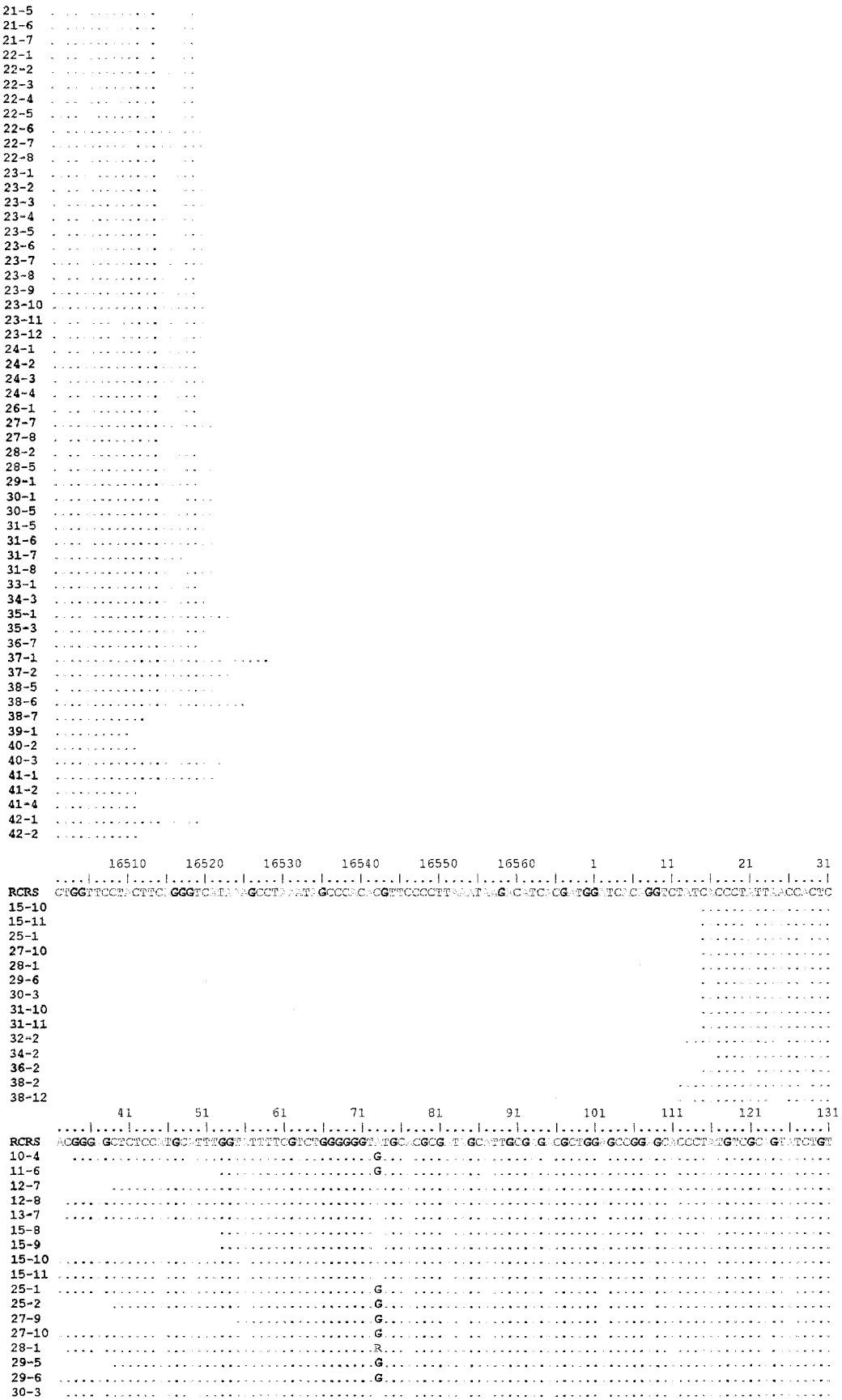


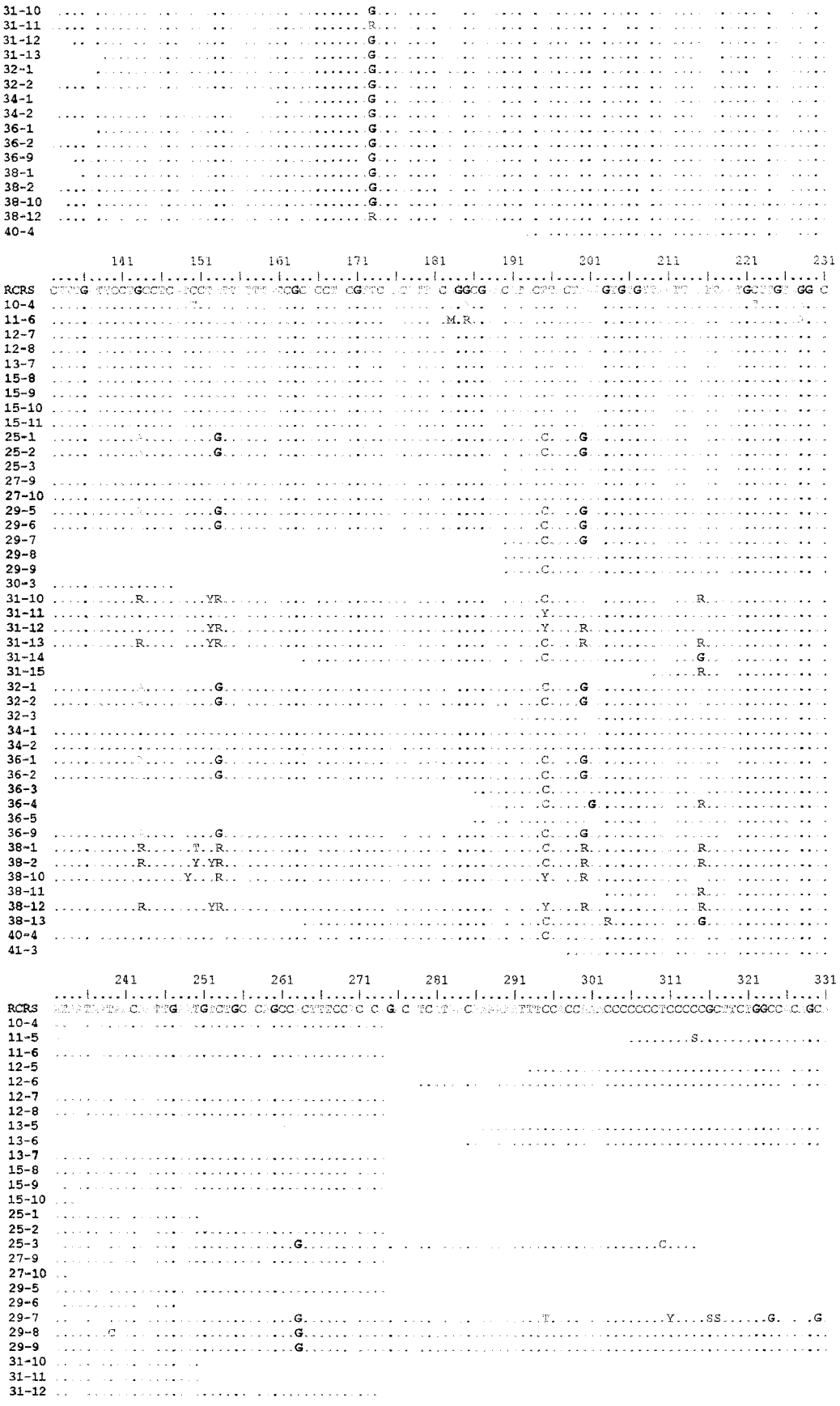


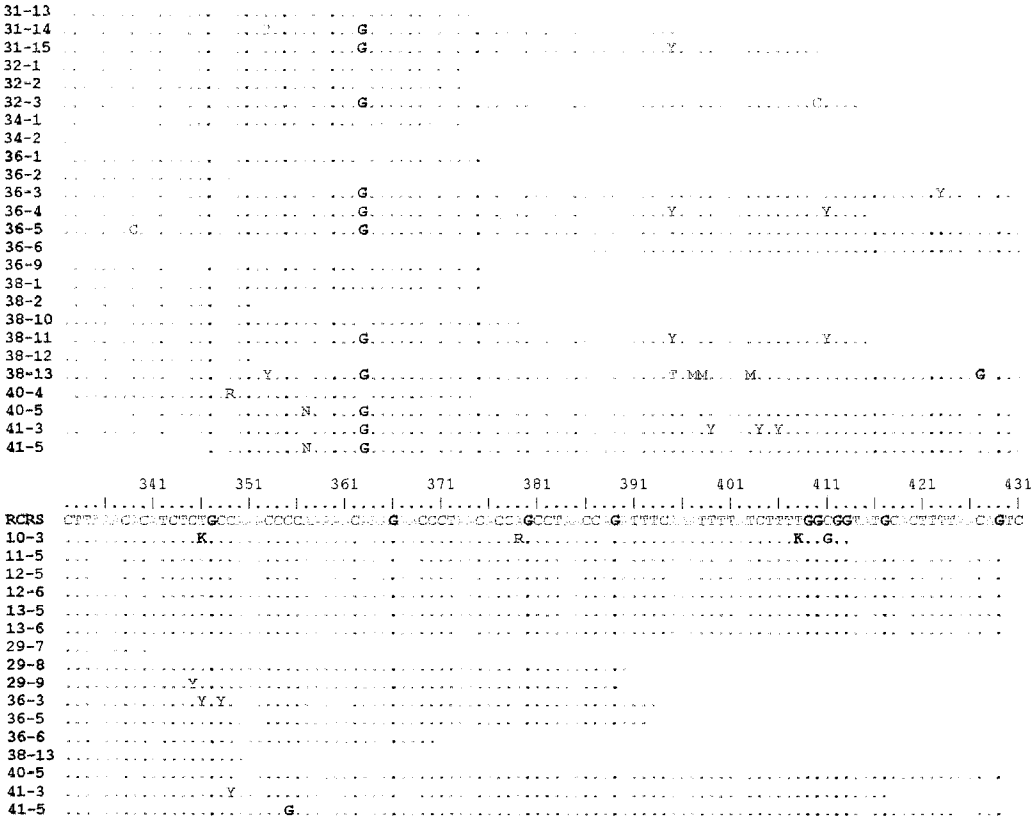












# XVII. Appendix 1B

Table of all polymorphisms observed within all aDNA sequences analyzed in this study.

| | | *Nucleotide in* RCRS → | 16069C | 16093T | 16126T | 16129G | 16144T | 16148C | 16151C | 16170A | 16171A | 16183A | 16186C | 16189T | 16192C | 16209T | 16213G | 16214C | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, C | A, C | A, C | A,C | T | None | G | G, T | A-DEL, AC, ACC, C, CC, G | A,T | C, TC, A | A, T | C | A | T | C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 1 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16218C | 16220A | 16223C | 16224T | 16226A | 16230A | 16231T | 16232C | 16233A | 16235A | 16241A | 16249T | 16255G | 16256C | 16257C | 16258A | 16259C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A, T | A-DEL, C, G | T | C | None | G | C | T | G | G | C, G | A, C | A | T | C-DEL, A, T | G, T | C-DEL, A, CA, T |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 1 | 1 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 2 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 3 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 4 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 5 | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . |
| 1 | 1 | 6 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 7 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 1 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 2 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 3 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 4 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 5 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 6 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 1 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 2 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 3 | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . |
| 1 | 3 | 4 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 5 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 6 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 4 | 1 | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . |
| 1 | 4 | 2 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 1 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 2 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 3 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 4 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 5 | 5 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 6 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 7 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 6 | 1 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 6 | 2 | – | – | – | . | . | . | . | . | . | . | . | . | . | . | T/C | . | . |
| 1 | 6 | 3 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 6 | 4 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 6 | 5 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 1 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 2 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 3 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 4 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 5 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 6 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 1 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 2 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 3 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 4 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 1 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 2 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T |
| 1 | 9 | 3 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16260C | 16261C | 16262C | 16263T | 16264C | 16265A | 16267C | 16268C | 16270C | 16271T | 16274G | 16277A | 16278C | 16281A | 16282C | 16283A | 16284A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | T | T | C | A, G, T | C, G, T | None | C-DEL, T | A, T | C, A | A, T | G, T | T | G | None | G | G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 1 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 5 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 1 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 4 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 1 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 2 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 3 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 4 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 5 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 6 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 4 | 1 | . | . | . | . | T | . | . | . | T | . | . | . | T | . | . | . | . |
| 1 | 4 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 5 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 6 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 6 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 6 | 3 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . |
| 1 | 6 | 4 | . | T | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 6 | 5 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 6 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . |
| 1 | 8 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 3 | C/T | C/T | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 2 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 3 | T | . | . | . | T/C | . | T | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16286C | 16287C | 16288T | 16290C | 16291C | 16292C | 16293A | 16294C | 16295C | 16296C | 16297T | 16298T | 16301C | 16304T | 16311T | 16319G | 16325T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Variations in Population →* | G, T | T | C | T | T | A, G, T | C, T, G | G, T | T | T | C | C | T | *C, G, TT* | C | G-DEL, A, C | T-DEL, A, C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 1 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 1 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 1 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 1 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 1 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 1 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 1 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 2 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 2 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 2 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 2 | 5 | . | . | . | C/T | C/T | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 2 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 3 | 1 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . |
| 1 | 3 | 2 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C |
| 1 | 3 | 3 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | – |
| 1 | 3 | 4 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C |
| 1 | 3 | 5 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C |
| 1 | 3 | 6 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C |
| 1 | 4 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 4 | 2 | . | . | . | . | . | . | . | C/T | . | . | . | . | C/T | . | . | . | C |
| 1 | 5 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 5 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 5 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 5 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 5 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C |
| 1 | 5 | 7 | . | . | . | . | . | . | . | C/T | . | . | . | . | C/T | . | . | . | C |
| 1 | 6 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 6 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 6 | 3 | . | T | . | . | . | . | C | . | . | . | . | . | . | . | . | . | – |
| 1 | 6 | 4 | . | . | . | T | . | T | C | . | . | . | . | . | . | . | . | . | – |
| 1 | 6 | 5 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C |
| 1 | 7 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 7 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 6 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C |
| 1 | 8 | 1 | . | T | . | . | . | . | C | . | . | . | . | . | . | . | . | . | – |
| 1 | 8 | 2 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | – |
| 1 | 8 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 8 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . |
| 1 | 9 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 1 | 9 | 2 | T/C | T/C | . | . | T | . | T | T | T | . | . | . | . | . | . | . | – |
| 1 | 9 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16327C | 16328C | 16330T | 16332C | 16343A | 16354C | 16355C | 16356T | 16360C | 16362T | 16363C | 16366C | 16368T | 16375C | 16376C | 16377C | 16378C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, T | C | None | C, G, T | T | T, A | C, G | T | G, C | G | T, C-DEL | C | A | None | None | None |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 1 | 1 | . | . | . | T | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 1 | 2 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 1 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 4 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 1 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 6 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 1 | 7 | . | . | . | . | . | . | . | . | . | C | T/C | T/C | . | T/C | . | . | . |
| 1 | 2 | 1 | . | . | . | . | . | . | . | . | . | C | . | T | . | . | . | . | . |
| 1 | 2 | 2 | . | . | . | . | . | . | . | . | T | C | . | . | . | . | . | . | . |
| 1 | 2 | 3 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 2 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 1 | C/T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 2 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 4 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 5 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 3 | 6 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 4 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | 2 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C/T | C/T | C/T |
| 1 | 5 | 1 | . | C/T | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 5 | 2 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 5 | 3 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 5 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 5 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 5 | 6 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 1 | 5 | 7 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | C/T | C/T | C/T |
| 1 | 6 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 5 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 7 | 6 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 8 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16380C | 16384G | 16390G | 16391G | 73A | 143G | 150C | 152T | 153A | 180G | 183A | 185G | 195T | 196T | 200A | 201A | 203G | 215A | 222C | 228G | 239T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | None | A | A | A | G | A, G-DEL | T | C, T-DEL, A | G | A | G | A, C, T | A, C | C | G | None | A | G | G, T | A, C | C, G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 1 | 1 | 1 | T | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 7 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | 2 | C/T | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 7 | T | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 249A | 253C | 257A | 263A | 295C | 297A | 298C | 299C | 303C | 304C | 306C | 310T | 311C | 316G | 317C | 324C | 346T | 348C | 349C | 355C | 379A | 408T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A-DEL, G | A | G, T | G, C | T, A | G | T | G | C-DEL, CC-INS | A | None | C, CTC, TC, TT | CC, T | A | T | T | None | None | None | None | None | A |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | | |
| 1 | 1 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 1 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 411C | Tissue Type | Geographical Location | Reference |
|---|---|---|---|---|---|---|
| | | Variations in Population → | None | | | |
| Population | Sample | Replicate ↓ | | | | |
| 1 | 1 | 1 | – | Tooth | California | Unpublished Data |
| 1 | 1 | 2 | – | Tooth | California | Unpublished Data |
| 1 | 1 | 3 | – | Tooth | California | Unpublished Data |
| 1 | 1 | 4 | – | Tooth | California | Unpublished Data |
| 1 | 1 | 5 | – | Tooth | California | Unpublished Data |
| 1 | 1 | 6 | – | Tooth | California | Unpublished Data |
| 1 | 1 | 7 | – | Tooth | California | Unpublished Data |
| 1 | 2 | 1 | – | Tooth | California | Unpublished Data |
| 1 | 2 | 2 | – | Tooth | California | Unpublished Data |
| 1 | 2 | 3 | – | Tooth | California | Unpublished Data |
| 1 | 2 | 4 | – | Tooth | California | Unpublished Data |
| 1 | 2 | 5 | – | Tooth | California | Unpublished Data |
| 1 | 2 | 6 | – | Tooth | California | Unpublished Data |
| 1 | 3 | 1 | – | Bone | California | Unpublished Data |
| 1 | 3 | 2 | – | Bone | California | Unpublished Data |
| 1 | 3 | 3 | – | Bone | California | Unpublished Data |
| 1 | 3 | 4 | – | Bone | California | Unpublished Data |
| 1 | 3 | 5 | – | Bone | California | Unpublished Data |
| 1 | 3 | 6 | – | Bone | California | Unpublished Data |
| 1 | 4 | 1 | – | Tooth | California | Unpublished Data |
| 1 | 4 | 2 | – | Tooth | California | Unpublished Data |
| 1 | 5 | 1 | – | Tooth | California | Unpublished Data |
| 1 | 5 | 2 | – | Tooth | California | Unpublished Data |
| 1 | 5 | 3 | – | Tooth | California | Unpublished Data |
| 1 | 5 | 4 | – | Tooth | California | Unpublished Data |
| 1 | 5 | 5 | – | Tooth | California | Unpublished Data |
| 1 | 5 | 6 | – | Tooth | California | Unpublished Data |
| 1 | 5 | 7 | – | Tooth | California | Unpublished Data |
| 1 | 6 | 1 | – | Tooth | California | Unpublished Data |
| 1 | 6 | 2 | – | Tooth | California | Unpublished Data |
| 1 | 6 | 3 | – | Tooth | California | Unpublished Data |
| 1 | 6 | 4 | – | Tooth | California | Unpublished Data |
| 1 | 6 | 5 | – | Tooth | California | Unpublished Data |
| 1 | 7 | 1 | – | Tooth | California | Unpublished Data |
| 1 | 7 | 2 | – | Tooth | California | Unpublished Data |
| 1 | 7 | 3 | – | Tooth | California | Unpublished Data |
| 1 | 7 | 4 | – | Tooth | California | Unpublished Data |
| 1 | 7 | 5 | – | Tooth | California | Unpublished Data |
| 1 | 7 | 6 | – | Tooth | California | Unpublished Data |
| 1 | 8 | 1 | – | Tooth | California | Unpublished Data |
| 1 | 8 | 2 | – | Tooth | California | Unpublished Data |
| 1 | 8 | 3 | – | Tooth | California | Unpublished Data |
| 1 | 8 | 4 | – | Tooth | California | Unpublished Data |
| 1 | 8 | 5 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 1 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 2 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 3 | – | Tooth | California | Unpublished Data |

