

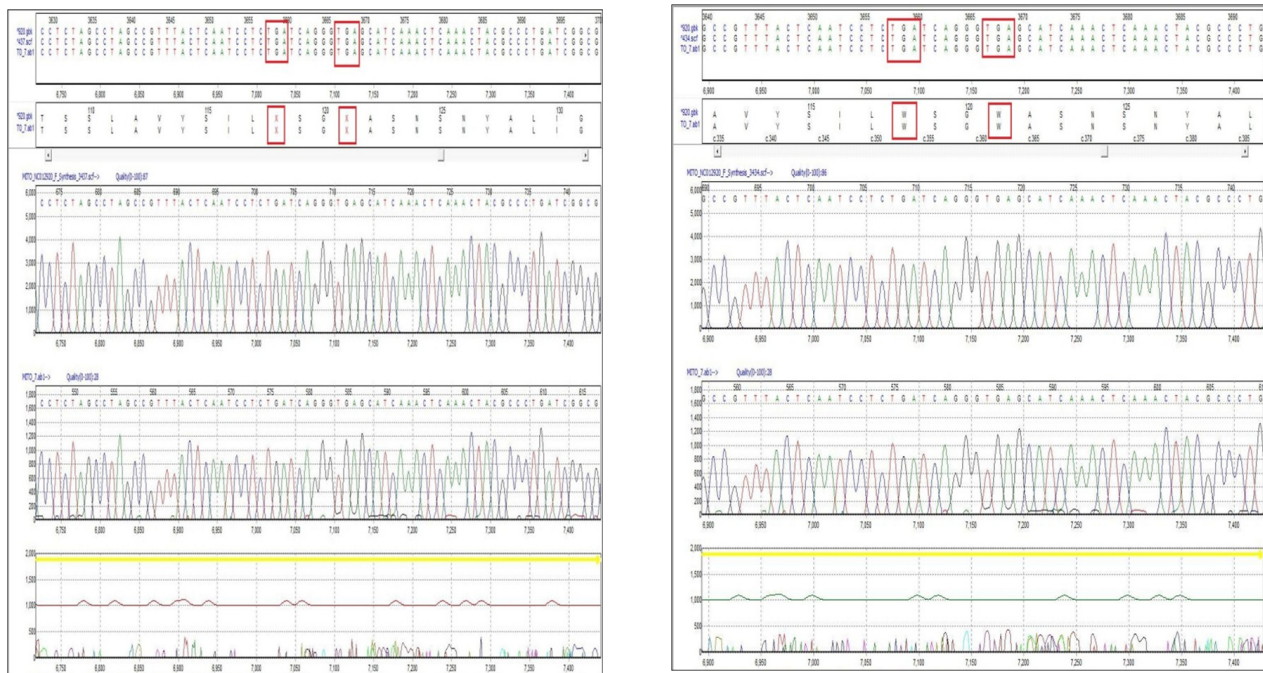
# Mitochondrial DNA Sequence Analysis using Mutation Surveyor® Software

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## Introduction

Mitochondria are the “powerhouses” of the human body that contain their own circular genome. Along with a unique circular genome, some of the genetic code or codons encode different amino acids than the human genetic code. Mutations in the mitochondrial genome have been known to cause mitochondrial myopathies, a group of diseases related to defects of the mitochondrion. This group includes Kearns-Sayre syndrome<sup>1</sup>, dementia<sup>2</sup>, deafness, and diabetes<sup>3</sup>. In order to study these diseases, researchers must study the mutations within the mitochondria genome. However, the difficulty of investigating the different mutations in mitochondrial sequences is due to the circular genome and different genetic code.

Mutation Surveyor software can solve both problems while offering high accuracy, speed, sensitivity, and custom reporting features that include specific regions of interest. When analyzing mitochondrial sequences, Mutation Surveyor software automatically recognizes the tag in the GenBank file and utilizes the mitochondrial genetic code without user intervention. In addition, the software can recognize the end and start position of the circular sequence and annotate the positions correctly across the origin/overlap region (**figures 1 and 2**).



**Figure 1:** Example of sequence data annotated with human and mitochondrial genetic code. Amino acid residues at positions 118 and 121 are displayed as stop codons with the standard genetic code (**left image**). Using the same sequence data and a mitochondrial GenBank file, the positions are annotated according to the mitochondrial genetic code and display Tryptophan (**right image**).

