PCR: EphA1 Reporter Mice Assay

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
mEphA1-F12946	GGAGTCCCAGAATACCCAGTG
mEphA1-R13260	CCCAGCTCCCTAGGAGTACC
mEphA1-IRES-MCS-R	GGGCTGCAGGAATTCGATA

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		20.0 mM MgCl2	5.0
dNTPs		2.0 mM	10.0
Primer: **mEphA1-F12946**		10.0 µM	
Primer: **mEphA1-R13260**		10.0 µM	
Primer: **mEphA1-IRES-MCS-R**		10.0 µM	
Water			17.5
Taq Polymerase			0.5
Reagent: 5mM MgCl2			5.0
Reagent: 5M Betaine			10.0

Comments on protocol: Final Concentration of MgCl2 is 2.5 mM

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	95.0	600
Denaturation	95.0	30
Annealing	50.0	30
Elongation	72.0	60
Number of cycles (repeat denature, anneal & elgonate): **35**		
Strand completion (i.e. 72°C, 10 min)		
Finish (i.e. 4°C, indefinite)	4.0	Hold

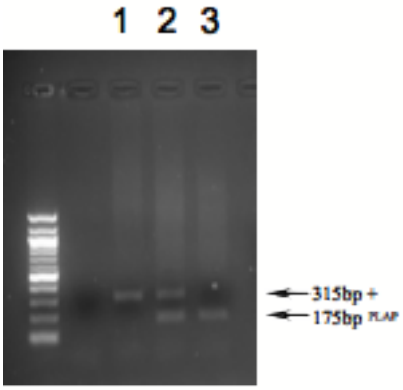
Electrophoresis

Running buffer:	TBE
% Agarose:	2.0
Volt:	80.0
Estimated running time (min):	30.0

Image

Bands

Number	Band (bp)	Genotype
1.	315	+/+
2.	315 / 175	-/PLAP
3.	175	PLAP/PLAP


1 2 3
315bp +
175bp PLAP