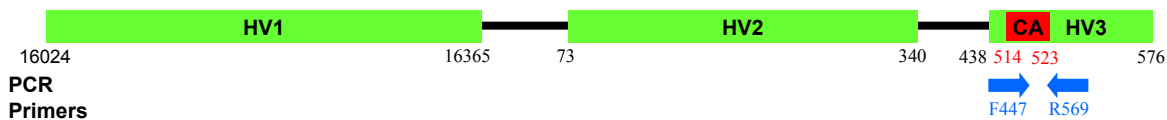




# mtDNA CA repeat length polymorphism analysis

## 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.
F447	HEX-CAT TAT TTT CCC CTC CCA CTC C	2.0 µM
R569	GGT GTC TTT GGG GTT TGG TTG	2.0 µM

### PCR Mixture:

PCR Component	Volume per Sample
dH <sub>2</sub> O	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:

95°C for 11 minutes, then:

94°C for 1 minutes

57°C for 1 minutes

72°C for 1 minutes

For 23 cycles, then:

60°C for 45 minutes

4°C 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 95°C 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 60°C.

### GeneScan Software: