PCR: Eed null

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
Eed 7F	ACACTGAATCTTGGGCCTTG
Eed 7R	GCCACCTACACATGGGTTCT

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		20.0 mM MgCl2	2.0
dNTPs		2.0 mM	2.0
Primer: **Eed 7F**		10.0 µM	
Primer: **Eed 7R**		10.0 µM	
Water			12.9
Taq Polymerase			0.1

Comments on protocol: The PCR product can be sequenced to find the Eed null point mutation, as below. Mutation is in brackets.

T is the wild-type allele and C the null allele.

Exon 7 sequence

CACTATGTTGGCCATGGAAATGCTATCAATGAGC[T/C]GAAATTCCACCCACGAGACCCAAACCTTCTCCTGTCAGT AAGTAAAG

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	94.0	300
Denaturation	94.0	30
Annealing	60.0	30
Elongation	72.0	60
Number of cycles (repeat denature, anneal & elgonate): **35**		
Strand completion (i.e. 72°C, 10 min)	72.0	420
Finish (i.e. 4°C, indefinite)		

Electrophoresis

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