| | | Nucleotide in RCRS → | 16069C | 16093T | 16126T | 16129G | 16144T | 16148C | 16151C | 16170A | 16171A | 16183A | 16186C | 16189T | 16192C | 16209T | 16213G | 16214C | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, C | A, C | A, C | A,C | T | None | G | G, T | A-DEL, AC, ACC, C, CC, G | A,T | C, TC, A | A, T | C | A | T | C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 9 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 1 | T | • | C | • | • | • | • | C/A | C/A | • | • | • | • | • | • | C/T | • |
| 2 | 11 | 2 | T | • | C | • | • | • | • | • | • | • | • | • | • | T/C | • | • | • |
| 2 | 11 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 12 | 2 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 12 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 13 | 2 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 13 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • |
| 3 | 14 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | T/C |
| 4 | 15 | 2 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | T/C |
| 4 | 15 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | • |
| 4 | 15 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • |
| 4 | 15 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • |
| 4 | 15 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | T/C |
| 4 | 15 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 16 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16218C | 16220A | 16223C | 16224T | 16226A | 16230A | 16231T | 16232C | 16233A | 16235A | 16241A | 16249T | 16255G | 16256C | 16257C | 16258A | 16259C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A, T | A-DEL, C, G | T | C | None | G | C | T | G | G | C, G | A, C | A | T | C-DEL, A, T | G, T | C-DEL, A, CA, T |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 9 | 4 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 5 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 6 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 7 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 8 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 9 | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . |
| 1 | 9 | 10 | – | – | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 10 | 1 | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 10 | 2 | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 10 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 1 | . | . | . | . | . | . | . | . | . | . | A/G | . | . | . | . | . | – |
| 2 | 11 | 2 | . | A/C | . | . | . | . | . | . | . | . | A/G | . | . | . | . | – | – |
| 2 | 11 | 3 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 11 | 4 | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 11 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 2 | 12 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 2 | 12 | 3 | – | – | – | | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 12 | 4 | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . |
| 2 | 12 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – |
| 2 | 13 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – |
| 2 | 13 | 3 | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 13 | 4 | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . |
| 2 | 13 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 1 | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . |
| 3 | 14 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 14 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 16 | 1 | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16260C | 16261C | 16262C | 16263T | 16264C | 16265A | 16267C | 16268C | 16270C | 16271T | 16274G | 16277A | 16278C | 16281A | 16282C | 16283A | 16284A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | T | T | C | A, G, T | C, G, T | None | C-DEL, T | A, T | C, A | A, T | G, T | T | G | None | G | G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 9 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 6 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 1 | 9 | 10 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 10 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 10 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 10 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 11 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 11 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 12 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 12 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 13 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 13 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 14 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 14 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 16 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16286C | 16287C | 16288T | 16290C | 16291C | 16292C | 16293A | 16294C | 16295C | 16296C | 16297T | 16298T | 16301C | 16304T | 16311T | 16319G | 16325T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | G, T | T | C | T | T | A, G, T | C, T, G | G, T | T | T | C | C | T | C, G, TT | C | G-DEL, A, C | T-DEL, A, C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 9 | 4 | . | . | . | . | . | C/T | C | . | . | . | . | . | . | . | . | . | – |
| 1 | 9 | 5 | . | . | . | C/T | C/T | C/T | C | T | . | T | C | . | . | . | . | . | – |
| 1 | 9 | 6 | . | . | . | C/T | C/T | . | C | T | . | . | . | . | . | . | . | . | – |
| 1 | 9 | 7 | . | . | . | . | . | . | C/T | . | . | . | . | . | . | . | . | . | – |
| 1 | 9 | 8 | . | . | . | C/T | . | . | C | . | . | . | . | . | . | . | . | . | – |
| 1 | 9 | 9 | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | – |
| 1 | 9 | 10 | . | . | . | . | . | . | . | C/T | . | . | . | . | C/T | . | . | . | C |
| 2 | 10 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . |
| 2 | 10 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . |
| 2 | 10 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . |
| 2 | 11 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | A | . |
| 2 | 11 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 3 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . |
| 2 | 12 | 4 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . |
| 2 | 12 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 3 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . |
| 2 | 13 | 4 | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . |
| 2 | 13 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 14 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 14 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 2 | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 15 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 16 | 1 | . | . | . | . | . | . | . | T | . | T | C | . | . | C | . | . | . |

| | | Nucleotide in RCRS → | 16327C | 16328C | 16330T | 16332C | 16343A | 16354C | 16355C | 16356T | 16360C | 16362T | 16363C | 16366C | 16368T | 16375C | 16376C | 16377C | 16378C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, T | C | None | C, G, T | T | T, A | C, G | T | G, C | G | T, C-DEL | C | A | None | None | None |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 1 | 9 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 10 | • | • | • | • | • | • | • | • | • | C | • | • | • | • | – | – | – |
| 2 | 10 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 10 | 2 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 10 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 3 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 11 | 4 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 11 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 3 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 12 | 4 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 12 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 3 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 13 | 4 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 13 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 1 | • | • | • | • | • | T | • | • | • | • | • | • | • | • | • | • | • |
| 3 | 14 | 2 | • | • | • | • | • | T | • | • | • | • | • | • | • | • | • | • | • |
| 3 | 14 | 3 | • | • | • | • | • | T | • | • | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 1 | • | • | • | • | • | C/T | • | • | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 3 | • | • | • | • | • | C/T | • | • | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 4 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 5 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 6 | • | • | • | • | • | C/T | • | T/C | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 7 | • | • | • | • | • | C/T | • | • | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 16 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |

| | | Nucleotide in RCRS → | 16380C | 16384G | 16390G | 16391G | 73A | 143G | 150C | 152T | 153A | 180G | 183A | 185G | 195T | 196T | 200A | 201A | 203G | 215A | 222C | 228G | 239T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | None | A | A | A | G | A, G-DEL | T | C, T-DEL, A | G | A | G | A, C, T | A, C | C | G | None | A | G | G, T | A, C | C, G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 1 | 9 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 2 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 4 | – | – | – | – | G | • | T | • | • | A | • | • | • | • | • | • | • | • | T | A | • |
| 2 | 11 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 3 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 4 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 6 | – | – | – | – | G | • | • | • | • | • | A/C | A/G | • | • | • | • | • | • | • | A | • |
| 2 | 12 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 3 | • | • | A | | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 4 | • | G/T | A | | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 7 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 12 | 8 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 13 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 3 | • | • | A | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 4 | • | • | A | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 7 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 3 | 14 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 2 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 3 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 3 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 4 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 5 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 6 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 7 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 8 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 9 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 4 | 15 | 10 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | – |
| 4 | 15 | 11 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | – |
| 5 | 16 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 249A | 253C | 257A | 263A | 295C | 297A | 298C | 299C | 303C | 304C | 306C | 310T | 311C | 316G | 317C | 324C | 346T | 348C | 349C | 355C | 379A | 408T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A-DEL, G | A | G, T | G, C | T, A | G | T | G | C-DEL, CC-INS | A | None | C, CTC, TC, TT | CC, T | A | T | T | None | None | None | None | None | A |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | | |
| 1 | 9 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 10 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | G/T | • | • | • | A/G | T/G |
| 2 | 10 | 4 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 11 | 5 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 11 | 6 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 5 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 12 | 6 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 12 | 7 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 12 | 8 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 13 | 5 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 13 | 6 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 2 | 13 | 7 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 14 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 8 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 9 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 15 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 16 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 411C | Tissue Type | Geographical Location | Reference |
|---|---|---|---|---|---|---|
| | | Variations in Population → | None | | | |
| Population | Sample | Replicate ↓ | | | | |
| 1 | 9 | 4 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 5 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 6 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 7 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 8 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 9 | – | Tooth | California | Unpublished Data |
| 1 | 9 | 10 | – | Tooth | California | Unpublished Data |
| 2 | 10 | 1 | – | Bone | Belgium | Unpublished Data |
| 2 | 10 | 2 | – | Bone | Belgium | Unpublished Data |
| 2 | 10 | 3 | G | Bone | Belgium | Unpublished Data |
| 2 | 10 | 4 | – | Bone | Belgium | Unpublished Data |
| 2 | 11 | 1 | – | Tooth | Belgium | Unpublished Data |
| 2 | 11 | 2 | – | Tooth | Belgium | Unpublished Data |
| 2 | 11 | 3 | – | Tooth | Belgium | Unpublished Data |
| 2 | 11 | 4 | – | Tooth | Belgium | Unpublished Data |
| 2 | 11 | 5 | • | Tooth | Belgium | Unpublished Data |
| 2 | 11 | 6 | – | Tooth | Belgium | Unpublished Data |
| 2 | 12 | 1 | – | Bone | Belgium | Unpublished Data |
| 2 | 12 | 2 | – | Bone | Belgium | Unpublished Data |
| 2 | 12 | 3 | – | Bone | Belgium | Unpublished Data |
| 2 | 12 | 4 | – | Bone | Belgium | Unpublished Data |
| 2 | 12 | 5 | • | Bone | Belgium | Unpublished Data |
| 2 | 12 | 6 | • | Bone | Belgium | Unpublished Data |
| 2 | 12 | 7 | – | Bone | Belgium | Unpublished Data |
| 2 | 12 | 8 | – | Bone | Belgium | Unpublished Data |
| 2 | 13 | 1 | – | Tooth | Belgium | Unpublished Data |
| 2 | 13 | 2 | – | Tooth | Belgium | Unpublished Data |
| 2 | 13 | 3 | – | Tooth | Belgium | Unpublished Data |
| 2 | 13 | 4 | – | Tooth | Belgium | Unpublished Data |
| 2 | 13 | 5 | • | Tooth | Belgium | Unpublished Data |
| 2 | 13 | 6 | • | Tooth | Belgium | Unpublished Data |
| 2 | 13 | 7 | – | Tooth | Belgium | Unpublished Data |
| 3 | 14 | 1 | – | Tooth | Israel | Unpublished Data |
| 3 | 14 | 2 | – | Bone | Israel | Unpublished Data |
| 3 | 14 | 3 | – | Bone | Israel | Unpublished Data |
| 4 | 15 | 1 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 2 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 3 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 4 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 5 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 6 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 7 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 8 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 9 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 10 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 4 | 15 | 11 | – | Tooth | North-Eastern Canada | Unpublished Data |
| 5 | 16 | 1 | – | Bone | Western Egypt | Unpublished Data |

| | | Nucleotide in RCRS → | 16069C | 16093T | 16126T | 16129G | 16144T | 16148C | 16151C | 16170A | 16171A | 16183A | 16186C | 16189T | 16192C | 16209T | 16213G | 16214C | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, C | A, C | A, C | A,C | T | None | G | G, T | A-DEL, AC, ACC, C, CC, G | A,T | C, TC, A | A, T | C | A | T | C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 5 | 16 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | · | · | · | · | · |
| 5 | 16 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | · | · | · | · | · |
| 5 | 17 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | · | · | · | · | · |
| 5 | 17 | 2 | – | – | – | – | – | – | – | – | – | – | – | · | · | · | · | · | · |
| 5 | 18 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | · | · | · | · | · |
| 5 | 18 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | · | · |
| 5 | 18 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | · | · | · | · | · |
| 5 | 18 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | · | · | · | · | · |
| 6 | 19 | 1 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 19 | 2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 19 | 3 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 19 | 4 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 19 | 5 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 19 | 6 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 20 | 1 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 20 | 2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 20 | 3 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 20 | 4 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 20 | 5 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 20 | 6 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 21 | 1 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 21 | 2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 21 | 3 | · | · | · | · | · | · | · | · | · | · | · | · | T | · | · | · | · |
| 6 | 21 | 4 | · | · | · | · | · | · | · | · | · | · | · | A | · | · | · | · | · |
| 6 | 21 | 5 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 21 | 6 | · | · | · | · | · | · | · | · | · | · | · | · | T | · | · | · | · |
| 6 | 21 | 7 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 22 | 1 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 22 | 2 | · | · | · | · | · | · | · | · | · | · | T | C | · | · | · | · | · |
| 6 | 22 | 3 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 22 | 4 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 22 | 5 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 22 | 6 | · | · | · | · | · | · | A | · | · | · | · | · | · | · | · | · | · |
| 6 | 22 | 7 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 22 | 8 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 1 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 3 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 4 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 5 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 6 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | C |
| 6 | 23 | 7 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 8 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 9 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 10 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 6 | 23 | 11 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |

