PCR: Vibes B –D15Mit86 (18cM)

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
D15Mit86 F	TACACTTATCTGTTGGAACATCCC
D15Mit86 R	ATATAGATAAACACACGCACACCA

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		20.0 mM MgCl2	5.0
dNTPs		10.0 mM	1.0
Primer: **D15Mit86 F**		6.6 µM	
Primer: **D15Mit86 R**		6.6 µM	
Water			38.45
Taq Polymerase			0.05
Reagent: Triton X-100 (10%)			0.5
Reagent: 25mM MgCl2			1.0

Comments on protocol: DNA Sample (ear punch).

Final concentration of 2.5mM MgCl2

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	94.0	120
Denaturation	94.0	20
Annealing	55.0	20
Elongation	72.0	20
Number of cycles (repeat denature, anneal & elgonate): **35**		
Strand completion (i.e. 72°C, 10 min)	72.0	180
Finish (i.e. 4°C, indefinite)	10.0	Hold

Electrophoresis

Running buffer:	
% Agarose:	3.0
Volt:	400.0
Estimated running time (min):	20.0

Bands

Number	Band (bp)	Genotype
1.	172	C57BL/6
2.	196	NOD