PCR: EL20

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
EL20 fwd	gaccaactgctcctggagag
EL20 rev	tctgcttcgtgtatgcatacttg

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer	Fisher Biotech		2.0
dNTPs			0.2
Primer: **EL20 fwd**		10.0 µM	
Primer: **EL20 rev**		10.0 µM	
Water			12.3
Taq Polymerase			0.5
Reagent: MgCl2			2.0

Comments on protocol: Samples degrade easily and for crisp bands, PCR products must be used straight away

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **Yes**		
Initiation/Melting	94.0	180
Denaturation	94.0	30
Annealing	60.0	60
Elongation	72.0	30
Number of cycles (repeat denature, anneal & elgonate): **40**		
Strand completion (i.e. 72°C, 10 min)	72.0	600
Finish (i.e. 4°C, indefinite)	4.0	indefinite

Electrophoresis

Running buffer:	TAE
% Agarose:	2.0
Volt:	90.0
Estimated running time (min):	60.0

Restriction Digest

Enzyme name:	DpnII
Company:	NEB
PCR Volume:	10.0 µl
Enzyme Volume:	0.5 µl
Buffer Volume:	2.0 µl
Temperature:	37.0 °C
Time:	120.0 mins

Bands

Number	Band (bp)	Genotype
1.	201	wildtype
2.	270	mutant