

PCR: To Distinguish the Cited2 wildtype allele from C2F Conditional Allele

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
C2fF	GTCTCAGCGTCTGCTCGTTT
C2fR	CTGCTGCTGTTGGTGATGAT

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		15.0 mM MgCl2	2.0
dNTPs		2.5 mM	2.0
Primer: C2fF		20.0 µM	
Primer: C2fR		20.0 µM	
Water			11.5
Taq Polymerase			0.5

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: No		
Initiation/Melting	95.0	60
Denaturation	95.0	30
Annealing	58.0	30
Elongation	72.0	30
Number of cycles (repeat denature, anneal & elgonate): 35		
Strand completion (i.e. 72°C, 10 min)	72.0	600
Finish (i.e. 4°C, indefinite)	11.0	

Electrophoresis

Running buffer:	
% Agarose:	2.0
Volt:	80.0
Estimated running time (min):	40.0

Bands

Number	Band (bp)	Genotype
1.	210	C2F
2.	166	C2 WT