PCR: Twimp_(Twp)

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
TwpF'	GAAAGAAACCACCATTTTCTGAT
TwpR'WT	TTCTGGACTTGGTACAGTATGACAGGCA
TwpR'M	GGGCAGAGATCTGCAGCAGAAGCCTGGACTTGGTACAGTATGACATACC

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer	10x 0289 PCR Buffer	0.0 mM MgCl2	5.0
dNTPs		10.0 mM	0.5
Primer: **TwpF'**		10.0 µM	
Primer: **TwpR'WT**		10.0 µM	
Primer: **TwpR'M**		10.0 µM	
Water			27.35
Taq Polymerase			0.15
Reagent: 100mM MgCl2			1.75

Comments on protocol: DNA Sample (ear punch).

Final conc. of 3.5mM MgCl2

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	94.0	120
Denaturation	94.0	20
Annealing	65.0	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):	30.0

Bands

Number	Band (bp)	Genotype
1.	195	Wt/Wt
2.	215	Twp/Twp