PCR: Smad3 (Mutant)

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
HA 73	TCCCAACAGTTGCGCAGCCTGAATG
HA 74	ATATCCTGATCTTCCAGATAACTGCCG

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			3.0
10x Buffer		20.0 mM MgCl2	1.5
dNTPs		10.0 mM	0.4
Primer: **HA 73**		10.0 µM	
Primer: **HA 74**		10.0 µM	
Water			5.3
Taq Polymerase			0.5
Reagent: 25mM MgCl2			1.8

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	94.0	300
Denaturation	94.0	60
Annealing	94.0	15
Elongation	72.0	60
Number of cycles (repeat denature, anneal & elgonate): **30**		
Strand completion (i.e. 72°C, 10 min)	72.0	600
Finish (i.e. 4°C, indefinite)	20.0	Hold

Electrophoresis

Running buffer:	
% Agarose:	2.0
Volt:	120.0
Estimated running time (min):	45.0

Bands

Number	Band (bp)	Genotype
1.	450	Mutant