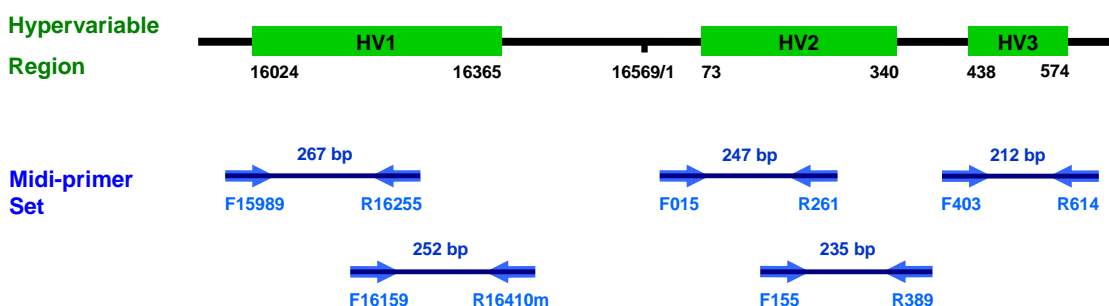


Midi-primer Set for mtDNA Sequence Analysis



Sequences of Midi-primer Set:

Region	Amplicon	Primer	Sequence (5'→3')
HV1	P11	F15989	CCC AAA GCT AAG ATT CTA AT
		R16255	CTT TGG AGT TGC AGT TGA TG
	P12	F16159	CAT AAA AAC CCA ATC CAC AT
		R16410m	GAG GAT GGT GGT CAA GGG A
HV2	P21	F015	CAC CCT ATT AAC CAC TCA CG
		R261	GCT GTG CAG ACA TTC AAT TGT T
	P22	F155	TAT TTA TCG CAC CTA CGT TC
		R389	CTG GTT AGG CTG GTG TTA GG
HV3	P31	F403	TCT TTT GGC GGT ATG CAC TTT
		R614	TTT CAG TGT ATT GCT TTG AGG A

Reagents Needed:

AmpliTaq 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	17.0 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
AmpliTaq Gold (5 U/µL)	0.5 µL	2.5 U
DNA Template	2.0 µL	
Total	25.0 µL	

Thermal Cycling:

95°C for 11 minutes, then:

95°C for 20 seconds

50°C for 20 seconds

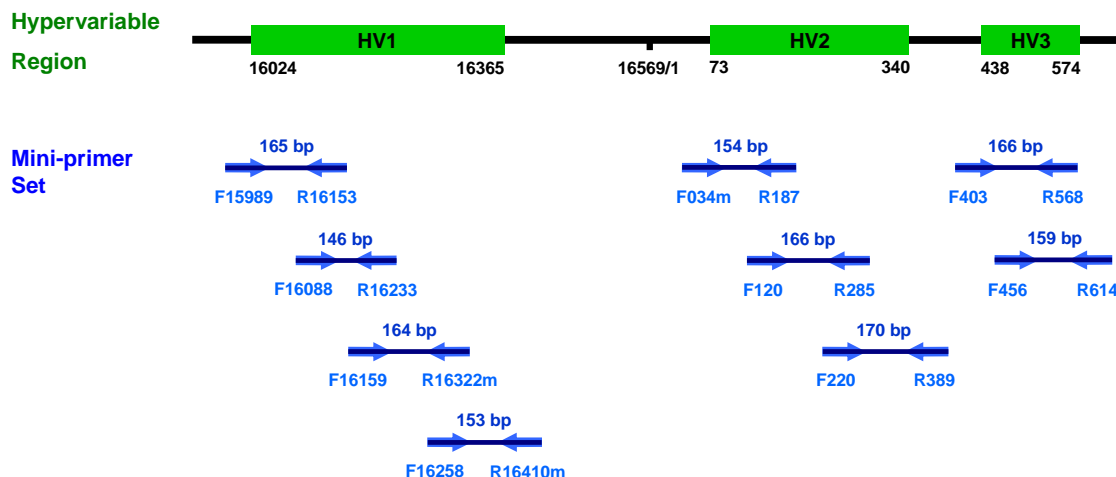
72°C for 30 seconds

for 40-42 cycles, then:

