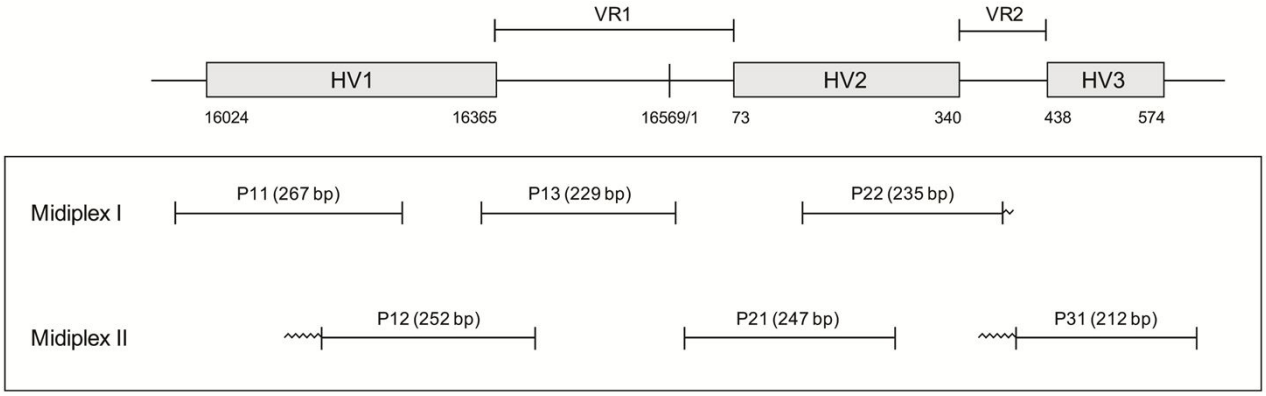
Multiplex-PCR System for mtDNA Sequence Analysis



Midi-primer Set (10X Primer Mix):

Multiplex	Amplicon	Primer	Sequence (5'→3')	Conc. (μM)
Midiplex I	P11	F15989	CCC AAA GCT AAG ATT CTA AT	8.0
		R16255	CTT TGG AGT TGC AGT TGA TG	8.0
	P13	F16347	TCA AAT CCC TTC TCG TCC C	2.0
		R006	GTG ATC CAT CGT GAT GTC TT	2.0
	P22	F155m	TAT TTA TCG CAC CTA CGT TCA	6.0
		R389-5t	ta$(tta)_1$ CTG GTT AGG CTG GTG TTA GG	6.0
Midiplex II	P12	F16159-15t	$(tat)_5$ CAT AAA AAC CCA ATC CAC AT	4.0
		R16410m	GAG GAT GGT GGT CAA GGG A	4.0
	P21	F015	CAC CCT ATT AAC CAC TCA CG	6.0
		R261	GCT GTG CAG ACA TTC AAT TGT T	6.0
	P31	F403-15t	$(aat)_5$ TCT TTT GGC GGT ATG CAC TTT	12.0
		R614	TTT CAG TGT ATT GCT TTG AGG A	12.0

Regents Needed:

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

PCR Mixture:

PCR Component		Final Conc.
dH_2O	17.4 μL	
Gold ST*R 10X Buffer	2.5 μL	1X
10X primer mix	2.5 μL	1X
AmpliTaq Gold (5 U/μL)	0.6 μL	3.0 U
DNA Template	2.0 μL	
Total	25.0 μL	

Thermal Cycling:

95°C for 11 minutes, then:

95°C for 20 seconds
55°C for 60 seconds
72°C for 30 seconds
for 40~42 cycles, then:

72°C for 7 minutes
4°C soak

Sequence Analysis of mtDNA Control Region

Enzyme Purification of the PCR Product

Reagent Needed:

PCR Product	20.0 µL
ExoSAP-IT® (USB, Cleveland, OH)	5.0 µL

Thermal Cycling:

37℃ for 45 minutes

80℃ for 15 minutes

Sequencing Reaction Using BigDye™ Kit

Reagent Needed:

ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems)

Single Sequencing Primer

BigDye® Terminator v1.1 & v3.1 5X Sequencing Buffer (Applied Biosystems)

Sequencing Reaction Mixture:

Reaction Component	1/4 rxn	1/8 rxn
dH_2O	4.0 µL	4.0 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
Purified PCR Product	1.0 µL	1.0 µL
Total	10.0 µL	10.0 µL

Thermal Cycling:

96℃ for 10 seconds
50℃ for 5 seconds
60℃ for 2 minutes

for 30 cycles