| | | Nucleotide in RCRS → | 16218C | 16220A | 16223C | 16224T | 16226A | 16230A | 16231T | 16232C | 16233A | 16235A | 16241A | 16249T | 16255G | 16256C | 16257C | 16258A | 16259C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A, T | A-DEL, C, G | T | C | None | G | C | T | G | G | C, G | A, C | A | T | C-DEL, A, T | G, T | C-DEL, A, CA, T |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 5 | 16 | 2 | . | . | . | . | . | N | . | N | N | N | . | . | . | . | . | . | . |
| 5 | 16 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 17 | 1 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 17 | 2 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 1 | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 2 | . | . | T | . | . | . | . | . | N | . | . | . | . | . | . | . | . |
| 5 | 18 | 3 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 4 | . | . | . | . | . | . | . | . | . | N | . | . | . | . | . | . | . |
| 5 | 18 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 3 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 1 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 2 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . |
| 6 | 21 | 4 | T | . | . | . | . | G | . | . | . | . | . | C | . | A | . | . | . |
| 6 | 21 | 5 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 1 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 3 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 4 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 5 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 8 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 6 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 10 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 11 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16260C | 16261C | 16262C | 16263T | 16264C | 16265A | 16267C | 16268C | 16270C | 16271T | 16274G | 16277A | 16278C | 16281A | 16282C | 16283A | 16284A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | T | T | C | A, G, T | C, G, T | None | C-DEL, T | A, T | C, A | A, T | G, T | T | G | None | G | G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 5 | 16 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | N | . | . | . |
| 5 | 16 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 17 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 17 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 4 | . | . | . | . | . | . | . | . | . | . | . | N | . | N | . | N | . |
| 5 | 18 | 5 | . | . | . | . | . | . | . | . | C/G | . | . | . | . | . | . | . | . |
| 6 | 19 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 5 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 3 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . |
| 6 | 21 | 4 | . | . | . | C | T | . | . | . | . | . | A | . | T | . | . | . | G |
| 6 | 21 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 10 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 11 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16286C | 16287C | 16288T | 16290C | 16291C | 16292C | 16293A | 16294C | 16295C | 16296C | 16297T | 16298T | 16301C | 16304T | 16311T | 16319G | 16325T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | G, T | T | C | T | T | A, G, T | C, T, G | G, T | T | T | C | C | T | C, G, TT | C | G-DEL, A, C | T-DEL, A, C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 5 | 16 | 2 | . | . | . | . | . | . | . | T | . | T | C | . | . | C | . | . | . |
| 5 | 16 | 3 | . | . | . | . | . | . | . | T | . | T | C | . | . | C | . | . | . |
| 5 | 17 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 17 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 1 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 6 | 20 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 6 | 20 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 6 | 20 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 4 | . | . | C | T | . | . | C | . | . | . | . | . | T | . | C | . | . |
| 6 | 21 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 2 | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 6 | 22 | 4 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 5 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 8 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 6 | 23 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . |
| 6 | 23 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . | . |
| 6 | 23 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 10 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 11 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16327C | 16328C | 16330T | 16332C | 16343A | 16354C | 16355C | 16356T | 16360C | 16362T | 16363C | 16366C | 16368T | 16375C | 16376C | 16377C | 16378C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, T | C | None | C, G, T | T | T, A | C, G | T | G, C | G | T, C-DEL | C | A | None | None | None |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 5 | 16 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 16 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 17 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 17 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 1 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 5 | 18 | 2 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 5 | 18 | 3 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 5 | 18 | 4 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 18 | 5 | . | . | . | . | . | T | . | . | . | . | G | . | . | . | . | . | . |
| 6 | 19 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 19 | 5 | . | . | . | . | . | . | . | . | A | . | . | . | . | . | . | . | . |
| 6 | 19 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 20 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 3 | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 4 | . | . | . | . | . | . | T | C | . | . | . | . | C | . | . | . | . |
| 6 | 21 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 21 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 22 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 10 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 23 | 11 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16380C | 16384G | 16390G | 16391G | 73A | 143G | 150C | 152T | 153A | 180G | 183A | 185G | 195T | 196T | 200A | 201A | 203G | 215A | 222C | 228G | 239T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | None | A | A | A | G | A, G-DEL | T | C, T-DEL, A | G | A | G | A, C, T | A, C | C | G | None | A | G | G, T | A, C | C, G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 5 | 16 | 2 | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 16 | 3 | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 17 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 17 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 4 | . | . | A | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 7 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 7 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 8 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 7 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 8 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 9 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 10 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 11 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 249A | 253C | 257A | 263A | 295C | 297A | 298C | 299C | 303C | 304C | 306C | 310T | 311C | 316G | 317C | 324C | 346T | 348C | 349C | 355C | 379A | 408T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A-DEL, G | A | G, T | G, C | T, A | G | T | G | C-DEL, CC-INS | A | None | C, CTC, TC, TT | CC, T | A | T | T | None | None | None | None | None | A |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | | |
| 5 | 16 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 16 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 17 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 17 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 18 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 19 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 20 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 21 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 22 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 23 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 411C | Tissue Type | Geographical Location | Reference |
|---|---|---|---|---|---|---|
| | | Variations in Population → | None | | | |
| Population | Sample | Replicate ↓ | | | | |
| 5 | 16 | 2 | – | Bone | Western Egypt | Unpublished Data |
| 5 | 16 | 3 | – | Bone | Western Egypt | Unpublished Data |
| 5 | 17 | 1 | – | Bone | Western Egypt | Unpublished Data |
| 5 | 17 | 2 | – | Bone | Western Egypt | Unpublished Data |
| 5 | 18 | 1 | – | Bone | Western Egypt | Unpublished Data |
| 5 | 18 | 2 | – | Bone | Western Egypt | Unpublished Data |
| 5 | 18 | 3 | – | Bone | Western Egypt | Unpublished Data |
| 5 | 18 | 4 | – | Bone | Western Egypt | Unpublished Data |
| 5 | 18 | 5 | – | Bone | Western Egypt | Unpublished Data |
| 6 | 19 | 1 | – | Bone | Western Egypt | Graver *et al.* 189 |
| 6 | 19 | 2 | – | Bone | Western Egypt | Graver *et al.* 189 |
| 6 | 19 | 3 | – | Bone | Western Egypt | Graver *et al.* 189 |
| 6 | 19 | 4 | – | Bone | Western Egypt | Graver *et al.* 189 |
| 6 | 19 | 5 | – | Bone | Western Egypt | Graver *et al.* 189 |
| 6 | 19 | 6 | – | Bone | Western Egypt | Graver *et al.* 189 |
| 6 | 20 | 1 | – | Bone | Western Egypt | Graver *et al.* 227 |
| 6 | 20 | 2 | – | Bone | Western Egypt | Graver *et al.* 227 |
| 6 | 20 | 3 | – | Bone | Western Egypt | Graver *et al.* 227 |
| 6 | 20 | 4 | – | Bone | Western Egypt | Graver *et al.* 227 |
| 6 | 20 | 5 | – | Bone | Western Egypt | Graver *et al.* 227 |
| 6 | 20 | 6 | – | Bone | Western Egypt | Graver *et al.* 227 |
| 6 | 21 | 1 | – | Bone | Western Egypt | Graver *et al.* 261 |
| 6 | 21 | 2 | – | Bone | Western Egypt | Graver *et al.* 261 |
| 6 | 21 | 3 | – | Bone | Western Egypt | Graver *et al.* 261 |
| 6 | 21 | 4 | – | Bone | Western Egypt | Graver *et al.* 261 |
| 6 | 21 | 5 | – | Bone | Western Egypt | Graver *et al.* 261 |
| 6 | 21 | 6 | – | Bone | Western Egypt | Graver *et al.* 261 |
| 6 | 21 | 7 | – | Bone | Western Egypt | Graver *et al.* 261 |
| 6 | 22 | 1 | – | Bone | Western Egypt | Graver *et al.* 269 |
| 6 | 22 | 2 | – | Bone | Western Egypt | Graver *et al.* 269 |
| 6 | 22 | 3 | – | Bone | Western Egypt | Graver *et al.* 269 |
| 6 | 22 | 4 | – | Bone | Western Egypt | Graver *et al.* 269 |
| 6 | 22 | 5 | – | Bone | Western Egypt | Graver *et al.* 269 |
| 6 | 22 | 6 | – | Bone | Western Egypt | Graver *et al.* 269 |
| 6 | 22 | 7 | – | Bone | Western Egypt | Graver *et al.* 269 |
| 6 | 22 | 8 | – | Bone | Western Egypt | Graver *et al.* 269 |
| 6 | 23 | 1 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 2 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 3 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 4 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 5 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 6 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 7 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 8 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 9 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 10 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 23 | 11 | – | Bone | Western Egypt | Graver *et al.* 322 |

| | | Nucleotide in RCRS → | 16069C | 16093T | 16126T | 16129G | 16144T | 16148C | 16151C | 16170A | 16171A | 16183A | 16186C | 16189T | 16192C | 16209T | 16213G | 16214C | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, C | A, C | A, C | A,C | T | None | G | G, T | A-DEL, AC, ACC, C, CC, G | A,T | C, TC, A | A, T | C | A | T | C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 6 | 23 | 12 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 25 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | A | . | . |
| 7 | 27 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 27 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 27 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 27 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 27 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 27 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 27 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 28 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | A | . | . |
| 7 | 28 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 29 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 29 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 29 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 30 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 30 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 1 | . | C | . | . | . | . | . | . | . | C | . | C | . | . | A | . | . |
| 7 | 31 | 2 | . | C/T | . | A | C/T | T/C | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 31 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . |
| 7 | 31 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . |
| 7 | 31 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . |
| 7 | 31 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16218C | 16220A | 16223C | 16224T | 16226A | 16230A | 16231T | 16232C | 16233A | 16235A | 16241A | 16249T | 16255G | 16256C | 16257C | 16258A | 16259C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A, T | A-DEL, C, G | T | C | None | G | C | T | G | G | C, G | A, C | A | T | C-DEL, A, T | G, T | C-DEL, A, CA, T |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 6 | 23 | 12 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . |
| 6 | 24 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 3 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 25 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 2 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 1 | . | . | T | . | . | . | . | . | . | . | G | . | . | . | . | . | . |
| 7 | 27 | 2 | . | . | T | . | . | . | . | . | . | . | G | . | . | . | . | . | . |
| 7 | 27 | 3 | . | . | T | . | . | . | . | . | . | . | G | . | . | . | . | . | . |
| 7 | 27 | 4 | . | . | T | . | . | . | . | . | . | . | G | . | . | . | . | . | . |
| 7 | 27 | 5 | . | . | T | . | . | . | . | . | . | . | G | . | . | . | . | . | . |
| 7 | 27 | 6 | . | . | T | . | . | . | . | . | . | . | G | . | . | . | . | . | . |
| 7 | 27 | 7 | – | – | – | – | – | – | – | . | . | . | G | . | . | . | . | . | . |
| 7 | 27 | 8 | – | – | – | – | – | – | – | . | . | . | G | . | . | . | . | . | . |
| 7 | 27 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 2 | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | 3 | . | . | T/C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | 4 | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | 5 | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 1 | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 1 | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | 2 | . | . | T/C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | 5 | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 1 | . | . | T | . | . | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 2 | . | . | T | . | . | . | . | . | . | . | G/A | . | . | . | . | . | . |
| 7 | 31 | 3 | . | . | . | . | . | . | C | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 4 | . | . | T | . | . | . | . | . | . | . | G | . | . | . | . | . | . |
| 7 | 31 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 6 | . | . | T | . | . | . | . | . | . | . | G | . | . | . | . | . | . |
| 7 | 31 | 7 | – | – | – | . | . | . | . | . | . | . | . | . | A | . | . | . | . |
| 7 | 31 | 8 | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16260C | 16261C | 16262C | 16263T | 16264C | 16265A | 16267C | 16268C | 16270C | 16271T | 16274G | 16277A | 16278C | 16281A | 16282C | 16283A | 16284A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | T | T | C | A, G, T | C, G, T | None | C-DEL, T | A, T | C, A | A, T | G, T | T | G | None | G | G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 6 | 23 | 12 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 25 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . |
| 7 | 27 | 1 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 27 | 2 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 27 | 3 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 27 | 4 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 27 | 5 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 27 | 6 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 27 | 7 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 27 | 8 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 27 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | C/T | . | . | . | . |
| 7 | 28 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . |
| 7 | 28 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . |
| 7 | 28 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . |
| 7 | 29 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | T/C | . | . | . | . |
| 7 | 30 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | T/C | . | . | . | . |
| 7 | 30 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 2 | . | . | . | . | . | C/A | . | . | . | . | A/G | . | . | . | . | . | . |
| 7 | 31 | 3 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 4 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 31 | 5 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 6 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 7 | 31 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16286C | 16287C | 16288T | 16290C | 16291C | 16292C | 16293A | 16294C | 16295C | 16296C | 16297T | 16298T | 16301C | 16304T | 16311T | 16319G | 16325T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | G, T | T | C | T | T | A, G, T | C, T, G | G, T | T | T | C | C | T | C, G, TT | C | G-DEL, A, C | T-DEL, A, C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 6 | 23 | 12 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 25 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 1 | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 26 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 27 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 27 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 27 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 27 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 27 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 27 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 27 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 27 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 7 | 31 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 31 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 7 | 31 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T/C | . | . |
| 7 | 31 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16327C | 16328C | 16330T | 16332C | 16343A | 16354C | 16355C | 16356T | 16360C | 16362T | 16363C | 16366C | 16368T | 16375C | 16376C | 16377C | 16378C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, T | C | None | C, G, T | T | T, A | C, G | T | G, C | G | T, C-DEL | C | A | None | None | None |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 6 | 23 | 12 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 6 | 24 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 25 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 26 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 1 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 2 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 3 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | – | – |
| 7 | 27 | 4 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 5 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 6 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | – | – |
| 7 | 27 | 7 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 8 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 28 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 29 | 1 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 7 | 29 | 2 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 7 | 29 | 3 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 7 | 29 | 4 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 7 | 29 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | [illegible] | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | [illegible] | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | [illegible] | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 30 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | [illegible] | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | [illegible] | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | [illegible] | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | [illegible] | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16380C | 16384G | 16390G | 16391G | 73A | 143G | 150C | 152T | 153A | 180G | 183A | 185G | 195T | 196T | 200A | 201A | 203G | 215A | 222C | 228G | 239T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | None | A | A | A | G | A, G-DEL | T | C, T-DEL, A | G | A | G | A, C, T | A, C | C | G | None | A | G | G, T | A, C | C, G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 6 | 23 | 12 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 24 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 24 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 24 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 24 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 1 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 7 | 25 | 2 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 7 | 25 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . |
| 7 | 26 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 1 | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 2 | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 4 | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 5 | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 7 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 8 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 9 | – | – | – | – | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 27 | 10 | – | – | – | – | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 7 | 28 | 1 | – | – | – | – | G/A | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 5 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 7 | 29 | 6 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 7 | 29 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | C | . | G | . | . | . | . | . | . |
| 7 | 29 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | C |
| 7 | 29 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | C | . | . | . | . | . | . | . | . |
| 7 | 30 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 3 | – | – | – | – | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 1 | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 2 | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 7 | . | . | . | T/G | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 8 | . | . | . | T/G | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 249A | 253C | 257A | 263A | 295C | 297A | 298C | 299C | 303C | 304C | 306C | 310T | 311C | 316G | 317C | 324C | 346T | 348C | 349C | 355C | 379A | 408T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A-DEL, G | A | G, T | G, C | T, A | G | T | G | C-DEL, CC-INS | A | None | C, CTC, TC, TT | CC, T | A | T | T | None | None | None | None | None | A |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | | |
| 6 | 23 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 24 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 24 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 24 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 24 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 2 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 25 | 3 | • | • | • | G | • | • | • | • | • | • | • | C | • | • | – | – | – | – | – | – | – | – |
| 7 | 26 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 26 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 9 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 27 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 28 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 5 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 6 | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 29 | 7 | • | • | • | G | T | • | • | • | • | • | • | • | T/C | G/C | G/C | G | – | – | – | – | – | – |
| 7 | 29 | 8 | • | • | • | G | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | – |
| 7 | 29 | 9 | • | • | • | G | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | – |
| 7 | 30 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 30 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 411C | Tissue Type | Geographical Location | Reference |
|---|---|---|---|---|---|---|
| | | Variations in Population → | None | | | |
| Population | Sample | Replicate ↓ | | | | |
| 6 | 23 | 12 | – | Bone | Western Egypt | Graver *et al.* 322 |
| 6 | 24 | 1 | – | Bone | Western Egypt | Graver *et al.* 329 |
| 6 | 24 | 2 | – | Bone | Western Egypt | Graver *et al.* 329 |
| 6 | 24 | 3 | – | Bone | Western Egypt | Graver *et al.* 329 |
| 6 | 24 | 4 | – | Bone | Western Egypt | Graver *et al.* 329 |
| 7 | 25 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 25 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 25 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 26 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 26 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 6 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 7 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 8 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 9 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 27 | 10 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 28 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 28 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 28 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 28 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 28 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 6 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 7 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 8 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 29 | 9 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 30 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 30 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 30 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 30 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 30 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 6 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 7 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 8 | – | Bone | Western Honduras | Unpublished Data |

| | | Nucleotide in RCRS → | 16069C | 16093T | 16126T | 16129G | 16144T | 16148C | 16151C | 16170A | 16171A | 16183A | 16186C | 16189T | 16192C | 16209T | 16213G | 16214C | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, C | A, C | A, C | A,C | T | None | G | G, T | A-DEL, AC, ACC, C, CC, G | A,T | C, TC, A | A, T | C | A | T | C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 7 | 31 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | • | A | • | • |
| 8 | 34 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 4 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 8 | 34 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • |
| 8 | 35 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • |
| 8 | 35 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 4 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | A | • | • |
| 8 | 36 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 8 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 8 | 36 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 10 | • | C | • | • | • | • | • | • | • | C | • | C | • | – | – | – | – |
| 8 | 37 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 3 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | G/A | • | • |
| 8 | 37 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 3 | • | C | • | • | • | • | • | • | • | C | • | C | • | – | – | – | – |
| 8 | 38 | 4 | • | C | • | • | • | • | • | • | • | C | • | C | • | – | – | – | – |
| 8 | 38 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • |
| 8 | 38 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 9 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 8 | 38 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16218C | 16220A | 16223C | 16224T | 16226A | 16230A | 16231T | 16232C | 16233A | 16235A | 16241A | 16249T | 16255G | 16256C | 16257C | 16258A | 16259C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A, T | A-DEL, C, G | T | C | None | G | C | T | G | G | C, G | A, C | A | T | C-DEL, A, T | G, T | C-DEL, A, CA, T |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 7 | 31 | 9 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 7 | 31 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 2 | • | • | T | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 34 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 4 | • | • | T | • | • | • | • | • | • | • | G | • | • | • | • | • | • |
| 8 | 34 | 5 | • | • | T | • | • | • | • | • | • | • | C | • | • | • | • | • | • |
| 8 | 35 | 1 | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 35 | 2 | • | • | C/T | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 35 | 3 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 35 | 4 | • | • | T | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 36 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 7 | – | – | – | – | – | – | – | • | • | • | • | • | • | • | • | • | • |
| 8 | 36 | 8 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 36 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 1 | – | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 37 | 2 | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 37 | 3 | • | • | C/T | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 37 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 5 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 38 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 5 | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 8 | 38 | 6 | • | • | T | • | • | • | • | • | • | • | G | • | • | • | • | • | • |
| 8 | 38 | 7 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 8 | 38 | 8 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 8 | 38 | 9 | • | • | T | • | • | • | • | • | • | • | G | • | • | • | • | • | • |
| 8 | 38 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16260C | 16261C | 16262C | 16263T | 16264C | 16265A | 16267C | 16268C | 16270C | 16271T | 16274G | 16277A | 16278C | 16281A | 16282C | 16283A | 16284A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | T | T | C | A, G, T | C, G, T | None | C-DEL, T | A, T | C, A | A, T | G, T | T | G | None | G | G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 7 | 31 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | C/T | . | . | . | . |
| 7 | 31 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . |
| 8 | 34 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 3 | – | – | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 8 | 34 | 4 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 8 | 34 | 5 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 8 | 35 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | C/T | . | . | . | . |
| 8 | 35 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | C/T | . | . | . | . |
| 8 | 35 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . |
| 8 | 35 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . |
| 8 | 36 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 36 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 36 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | C/T | . | . | . | . |
| 8 | 37 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | C/T | . | . | . | . |
| 8 | 37 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | T/C | . | . | . | . |
| 8 | 37 | 4 | – | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 37 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 6 | . | . | . | . | . | . | . | . | . | . | A | . | . | . | . | . | . |
| 8 | 38 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 9 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 8 | 38 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16286C | 16287C | 16288T | 16290C | 16291C | 16292C | 16293A | 16294C | 16295C | 16296C | 16297T | 16298T | 16301C | 16304T | 16311T | 16319G | 16325T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | G, T | T | C | T | T | A, G, T | C, T, G | G, T | T | T | C | C | T | C, G, TT | C | G-DEL, A, C | T-DEL, A, C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 7 | 31 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 1 | – | – | – | – | – | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 33 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 34 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 8 | 34 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 8 | 34 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 8 | 35 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 35 | 2 | . | . | . | C/T | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 35 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 35 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 36 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 36 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 36 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 37 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 37 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 37 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 37 | 5 | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | A | . |
| 8 | 38 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 6 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 8 | 38 | 7 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 8 | 38 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16327C | 16328C | 16330T | 16332C | 16343A | 16354C | 16355C | 16356T | 16360C | 16362T | 16363C | 16366C | 16368T | 16375C | 16376C | 16377C | 16378C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, T | C | None | C, G, T | T | T, A | C, G | T | G, C | G | T, C-DEL | C | A | None | None | None |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 7 | 31 | 9 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 7 | 31 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 33 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 34 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 3 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 34 | 4 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 34 | 5 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 35 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 35 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 35 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 35 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 36 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 7 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 8 | 36 | 8 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 8 | 36 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 37 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 37 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 37 | 4 | . | . | T/C | . | . | . | . | . | . | T/C | . | . | . | . | . | . | . |
| 8 | 37 | 5 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – |
| 8 | 38 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 6 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 7 | . | . | . | . | . | . | . | . | . | . | C/T | . | . | . | . | . | . |
| 8 | 38 | 8 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 9 | . | . | . | . | G | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16380C | 16384G | 16390G | 16391G | 73A | 143G | 150C | 152T | 153A | 180G | 183A | 185G | 195T | 196T | 200A | 201A | 203G | 215A | 222C | 228G | 239T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | None | A | A | A | G | A, G-DEL | T | C, T-DEL, A | G | A | G | A, C, T | A, C | C | G | None | A | G | G, T | A, C | C, G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 7 | 31 | 9 | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 10 | – | – | – | – | G | G/A | . | T/C | A/G | . | . | . | C | . | . | . | . | A/G | . | . | . |
| 7 | 31 | 11 | – | – | – | – | G/A | . | . | . | . | . | . | . | C/T | . | . | . | . | . | . | . | . |
| 7 | 31 | 12 | – | – | – | – | G | . | . | T/C | A/G | . | . | . | C/T | . | G/T | . | . | . | . | . | . |
| 7 | 31 | 13 | – | – | – | – | G | G/A | . | T/C | A/G | . | . | . | C | . | T/G | . | . | A/G | . | . | . |
| 7 | 31 | 14 | – | – | – | – | – | – | – | – | – | . | . | . | C | . | . | . | . | G | . | . | . |
| 7 | 31 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | A/G | . | . | . |
| 8 | 32 | 1 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 8 | 32 | 2 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 8 | 32 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . | . |
| 8 | 33 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 1 | – | – | – | – | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 34 | 2 | – | – | – | – | G | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | – |
| 8 | 34 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 5 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 1 | . | G/A | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 4 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 1 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 8 | 36 | 2 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 8 | 36 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | C | . | . | . | . | . | . | . | . |
| 8 | 36 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | C | . | . | G | . | A/G | . | . | . |
| 8 | 36 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | . | T/C | . | . | . | . | . | . | C |
| 8 | 36 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 7 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 8 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 9 | – | – | – | – | G | A | . | . | G | . | . | . | C | . | G | . | . | . | . | . | . |
| 8 | 36 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 1 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 2 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 3 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 4 | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 1 | – | – | – | – | G | G/A | T/C | . | G/A | . | . | . | C | . | G/A | . | . | A/G | . | . | . |
| 8 | 38 | 2 | – | – | – | – | G | G/A | T/C | T/C | A/G | . | . | . | C | . | A/G | . | . | A/G | . | . | . |
| 8 | 38 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 5 | . | . | . | N | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 6 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 7 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 8 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 9 | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 10 | – | – | – | – | G | . | C/T | . | A/G | . | . | . | T/C | . | A/G | . | . | . | . | . | . |
| 8 | 38 | 11 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | . | A/G | . | . | . |

| | | Nucleotide in RCRS → | 249A | 253C | 257A | 263A | 295C | 297A | 298C | 299C | 303C | 304C | 306C | 310T | 311C | 316G | 317C | 324C | 346T | 348C | 349C | 355C | 379A | 408T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A-DEL, G | A | G, T | G, C | T, A | G | T | G | C-DEL, CC-INS | A | None | C, CTC, TC, TT | CC, T | A | T | T | None | None | None | None | None | A |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | | |
| 7 | 31 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 10 | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 11 | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 12 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 13 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 14 | • | T | • | G | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 31 | 15 | • | • | • | G | T/C | • | • | • | • | • | • | • | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 2 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 32 | 3 | • | • | • | G | • | • | • | • | • | • | • | C | • | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 33 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 34 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 35 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 3 | • | • | • | G | • | • | • | • | • | • | • | • | • | • | • | C/T | T/C | C/T | • | • | • | – |
| 8 | 36 | 4 | • | • | • | G | T/C | • | • | • | • | • | • | • | C/T | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 5 | • | • | • | G | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | – |
| 8 | 36 | 6 | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • | • | • | – | – | – | – |
| 8 | 36 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 9 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 36 | 10 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 37 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 2 | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 8 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 10 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 11 | • | • | • | G | C/T | • | • | • | • | • | • | • | C/T | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 411C | Tissue Type | Geographical Location | Reference |
|---|---|---|---|---|---|---|
| | | Variations in Population → | None | | | |
| Population | Sample | Replicate ↓ | | | | |
| 7 | 31 | 9 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 10 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 11 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 12 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 13 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 14 | – | Bone | Western Honduras | Unpublished Data |
| 7 | 31 | 15 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 32 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 32 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 32 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 33 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 33 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 34 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 34 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 34 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 34 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 34 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 35 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 35 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 35 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 35 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 6 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 7 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 8 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 9 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 36 | 10 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 37 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 37 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 37 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 37 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 37 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 4 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 5 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 6 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 7 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 8 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 9 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 10 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 11 | – | Bone | Western Honduras | Unpublished Data |

| | | Nucleotide in RCRS → | 16069C | 16093T | 16126T | 16129G | 16144T | 16148C | 16151C | 16170A | 16171A | 16183A | 16186C | 16189T | 16192C | 16209T | 16213G | 16214C | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | A, C | A, C | A, C | A,C | T | None | G | G, T | A-DEL, AC, ACC, C, CC, G | A,T | C, TC, A | A, T | C | A | T | C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 8 | 38 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 14 | . | T/C | . | A/G | C/T | T/C | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 15 | . | T/C | . | G/A | C | T | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 38 | 16 | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . |
| 8 | 39 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 9 | 40 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 16218C | 16220A | 16223C | 16224T | 16226A | 16230A | 16231T | 16232C | 16233A | 16235A | 16241A | 16249T | 16255G | 16256C | 16257C | 16258A | 16259C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A, T | A-DEL, C, G | T | C | None | G | C | T | G | G | C, G | A, C | A | T | C-DEL, A, T | G, T | C-DEL, A, CA, T |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 8 | 38 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 14 | • | • | T | • | • | • | • | • | • | • | G/A | • | • | • | • | • | • |
| 8 | 38 | 15 | • | • | T | • | • | • | • | • | • | • | A/G | • | • | • | • | • | • |
| 8 | 38 | 16 | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • | • | • |
| 8 | 39 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • |
| 8 | 39 | 2 | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | N | • |
| 8 | 39 | 3 | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | • |
| 9 | 40 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 9 | 40 | 2 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 9 | 40 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 1 | – | – | – | – | – | • | • | • | • | • | • | • | • | • | • | • | • |
| 9 | 41 | 2 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 9 | 41 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 4 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |
| 9 | 41 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 1 | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | • |
| 9 | 42 | 2 | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • |

| | | Nucleotide in RCRS → | 16260C | 16261C | 16262C | 16263T | 16264C | 16265A | 16267C | 16268C | 16270C | 16271T | 16274G | 16277A | 16278C | 16281A | 16282C | 16283A | 16284A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | T | T | T | C | A, G, T | C, G, T | None | C-DEL, T | A, T | C, A | A, T | G, T | T | G | None | G | G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 8 | 38 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 14 | . | . | . | . | . | C/A | . | . | . | . | A/G | . | . | . | . | . | . |
| 8 | 38 | 15 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 8 | 38 | 16 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 39 | 1 | . | . | . | . | . | C | . | . | . | . | A | . | . | . | . | . | . |
| 8 | 39 | 2 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | N | . |
| 8 | 39 | 3 | . | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 9 | 40 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 9 | 40 | 2 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 9 | 40 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 1 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 9 | 41 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 9 | 41 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 4 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 9 | 41 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 1 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 9 | 42 | 2 | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |

| | | Nucleotide in RCRS → | 16286C | 16287C | 16288T | 16290C | 16291C | 16292C | 16293A | 16294C | 16295C | 16296C | 16297T | 16298T | 16301C | 16304T | 16311T | 16319G | 16325T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | G, T | T | C | T | T | A, G, T | C, T, G | G, T | T | T | C | C | T | C, G, TT | C | G-DEL, A, C | T-DEL, A, C |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 8 | 38 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 14 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | – |
| 8 | 38 | 15 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 8 | 38 | 16 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 8 | 39 | 1 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | C | · | · |
| 8 | 39 | 2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | – |
| 8 | 39 | 3 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | – |
| 9 | 40 | 1 | · | · | · | · | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 9 | 40 | 3 | – | – | – | – | – | – | – | · | · | · | · | C | · | · | C | · | · |
| 9 | 40 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 1 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 9 | 41 | 2 | · | · | · | · | T | · | · | · | · | · | · | C | · | · | C | · | · |
| 9 | 41 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 4 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 9 | 41 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 1 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 9 | 42 | 2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |

| | | Nucleotide in RCRS → | 16327C | 16328C | 16330T | 16332C | 16343A | 16354C | 16355C | 16356T | 16360C | 16362T | 16363C | 16366C | 16368T | 16375C | 16376C | 16377C | 16378C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Variations in* Population → | T | A, T | C | None | C, G, T | T | T, A | C, G | T | G, C | G | T, C-DEL | C | A | None | None | None |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | |
| 8 | 38 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 16 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 8 | 39 | 1 | · | · | · | · | G | · | · | N | · | · | · | · | · | · | · | · | · |
| 8 | 39 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 2 | · | · | · | · | · | · | · | C | · | C | · | · | · | · | · | · | · |
| 9 | 40 | 3 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 9 | 40 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 1 | · | · | · | · | · | · | · | C | · | C | · | · | · | · | · | · | · |
| 9 | 41 | 2 | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · | · |
| 9 | 41 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 4 | · | · | · | · | · | · | · | C | · | C | · | · | · | · | · | · | · |
| 9 | 41 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 1 | · | · | · | · | · | · | · | C | · | C | · | · | · | · | · | · | · |
| 9 | 42 | 2 | · | · | · | · | · | · | · | C | · | C | · | · | · | · | · | · | · |

| | | Nucleotide in RCRS → | 16380C | 16384G | 16390G | 16391G | 73A | 143G | 150C | 152T | 153A | 180G | 183A | 185G | 195T | 196T | 200A | 201A | 203G | 215A | 222C | 228G | 239T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | None | A | A | A | G | A, G-DEL | T | C, T-DEL, A | G | A | G | A, C, T | A, C | C | G | None | A | G | G, T | A, C | C, G |
| Population | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 8 | 38 | 12 | – | – | – | – | A/G | G/A | • | T/C | A/G | • | • | • | T/C | • | A/G | • | • | A/G | • | • | • |
| 8 | 38 | 13 | – | – | – | – | – | – | – | – | – | • | • | • | C | • | • | • | G/A | G | • | • | • |
| 8 | 38 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 16 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 2 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 3 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 4 | – | – | – | – | – | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 9 | 40 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | • |
| 9 | 41 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 2 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | • |
| 9 | 41 | 4 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 1 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 2 | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 249A | 253C | 257A | 263A | 295C | 297A | 298C | 299C | 303C | 304C | 306C | 310T | 311C | 316G | 317C | 324C | 346T | 348C | 349C | 355C | 379A | 408T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Variations in Population → | A-DEL, G | A | G, T | G, C | T, A | G | T | G | C-DEL, CC-INS | A | None | C, CTC, TC, TT | CC, T | A | T | T | None | None | None | None | None | A |
| lation | Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | | |
| 8 | 38 | 12 | • | – | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 13 | • | C/T | • | G | T | A/C | C/A | • | C/A | • | • | • | • | • | • | • | • | • | – | – | – | – |
| 8 | 38 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 38 | 16 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 8 | 39 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 1 | • | – | • | – | – | – | – | • | – | • | • | – | – | – | – | – | – | – | • | • | – | – |
| 9 | 40 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 4 | G/A | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 40 | 5 | • | • | N | G | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| 9 | 41 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 3 | • | • | • | G | • | • | • | T/C | • | T/C | T/C | • | • | • | • | • | • | • | T/C | • | • | • |
| 9 | 41 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 41 | 5 | • | • | N | G | • | • | • | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 9 | 42 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 9 | 42 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Nucleotide in RCRS → | 411C | Tissue Type | Geographical Location | Reference |
|---|---|---|---|---|---|---|
| | | Variations in Population → | None | | | |
| Population | Sample | Replicate ↓ | | | | |
| 8 | 38 | 12 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 13 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 14 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 15 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 38 | 16 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 39 | 1 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 39 | 2 | – | Bone | Western Honduras | Unpublished Data |
| 8 | 39 | 3 | – | Bone | Western Honduras | Unpublished Data |
| 9 | 40 | 1 | – | Tooth | Northern France | Unpublished Data |
| 9 | 40 | 2 | – | Tooth | Northern France | Unpublished Data |
| 9 | 40 | 3 | – | Tooth | Northern France | Unpublished Data |
| 9 | 40 | 4 | – | Tooth | Northern France | Unpublished Data |
| 9 | 40 | 5 | • | Tooth | Northern France | Unpublished Data |
| 9 | 41 | 1 | – | Tooth | Northern France | Unpublished Data |
| 9 | 41 | 2 | – | Tooth | Northern France | Unpublished Data |
| 9 | 41 | 3 | • | Tooth | Northern France | Unpublished Data |
| 9 | 41 | 4 | – | Tooth | Northern France | Unpublished Data |
| 9 | 41 | 5 | • | Tooth | Northern France | Unpublished Data |
| 9 | 42 | 1 | – | Tooth | Northern France | Unpublished Data |
| 9 | 42 | 2 | – | Tooth | Northern France | Unpublished Data |

# XVIII. Appendix 1C

Table of all potential damage or error sites for aDNA sequences analyzed in this study along with the substitution observed.

| Population | Sample | Replicate | Polymorphism | Tissue Type | Variation Type | % Transitions & Transversions |
|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 16332C-T | Tooth | Transition | 100% Transitions |
| 1 | 1 | 7 | 16363C-T; 16375C-T | Tooth | Transition | |
| 1 | 1 | 1 | 16380C-T | Tooth | Transition | |
| 1 | 4 | 2 | 16376C-T; 16377C-T; 16378C-T | Tooth | Transition | |
| 1 | 4 | 2 | 16380C-T | Tooth | Transition | |
| 1 | 5 | 7 | 16376C-T; 16377C-T; 16378C-T | Tooth | Transition | |
| 1 | 5 | 7 | 16380C-T | Tooth | Transition | |
| 1 | 6 | 3 | 16282C-T | Tooth | Transition | |
| 1 | 8 | 1 | 16282C-T | Tooth | Transition | |
| 1 | 9 | 3 | 16267C-T | Tooth | Transition | |
| 2 | 10 | 3 | 346T-G; 379A-G; 408T-G; 411C-G | Bone | Transversion, Transition, Transversion, Transversion | 87.5% Transversions; 12.5% Transitions |
| 2 | 11 | 1 | 16170A-C; 16171A-C | Tooth | Transversion, Transversion | |
| 2 | 11 | 6 | 183A-C | Tooth | Transversion | |
| 2 | 12 | 4 | 16384G-T | Bone | Transversion | |
| 5 | 18 | 5 | 16270C-G | Bone | Transversion | 100% Transversions |
| 6 | 19 | 5 | 16360C-A | Bone | Transversion | 100% Transversions |
| 6 | 21 | 4 | 16256C-A | Bone | Transversion | |
| 6 | 22 | 6 | 16151C-A | Bone | Transversion | |
| 7 | 29 | 7 | 316G-C; 317C-G; 324C-G | Bone | Transversion, Transversion, Transversion | 83.3% Transversions; 16.7% Transitions |
| 7 | 31 | 7 | 16391G-T | Bone | Transversion | |
| 7 | 31 | 8 | 16391G-T | Bone | Transversion | |
| 7 | 31 | 14 | 253C-T | Bone | Transition | |
| 8 | 36 | 4 | 201A-G | Bone | Transition | 62.5% Transitions; 37.5% Transversions |
| 8 | 36 | 3 | 346T-C; 348C-T | Bone | Transition, Transition | |
| 8 | 38 | 7 | 16363C-T | Bone | Transition | |
| 8 | 38 | 13 | 253C-T; 297A-C; 298C-A; 303C-A | Bone | Transition, Transversion, Transversion, Transversion | |
| 9 | 41 | 3 | 299C-T; 304C-T; 306C-T; 349C-T | Tooth | Transitions | 80% Transitions; 20% Transversions |
| 9 | 41 | 5 | 355C-G | Tooth | Transversion | |

# XIX. Appendix 1D

Table of all polymorphisms observed within all modern DNA sequences analyzed in this study.

| | Nucleotide in RCRS → | 16069C | 16086T | 16093T | 16126T | 16129G | 16136T | 16140T | 16144T | 16148C | 16155A | 16157T | 16172T | 16182A | 16183A | 16187C | 16189T | 16203A | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | T | C | A, C | A, C | A, C | C | C | A, C | T | G | A, C | C | C, G | G, C, CC | G, T | C, TC, A | G | C |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | |
| 1 | 1 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • |
| 1 | 2 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • |
| 1 | 3 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • |
| 1 | 4 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • |
| 1 | 5 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • |
| 1 | 6 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • |
| 1 | 7 | • | • | C | • | • | • | • | • | • | A/G | • | • | • | • | • | C | • | • |
| 1 | 8 | • | • | C | • | • | • | • | • | • | A/G | • | • | • | • | • | C | • | • |
| 1 | 9 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | – | – | – |
| 1 | 10 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • |
| 1 | 11 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • |
| 1 | 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 13 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 14 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 15 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 16 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 17 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 18 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 19 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 20 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 21 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | • | • |
| 1 | 22 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 23 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 24 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 25 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 26 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 27 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 28 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 29 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 30 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 31 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 32 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 33 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 34 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 35 | | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 36 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | – | – |
| 1 | 37 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | – | – |
| 1 | 38 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | – | – |
| 1 | 39 | • | • | C | • | • | • | • | • | • | • | • | • | • | • | • | C | – | – |

| | Nucleotide in RCRS → | 16223C | 16231T | 16241A | 16243T | 16247A | 16256C | 16257C | 16261C | 16265A | 16266C | 16270C | 16274G | 16278C | 16288T | 16291C | 16292C | 16294C | 16295C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | T | C | C, G | C | G | T | DEL, A, T | T | C, G, T | A, G, T | A, T | A | T | C | T | A, G, T | G, T | T |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | |
| 1 | 1 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 2 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 3 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 4 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 5 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 6 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 7 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 8 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 10 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 11 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 12 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 13 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 14 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 15 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 16 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 17 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 18 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 19 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 20 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 21 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 22 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 23 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 24 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 25 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 26 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 27 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 28 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 29 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 30 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 31 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 32 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 33 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 34 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 35 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 36 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 37 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 38 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 39 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | Nucleotide in RCRS → | 16297T | 16298T | 16304T | 16311T | 16325T | 16330T | 16335A | 16343A | 16354C | 16362T | 16390G | 16427C | 16519T | 7 A | 89T | 92G | 103G | 105C | 126A | 133T | 146T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | C | T | C, G, TT | C | A, C, DEL | C | G | C, G, T | T | C, G | A | NONE | C | G | C | A | A | T, DEL, G | C | DEL | C, A |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 1 | 1 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | . | . | . | . | . | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 10 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 11 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 12 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 13 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 14 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 15 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 16 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 17 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 18 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 19 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 20 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 21 | . | . | . | . | . | . | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 22 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 23 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 24 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 25 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 26 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | C/G | . | . | . |
| 1 | 27 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 28 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 29 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 30 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 31 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 32 | – | – | – | – | – | – | – | – | – | – | – | – | – | G | . | . | . | . | . | . | . |
| 1 | 33 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | . | A/G | . | . |
| 1 | 34 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . |
| 1 | 35 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | . | . | . | . |
| 1 | 36 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 37 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 38 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 39 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | Nucleotide in RCRS → | 147C | 150C | 152T | 194C | 195 | 199T | 204T | 207G | 210A | 222C | 251G | 255G | 263A | 311C | 325C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | A, T | T | C, A | T | A, C | C | A, C, G | A | G | G, T | T, A | None | G, C | CC, T | T |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | |
| 1 | 1 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 2 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 3 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 4 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 5 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 6 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 7 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 8 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 9 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 10 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 11 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 12 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 13 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 14 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 15 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 16 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 17 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 18 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 19 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 20 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 21 | – | – | – | | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 22 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 23 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 24 | • | T | • | • | • | • | • | • | • | • | • | G/C | G | • | • |
| 1 | 25 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 26 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 27 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 28 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 29 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 30 | | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 31 | | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 32 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 33 | • | T | • | G/C | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 34 | C/G | T | • | • | • | • | • | • | • | • | • | • | G | C/T | • |
| 1 | 35 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 36 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 37 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 38 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 39 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

| | Nucleotide in RCRS → | 16069C | 16086T | 16093T | 16126T | 16129G | 16136T | 16140T | 16144T | 16148C | 16155A | 16157T | 16172T | 16182A | 16183A | 16187C | 16189T | 16203A | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | T | C | A, C | A, C | A, C | C | C | A, C | T | G | A, C | C | C, G | G, C, CC | G, T | C, TC, A | G | C |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | |
| 1 | 40 | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | C | – | – |
| 1 | 41 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | . | . |
| 1 | 42 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | . | . |
| 1 | 43 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | . | . |
| 1 | 44 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | . | . |
| 1 | 45 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | C | . | . |
| 1 | 46 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 47 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 48 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 2 | – | – | – | – | | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 1 | . | . | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 2 | . | . | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 1 | . | . | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | . | . |
| 7 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 8 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | C | . | C |
| 9 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 10 | 1 | . | . | . | . | A | . | . | . | . | . | . | C | . | . | . | . | . | . |
| 11 | 1 | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 12 | 1 | . | . | . | . | A | . | . | C | T | . | . | C | . | . | . | . | . | . |
| 13 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 14 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 15 | 1 | . | . | . | . | A | . | . | C | T | . | . | C | . | . | . | . | . | . |
| 16 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 17 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | C | C | . | C | . | C |
| 18 | 1 | . | . | . | . | A | . | . | C | T | . | . | C | . | . | . | . | . | . |
| 19 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . |
| 20 | 1 | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 21 | 1 | . | . | . | . | . | C | . | . | . | . | . | . | . | C | . | C | . | C |
| 22 | 1 | . | . | . | . | A | . | . | . | . | . | . | C | . | . | . | . | . | . |
| 23 | 1 | . | . | . | . | A | . | . | . | . | . | . | C | . | . | . | . | . | . |
| 24 | 1 | . | . | . | . | A | . | . | | . | . | . | C | . | . | . | . | . | . |
| 25 | 1 | . | . | . | . | . | . | . | | . | . | C | . | . | . | . | . | . | . |
| 26 | 1 | . | . | . | . | . | . | . | | . | . | . | . | C | C | . | C | G | C |
| 27 | 1 | . | . | C | . | . | . | . | . | . | . | . | C | . | C | . | C | . | . |

| | Nucleotide in RCRS → | 16223C | 16231T | 16241A | 16243T | 16247A | 16256C | 16257C | 16261C | 16265A | 16266C | 16270C | 16274G | 16278C | 16288T | 16291C | 16292C | 16294C | 16295C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | T | C | C, G | C | G | T | DEL, A, T | T | C, G, T | A, G, T | A, T | A | T | C | T | A, G, T | G, T | T |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | |
| 1 | 40 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 41 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 42 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 43 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 44 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 45 | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . |
| 1 | 46 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 47 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 48 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 2 | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . |
| 2 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 2 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 2 | – | – | – | – | – | – | – | – | – | – | . | . | . | . | . | . | . | . |
| 3 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 3 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 6 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 4 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 5 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 5 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 7 | 1 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T |
| 8 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 9 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 10 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 11 | 1 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 12 | 1 | T | . | G | . | . | . | . | . | C | . | . | . | . | C | . | . | . | . |
| 13 | 1 | T | . | . | . | G | . | A | T | . | . | . | . | . | . | . | . | T | . |
| 14 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 15 | 1 | T | . | G | . | . | . | . | . | C | . | . | . | . | C | . | . | . | . |
| 16 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 17 | 1 | . | . | . | . | G | . | . | T | . | . | . | . | . | . | . | T | . | . |
| 18 | 1 | T | . | G | . | . | . | . | . | C | . | . | . | . | C | . | . | . | . |
| 19 | 1 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . |
| 20 | 1 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 21 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 22 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . |
| 23 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . |
| 24 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . |
| 25 | 1 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 26 | 1 | . | . | . | . | G | . | . | T | . | . | . | . | . | . | . | . | . | . |
| 27 | 1 | T | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . | . | . |

| | Nucleotide in RCRS → | 16297T | 16298T | 16304T | 16311T | 16325T | 16330T | 16335A | 16343A | 16354C | 16362T | 16390G | 16427C | 16519T | 73A | 89T | 92G | 103G | 105C | 126A | 133T | 146T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | C | T | C, G, TT | C | A, C, DEL | C | G | C, G, T | T | C, G | A | NONE | C | G | C | A | A | T, DEL, G | C | DEL | C, A |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 1 | 40 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 41 | • | • | • | • | • | • | • | • | • | • | • | • | – | – | – | – | – | – | – | – | – |
| 1 | 42 | • | • | • | • | • | • | • | • | • | • | • | • | – | – | – | – | – | – | – | – | – |
| 1 | 43 | • | • | • | • | • | • | • | • | • | • | • | • | – | – | – | – | – | – | – | – | – |
| 1 | 44 | • | • | • | • | • | • | • | • | • | • | • | • | – | – | – | – | – | – | – | – | – |
| 1 | 45 | • | • | • | • | • | • | • | • | • | • | • | • | – | – | – | – | – | – | – | – | – |
| 1 | 46 | – | – | – | – | – | – | – | – | – | – | – | – | • | G | • | • | • | • | • | • | • |
| 1 | 47 | – | – | – | – | – | – | – | – | – | – | – | – | • | G | • | • | • | • | • | • | • |
| 1 | 48 | – | – | – | – | – | – | – | – | – | – | – | – | • | G | • | • | • | • | • | • | • |
| 2 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 2 | 2 | • | • | • | • | • | • | • | • | • | • | • | • | – | – | – | – | – | – | – | – | – |
| 2 | 3 | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 2 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | • | • |
| 2 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 3 | 2 | • | • | • | • | • | T/G | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 4 | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 3 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | • | • |
| 3 | 6 | – | – | – | – | – | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 4 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 4 | 2 | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 4 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | • | • |
| 4 | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | • | • |
| 5 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | • | • | • | • | • | • | • | • |
| 5 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | • | • | • | • | • | • | G/T | • |
| 5 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 6 | 1 | • | • | • | • | • | • | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 1 | • | • | • | • | • | • | • | • | • | C | • | • | C | G | • | • | • | • | • | • | C |
| 8 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | G | • | • | • | • | • | • | • |
| 9 | 1 | • | • | • | C | • | • | • | • | • | • | • | • | • | G | • | • | • | • | • | • | • |
| 10 | 1 | • | • | C | C | • | • | • | • | • | • | • | • | • | G | • | • | • | • | • | • | • |
| 11 | 1 | • | • | C | • | • | • | G | • | • | • | • | • | • | G | • | • | • | • | • | • | • |
| 12 | 1 | • | • | • | C | • | • | • | G | • | • | • | • | • | G | C | A | • | • | • | • | C |
| 13 | 1 | • | • | • | • | C | • | • | • | • | • | • | • | C | G | • | • | • | • | • | • | • |
| 14 | 1 | • | • | • | C | • | • | • | • | • | • | • | • | • | G | • | • | • | • | • | • | • |
| 15 | 1 | • | • | • | C | • | • | • | G | • | • | • | • | • | G | C | A | • | • | • | • | C |
| 16 | 1 | • | • | • | C | • | • | • | • | • | • | • | • | • | G | • | • | • | • | • | • | • |
| 17 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | G | • | • | • | • | • | • | C |
| 18 | 1 | • | • | • | C | • | • | • | G | • | • | • | • | • | G | C | A | • | • | • | • | C |
| 19 | 1 | • | • | • | • | • | • | • | • | • | C | A | • | C | G | • | • | • | • | • | • | • |
| 20 | 1 | • | • | C | • | • | • | G | • | • | • | • | • | • | G | • | • | • | • | • | • | • |
| 21 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | G | • | • | • | • | • | • | • |
| 22 | 1 | • | • | C | • | • | • | • | • | • | C | • | • | C | G | • | • | • | • | • | • | • |
| 23 | 1 | • | • | C | • | • | • | • | • | • | C | • | • | C | G | • | • | • | • | • | • | • |
| 24 | 1 | • | • | C | • | • | • | • | • | • | C | • | • | C | G | • | • | • | • | • | • | • |
| 25 | 1 | • | • | C | • | • | • | G | • | • | • | • | • | • | G | • | • | • | • | • | • | • |
| 26 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | G | • | • | • | • | • | • | C |
| 27 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | C | G | • | • | • | • | • | • | • |

| | Nucleotide in RCRS → | 147C | 150C | 152T | 194C | 195T | 199T | 204T | 207G | 210A | 222C | 251G | 255G | 263A | 311C | 325C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | A, T | T | C, A | T | A, C | C | A, C, G | A | G | G, T | T, A | None | G, C | CC, T | T |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | |
| 1 | 40 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 41 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 42 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 43 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 44 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 45 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 1 | 46 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 47 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 1 | 48 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 2 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 2 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 2 | 3 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 2 | 4 | • | • | • | • | • | • | • | • | • | C/G | • | • | G | • | • |
| 2 | 5 | – | – | – | – | – | – | – | – | – | – | – | – | G | • | • |
| 3 | 1 | • | • | • | • | • | • | • | • | • | • | A | • | G | – | – |
| 3 | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | 3 | – | – | – | • | • | • | • | • | • | • | A | • | G | – | – |
| 3 | 4 | • | • | • | • | • | • | • | • | • | • | A | • | G | – | – |
| 3 | 5 | • | • | • | • | • | • | • | • | • | • | A | • | G | – | – |
| 3 | 6 | • | • | • | • | • | • | • | • | • | • | A | • | G | – | – |
| 4 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | – | – |
| 4 | 2 | • | • | • | • | • | • | • | • | • | • | • | • | G | – | – |
| 4 | 3 | • | • | • | • | • | • | • | • | • | • | • | • | G | – | – |
| 4 | 4 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 5 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 5 | 2 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 5 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | G | • | • |
| 6 | 1 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| 7 | 1 | • | • | • | • | • | C | • | • | • | • | • | • | G | • | • |
| 8 | 1 | • | • | • | • | • | • | • | A | • | • | • | • | G | • | • |
| 9 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 10 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 11 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 12 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 13 | 1 | • | T | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 14 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 15 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 16 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 17 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 18 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 19 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 20 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 21 | 1 | • | • | • | • | • | • | • | A | • | • | • | • | G | • | • |
| 22 | 1 | • | • | C | • | • | • | • | • | • | • | • | • | G | • | • |
| 23 | 1 | • | • | C | • | • | • | • | • | • | • | • | • | G | • | • |
| 24 | 1 | • | • | C | • | • | • | • | • | • | • | • | • | G | • | • |
| 25 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 26 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |
| 27 | 1 | • | • | • | • | • | • | • | • | • | • | • | • | G | • | • |

