



Coding region SNPs for East Asian mtDNA haplogroup

Multiplex PCR I for M9, N9, M11, M10, A, D5, M and D4 haplogroup

Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
 GeneAmp® 10X PCR Buffer (Applied Biosystems, Foster City, CA)
 GeneAmp® dNTP Blend, 10 mM (Applied Biosystems, Foster City, CA)
 5X Primer Mix for PCR
 ExoSAP-IT® (USB, Cleveland, OH)

5X Primer Mix for PCR:

	Primer Sequence (5'-3')	Haplogroup	Conc.
F4491	CAC CTA TCA CAC CCC ATC CT	M9	2.0 µM
R4491	GAT GAG TGT GCC TGC AAA GA		2.0 µM
F5417	CAC CAT CAC CCT CCT TAA CC	N9	2.0 µM
R5417	GAT GAG TGT GGG GAG GAA TG		2.0 µM
F7642	ACA TGC AGC GCA AGT AGG TC	M11	0.6 µM
R7642	GGG CAT ACA GGA CTA GGA AGC		0.6 µM
F8793/8794	CAA CAC TAA AGG ACG AAC CTG A	M10/A	4.0 µM
R8793/8794	GAT GGC CAT GGC TAG GTT TA		4.0 µM
F10397/10400	GCC CTA AGT CTG GCC TAT GA	D5/M	1.6 µM
R10397/10400	TGT AAA TGA GGG GCA TTT GG		1.6 µM
F14668	ACC CCA CAA ACC CCA TTA CT	D4	4.0 µM
R14668	TTG GGG TCA TTG GTG TTC TT		4.0 µM

PCR Mixture:

PCR Component	Volume/Sample
dH ₂ O	13.1 µL
10X PCR Buffer	3.5 µL
dNTP Blend	2.0 µL
5X Primer Mix	5.0 µL
AmpliTaq Gold (5U/µL)	0.4 µL
DNA Template (1ng/µL)	1.0 µL
Total	25.0 µL

Thermal Cycling:

95°C for 10 minutes, then:

94°C for 20 seconds
 58°C for 20 seconds
 72°C for 30 seconds
 for 35 cycles, then:

72°C for 7 minutes
 4°C soak

Enzyme purification of the PCR product:

PCR Product	5.0 µL
ExoSAP-IT®	1.0 µL

Thermal Cycling:

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



Coding region SNPs for East Asian mtDNA haplogroup

Multiplex SBE I for M9, N9, M11, M10, A, D5, M and D4 haplogroup

Reagents Needed:

ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA)
 5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10mM MgCl₂
 10X Primer Mix for SNaPshot Reaction
 SAP (USB, Cleveland, OH) or CIP (Promega, Madison, WI)

10X Primer Mix for SBE:

	Primer Sequence (5'-3')	Haplogroup	Variation	Conc.
S4491	(T) ₂₆ GAATCCCCTGGCCCAACCC	M9	G→A	1.2 μM
S5417	(T) ₃₅ CATATCTAACAACGTAAAAATAAAATGACA	N9	G→A	1.2 μM
S7642	(T) ₃₁ GCTACTTCCCCTATCATAGAAGA	M11	G→A	2.4 μM
S8793	(T) ₂₆ CTAACCTCCTCGGACTCCTGCC	M10	T→C	14.0 μM
S8794	(T) ₃₈ GTGGTTGGTGTAATGAGT	A	C→T	1.0 μM
S10397	(T) ₈ CTATGAGTGACTACAAAAGGATTAGACTG	D5	A→G	4.4 μM
S10400	ATTCGTTTTGTTAAACTATATACCAATTC	M	C→T	0.4 μM
S14668	(T) ₁₁ CCACACTCAACAGAAACAAAGCATA	D4	C→T	2.0 μM

SBE Mixture:

PCR Component	Volume/Sample
dH ₂ O	5.0 μL
5X Sequencing Buffer	2.0 μL
SNaPshot Multiplex Ready Reaction Mix	1.0 μL
10X Primer Mix	1.0 μL
PCR Product	1.0 μL
Total	10.0μL

Thermal Cycling:

96°C for 10 seconds
 50°C for 5 seconds
 60°C for 30 seconds
 for 25 cycles, then:
 4°C soak

Post-Extension Treatment:

SNaPshot Reaction Product	10.0 μL
SAP or CIP	0.5 μL

Thermal Cycling:

