PCR-SSP Protocol for HPA-1 to -5 + -15

This consensus protocol was compiled from the methods used by the participants of the HPA-1-5 genotyping exercises organised by NIBSC. However, it may not perform any better than the original published method (Cavanagh *et al* Transfusion Medicine 1997; **7**, 41-45). The HPA-15 (Gov) system was added to the protocol after publication of the following paper; Schuh *et al* Blood 2002, **99**, 1692-1698.

The protocol has been used successfully at NIBSC but it should be validated locally.

Reagents (store all reagents at -40^{o}C if possible, otherwise -20^{o}C)

10x buffer Use $(NH_4)_2SO_4$ based buffer with 15mM $MgCl_2$ (final conc. 1.5mM) Alternatively a KCl based buffer can be used.

Taq Must lack 3' to 5'exonuclease activity.

dNTPs Mix equal volumes of four 10mM stock solutions, then freeze in 50uL aliquots. Note that when used as described below the dNTP concentration is 1/4 of the usual recommended concentration (to increase specificity).

DNA Prepare DNA at 100ug/mL (= ng/µL).

Thermal cycler The PCR machine must use tube control or simulated tube control. Beware of machines that use 'block control' to monitor temperature, as they start counting down the time before the tubes have reached the critical temperature and the contents of the tube do not always reach the temperature of the block.

Primers should have the following sequences and should be made up so that the following concentrations are achieved in the final reaction mix (i.e. the conc. in the stock primer mix must be 2x final conc. because 5uL reaction mix is added to 5uL primer mix). Aliquot 5uL of each primer mix into several sets of 12 tubes (0.2mL thin walled tubes in strips or plates) and store at -40^{o}C until use. Do not use traditional 0.5mL Epppendorf-type PCR tubes (the walls are too thick).

Primer	Sequence	Product size	Final conc.
HPA-1a	5’ TCACAGCGAGGTGAGGCCA		
HPA-1b	5’ TCACAGCGAGGTGAGGCCG 3’	90bp	0.35uM
common	5’ GGAGGTAGAGAGTCGCCATAG 3’		
HPA-2a	5’ GCCCCCAGGGCTCCTGAC 3’		
HPA-2b	5’ GCCCCCAGGGCTCCTGAT 3’	258bp	0.35uM
common	5’ TCAGCATTGTCCTGCAGCCA 3’		
HPA-3a	5’ TGGACTGGGGGCTGCCCAT 3’		
HPA-3b	5’ TGGACTGGGGGCTGCCCAG 3’	267bp	0.5uM
common	5’ TCCATGTTCACTTGAAGTGCT 3’		
HPA-4a	5’ GCTGGCCACCCAGATGCG 3’		
HPA-4b	5’ GCTGGCCACCCAGATGCA 3’	120bp	0.35uM
common	5’ CAGGGGTTTTCGAGGGCCT 3’		
HPA-5a	5’ AGAGTCTACCTGTTTACTATCAAAG 3’		
HPA-5b	5’ AGAGTCTACCTGTTTACTATCAAAA 3’	250bp	0.5uM
common	5’ CTCTCATGGAAAATGGCAGTACA 3’		
HPA-15a Gov[b]	5’ TTCAAATTCTTGGTAAATCCTGG 3’		
HPA-15b Gov[a]	5’ TTCAAATTCTTGGTAAATCCTGT 3’	225bp	0.5uM
common	5’ ATGACCTTATGATGACCTATTC 3’		
HGH controls	5’ GCCTTCCCAACCATTCCCTTA 3’ 5’ TCACGGATTTCTGTTGTGTTTC 3’	429bp	HPA-1-4; 0.2uM HPA-5 & -15; 0.1uM

N.B. The sequence given above for the HPA-5 primers is different from that in the original publication.

Method

1. For each sample to be tested, thaw one strip of 12 tubes each containing 5uL of the various primer mixes.
2. For each sample to be tested, make up a master mix of 14x all of the reagents as shown below;

	1 tube	14 tubes
dH_2O	1.8	25.2
10x reaction buffer	1.0	14.0
dNTP mix (each dNTP @ 2.5mM)	0.2	2.8
polymerase (5 units/ul)	0.07	0.98
DNA (approx 100ug/ml = ng/ul)	2.0	28.0
Primer mix	5.0	
Total volume	10.07uL	

3. Add 5uL of the master mix to each of the 12 tubes from step 1.

4. If using a PCR machine without a heated lid, add 25uL oil to each tube.

5. Cycle as below;

1 cycle 96^{o}/60 sec

5 cycles 96^{o}/25 sec, 70^{o}/45 sec, 72^{o}/30 sec

20 cycles 96^{o}/25 sec, 65^{o}/45 sec, 72^{o}/30 sec

8 cycles 96^{o}/25 sec, 55^{o}/45 sec, 72^{o}/30 sec

1 cycle 72^{o}/3min

The above protocol works well in some laboratories but not all, and this is presumably due to local differences in PCR machines or reagents. The protocol below has been used by several laboratories for HPA-1-5 but has not been tested for HPA-15.

1 cycle; 96°/60 sec

5 cycles; 96°/25 sec, 68°/45 sec, 72° /30 sec

28 cycles; 96°/25 sec, 61°/45 sec, 72° /30 sec

1 cycle; 72°/3min

6. Add 2.5uL loading buffer to each tube and run 2.5-5uL PCR product in a 1.5% agarose gel.

N.B. if insufficient PCR product is obtained try the following PCR parameters and then gradually increase the annealing temperatures to improve specificity;

1 cycle; 96°/60 sec

5 cycles; 96°/25 sec, 68°/45 sec, 72°/30 sec

20 cycles; 96°/25 sec, 61°/45 sec, 72°/30 sec

8 cycles; 96°/25 sec, 51°/1min, 72°/2min