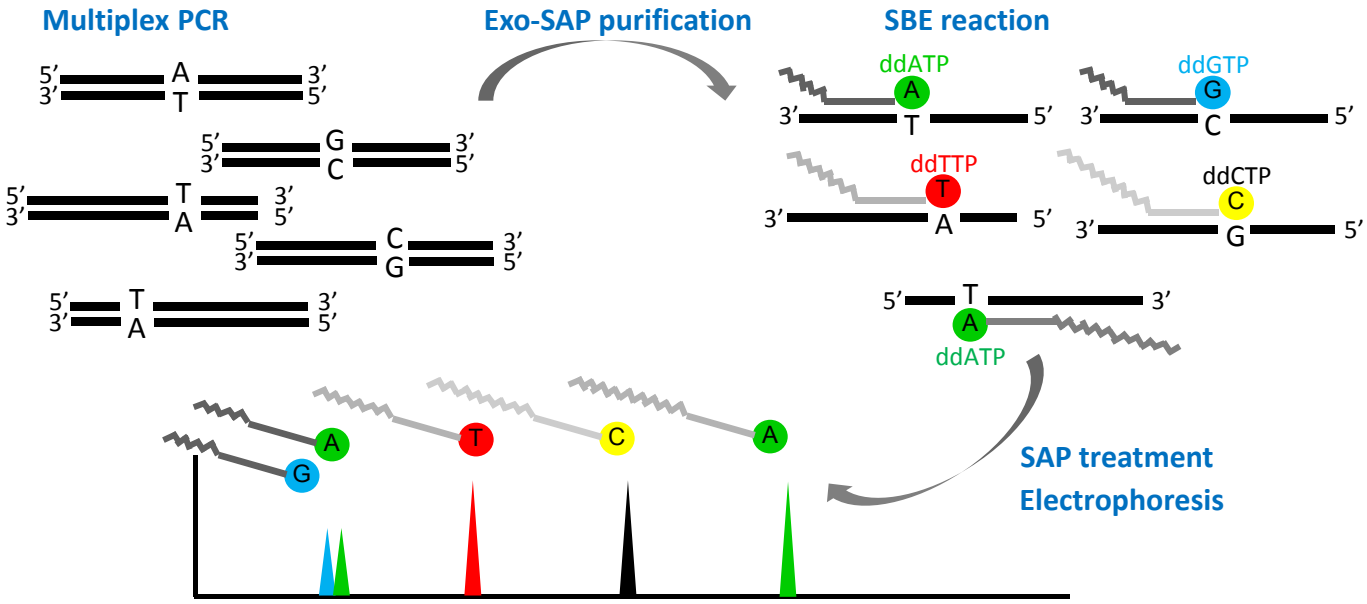
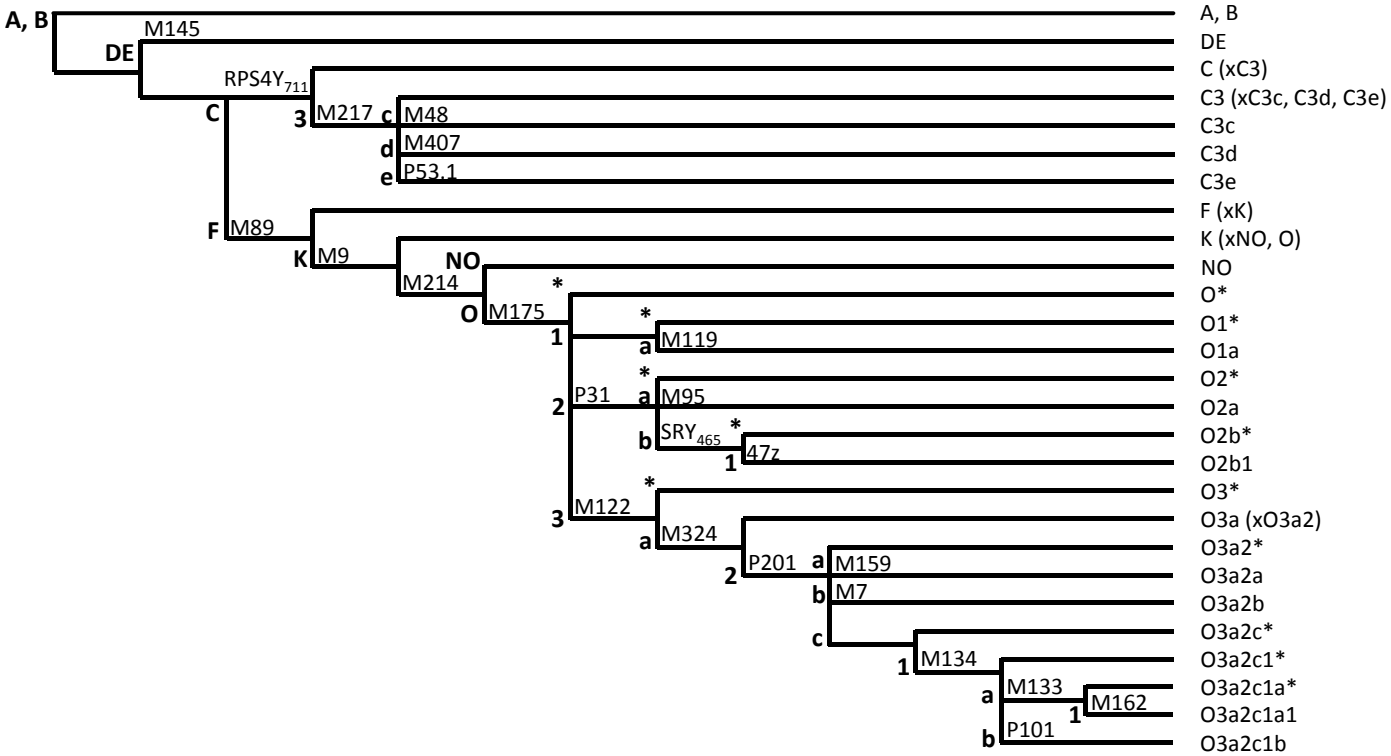
Y-chromosomal haplogroup typing – Using SBE reaction

