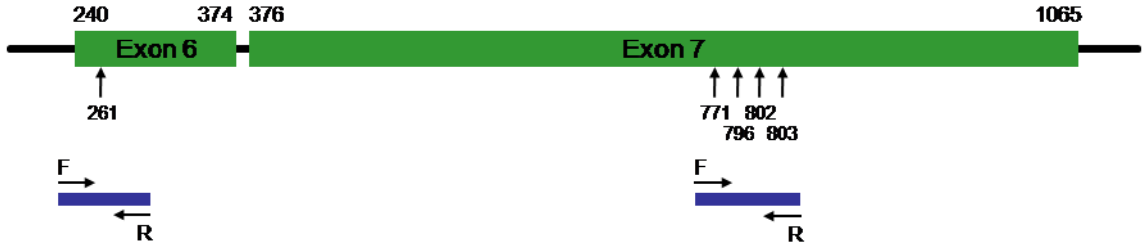
ABO blood typing system

Monoplex PCR for Exon 6 and 7



Reagents Needed:

AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)

Gold ST*R 10X Buffer (Promega, Madison, WI)

10X Primer Mix for Monoplex PCR

ExoSAP-IT® (USB, Cleveland, OH)

10X primer Mix for Monoplex PCR :

Region		Primer Sequence (5'→3')	Concentration	Amplicon Size
Exon 6	F	CTC CAT GTG CAG TAG GAA GGA	5.0 µM	72 bp
	R	AAT GTG CCC TCC CAG ACA A	5.0 µM	
Exon 7	F	CCA GTC CCA GGC CTA CAT C	4.0 µM	84 bp
	R	TGC AYC TCT TGC ACC GAC	4.0 µM	

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	18.8 µL
Gold ST*R 10X Buffer	2.5 µL
10X Primer Mix	2.5 µL
AmpliTaq Gold (5 U/µL)	0.2 µL
DNA Template (1 ng/µL)	1.0 µL
Total	25.0 µL

Thermal Cycling:

95°C for 11 minutes, then:

94°C for 20 seconds
63°C for 1 minute
72°C for 30 seconds
for 36 cycles, then:

72°C for 7 minutes
4°C soak

Enzyme purification of the PCR product:

PCR Product of Exon 6	2.5 µL
Exon 7	2.5 µL
ExoSAP-IT®	1.0 µL

Thermal Cycling:

37°C for 45 minutes
80ºC for 15 minutes

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Multiplex SBE for Exon 6 and 7

Reagents Needed:

ABI PRISM® SNaPshot® Multiplex Kit (Applied Biosystems, Foster City, CA)

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

10X Primer Mix for SNaPshot Reaction

SAP (USB, Cleveland, OH) or CIAP (Promega, Madison, WI)

10X Primer Mix for SBE:

Region	Primer Sequence (5'→3')	Variation	Concentration
ABO261	AGG AAG GAT GTC CTC GTG GT	G → ΔG	2.3 µM
ABO771	$(T)_{14}$GTC CCA GGC CTA CAT CCC	C → T	3.0 µM
ABO796	$(T)_{16}$GGA CGA GGG CGA TTT CTA CTA C	C → A	2.7 µM
ABO802	$(T)_{24}$GGC GAT TTC TAC TAC ATG GGG	G → A	3.0 µM
	$(T)_{25}$GGC GAT TTC TAC TAC CTG GGG		2.0 µM
ABO803	$(T)_{36}$CAC CGA CCC CCC GAA GAA C	G → C	3.5 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	10.0 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 CIAP	0.5 µL

Thermal Cycling:

37°C for 45 minutes
80ºC for 15 minutes

Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

Multiplex SBE for Exon 6 and 7

Materials & Reagents Needed:

Dry heating block, water bath, or thermal cycler

310 capillaries, 47 cm x 50 µm (Applied Biosystems, Foster City, CA)

Performance Optimized Polymer (POP-4™ Polymer, Applied Biosystems, Foster City, CA)

ABI PRISM® dRhodamine Matrix Standards Kit (Applied Biosystems, Foster City, CA)

Run module GS STR POP4 (1mL) E5

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

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. Prepare a loading cocktail by combining and mixing the 0.2 µL GeneScan™ 120 LIZ™ Size Standard and 20.0 µL Hi-Di™ Formamide per sample.
2. Vortex for 10 seconds.
3. Combine 20.0 µL of the prepared loading cocktail and 1.0 µL of the SNaPshot product.
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:

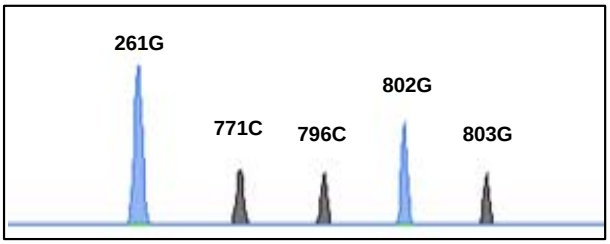
Prepared the samples are run using the Run module **GS STR POP4 (1mL) E5** and a described above **matrix**.

