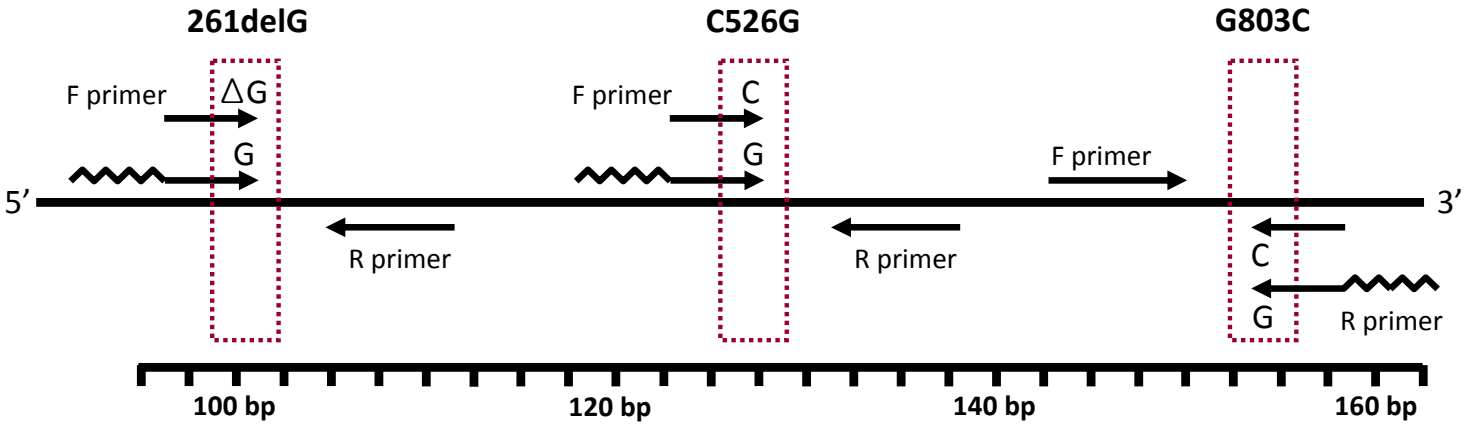
Rapid direct PCR for ABO blood typing

Schematic of allele specific multiplex PCR



Size	98	102	127	130	154	158
SNP	ΔG	G	C	G	G	C
Blood Type	O	Non-O	A or O	B or O03	A or O	B or *cis*AB

Sample preparation

Materials & Reagents Needed:

- EDTA Tube
- Cotton Swab
- Indicating FTA Classic Card (Whatman, Florham Park, NJ)
- Harris Micro Punch 1.2 mm (Whatman, Florham Park, NJ)
- Dry Heating block, Water Bath or Thermal Cycler

Whole blood

1. Prepare fresh blood by lancet into a EDTA tube.
2. Transfer 1 µL of blood to PCR reaction.

Blood and Saliva stains

1. Prepare the stains by spotting the blood and by smearing buccal swabs onto a 2.5-cm-diameter Indicating FTA Classic Card.
2. Punch out a 1.2 mm disc from the stain on the FTA card using Punch and wash twice with 20 µL H_2O at 50°C for 3 minutes.
3. After removing the H_2O, directly transfer the rinsed punch to PCR reaction.

Hair root

1. Cut off a 0.5 cm piece starting from the hair bulb.
2. Transfer the hair root to PCR reaction.

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Pre-amplification

Materials & Reagents Needed:

Veriti 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA)
10X Primer Mix
Phire™ Hot Start DNA Polymerase (Finnzymes Oy, Espoo, Finland)
5X Phire™ Reaction Buffer (Finnzymes Oy, Espoo, Finland)
GeneAmp® 10 mM dNTP Mix with dTTP (Applied Biosystems, Foster City, CA)

10X Primer Mix:

SNP		Primer	Primer Sequence (5'- 3')	Conc.
	ΔG	ABO261d-F	AGGAAGGATGTCCTCGTGTT**A**C	3.7 µM
261	G	ABO261G-F	ttaAGGAAGGATGTCCTCGTTGT**G**	4.5 µM
	-	ABO261-R	6FAM-GTTCTGGAGCCTGAACTGCT	8.2 µM
	C	ABO526C-F	AGCTGTCAGTGCTGGAGATG**C**	2.1 µM
526	G	ABO526G-F	ttatGCTGTCAGTGCTGGAGGAG**G**	1.9 µM
	-	ABO526-R	6FAM-TCCACGCACACCAGGTAATC	4.0 µM
	G	ABO803G-R	CCGACCCCCCGAAGTAC**C**	2.7 µM
803	C	ABO803C-R	atatCCGACCCCCCGAAGATC**G**	3.0 µM
	-	ABO803-F	6FAM-GAGATCCTGACTCCGCTGTT	5.7 µM

Two alleles of a SNP at the 3' end of allele-specific primers are indicated in bold.
Additional mismatches included at the third or other position from the 3' end are underlined.
The tails inserted at the 5' end of one of the two allele specific primers are written in lower case.

PCR Mixture:

PCR Component	Volume/Sample
dH_2O	Add to 20 µL
5X Phire™ Reaction Buffer	4.0 µL
dNTP Mix	1.6 µL
10X Primer Mix	2.0 µL
Phire™ Hot Start DNA Polymerase	0.4 µL
DNA Template	1.0 µL
Total	20.0 µL

Thermal Cycling:

98ºC for 5 minutes, then:

98ºC for 5 seconds
65ºC for 10 seconds
72ºC for 10 seconds
For 28~30 cycles, then:

72ºC for 1 minute
4ºC soak

Electrophoresis on the ABI PRISM® 310 Genetic Analyzer

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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-33 Matrix Standard Set [6FAM™, VIC®, NED™, PET®, and LIZ® dyes] For The ABI PRISM® 310/377 Systems (Applied Biosystems, Foster City, CA)

GS STR POP4 (1mL) G5v2.md5

GeneScan™ 500 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™ 500 LIZ™ Size Standard and 20 µL Hi-Di Formamide per sample.
2. Vortex for 10 seconds.
3. Combine 20.2 µL of the prepared loading cocktail and 1.0 µL of the PCR product.
4. Preparing the allelic ladder, combine 20.2 µL of the prepared loading cocktail and 1.0 µl of the allelic ladder mix. Vortex the allelic ladder mix prior to pipetting.
5. Denature the samples and ladder by heating at 95ºC for 5 minutes and immediately chill on crushed ice. Denature the samples just prior to loading.
6. 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) G5v2.md5** and a described above **matrix**.

Samples are injected for 5 seconds at 15,000 V and separate at 15,000V for 24 minutes with run temperature of 60ºC.

Genotyper Macro:

ABO.gta

Electropherograms of Six Common ABO Genotypes