Schematic of multiplex PCR followed by SBE reaction



Phylogenetic tree of the 24 Y-chromosomal binary polymorphisms



Y-chromosomal haplogroup typing – Using SBE reaction

Major haplogroup

Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
Gold ST*R 10X Buffer (Promega, Madison, WI)
Primers
ExoSAP-IT® (USB, Cleveland, OH)

5X primer mix for PCR:

SNP	Haplogroup		Primer Sequence (5'-3')	Conc.
M145	DE	F142	GCC TCC ACG ACT TTC CTA GAC	1.0 µM
		R227	AGG TTC CTC CCA CTC CTT TTT	1.0 µM
$RPS4Y_{711}$	C	F136	CAG GGC AAT AAA CCT TGG AT	5.0 µM
		R228	GTG GCC AGC CTC TTA TCT CTC	5.0 µM
M89	F	F096	AGC TTC CTG GAT TCA GCT CTC	1.0 µM
		R188	CAG GAT CAC CAG CAA AGG TAG	1.0 µM
M9	K	F110	GGA CCC TGA AAT ACA GAA CTG C	5.0 µM
		R194	CGT TTG AAC ATG TCT AAA TTA AAG AAA A	5.0 µM
M214	NO	F108	CAC TGG AAA GAA AAA GAA TGC TG	2.0 µM
		R206	AGC CTG GGA GAC AGT GTG AG	2.0 µM
M175	O	F104	ACC CAA ATC AAC TCA ACT CCA	2.0 µM
		R199	TGA TAC CTT TGT TTC TGT TCA TTC TT	2.0 µM

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	16.1 µL
Gold ST*R 10X Buffer	2.5 µL
10X 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:

95ºC for 11 minutes, then:

94ºC for 20 seconds
60ºC for 1 minute
72ºC for 30 seconds
for 33 cycles, then:

72ºC for 7 minutes
4ºC soak

Enzyme purification of the PCR product:

PCR Product	5.0 µL
ExoSAP-IT®	1.0 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Major haplogroup

Reagents Needed:

ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA)
5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10mM $MgCl_2$
10X Primer Mix for SNaPshot Reaction
SAP (USB, Cleveland, OH) or CIP (Promega, Madison, WI)

10X Primer Mix for SBE:

Primer	Variation	Primer Sequence (5'-3')	Conc.
DE-F151-22	G → A	$(T)_1$CTA GAC ACC AGA AAG AAA GGC	1.0 µM
C-F143-31	C → T	$(T)_9$AGG GCA ATA AAC CTT GGA TTT C	10.0 µM
F-R164-39	C → T	$(T)_{17}$CAA CTC AGG CAA AGT GAG AGA T	1.6 µM
K-F137-48	C → G	$(T)_{27}$AAC GGC CTA AGA TGG TTG AAT	1.1 µM
NO-F142-55	T → C	$(T)_{29}$TGG TTA CTT TCG TTC GTT TAT TTT TC	1.0 µM
O-F148-63	TTCTC → ΔTTCTC	$(T)_{42}$GCA CAT GCC TTC TCA CTT CTC	2.6 µM

SBE Mixture:

PCR Component	Volume/Sample
dH_2O	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:

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

4ºC soak

Post-Extension Treatment:

SNaPshot Reaction Product	10.0 µL
SAP or CIP	0.5 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Subhaplogroup C

Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
Gold ST*R 10X Buffer (Promega, Madison, WI)
Primers
ExoSAP-IT® (USB, Cleveland, OH)

5X primer mix for PCR:

SNP	Haplogroup		Primer Sequence (5'-3')	Conc.
M217	C3	F100	GGA GAA TGA AAA AGT TGG GTG	2.0μM
		R181	AAG CTG CTG TGG CTT TCA TC	2.0 μM
M48	C3c	F098	TCC CTT CCA CTC TTA GCT TGA C	5.0 μM
		R190	CTG AGG GCA ACT ATT AAG GCA	5.0 μM
M407	C3d	F124	CTG AAA GTT GGG GAC AGT CAT	2.5 μM
		R205	TGG CAC TAA ATC AAC TTC TCC TT	2.5 μM
P53.1	C3e	F067	CAA CGA GGC TGC AGG TCT TA	3.0 μM
		R166	GAA CCA ATC CCA CCC TAT CA	3.0 μM

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	16.1 μL
Gold ST*R 10X Buffer	2.5 μL
10X 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:

95ºC for 11 minutes, then:

94ºC for 20 seconds
60ºC for 1 minute
72ºC for 30 seconds
for 33 cycles, then:

72ºC for 7 minutes
4ºC soak

Enzyme purification of the PCR product:

PCR Product	5.0 μL
ExoSAP-IT®	1.0 μL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Subhaplogroup C

Reagents Needed:

ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA)
5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10mM $MgCl_2$
10X Primer Mix for SNaPshot Reaction
SAP (USB, Cleveland, OH) or CIP (Promega, Madison, WI)

10X Primer Mix for SBE:

Primer	Variation	Primer Sequence (5'-3')	Conc.
C3-F102-23	A → C	AGA ATG AAA AAG TTG GGT GAC AC	3.0 µM
C3c-F112-29	A → G	AGC TTG ACA ATT AGG ATT AAG AAT ATG AT	8.0 µM
C3d-R204-38	A → G	$(T)_{14}$GCA CTA AAT CAA CTT CTC CTT TGG	8.0 µM
C3e-R156-48	T → C	$(T)_{25}$CAC CCT ATC ACT ATG CTT GTC TC	1.2 µM

SBE Mixture:

PCR Component	Volume/Sample
dH_2O	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:

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

4ºC soak

Post-Extension Treatment:

SNaPshot Reaction Product	10.0 µL
SAP or CIP	0.5 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Subhaplogroups O

Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
Gold ST*R 10X Buffer (Promega, Madison, WI)
Primers
ExoSAP-IT® (USB, Cleveland, OH)

5X primer mix for PCR:

SNP	Haplogroup		Primer Sequence (5'-3')	Conc.
M119	O1a	F102	CAA ACC GCA GTG CTA TGT GT	1.0 µM
		R195	ATG GGT TAT TCC AAT TCA GCA	1.0 µM
P31	O2	F074	TGG GGA ACA GGT AGG TGG TA	4.0 µM
		R161	GTG TGA GAC TCC ATC GCA AA	4.0 µM
M95	O2a	F105	GGG ATC AAA TGG AGT TCC TG	1.0 µM
		R183	GCC TAC AGG TTG GAA AGG CTA	1.0 µM
SRY_{465}	O2b	F108	ATC CCG CTT CGG TAC TCT G	1.0 µM
		R190	TCT TGA GTG TGT GGC TTT CGT	1.0 µM
47z	O2b1	F088	TCT CCT GAC CTY GTG ATT CG	5.0 µM
		R174	TCA TTG ACA TGG GCT GGA CT	5.0 µM
M122	O3	F102	CTT AGT TGC CTT TTG GAA ATG AA	2.0 µM
		R189	GCT TTA TTC AGA TTT TCC CCT GA	2.0 µM

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	16.1 µL
Gold ST*R 10X Buffer	2.5 µL
10X 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:

95ºC for 11 minutes, then:

94ºC for 20 seconds
60ºC for 1 minute
72ºC for 30 seconds
for 33 cycles, then:

72ºC for 7 minutes
4ºC soak

Enzyme purification of the PCR product:

PCR Product	5.0 µL
ExoSAP-IT®	1.0 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Subhaplogroups O

Reagents Needed:

ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA)
5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10mM $MgCl_2$
10X Primer Mix for SNaPshot Reaction
SAP (USB, Cleveland, OH) or CIP (Promega, Madison, WI)

10X Primer Mix for SBE:

Primer	Variation	Primer Sequence (5'-3')	Conc.
O1a-R192-25	A → C	GGT TAT TCC AAT TCA GCA TAC AGG C	10.0 µM
O2-F097-33	T → C	$(A)_3$GGT TAC ATA AAT AAG GTT TTT TTT TGG TTG	3.5 µM
O2a-F123-42	C → T	$(T)_{12}$TGA GGA TAA GGA AAG ACT ACC ATA TTA GTG	2.5 µM
O2b-R151-50	C → T	$(T)_{29}$CCT GTT GTC CAG TTG CAC TTC	1.0 µM
O2b1-R165-58	G → C	$(T)_{37}$TGG GCT GGA CTT GGT GGC TCA	10.0 µM
O3-R183-66	T → C	$(T)_{45}$TTC AGA TTT TCC CCT GAG AGC	4.0 µM

SBE Mixture:

PCR Component	Volume/Sample
dH_2O	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:

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

4ºC soak

Post-Extension Treatment:

SNaPshot Reaction Product	10.0 µL
SAP or CIP	0.5 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Subhaplogroups O3

Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
Gold ST*R 10X Buffer (Promega, Madison, WI)
Primers
ExoSAP-IT® (USB, Cleveland, OH)

5X primer mix for PCR:

SNP	Haplogroup		Primer Sequence (5'-3')	Conc.
M324	O3a	F109	TGA TTT GAT CTA CCT GCC CTT T	2.0 µM
		R178	AAG GGA ACA AAT TGA TTT CCA G	2.0 µM
P201	O3a2	F078	TGT GCT GTG CAA GTT GTG TG	2.5 µM
		R174	TGG GTG CAG TTA AGC AAT GA	2.5µM
M159	O3a2a	F063	TTC AGC CTT CTT CTG GTA CTT TTT A	2.5 µM
		R161	TCC TCT GGA GTC GAA AGA GTG	2.5 µM
M7	O3a2b	F131	CAA AGG GCA TGT AAT CAT TCC T	2.5 µM
		R230	TGA TCC AAT TAT TTC CAT TGT GTT	2.5 µM
M134	O3a2c1	F076	ATC AAA CCC AGA AGG GTT AAA GA	2.0 µM
		R147	GAG ATA CTT TTG ATC CCC ACC A	2.0 µM
M133	O3a2c1a	F092	AAG GTG GGG CTT TCT GAA G	4.0 µM
		R189	GAT TGT CTG GTT GTG GGG AA	4.0 µM

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	16.1 µL
Gold ST*R 10X Buffer	2.5 µL
10X 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:

95ºC for 11 minutes, then:

94ºC for 20 seconds
60ºC for 1 minute
72ºC for 30 seconds
for 33 cycles, then:

72ºC for 7 minutes
4ºC soak

Enzyme purification of the PCR product:

PCR Product	5.0 µL
ExoSAP-IT®	1.0 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Subhaplogroups O3

Reagents Needed:

ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA)
5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10mM $MgCl_2$
10X Primer Mix for SNaPshot Reaction
SAP (USB, Cleveland, OH) or CIP (Promega, Madison, WI)

10X Primer Mix for SBE:

