

PCR: BLOBBY

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
Blobby F	AAAGCCCCACATGTAGATCG
Blobby R	TGAGGTATATGCTGAACCTCTTAT

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			0.1
10x Buffer		20.0 mM MgCl2	5.0
dNTPs		10.0 mM	1.0
Primer: Blobby F		10.0 µM	
Primer: Blobby R		10.0 µM	
Water			40.45
Taq Polymerase			0.05
Reagent: Triton			0.5

Comments on protocol: DNA Sample (from ear punch).

Mutagenic primer introduces Psi1 restriction site in the wt but not the mutant Alms1 sequence.

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: No		
Initiation/Melting	94.0	120
Denaturation	94.0	30
Annealing	59.0	60
Elongation	72.0	120
Number of cycles (repeat denature, anneal & elgonate): 38		
Strand completion (i.e. 72°C, 10 min)	72.0	300
Finish (i.e. 4°C, indefinite)	10.0	Hold

Electrophoresis

Running buffer:	
% Agarose:	4.0
Volt:	300.0
Estimated running time (min):	30.0

Restriction Digest

Enzyme name:	Psil
Company:	
PCR Volume:	20.0 µl
Enzyme Volume:	1.0 µl
Buffer Volume:	3.0 µl
Water Volume:	3.0 µl
Temperature:	37.0 °C
Time:	120.0 mins
Comments:	Additional reagent: 10x BSA, 3l. 10x Buffer (SEB Buffer B).

Bands

Number	Band (bp)	Genotype
1.	200	all uncut
2.	190	Psil cut wt
3.	200	Psil cut Mutant