Resequencing Human Mitochondrial DNA with the mitoSEQr™

mitoSEQr™ Resequencing Primers

The mitoSEQr™ system is designed for detecting sequence variants in human mitochondrion. Two mitoSEQr Resequencing sets (RSS) are available:

- RSS000056015_01 (mitoALL™) for the complete mitochondrial genome (46 primer pairs)
- RSS000056016_01 (mitoCR™) for the control region including Hypervariable regions I/II (9 primer pairs)

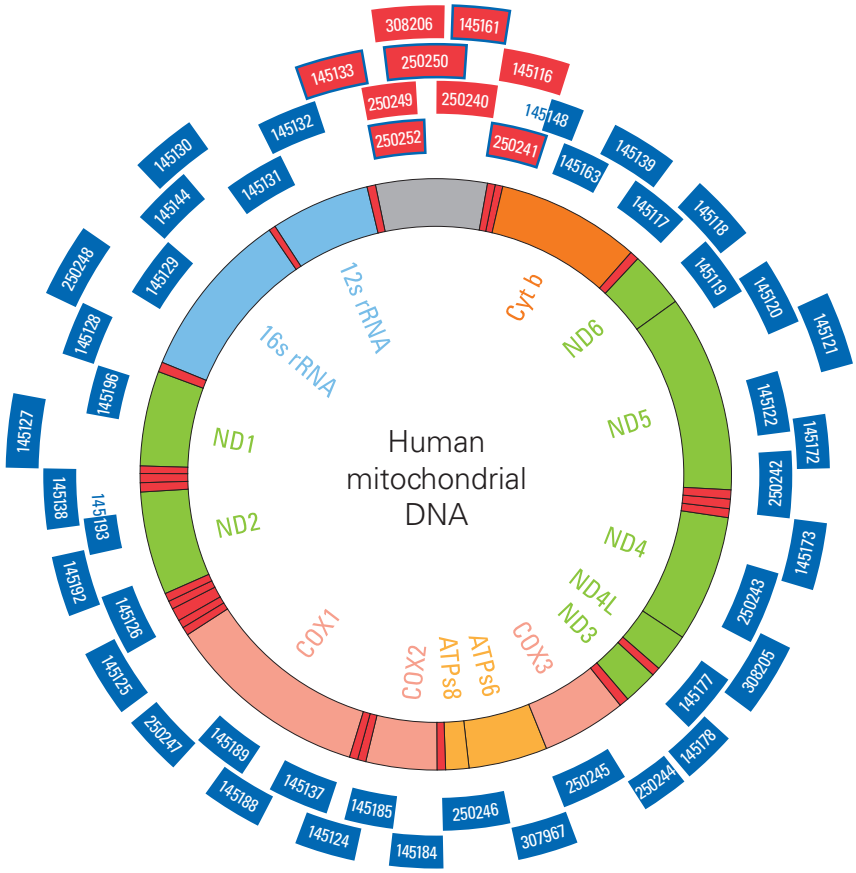
Each primer is tailed with a universal M13 sequence and are designed for universal PCR and Sequencing conditions. Please note, mitoSEQr has not been validated for use in human identity applications.

mtDNA Sequence Analysis

Sequence analysis of PCR-amplified mitochondrial DNA (mtDNA) is rapidly becoming an accepted tool for determination of phylogenetic origins, for confirmatory identification of human remains and for the detection of variants associated with specific diseases.

First sequenced by Sanger in 1981, the mitochondrial genome contains 16,569 base pairs coding for subunits of NADH dehydrogenase (7 subunits), 1 subunit of ubiquinol-cytochrome C oxidoreductase, 3 subunits of cytochrome C oxidase, 2 subunits of ATP synthase, 2 rRNA and 22 tRNA molecules. A non-coding region, the D-loop or control region, contains the Hypervariable regions 1 and 2 (HV1 & HV2).

mtDNA sequence analysis was used for the identification of remains of Czar Nicholas II, Neanderthal man and Louis XXIV.



mitoSEQr™ Resequencing Amplicons for mtDNA

- RSS000056015_01 (MitoALL™) resequencing amplicons (RSA's) for the complete mitochondrial genome.
- RSS000056016_01 (MitoCR™) RSA's for the control region.
- Five amplicons are shared by the two Resequencing sets.