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



Coding region SNPs for East Asian mtDNA haplogroup

Multiplex PCR II for R9, G, D, M8, B, M7 and R haplogroup

Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
GeneAmp® 10X PCR Buffer (Applied Biosystems, Foster City, CA)
GeneAmp® dNTP Blend, 10 mM (Applied Biosystems, Foster City, CA)
5X Primer Mix for PCR
ExoSAP-IT® (USB, Cleveland, OH)

5X Primer Mix for PCR:

	Primer Sequence (5'-3')	Haplogroup	Conc.
F3970	GGC TTC AAC ATC GAA TAC GC	R9	2.0 µM
R3970	CAG GGG AGA GTG CGT YAT A		2.0 µM
F4833/4883	GTC CCA GAG GTT ACC CAA GG	G/D	2.0 µM
R4833/4883	GGC TTA CGT TTA GTG AGG GAG A		2.0 µM
F7196	AGA CCA AAC CTA CGC CAA AA	M8	2.0 µM
R7196	CAT CGG GGT AGT CCG AGT AA		2.0 µM
F8281-8289d	AGG GCC CGT ATT TAC CCT AT	B	4.0 µM
R8281-8289d	TTT AGT TGG GGC ATT TCA CTG		4.0 µM
F9824	GGC ATC TAC GGC TCA ACA TT	M7	2.0 µM
R9824	ATT AGT TGG CGG ATG AAG CA		2.0 µM
F12705	TGT TCG TTA CAT GGT CYA TCA	R	2.5 µM
R12705	TCT CAG CCG ATG AAC AGT TG		2.5 µM

PCR Mixture:

PCR Component	Volume/Sample
dH ₂ O	13.1 µL
10X PCR Buffer	3.5 µL
dNTP Blend	2.0 µL
5X Primer Mix	5.0 µL
AmpliTaq Gold (5U/µL)	0.5 µL
DNA Template (1ng/µL)	1.0 µL
Total	25.0 µL

Thermal Cycling:

95°C for 10 minutes, then:

94°C for 20 seconds
58°C for 20 seconds
72°C for 30 seconds
for 35 cycles, then:

72°C for 7 minutes
4°C soak

Enzyme purification of the PCR product:

PCR Product	5.0 µL
ExoSAP-IT®	1.0 µL

Thermal Cycling:

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



Coding region SNPs for East Asian mtDNA haplogroup

Multiplex SBE II for R9, G, D, M8, B, M7 and R haplogroup

Reagents Needed:

ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA)
5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10mM MgCl₂
10X Primer Mix for SNaPshot Reaction
SAP (USB, Cleveland, OH) or CIP (Promega, Madison, WI)

10X Primer Mix for SBE:

	Primer Sequence (5'-3')	Haplogroup	Variation	Conc.
S3970	(T) ₂₇ GGTGTATTCGGCTATGAAGAATA	R9	C→T	1.0 μM
S4833	(T) ₈ GTCCCAGAGGTTACCCAAGGC	G	A→G	5.0 μM
S4883	(T) ₄ CACATGACAAAACTAGCCCC	D	C→T	1.0 μM
S7196	(T) ₁₅ TAACTTTCTTCCCACAACACTTTCT	M8	C→A	2.0 μM
S8281-8289d	(T) ₃₅ GTGGGCTCTAGAGGGGGT	B	Del 9bp	6.0 μM
S9824	(T) ₉ GAAAGTTGAGCCAATAATGACGTG	M7	T→C	9.0 μM
S12705	(T) ₁₂ AAACATTAATCAGTTCTTCAAATATCTACTCAT	R	T→C	2.0 μM

SBE Mixture:

PCR Component	Volume/Sample
dH ₂ O	5.0 μL
5X Sequencing Buffer	2.0 μL
SNaPshot Multiplex Ready Reaction Mix	1.0 μL
10X Primer Mix	1.0 μL
PCR Product	1.0 μL
Total	10.0 μL

Thermal Cycling:

96°C for 10 seconds
50°C for 5 seconds
60°C for 30 seconds
for 25 cycles, then:

4°C soak

Post-Extension Treatment:

SNaPshot Reaction Product	10.0 μL
SAP or CIP	0.5 μL

Thermal Cycling:

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



Coding region SNPs for East Asian mtDNA haplogroup

Multiplex PCR III for D4, D4h, D4b, D4g, D4e, D4j and D4a haplogroup

Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
GeneAmp® 10X PCR Buffer (Applied Biosystems, Foster City, CA)
GeneAmp® dNTP Blend, 10 mM (Applied Biosystems, Foster City, CA)
5X Primer Mix for PCR
ExoSAP-IT® (USB, Cleveland, OH)

5X Primer Mix for PCR:

	Primer Sequence (5'-3')	Haplogroup	Conc.
F3010	GGG ATA ACA GCG CAA TCC TA	D4	2.0 µM
R3010	TCG TTG AAC AAA CGA ACC TT		2.0 µM
F5048	CCA TCA TAG CAG GCA GTT GA	D4h	2.0 µM
R5048	TGG TTA TGT TAG GGT TGT ACG G		2.0 µM
F8020	CGA CTA CGG CGG ACT AAT CT	D4b	4.0 µM
R8020	TTA TAC GAA TGG GGG CTT CA		4.0 µM
F8701	CGA CTA ATC ACC ACC CAA CA	D4g	0.5 µM
R8701	TCC GAG GAG GTT AGT TGT GG		0.5 µM
F11215	CCA CAC TTA TCC CCA CCT TG	D4e	1.0 µM
R11215	AGT GCG ATG AGT AGG GGA AG		1.0 µM
F11696	CAG CCA TTC TCA TCC AAA CC	D4j	3.0 µM
R11696	GCG TTC GTA GTT TGA GTT TGC		3.0 µM
F14979	GCC ATG CAC TAC TCA CCA GA	D4a	2.0 µM
R14979	AGA ATA TTG AGG CGC CAT TG		2.0 µM

PCR Mixture:

PCR Component	Volume/Sample
dH ₂ O	12.5 µL
10X PCR Buffer	3.5 µL
dNTP Blend	2.0 µL
5X Primer Mix	5.0 µL
AmpliTaq Gold (5U/µL)	0.5 µL
DNA Template (1ng/µL)	1.0 µL
Total	25.0 µL

Thermal Cycling:

95°C for 10 minutes, then:

94°C for 20 seconds
58°C for 20 seconds
72°C for 30 seconds
for 35 cycles, then:

72°C for 7 minutes
4°C soak

Enzyme purification of the PCR product:

PCR Product	5.0 µL
ExoSAP-IT®	1.0 µL

Thermal Cycling:

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



Coding region SNPs for East Asian mtDNA haplogroup

Multiplex SBE III for D4, D4h, D4b, D4g, D4e, D4j and D4a haplogroup

Reagents Needed:

ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA)
5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10mM MgCl₂
10X Primer Mix for SNaPshot Reaction
SAP (USB, Cleveland, OH) or CIP (Promega, Madison, WI)

10X Primer Mix for SBE:

	Primer Sequence (5'-3')	Haplogroup	Variation	Conc.
S3010	(T) ₅ TTTAATAGCGGCTGCACCAT	D4	G→A	0.5 μM
S5048	(T) ₂₂ TTACCCACATAGGATGAATAATAGCAGT	D4h	T→C	2.0 μM
S8020	(T) ₁₃ TTATACGAATGGGGGCTTCAAT	D4b	G→A	5.0 μM
S8701	(T) ₁₈ CTAATCAAACCTCAAACAAATGATA	D4g	G→A	5.0 μM
S11215	(T) ₁₇ CGCAGGCACATACTTCCTATTCTA	D4e	C→T	2.0 μM
S11696	(T) ₃₂ CGTGGGCGATTATGAGAATGA	D4j	G→A	2.0 μM
S14979	(T) ₅ CATTGGCGTGAAGGTAGCGGATG	D4a	T→C	2.0 μM

SBE Mixture:

PCR Component	Volume/Sample
dH ₂ O	5.0 μL
5X Sequencing Buffer	2.0 μL
SNaPshot Multiplex Ready Reaction Mix	1.0 μL
10X Primer Mix	1.0 μL
PCR Product	1.0 μL
Total	10.0 μL

Thermal Cycling:

96°C for 10 seconds
50°C for 5 seconds
60°C for 30 seconds
for 25 cycles, then:
4°C soak

Post-Extension Treatment:

SNaPshot Reaction Product	10.0 μL
SAP or CIP	0.5 μL

Thermal Cycling:

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



Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

Cording region SNPs for East Asian mtDNA haplogroup

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)
ABI PRISM® dRhodamine Matrix Standards Kit (Applied Biosystems, Foster City, CA)
Run module GS POP4 (1 mL) E
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. Add 20 µL of Hi-Di Formamide into each tube.
2. Add 1.0 µL of the SNaPshot product into each tube.
3. Vortex briefly and quick spin.
4. Denature the samples by heating at 95°C for 5 minutes and immediately chill on crushed ice. Denature the samples just prior to loading.
5. 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 POP4 (1 mL) E** and a described above **matrix**.

Samples are injected for 5 seconds at 15,000 V and separate at 15,000V for 18 minutes with run temperature of 60°C.

GeneScan Software: