PCR: Ly5a/b (amplifluor)

Amplifluor: Yes

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
Ly5a/b-1	GAAGGTCGGAGTCAACGGATTTCTTGGCCTGAGCCTGC
Ly5a/b-2	GAAGGTGACCAAGTTCATGCTTCTCTTGGCCTGAGCCT GT
Ly5a/b-R	GCATAAAACATATCCATGGGGTTTAGA

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	20x Primer mix concentration (µM)	Volume (µL)
DNA Sample				1.0
10x Buffer	10x Reaction Mix S Plus			1.0
dNTPs		2.5 mM		0.8
20x amplifluor SNP FAM Primer				
20x amplifluor SNP JOE Primer				
Primer: **Ly5a/b-1**			0.5	
Primer: **Ly5a/b-2**			0.5	
Primer: **Ly5a/b-R**			7.5	
Amplifluor primer volume				1.5
Water				4.6
Taq Polymerase	Platinum Taq (5U/µl)			0.1

Comments on protocol: DNA Sample (cleaned ear punch in dH20-see cleanup protocol).

Reactions to be done at room temperature with reagents added in order.

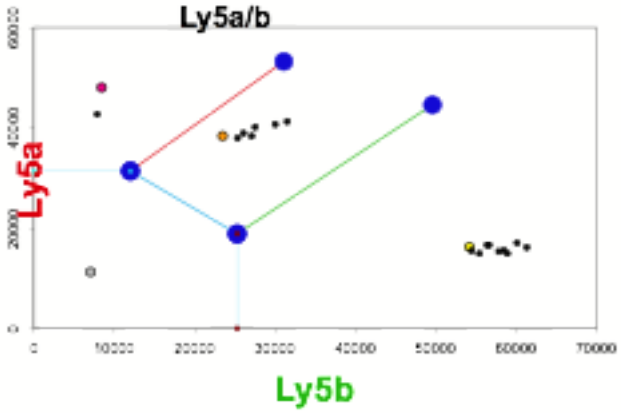
Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **Yes**		
Initiation/Melting	94.0	240
Denaturation	94.0	10
Annealing	60.0	20
Elongation	72.0	40
Number of cycles (repeat denature, anneal & elgonate): **35**		
Strand completion (i.e. 72°C, 10 min)	72.0	180
Finish (i.e. 4°C, indefinite)	10.0	Hold

Electrophoresis

Running buffer:	
% Agarose:	
Volt:	
Estimated running time (min):	

Image


Ly5a/b
Ly5a
Ly5b
0
20000
40000
60000
0
10000
20000
30000
40000
50000
60000
70000