

PCR: Unmodulated (Piggy)

Amplifluor: No

Primers

Nucleotide sequence (5' --> 3')

Primer	Sequence
T18MSForward	TTCCGCCACGAAATCTTCTGC
T18MSRevWT	TCTAGCGCCTCCTTCCGGTCATGTTACA
T18MSRevMut	TTGACCAGTTCCTGCGGTCCCTTAGCGCCTCCTTCCGGTCATGTGCCT

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		20.0 mM MgCl ₂	5.0
dNTPs		10.0 mM	0.5
Primer: T18MSForward		10.0 µM	
Primer: T18MSRevWT		10.0 µM	
Primer: T18MSRevMut		10.0 µM	
Water			33.4
Taq Polymerase			0.1
Reagent: 25mM MgCl ₂			3.0

Comments on protocol: DNA Sample (ear punch).

works much better with lab 10x buffer (20mM MgCl₂) plus 3 ul per reaction of 25 mM MgCl₂ than original 0289 buffer.

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 & elongate): 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:	3.5
Volt:	400.0
Estimated running time (min):	20.0

Bands

Number	Band (bp)	Genotype
1.	190	Mutant
2.	190 / 169	Heterozygous
3.	169	WildType