PCR: EphA1 Conditional Mice (Knockout) Assay

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
mEphA1-F19118	ACCATCAGAGACCTGGCATC
mEphA1-R19399	GTGCATTCGTGGTGTACCTG
mEphA1-F16670	CTCAGAAGGCTTTGAAAGTGG
mEphA1-R20118	GGTGTCCATTTCCTCTCACC

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-F19118**		10.0 µM	
Primer: **mEphA1-R19399**		10.0 µM	
Primer: **mEphA1-F16670**		10.0 µM	
Primer: **mEphA1-R20118**		10.0 µM	
Water			17.2
Taq Polymerase			0.5
Reagent: 5mM MgCl2			5.0
Reagent: 5M Betaine			10.0

Comments on protocol: Assay was developed using a final concentration of 2.5mM for MgCl2. We, however, foresee no problem using 2.0mM as a final concentration.

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **Yes**		
Initiation/Melting	95.0	300
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

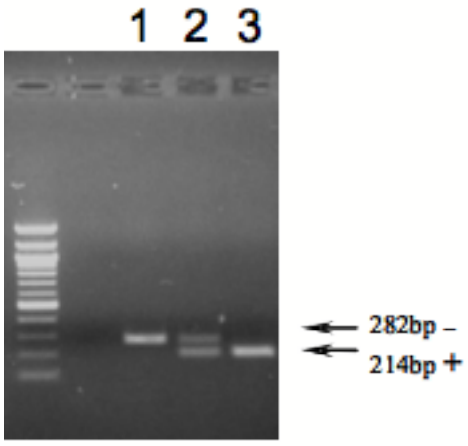
Electrophoresis

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

Image

Bands

Number	Band (bp)	Genotype
1.	282	-/-
2.	282 / 214	+/-
3.	214	+/+


1 2 3
282bp –
214bp +