Inheritance of mtDNA associated traits is non-mendelian and passes from mother to offspring, though paternal inheritance has also been reported. mtDNA mutations are linked to neurologic disease, cardiomyopathy, skeletal myopathy, and maternally inherited diabetes. Multiple genotypes of mitochondria may be found in the same cell (heteroplasmy). Tissue-specific and age-related heteroplasmy has been reported. Mutant mtDNA variants may exceed 85 percent of the total pool of mtDNA in ischemic heart disease, Parkinson's disease, etc. The range of clinical manifestations may depend on the proportion of abnormal mtDNA present.

mitoSEQr™ Protocol

1. Genomic DNA

- Prepare genomic DNA according to standard protocols
- Determine the concentration by A260 or fluorescence

2. PCR reaction (for 10-µL reactions)

- Mix AmpliTaq Gold® PCR Master Mix (2X) 5.0 µL; 50% of UltraPure™ glycerol 1.6 µL, Forward mitoSEQr™ RSA primer, 1.0 µL (0.6 µM/µL); Reverse mitoSEQr RSA primer, 1.0 µL (0.6 µM/µL), Genomic DNA 1.0 µL (0.5–1.0 ng/µL). Recommended thermocycling conditions with the AB 9700 are: Heat activation 96°C for 5 min, followed by 40 cycles of 94°C for 30 sec, 60°C for 45 sec, and 72°C for 45 sec. Final extension of 72°C for 10 min. Hold at 4°C.

3. PCR Reaction Clean-up

- Add 2 µL of ExoSAP-IT® (USB Corporation), incubate at 37 °C for 30 min followed by heat inactivate at 80 °C for 15 min.

4. Sequencing Reaction

- Prepare a forward and reverse sequencing reaction mix. The forward sequencing master mix contains the M13 forward primer and the reverse contains the M13 reverse primer. For 10-µL sequencing reactions use: 4.0 µL of BigDye® Terminator Ready Reaction Mix v3.1, 1.0 µL of the M13 forward or reverse primer (3.2 pmol/µL), 3.0 µL of deionized water 2.0 µL of the PCR product from the PCR reaction (step 3)
- Thermocycling conditions with the AB 9700 are: Heat activation of 96 °C for 1 min, followed by 25 cycles of 96 °C for 10 sec, 50°C for 5 sec, and 60 °C for 4 min. Hold at 4 °C

5. Sequencing Reaction Clean-up

- Add 2.5 µL of 125 mM EDTA and mix. Add 30 µL of 100% ethanol and mix. Incubate at room temperature for 15 min.
- Centrifuge at 2000 x g for 45 min. Remove supernatant. Add 30 µL of 70% ethanol and centrifuge at 2000 x g for 15 min. Remove supernatant and air dry. Resuspend in 10 µL of Hi-Di™ Formamide

6. Electrophoresis

- To perform electrophoresis on the AB 3130*xl* (POP-6™) Genetic Analyzer running buffer with EDTA; 36-cm array: RapidSeq36_POP6_1. Run file; KB_3130_POP6_BDTv3.mob; KB.bcp
- To perform electrophoresis on the AB 3730/3730*xl* (POP-7™) DNA Analyzer Running buffer with EDTA; 36-cm array: RapidSeq36_POP7_1. Run file; Mobility file KB_3730_POP7_BDTv3.mob; KB.bcp

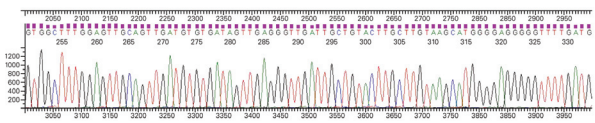
7. Data Analysis

- Import the SeqScape® template and sample files into a new project. Click "Analyze".
- Review and examine variants
- Create the mutation/genotyping report

Genomic DNA Prep
↓
PCR Amplification
↓
DNA Sequencing
↓
Electrophoresis
↓
Data Analysis

Product Description	Package	Part Number
RSS000056015_01 mitoSEQr™ Resequencing Primers for the complete human mitochondrial genome 46 RSA's	500 reactions	4348846
RSS000056016_01 mitoSEQr™ Resequencing Primers for the human mitochondrial Control Region 9 RSA's	500 reactions	4348809

For additional Resequencing Primer Sets for Human Genes visit www.appliedbiosystems.com



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