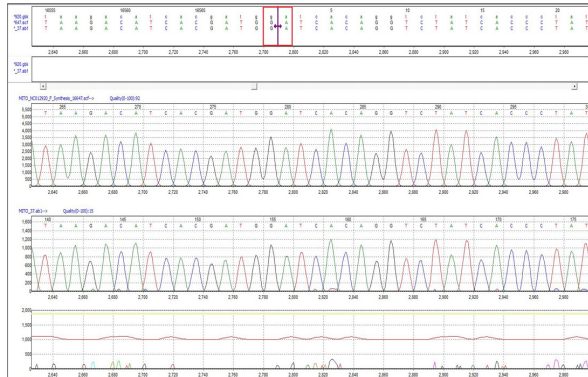
## Procedure

### Data Entry

1. Add 1-Directional or 2-Directional mitochondrial DNA sample files
2. Add mitochondrial GenBank file or appropriate reference sequence files
3. Click Process → Run to begin the analysis

## Analysis

Mutation Surveyor software analyzes mitochondrial sequence data in the same way mutations are detected using nuclear DNA sequence data (**figure 3**). The software uses a patented physical comparison “anti correlation” technology (U.S. Patent No. 7,400,980) to detect Single Nucleotide Polymorphisms (SNPs) and various other mutations. In addition, a multi-step alignment algorithm is used to detect insertions and deletions (INDELS).

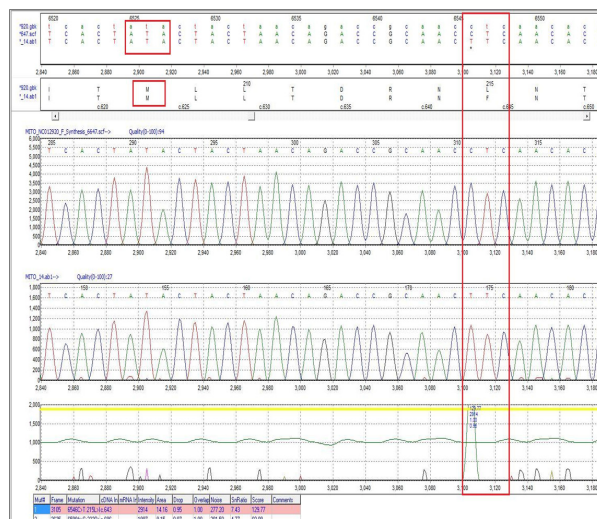


**Figure 2:** Graphic Analysis Display (GAD) of mitochondrial sequence data. The amplicon sequence in this illustration spans the origin of the mitochondrion circular genome. Mutation Surveyor software is able to align and annotate the sequence correctly, and the position of overlap/origin is indicated with a purple arrow.

## Results

Mitochondrial DNA does not follow the standard vertebrate genetic code and instead has different amino acid codons. Mutation Surveyor automatically uses the mitochondrial genetic code by reading a tag in the mitochondrion GenBank file. The nucleotide sequence and corresponding amino acid translation are displayed in the panes at the top of the Graphic Analysis Display (**figure 3**).

In this example, a GenBank sequence file was loaded into the software along with mtDNA sample files. Synthetic reference files were automatically generated using the sequence from the GenBank file and the best quality sample trace from the sample files. A homozygous single nucleotide polymorphism (SNP) of a cytosine to a thymine was detected at position 6456 relative to the GenBank file. The mutation results in a change of the amino acid sequence at residue 215. The software picks up the SNP as well as the amino acid change, indicating a change from Leucine (L) to Phenylalanine (F).



**Figure 3:** Mitochondrial analysis with Mutation Surveyor. The “ATA” that codes for Isoleucine (I) with the Standard Vertebrate Genetic Code is read as Methionine (M) for the Mitochondrial Genetic Code. A homozygous point mutation from Cytosine (C) to Thymine (T) has been detected at position 6456 in the GenBank file. The SNP was detected in the coding region indicated by the yellow band, and caused an amino acid change that is indicated by the software.

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Differences in stop codons between the standard genetic code and mitochondrial genetic code are also accounted for in Mutation Surveyor. As displayed in figure 1, mitochondrial sequence data analyzed with the standard code in Mutation Surveyor shows two stop (X) positions at residues 118 and 121 for the triplet TGA. With a GenBank file containing the mtDNA genetic code, Mutation Surveyor aligns the same sequence data and displays the amino acid Tryptophan at residues 118 and 121 in place of the stop sequence.

## Notes

Mutation Surveyor software offers accurate detection and quantification of low frequency mutations such as somatic or heteroplasmic mutations. The “Mutation Quantifier” function quantifies the presence of multiple alleles at a specific location in the genome.

In order for Mutation Surveyor software to successfully import mitochondrial DNA annotation information, the mitochondrial GenBank file must have the correct translation table listed in its annotation. The table is located in the CDS section of the GenBank file and is listed as “/transl\_table=2”. Mutation Surveyor software checks this file and will use mitochondrial annotation when the number “2” is located. Older GenBank files may not contain translation table information. Newer GenBank files automatically include this information.

## References

1. Prigione et al. “Mitochondrial DNA Deletions and Chloramphenicol Treatment Stimulate the Autophagic Transcript ATG12.” 2007. *Autophagy*. 3:4, 377-380.
2. Grazina et al. “Genetic Basis of Alzheimer’s Dementia: Role of mtDNA Mutations.” 2006/ *Genes, Brain and Behavior*. 5:2; 92-107
3. Bannwarth et al. “A Novel Unstable Mutation in Mitochondrial DNA Responsible for Maternally Inherited Diabetes and Deafness”. 2011. *Diabetes Care*. 34:12. 2591-2593