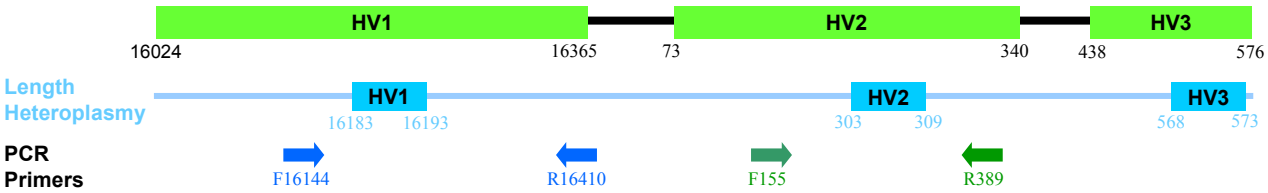
mtDNA HV1 and HV2 length heteroplasmies analysis

mtDNA Hypervariable Regions



Pre-amplification

Reagents Needed:

Primer Mix
AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA)
Gold ST*R 10X Buffer (Promega, Madison, WI)

10 X Primer Mix:

Primer	Primer Sequence (5'-3')	Conc.
F16144	TGA CCA CCT GTA GTA CAT AA	2.0 µM
R16410	6FAM-GAG GAT GGT GGT CAA GGG AC	2.0 µM
F155	TAT TTA TCG CAC CTA CGT TC	3.0 µM
R389	HEX-CTG GTT AGG CTG GTG TTA GG	3.0 µM

PCR Mixture:

PCR Component	Volume per Sample
dH_2O	6.9 µL
Gold ST*R 10 X Buffer	1.0 µL
10 X Primer Mix	1.0 µL
AmpliTaq Gold (5U/µL)	0.1 µL
DNA Template (1ng/µL)	1.0 µL
Total	10.0 µL

Thermal Cycling:

95ºC for 11 minutes, then:

94ºC for 1 minutes
56ºC for 1 minutes
72ºC for 1 minutes
For 25 cycles, then:

60ºC for 45 minutes
4ºC soak

Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

mtDNA Hypervariable Regions

Materials & Reagents Needed:

Dry heating block, water bath, or thermal cycler

310 capillaries, 47cm x 50 µm (Applied Biosystems, Foster City, CA)

Performance Optimized Polymer (POP4, Applied Biosystems, Foster City, CA)

Flourescent Amidite Matrix Standards [6FAM™, TET™, HEX™, TAMRA™, ROX™]
(Applied Biosystems, Foster City, CA)

Run module GS STR POP4 (1 mL) D

GeneScan™ 400HD ROX™ Size Standard (Applied Biosystems, Foster City, CA)

Hi-Di™ Formamide (Applied Biosystems, Foster City, CA)

Creating Matrix:

According to the ABI PRISM® 310 Genetic Analyzer User's Manual

Preparing the Sample:

1. Prepare a loading cocktail by combining and mixing the 0.2 µL GeneScan™ 400HD ROX™ Size Standard and 20 µL Hi-Di Formamide per sample.
2. Vortex for 10 seconds.
3. Combine 20.2 µL of the prepared loading cocktail and 1.0 µL of the PCR product.
4. Preparing the allelic ladder, combine 20.2 µL of the prepared loading cocktail and 1.0 µl of the allelic ladder mix. Vortex the allelic ladder mix prior to pipetting.
5. Denature the samples and ladder by heating at 95ºC for 5 minutes and immediately chill on crushed ice. Denature the samples just prior to loading.
6. Assemble the tubes in the appropriate autosampler, and place the autosampler tray in the instrument.

310 Data Collection Software:

Prepared the samples are run using the Run module **GS STR POP4 (1 mL) D** and a described above **matrix**.

Samples are injected for 5 seconds at 15,000 V and separate at 15,000V for 24 minutes with run temperature of 60ºC.

GeneScan Software: