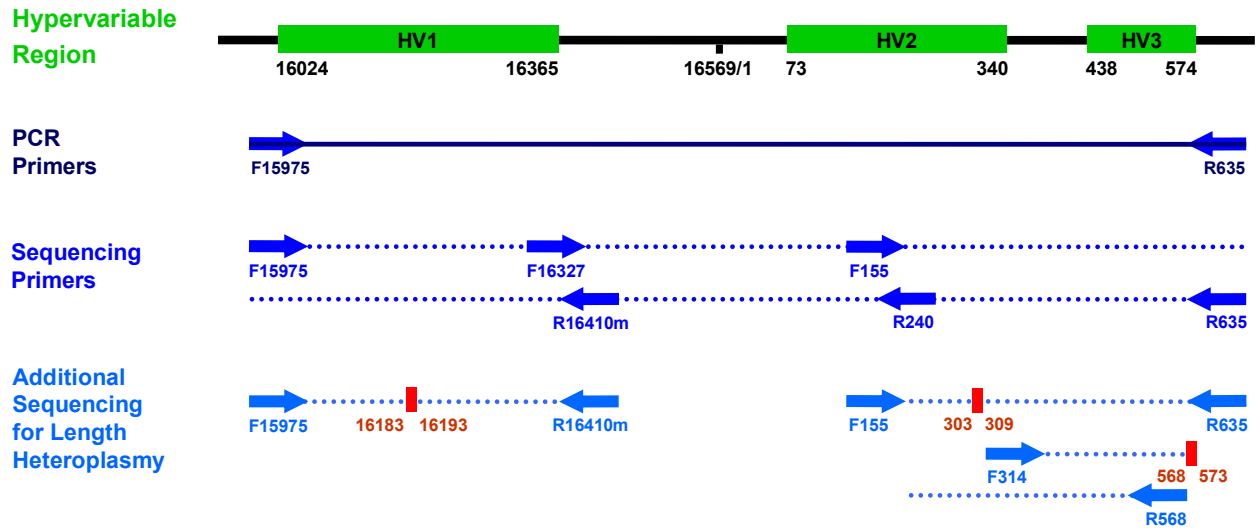


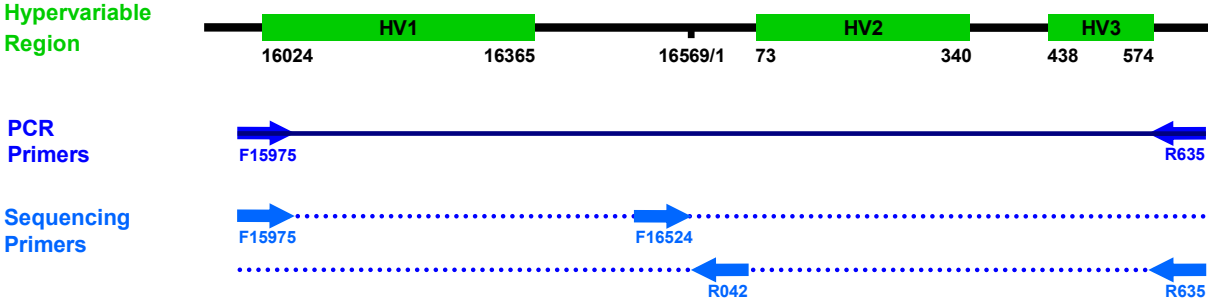
Primers for mtDNA Control Region Sequence Analysis



Primers for PCR and Sequencing:

Primer	Sequence (5'→3')	Usage
F15975	CTC CAC CAT TAG CAC CCA AA	PCR and Sequencing
F16327	CCG TAC ATA GCA CAT TAC AGT C	Sequencing
F155	TAT TTA TCG CAC CTA CGT TC	Sequencing
R16410m	GAG GAT GGT GGT CAA GGG A	Sequencing
R240	TAT TAT TAT GTC CTA CAA GCA	Sequencing
R635	GAT GTG AGC CCG TCT AAA CA	PCR and Sequencing
F314	CCG CTT CTG GCC ACA GCA CT	Sequencing
R568	GTG TCT TTG GGG TTT GGT TG	Sequencing

Primers for mtDNA Control Region Sequence Analysis



Primers for mtDNA HV Region Sequence Analysis



Primers for PCR and Sequencing:

Primer	Sequence (5'→3')	Usage
F15975	CTC CAC CAT TAG CAC CCA AA	PCR and Sequencing
F16524	AAG CCT AAA TAG CCC ACA CG	"
F015	CAC CCT ATT AAC CAC TCA CG	"
F403	TCT TTT GGC GGT ATG CAC TTT	"
R16410m	GAG GAT GGT GGT CAA GGG A	"
R042	AGA GCT CCC GTG AGT GGT TA	"
R389	CTG GTT AGG CTG GTG TTA GG	"
R635	GAT GTG AGC CCG TCT AAA CA	"

mtDNA Control Region Sequence Analysis

mtDNA Control Region Amplification

Reagent Needed:

AmpliAq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
Gold ST*R 10X Buffer (Promega, Madison, WI)

PCR Mixture:

PCR Component		Final Conc.
dH ₂ O	18.2 µL	
Gold ST*R 10X Buffer	2.5 µL	1X
Forward Primer (10 pmol/µL)	1.5 µL	0.6 µM
Reverse Primer (10 pmol/µL)	1.5 µL	0.6 µM
AmpliAq Gold (5 U/µL)	0.3 µL	1.5 U
Template DNA (1 ng/µL)	1.0 µL	
Total	25.0 µL	

Thermal Cycling:

95°C for 11 minutes, then:

95°C for 30 seconds
56°C for 30 seconds
72°C for 60~90 seconds
for 35 cycles, then:

72°C for 7 minutes
4°C soak

Enzyme Purification of the PCR Product

Reagent Needed:

PCR Product	20.0 µL
ExoSAP-IT® (USB, Cleveland, OH)	2.0 µL

Thermal Cycling:

37°C for 45 minutes
80°C for 15 minutes

Sequencing Reaction Using BigDye™ Kit

Reagent Needed:

ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit
(Applied Biosystems, Foster City, CA)
Single Sequencing Primer
5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10 mM MgCl₂

Sequencing Reaction Mixture:

Reaction Component	1/4 rxn	1/8 rxn
dH ₂ O	3.0 µL	3.0 µL
5X Sequencing Buffer	1.0 µL	2.0 µL
BigDye RR Mix	2.0 µL	1.0 µL
Primer (1.6 pmol/µL)	2.0 µL	2.0 µL
PCR Product	2.0 µL	2.0 µL
Total	10.0 µL	10.0 µL

Thermal Cycling:

96°C for 10 seconds
50°C for 5 seconds
60°C for 2 minutes

for 30 cycles