Primer	Variation	Primer Sequence (5'-3')	Conc.
O3a-F114-23n	G → C	$(T)(A)_2$TGA TCT ACC TGC CCT TTC CT	1.0 µM
O3a2-F097-30	T → C	$(T)_2(A)$GAT CTT GGT TAA GTC ATT TGA TCT CAG	1.0 µM
O3a2a-F115-39	A → C	$(T)_{13}$AGT TTT ATT ATT GAT GCA AGC CCT AA	1.5 µM
O3a2b-R209-47	C → G	$(T)_{14}(A)$TTA AAT TTT GTA GTT GAG TTA CTG TTC TTC TT	2.0 µM
O3a2c1-F102-55	G → ΔG	$(T)_{34}$AGA AAA GGC CCA GGA AAG TAT	1.0 µM
O3a2c1a-F096-67	T → ΔT	$(A)_{29}$TGG GGC TTT CTG AAG CAA ATA CCA GCT TTA AAA AAA AA	10.0 µM

SBE Mixture:

PCR Component	Volume/Sample
dH_2O	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:

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

4ºC soak

Post-Extension Treatment:

SNaPshot Reaction Product	10.0 µL
SAP or CIP	0.5 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Subhaplogroups O3a2c1

Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)
Gold ST*R 10X Buffer (Promega, Madison, WI)
Primers
ExoSAP-IT® (USB, Cleveland, OH)

5X primer mix for PCR:

SNP	Haplogroup		Primer Sequence (5'-3')	Conc.
P101	O3a2c1b	F113	TCT CCT AAC CTT GTG ATC TGC C	1.0 µM
		R209	AGG AAC ACC ATT ATC TTT TTC AGC	1.0 µM
M162	O3a2c1a1	F137	CAG GAA AAT AGA TGC CTG CAA	2.0 µM
		R222	CCT GAC AAC AGA GAC AGC ACA	2.0 µM
M133	O3a2c1a	F092	AAG GTG GGG CTT TCT GAA G	4.0 µM
		R189	GAT TGT CTG GTT GTG GGG AA	4.0 µM

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	16.3 µL
Gold ST*R 10X Buffer	2.5 µL
10X primer mix	5.0 µL
AmpliTaq Gold (5U/µL)	0.2 µL
DNA Template (1ng/µL)	1.0 µL
Total	25.0 µL

Thermal Cycling:

95ºC for 11 minutes, then:

94ºC for 20 seconds
60ºC for 1 minute
72ºC for 30 seconds
for 33 cycles, then:

72ºC for 7 minutes
4ºC soak

Enzyme purification of the PCR product:

PCR Product	5.0 µL
ExoSAP-IT®	1.0 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Y-chromosomal haplogroup typing – Using SBE reaction

Subhaplogroups O3a2c1

Reagents Needed:

ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA)
5X Sequencing Buffer: 400 mM Tris-Cl (pH 9.0), 10mM $MgCl_2$
10X Primer Mix for SNaPshot Reaction
SAP (USB, Cleveland, OH) or CIP (Promega, Madison, WI)

10X Primer Mix for SBE:

Primer	Variation	Primer Sequence (5'-3')	Conc.
O3a2c1b-R178-20	G → A	GGT GGC TCC CGT CTG TAA TC	1.0 µM
O3a2c1a1-F139-30	C → C/T	$(A)_7(T)_1$GGA AAA TAG ATG CCT GCA AAA A	8.0 µM
O3a2c1a-F096-67	T → ΔT	$(A)_{29}$TGG GGC TTT CTG AAG CAA ATA CCA GCT TTA AAA AAA AA	6.0 µM

SBE Mixture:

PCR Component	Volume/Sample
dH_2O	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:

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

4ºC soak

Post-Extension Treatment:

SNaPshot Reaction Product	10.0 µL
SAP or CIP	0.5 µL

Thermal Cycling:

37ºC for 45 minutes
80ºC for 15 minutes

Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

Multiplex SBE reactions

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)

DS-02 Matrix Standards Kit (Applied Biosystems, Foster City, CA)

GeneScan™ 120 LIZ™ Size Standard (Applied Biosystems, Foster City, CA)

Run module GS STR POP4 (1 mL) E5

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 95ºC 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 STR POP4 (1 mL) E5** 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 60ºC.

GeneMapper Software: