ABO blood typing using AmpliTaq Gold® DNA polymerase

Materials & Reagents Needed:

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

10X Primer Mix:

SNP		Primer	Primer Sequence (5'- 3')	Conc.
261	ΔG	ABO261d-F	AGGAAGGATGTCCTCGTGTT**A**C	1.5 µM
	G	ABO261G-F	ttaAGGAAGGATGTCCTCGTTGT**G**	2.6 µM
	-	ABO261-R	6FAM-GTTCTGGAGCCTGAACTGCT	4.1 µM
526	C	ABO526C-F	AGCTGTCAGTGCTGGAGATG**C**	3.3 µM
	G	ABO526G-F	ttatGCTGTCAGTGCTGGAGGAG**G**	2.7 µM
	-	ABO526-R	6FAM-TCCACGCACACCAGGTAATC	6.0 µM
803	G	ABO803G-R	CCGACCCCCCGAAGTAC**C**	3.8 µM
	C	ABO803C-R	atatCCGACCCCCCGAAGATC**G**	4.6 µM
	-	ABO803-F	6FAM-GAGATCCTGACTCCGCTGTT	8.4 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 10.0 µL
Gold ST*R 10X Buffer	1.0 µL
10X Primer Mix	1.0 µL
AmpliTaq Gold® DNA polymerase	0.5 µL
DNA Template (1ng/µL)	1.0 µL
Total	10.0 µL

Thermal Cycling:

95ºC for 11 minutes, then:

95ºC for 20 seconds
59ºC for 30 seconds
72ºC for 30 seconds
For 30 cycles, then:

60ºC for 60 minutes
4ºC soak

Electropherograms of Six Common ABO Genotypes