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

GeneScan Software:

Electropherogram of ABO Blood Genotype

AA



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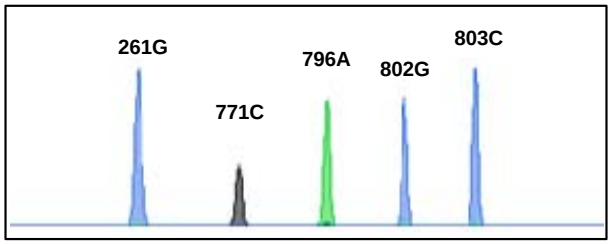

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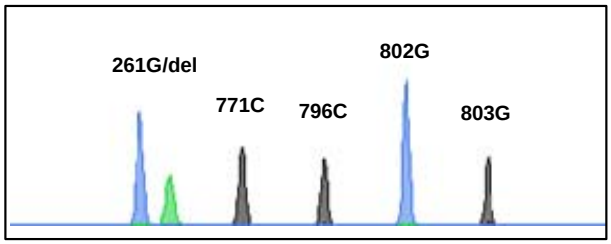

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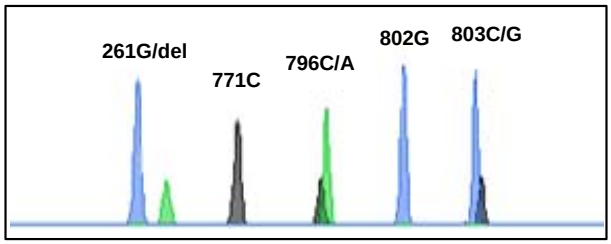

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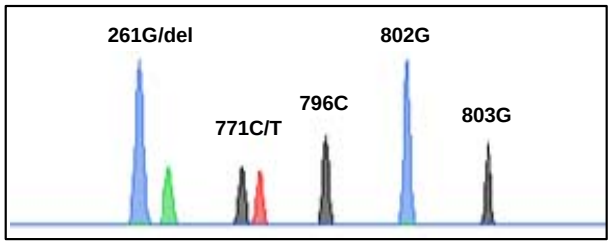

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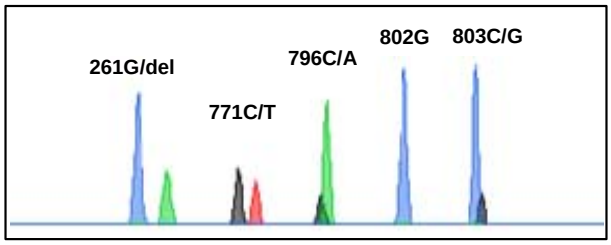
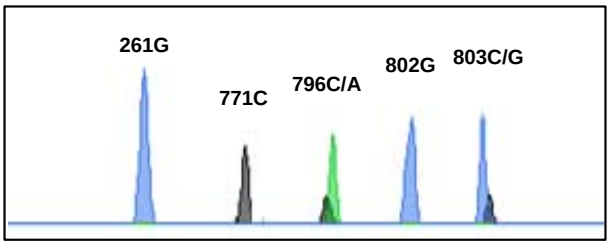


AB



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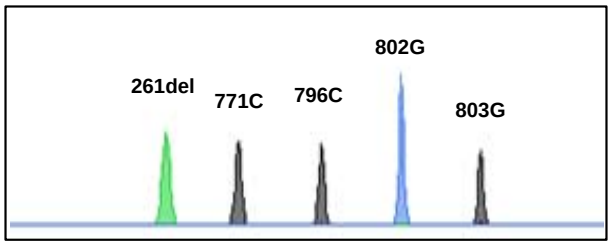

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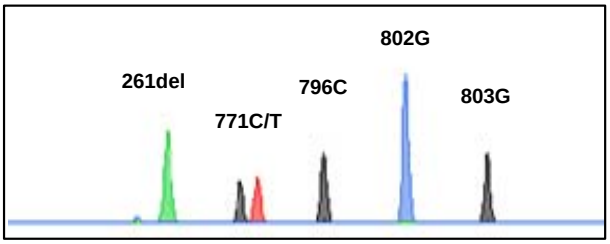

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