| | Nucleotide in RCRS → | 16069C | 16086T | 16093T | 16126T | 16129G | 16136T | 16140T | 16144T | 16148C | 16155A | 16157T | 16172T | 16182A | 16183A | 16187C | 16189T | 16203A | 16217T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | T | C | A, C | A, C | A, C | C | C | A, C | T | G | A, C | C | C, G | G, C, CC | G, T | C, TC, A | G | C |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | |
| 28 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | C | C | . | C | . | C |
| 29 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 30 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | C | . | . |
| 31 | 1 | . | . | . | . | . | . | C | . | . | . | . | . | . | C | . | C | . | . |
| 32 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 33 | 1 | . | . | . | . | A | . | . | . | . | . | . | C | . | . | . | . | . | . |
| 34 | 1 | . | . | . | . | . | . | . | . | . | . | . | C | C | C | . | C | . | C |
| 35 | 1 | . | . | . | . | A | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 36 | 1 | . | . | . | . | . | . | C | . | . | . | . | . | . | C | . | C | . | . |
| 37 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 38 | 1 | . | . | . | . | . | . | C | . | . | . | . | . | . | C | . | C | . | . |
| 39 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 40 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 41 | 1 | . | . | . | . | A | . | . | . | . | . | . | C | . | . | . | . | . | . |
| 42 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 43 | 1 | T | . | . | . | . | . | C | . | . | . | . | . | C | C | . | C | . | . |
| 44 | 1 | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 45 | 1 | . | . | . | . | . | C | . | . | . | . | . | . | . | C | . | C | . | C |
| 46 | 1 | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 47 | 1 | . | . | . | . | A | . | . | . | . | . | . | C | . | . | . | . | . | . |
| 48 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 49 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 50 | 1 | . | . | . | . | . | . | . | . | . | . | C | . | . | . | . | . | . | . |
| 51 | 1 | . | . | . | . | A | . | . | . | . | . | . | C | . | . | . | . | . | . |
| 52 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | Nucleotide in RCRS → | 16223C | 16231T | 16241A | 16243T | 16247A | 16256C | 16257C | 16261C | 16265A | 16266C | 16270C | 16274G | 16278C | 16288T | 16291C | 16292C | 16294C | 16295C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | T | C | C, G | C | G | T | DEL, A, T | T | C, G, T | A, G, T | A, T | A | T | C | T | A, G, T | G, T | T |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | |
| 28 | 1 | . | . | . | . | G | . | . | T | . | . | . | . | . | . | . | . | . | . |
| 29 | 1 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . |
| 30 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 31 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 32 | 1 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . |
| 33 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . |
| 34 | 1 | . | . | . | . | G | . | . | T | . | . | . | . | . | . | . | . | . | . |
| 35 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . |
| 36 | 1 | . | . | . | . | . | . | . | . | . | A | . | . | . | . | . | . | . | . |
| 37 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 38 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 39 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 40 | 1 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . | . | . |
| 41 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . |
| 42 | 1 | T | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . |
| 43 | 1 | . | . | . | . | . | . | . | . | . | . | . | A | . | . | . | . | . | . |
| 44 | 1 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 45 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 46 | 1 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 47 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T | . |
| 48 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 49 | 1 | . | C | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 50 | 1 | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 51 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 52 | 1 | T | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | T |
| 53 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

| | Nucleotide in RCRS → | 16297T | 16298T | 16304T | 16311T | 16325T | 16330T | 16335A | 16343A | 16354C | 16362T | 16390G | 16427C | 16519T | 73A | 89T | 92G | 103G | 105C | 126A | 133T | 146T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | C | T | C, G, TT | C | A, C, DEL | C | G | C, G, T | T | C, G | A | NONE | C | G | C | A | A | T, DEL, G | C | DEL | C, A |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | | | | | | | |
| 28 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | C | G | . | . | . | . | . | . | C |
| 29 | 1 | . | . | . | . | . | . | . | . | . | C | A | . | C | G | . | . | . | . | . | . | . |
| 30 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 31 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | C | G | . | . | A | . | . | . | . |
| 32 | 1 | . | . | . | . | . | . | . | . | . | C | A | . | C | G | . | . | . | . | . | . | . |
| 33 | 1 | . | . | C | . | . | . | . | . | . | C | | . | C | G | . | . | . | . | . | . | . |
| 34 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | C | G | . | . | . | . | . | . | C |
| 35 | 1 | . | . | C | . | . | . | . | . | . | C | . | . | C | G | . | . | . | . | . | . | . |
| 36 | 1 | C | C | . | . | . | . | . | . | . | . | . | . | C | G | . | . | . | . | . | . | . |
| 37 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 38 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | C | G | . | . | A | . | . | . | . |
| 39 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 40 | 1 | . | . | . | . | . | . | . | . | . | C | A | . | C | G | . | . | . | . | . | . | . |
| 41 | 1 | . | . | C | . | . | . | . | . | . | C | . | . | C | G | . | . | . | . | . | . | . |
| 42 | 1 | . | . | . | . | . | . | . | . | . | C | A | . | C | G | . | . | . | . | . | . | . |
| 43 | 1 | . | . | . | . | . | . | G | . | . | . | A | . | C | G | . | . | . | . | . | . | C |
| 44 | 1 | . | . | C | . | . | . | G | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 45 | 1 | . | . | . | . | . | . | . | . | . | . | . | A | C | G | . | . | . | . | . | . | . |
| 46 | 1 | . | . | C | . | . | . | G | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 47 | 1 | . | . | C | . | . | . | . | . | . | C | . | . | C | G | . | . | . | . | . | . | . |
| 48 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 49 | 1 | . | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 50 | 1 | . | . | C | . | . | . | G | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 51 | 1 | . | . | C | C | . | . | . | . | . | . | . | . | . | G | . | . | . | . | . | . | . |
| 52 | 1 | . | . | . | . | . | . | . | . | . | C | . | . | C | G | . | . | . | . | . | . | C |
| 53 | 1 | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 2 | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 3 | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 4 | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 5 | . | . | . | . | . | . | . | . | T | . | . | . | . | . | . | . | . | . | . | . | . |

| | Nucleotide in RCRS → | 147C | 150C | 152T | 194C | 195T | 199T | 204T | 207G | 210A | 222C | 251G | 255G | 263A | 311C | 325C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Variations in Population → | A, T | T | C, A | T | A, C | C | A, C, G | A | G | G, T | T, A | None | G, C | CC, T | T |
| Sample | Replicate ↓ | | | | | | | | | | | | | | | |
| 28 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 29 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 30 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 31 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 32 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 33 | 1 | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . |
| 34 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 35 | 1 | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . |
| 36 | 1 | . | . | . | . | . | . | . | . | G | . | . | . | G | . | . |
| 37 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 38 | 1 | . | . | C | . | . | . | C | . | . | . | . | . | G | . | . |
| 39 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 40 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 41 | 1 | . | . | C | . | . | . | . | . | . | . | . | . | G | . | T |
| 42 | 1 | . | . | C | . | . | . | . | . | . | . | . | . | G | . | . |
| 43 | 1 | . | T | . | . | C | . | . | . | . | . | . | . | G | . | . |
| 44 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 45 | 1 | . | . | . | . | . | . | . | A | . | . | . | . | G | . | . |
| 46 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 47 | 1 | . | . | C | . | . | . | . | . | . | . | . | . | G | . | T |
| 48 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 49 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 50 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 51 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | G | . | . |
| 52 | 1 | . | . | . | . | . | C | . | . | . | . | . | . | G | . | . |
| 53 | 1 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 3 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 4 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |
| 53 | 5 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | . |

## XX. Appendix 1E

Table of all potential damage and error sites observed among all modern DNA sequences analyzed in this study. The types of substitutions observed are also shown here.

| Sample | Replicate | Polymorphism | Variation Type | |
|---|---|---|---|---|
| | | | | |
| 1 | 7 | 16155A-A/G** | Transition | 50% Transitions; 50% Transversions |
| 1 | 8 | 16155A-A/G** | Transition | |
| 1 | 24 | 255G-C* | Transversion | |
| 1 | 26 | 105C-C/G** | Transversion | |
| 1 | 33 | 126A-G*; 194C-G* | Transition, Transversion | |
| 1 | 34 | 147C-G*; 311C-C/T** | Transversion, Transition | |
| 2 | 4 | 222C-C/G** | Transversion | 100% Transversion |
| 3 | 2 | 16330T-G* | Transversion | 100% Transversion |
| 5 | 2 | 133T-G* | Transversion | 100% Transversion |
| 36 | 1 | 16298T-C* | Transition | 100% Transition |
| 45 | 1 | 16427C-A* | Transversion | 100% Transversion |
| | | 13 polymorphisms of interest | Total | 61.5% Transversions; 38.5% Transitions |
| | | | | |
| | | *Not found in Human Population | | |
| | | **Not found in other replicates | | |

# XXI. Appendix 2A

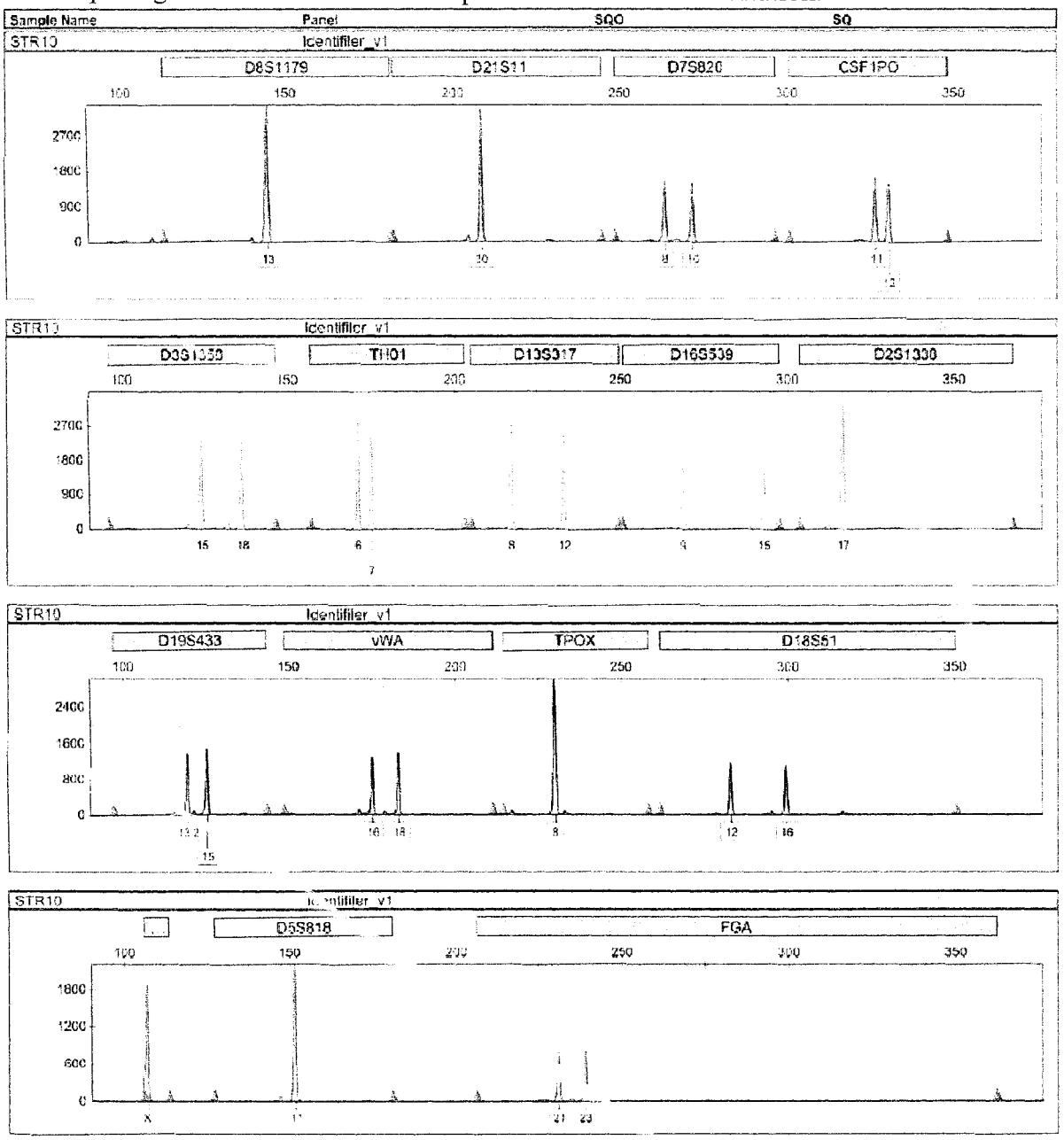
Raw Data for Ancient Y-STR Analysis – The number in each cell indicates the allele length amplified; OL indicates off-ladder alleles.

| Individual | Replicate | DYS19 | DYS385 a/b | DYS389 I | DYS389 II | DYS390 | DYS391 | DYS392 | DYS393 | DYS437 | DYS438 | DYS439 | DYS448 | DYS456 | DYS458 | YGATAH4 | AMEL. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | | 15 | | | 22 | 12 | 10 | | | | | | | | | |
| 1 | 2 | | | | | | | | | | <8 | | | | | | |
| 1 | 3 | | | | | | | | OL(11) | | | | | | | | |
| 1 | 4 | | | | | | | | | | 13 | | | | | | |
| 1 | 5 | 13 | | | | 23 | | 10 | | | | | | | | | |
| 1 | 6 | | | | | | | | 8 | | | | | | | | |
| 1 | 7 | | 11 | 11 | | | 10 | | 13 | | | | | 13 | 17 | 13 | |
| 1 | 8 | | | | | | | | | | 11 | | | | | | |
| 1 | 9 | | 11 | | | | | | | | | | | 16 | | | |
| 1 | 10 | | | | | | | | 13 | | OL | | | | | | |
| 1 | 11 | | | | | 23 | | | 13 | 13,14 | | | | | | | |
| 1 | 12 | | | | | | 10 | | | 15 | | | | | | | |
| 1 | 13 | | | | | | | 11 | 11 | | | | | | | | |
| 1 | 14 | | 11 | | | 21 | | 12 | 15 | | | | | | | | |
| 1 | 15 | | | | | | | 11 | OL(11) | | | | | | | | Y |
| 1 | 16 | | | 13 | | | | 11 | | | | | | | | | |
| 1 | 17 | | 11, 14 | 13 | | | OL(10) | | OL(13) | 15 | | | | 16 | 18 | 12 | |
| 1 | 18 | | 11 | 11/12/ 13 | | | 10 | 13 | OL(12/13) | | 12 | (OL),13 | | 16 | 17/18 | OL,12 | |
| 1 | 19 | | | 12/13 | | | | | | | | | | | | | |
| 1 | 20 | | | | | | <10 | | 13 | | | | <19 | | 18 | | |
| 1 | 21 | <14 | 11 | 13 | | 23 | | | 13 | 15 | | | | 16 | 18 | | |
| 1 | 22 | 13 | 14 | 13 | | | 10 | | <13 | 15 | | | | <17 | 18 | | |
| 2 | 1 | OL | | | | | | | OL(11) | | | | | | | | |
| 2 | 2 | | | | | | | | | | | | | | | | Y |
| 2 | 3 | | | | | | 10 | | | 14 | 9 | | | | | | |
| 2 | 4 | | | | | | 10 | | | | | | | | | | |
| 2 | 5 | | | | | | | | | | | | | | | | X |
| 2 | 6 | | | | | | | 11 | | | | | | | | | |
| 2 | 7 | | | | | 18 | | | | | | | | | | | |
| 2 | 9 | | | | | | | <7 | OL(10-11) | | | | | | | | |
| 3 | 1 | | | | | 24 | | | | | | | | | | | |

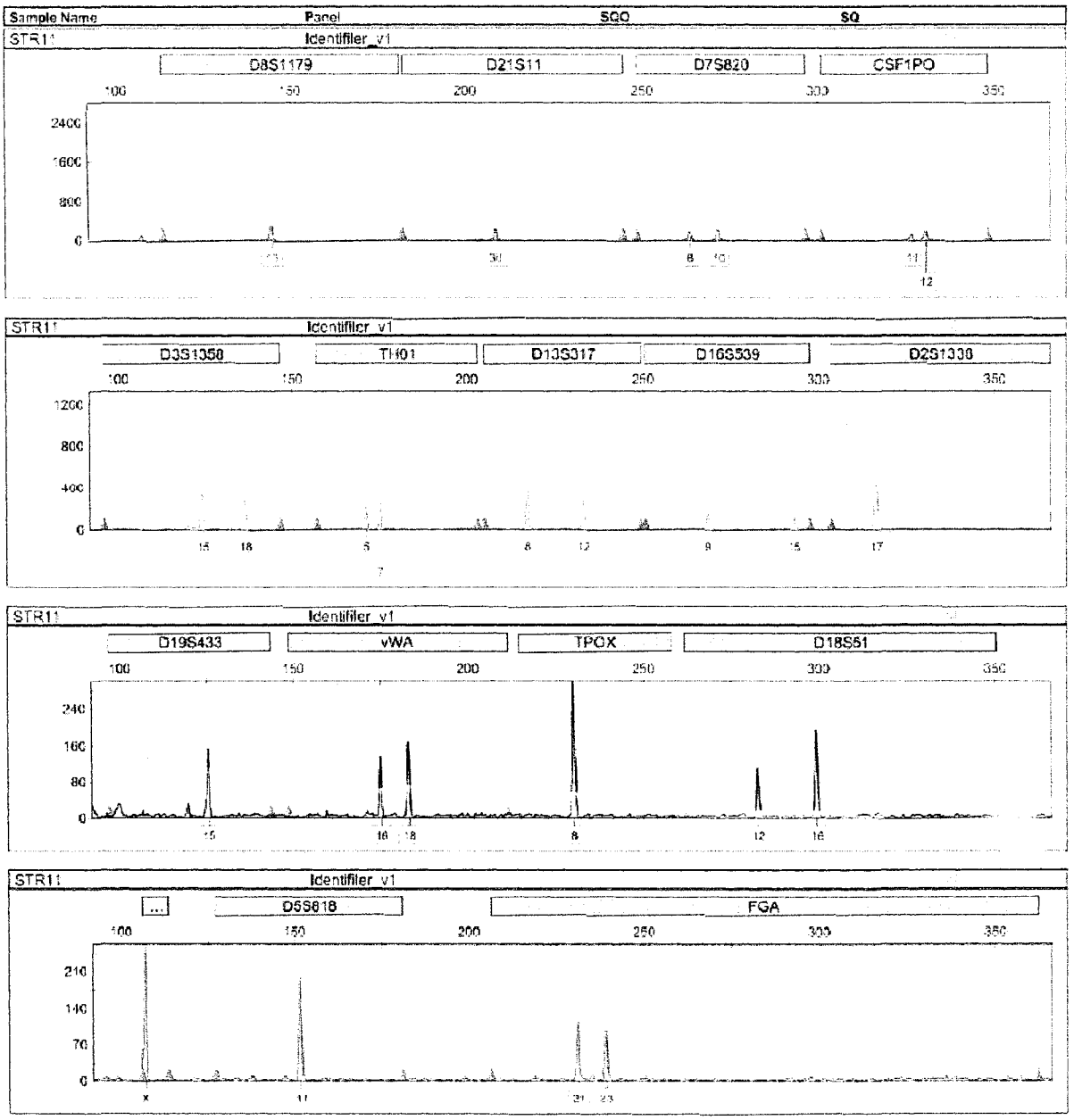
| Individual | Replicate | DYS19 | DYS385 a/b | DYS389 I | DYS389 II | DYS390 | DYS391 | DYS392 | DYS393 | DYS437 | DYS438 | DYS439 | DYS448 | DYS456 | DYS458 | YGATAH4 | AMEL. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 2 | >12 | | <13 | | <24 | | | | | | | | | | | OL |
| 3 | 3 | | | | | | | 11 | OL(11,16) | | | | | | | | |
| 3 | 4 | | <10 & <17 | | | >21 | | <13 | | | 12 | | | | | | |
| 3 | 5 | | | | | | | | | | <13 | | | | | | |
| 3 | 6 | | | | 28 | | | 12 | | | 10 | | | | | | |
| 4 | 1 | | | | 30 | 20 | | | | | | | | | | | |
| 4 | 2 | | >10 | | <30 | <20 | | | | | >11 | | | | | | |
| 4 | 3 | | | | | | | >12&<14 | | | | | | | | | |
| 4 | 4 | | | | | | | | | | >12 | | | | | | |
| 4 | 5 | | | | | | | | | | >12 <13 | | | | | | |
| 4 | 6 | | | 13 | | | | | | | | | | | | | |
| 4 | 7 | | | | | | | | | | | | | | | 8 | |
| 4 | 8 | | | | | | | | (9),13 | | OL(8/9) | | | | | 12 | |
| 4 | 9 | | | | | | | | | | | | | | | 13 | |
| 5 | 1 | | 10 | | | | | | | | | | | | | | |
| 5 | 2 | | 10 | | | | | | | | | OL | | | | | |
| 5 | 3 | | | | | | | 12 | | | | | | | | | |
| 5 | 4 | | | | | | | | | | 16 | | | | | | |
| 5 | 5 | | | | | | | | 9 | | | | | | | | |
| 6 | 1 | | | | | | | | | | 12 | | | | | | |
| 6 | 2 | | | | | | | 12 | | | | | | | | | |
| 6 | 3 | | | 13 | | | | | | | | | | | | | |
| 6 | 4 | | | | | | | 9 | 12 | 14 | | | | 13 | 15 | 11 | |
| 6 | 5 | | | | | | 10 | | | | | | | | | | |
| 6 | 6 | | 17 | | | | | | 12 | | | 9 | | | | | |
| 6 | 7 | | | | | | | | | | | | | 13 | 16 | | |
| 6 | 8 | | 17 | | | | | | | | | | | | 16 | | |
| 6 | 9 | | | | | | OL | | 10 | | | | | | | | |
| 6 | 10 | | 17 | | | | | | | | 9 | | | | | | |
| 6 | 11 | | 17 | | | | 10 | | 12 | | 9 | | | | | | |
| 6 | 12 | | | OL 10/11 | | | | | | | | | | | OL 17/18 | OL>13 | |
| 6 | 13 | | | | | | | | | | | | | | 16 | | |

## XXII. Appendix 3A

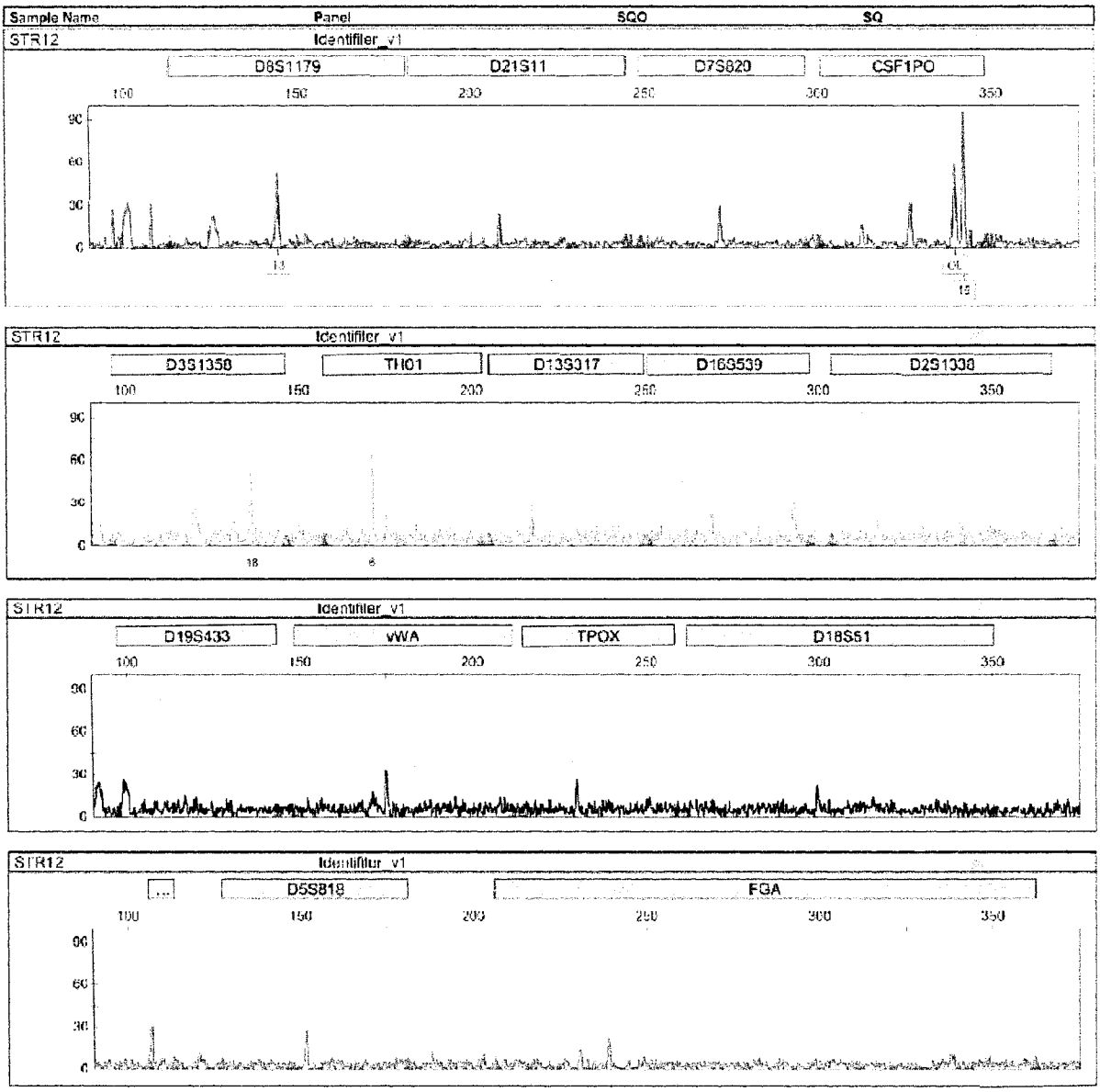
Electropherogram of the initial STR amplification for the 1:10 dilution.



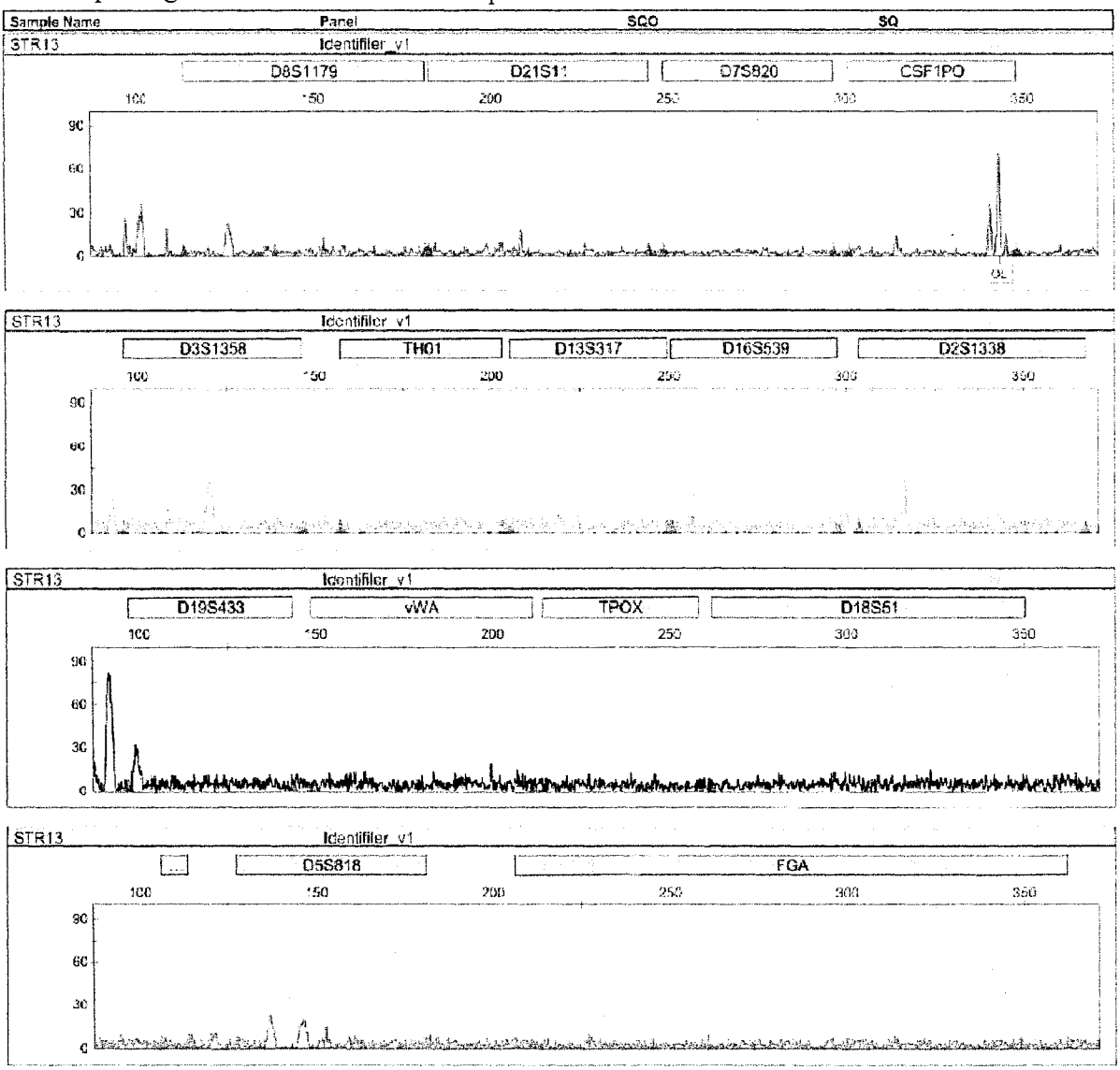
Electropherogram of the initial STR amplification for the 1:100 dilution.



Electropherogram of the initial STR amplification for the 1:1 000 dilution.

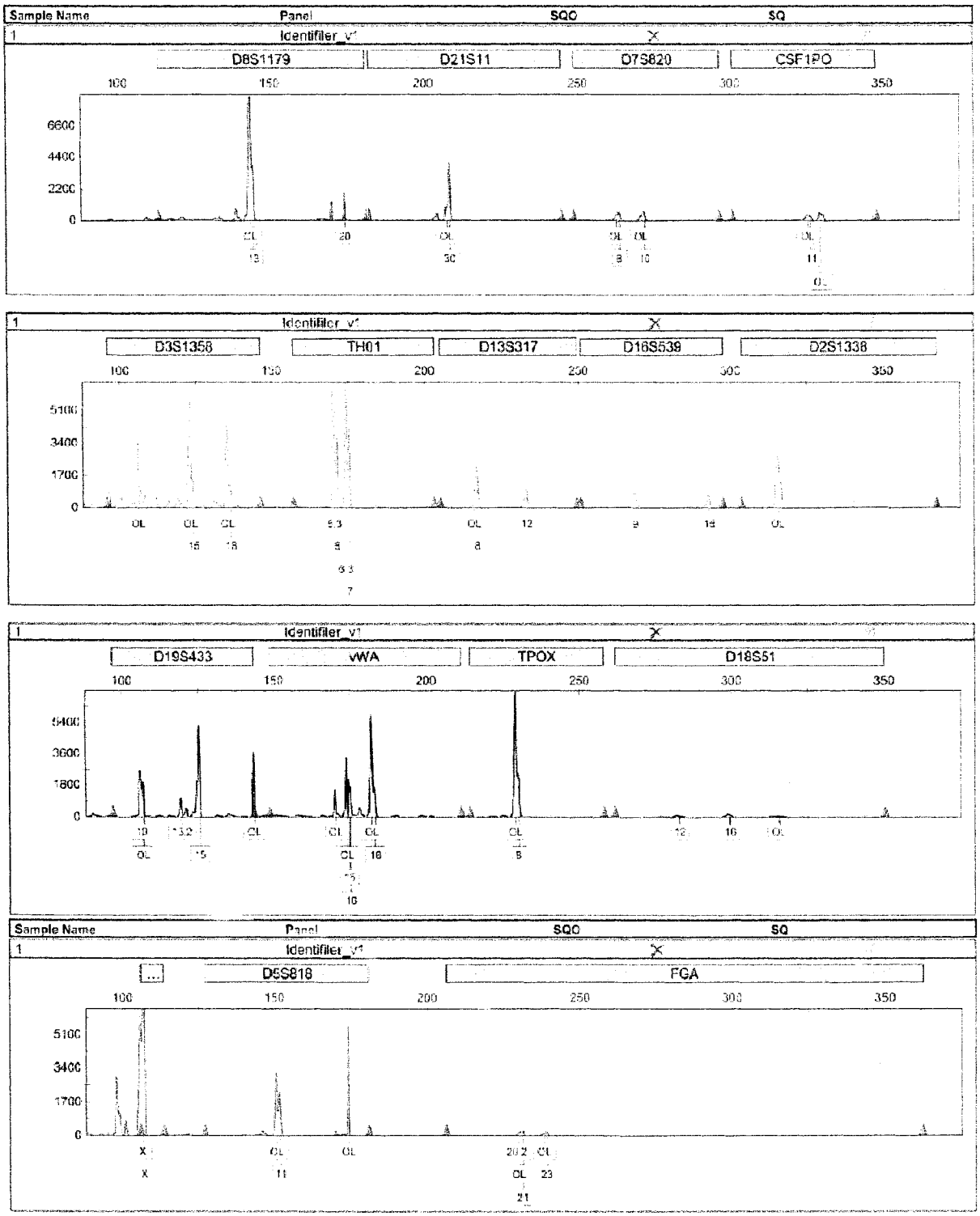


Electropherogram of the initial STR amplification for the 1:10 000 dilution.

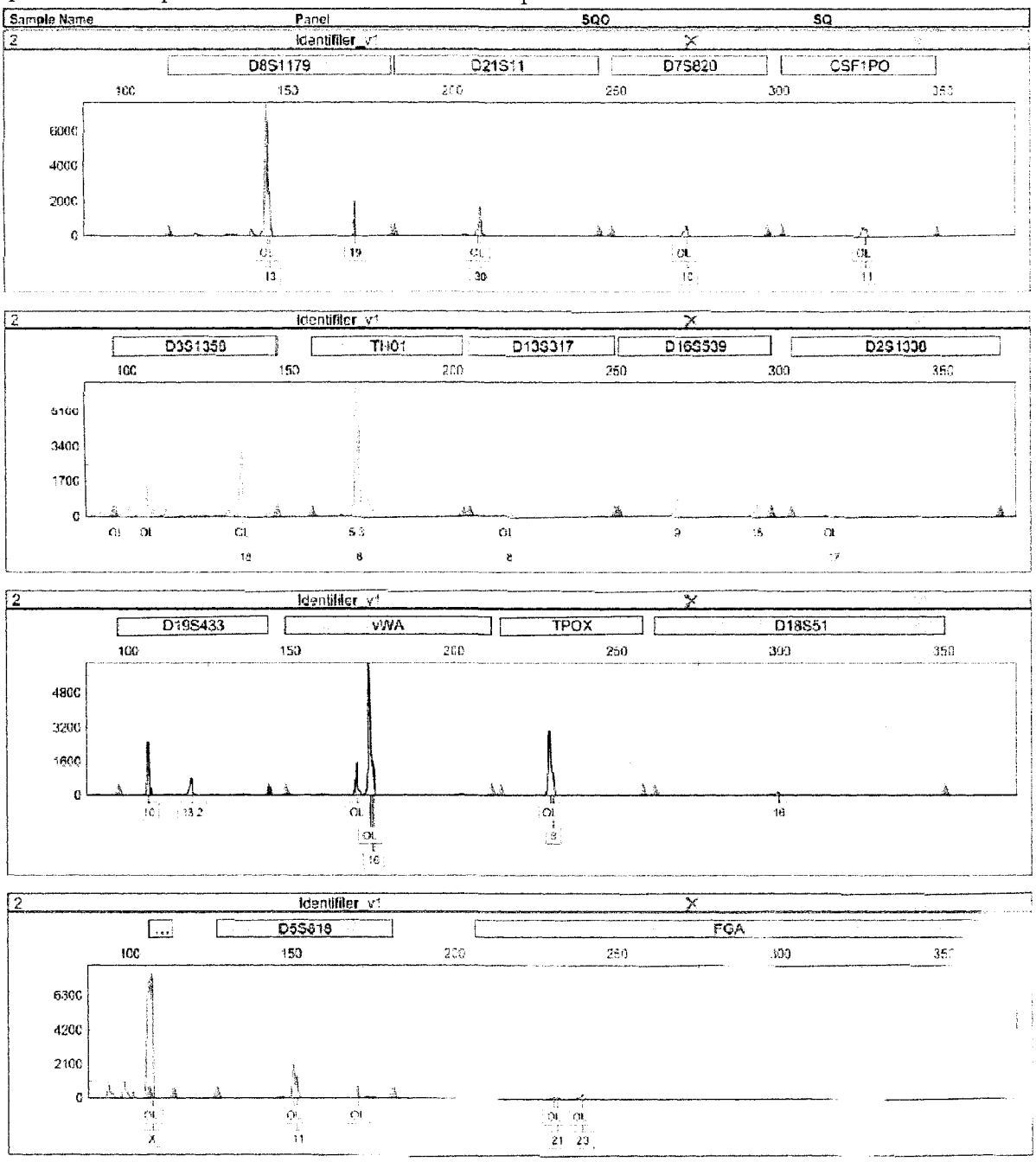


## XXIII. Appendix 3B

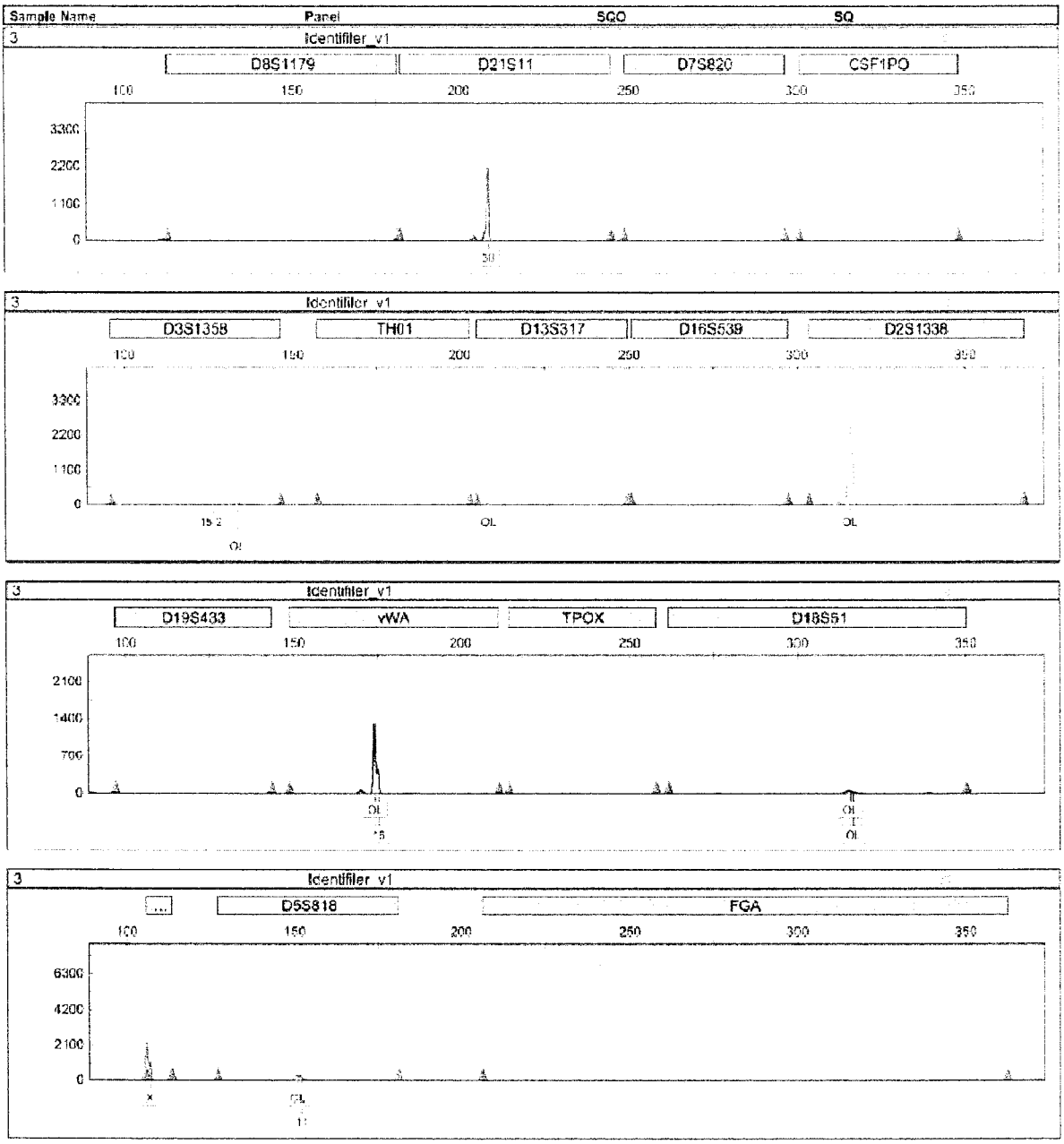
Electropherogram of the Booster STR amplification for the 1:100 dilution using 2.5 µL of purified PCR product from the initial STR amplification.



Electropherogram of the Booster STR amplification for the 1:1 000 dilution using 2.5 µL of purified PCR product from the initial STR amplification.

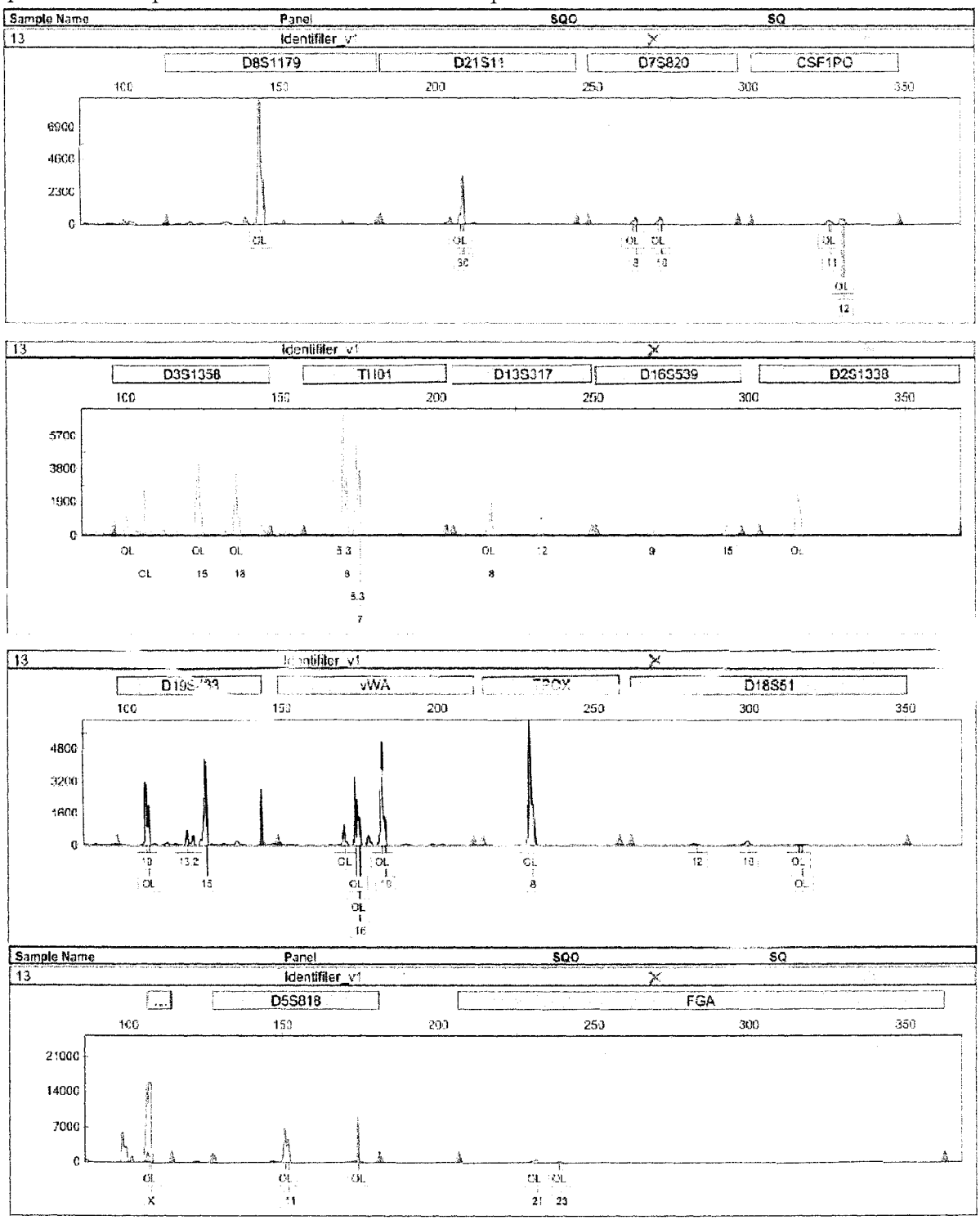


Electropherogram of the Booster STR amplification for the 1:10 000 dilution using 2.5 µL of purified PCR product from the initial STR amplification.

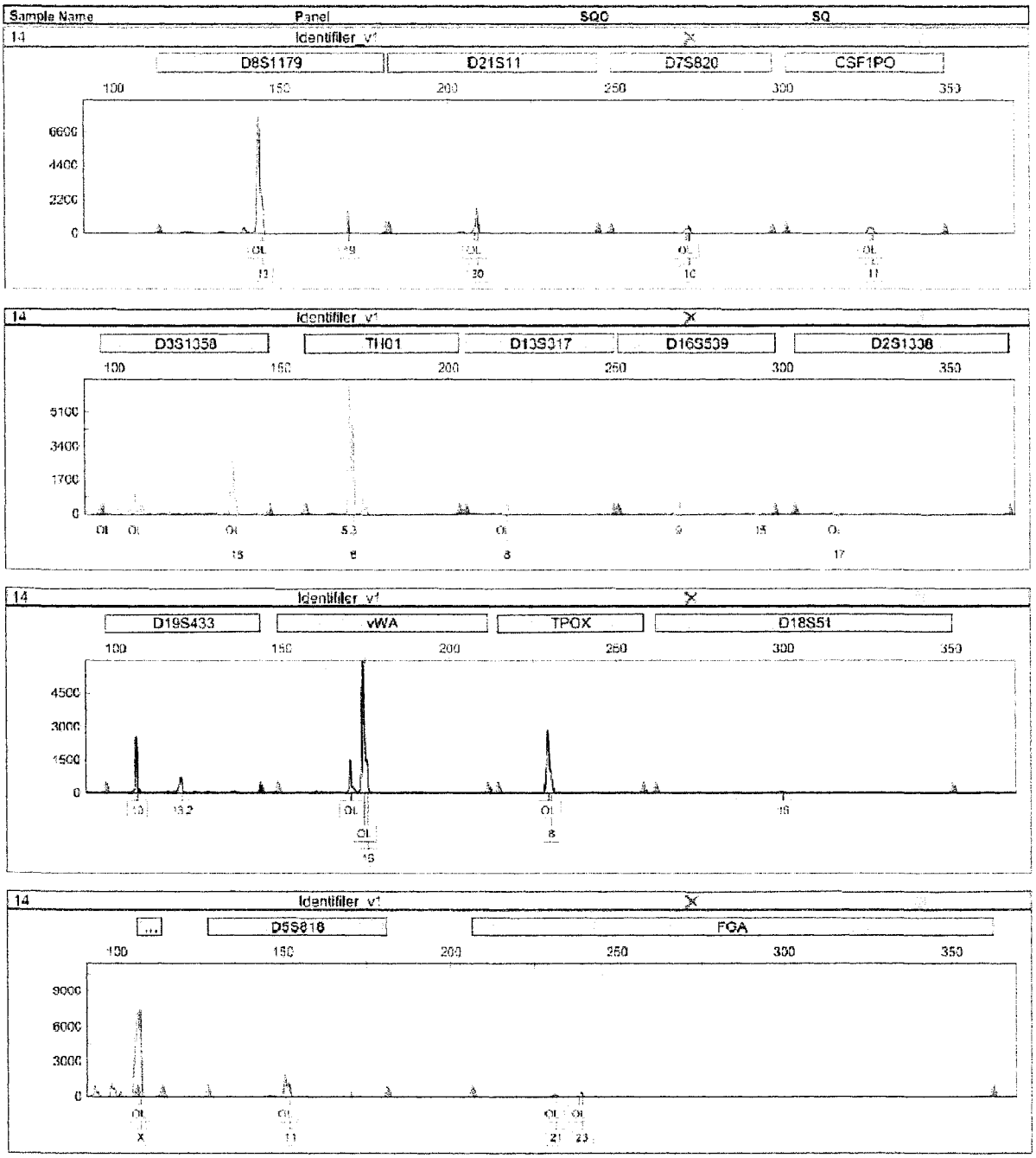


## XXIV. Appendix 3C

Electropherogram of the Booster STR amplification for the 1:100 dilution using 5.0 µL of purified PCR product from the initial STR amplification.



Electropherogram of the Booster STR amplification for the 1:1 000 dilution using 5.0 µL of purified PCR product from the initial STR amplification.



Electropherogram of the Booster STR amplification for the 1:10 000 dilution using 5.0 µL of purified PCR product from the initial STR amplification