PCR: c-Cbl G304E genotyping

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
4.2RI	TTCTTAGCTCTCAATGTTTCTACTCTCC
VM269R	CATGTAACCAGGGTGAGTTAC

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			2.5
10x Buffer		20.0 mM MgCl2	3.5
dNTPs		2.0 mM	1.75
Primer: **4.2RI**		10.0 µM	
Primer: **VM269R**		10.0 µM	
Water			24.55
Taq Polymerase	5U/µl		0.2

Comments on protocol: We use 1.5mM MgCl2, not 2mM final

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	94.0	180
Denaturation	94.0	30
Annealing	55.0	45
Elongation	72.0	45
Number of cycles (repeat denature, anneal & elgonate): **40**		
Strand completion (i.e. 72°C, 10 min)	72.0	180
Finish (i.e. 4°C, indefinite)		

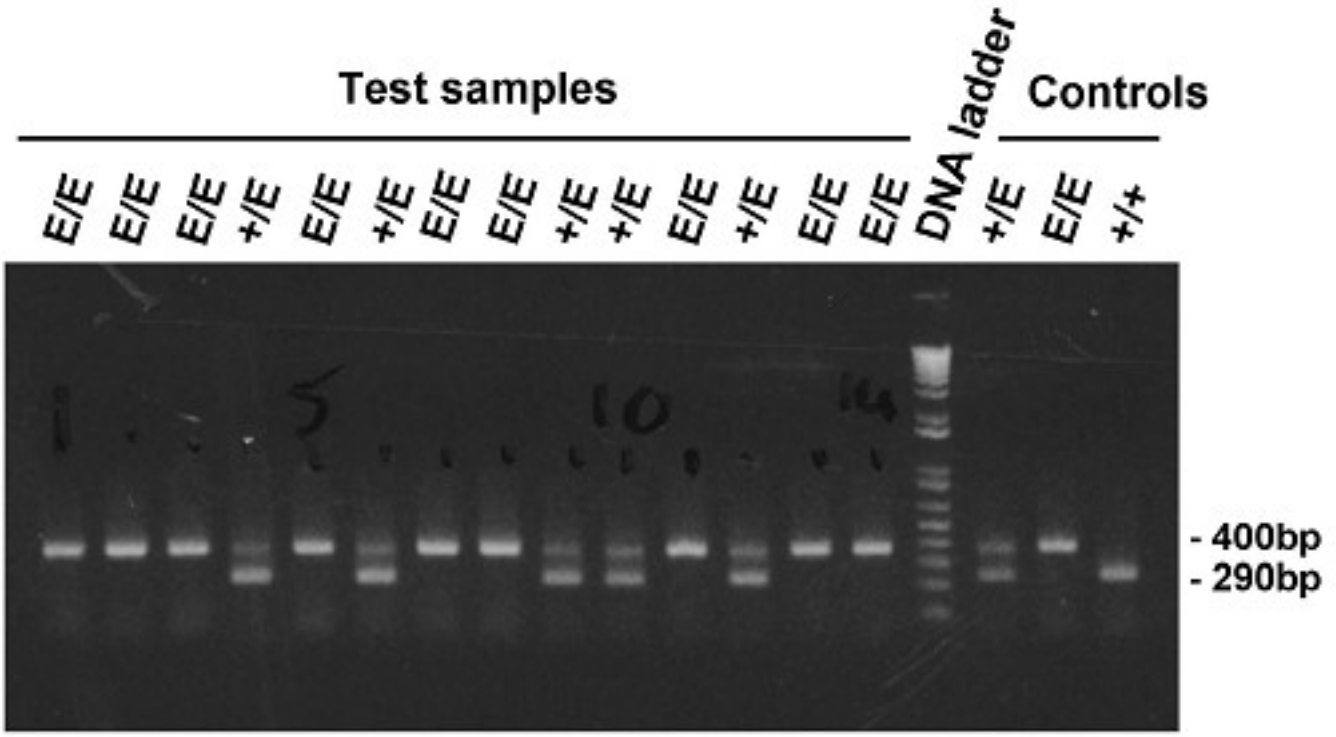
Electrophoresis

Running buffer:	
% Agarose:	1.2
Volt:	100.0
Estimated running time (min):	18.0

Image

Bands

Number	Band (bp)	Genotype
1.	400	Wild Type
2.	290	G304E
3.	400 / 290	Het


Test samples
Controls
DNA ladder
E/E
E/E
E/E
+/E
E/E
+/E
E/E
E/E
+/E
+/E
E/E
+/E
E/E
E/E
+/E
E/E
+/+
- 400bp
- 290bp