72°C for 7 minutes

4°C soak

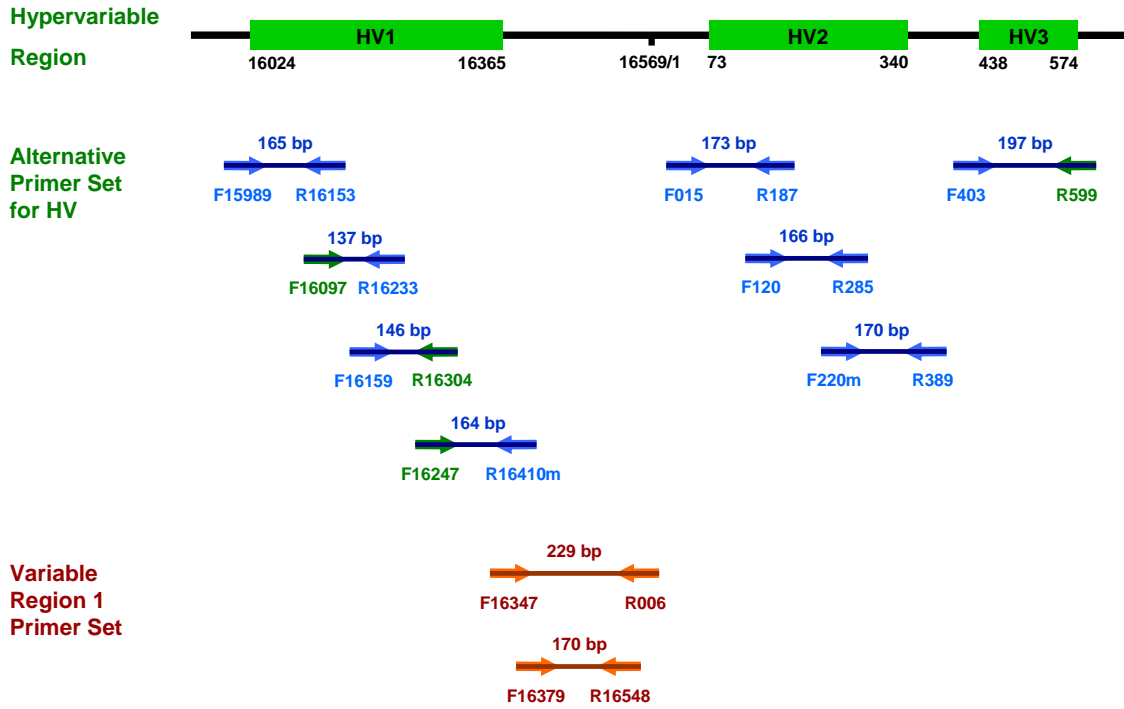
Mini-primer Set for mtDNA Sequence Analysis



Sequences of Mini-primer Set:

Region	Amplicon	Primer	Sequence (5'→3')
HV1	M11	F15989	CCC AAA GCT AAG ATT CTA AT
		R16153	CAG GTG GTC AAG TAT TTA TGG
	M12	F16088	TGT ATT TCG TAC ATT ACT GC
		R16233	TGA TAG TTG AAG GTT GAT TGC TGT
	M13	F16159	CAT AAA AAC CCA ATC CAC AT
		R16322m	TGG CTT TAT GTA CTA TGT ACT G
M14	F16258	ACC CCT CAC CCA CTA GGA TA	
	R16410m	GAG GAT GGT GGT CAA GGG A	
HV2	M21	F034m	GGG AGC TCT CCA TGC ATT T
		R187	CGC CTG TAA TAT TGA ACG TA
	M22	F120	CGC AGT ATC TGT CTT TGA TTC C
		R285	GTT ATG ATG TCT GTG TGG AA
	M23	F220	TGC TTG TAG GAC ATA ATA AT
		R389	CTG GTT AGG CTG GTG TTA GG
HV3	M31	F403	TCT TTT GGC GGT ATG CAC TTT
		R568	GTG TCT TTG GGG TTT GGT TG
	M32	F456	CCC CTC CCA CTC CCA TAC T
		R614	TTT CAG TGT ATT GCT TTG AGG A

Additional Primer Set for mtDNA Sequence Analysis



Sequences of Additional Primer Set:

Region	Amplicon	Primer	Sequence (5'→3')
HV1	M12	F16097	TAC ATT ACT GCC AGC CAC CA
		R16233	TGA TAG TTG A <u>A</u> G GTT GAT TGC TGT
	M13	F16159	CAT AAA AAC CCA ATC CAC AT
		R16304	ACT GTT AAG GGT GGG TAG GT
M14	F16247	ACT CCA AAG CCA CCC CTC A	
	R16410m	GAG GAT GGT GGT CAA GGG A	
HV2	M23	F220m	TGC TTG TAG GAC ATA ATA ATA ACA
		R389	CTG GTT AGG CTG GTG TTA GG
HV3	P31	F403	TCT TTT GGC GGT ATG CAC TTT
		R599	TTG AGG AGG TAA GCT ACA TA
VR1	P13	F16347	TCA AAT CCC TTC TCG TCC C
		R006	GTG ATC CAT CGT GAT GTC TT
	M15	F16379	CCT CAG ATA GGG GTC CCT TG
		R16548	GGG AAC GTG TGG GCT ATT TA

mtDNA Control Region Sequence Analysis

PCR Product Purification Using QIAquick® PCR Purification Kit

Material:

QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany)

Protocol:

1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix.
For example, add 100 µL of Buffer PB to 20 µL PCR sample.
2. Place a QIAquick spin column in a provided 2 mL collection tube.
3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30-60 seconds.
4. Discard flow-through. Place the QIAquick column in a clean 2 mL collection tube.
5. To wash, add 0.75 mL Buffer PE to the QIAquick column and centrifuge for 30-60 seconds.
6. Discard flow-through and place the QIAquick column in a clean 2 mL collection tube.

Centrifuge the column for an additional 1 minute.
7. Place QIAquick column in a clean 1.5 mL microcentrifuge tube.
8. To elute DNA, add 30 µL dH₂O to the center of the QIAquick membrane and centrifuge the column for 1 minute.

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