PCR: SCID PCR

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
F	GAGAAAAGGAGGATCATGGATTCAAGAAATAAATGTAACG
WR	TGGCCCCTGCTAACTTTCTCTTAGCA
MF	TGGTATCCACAACATAAAATACGCTAA
R	CCTAAGAGTCACTTTCTCCATTTACACAGTGAAGTGCC

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			5.0
10x Buffer		20.0 mM MgCl2	3.75
dNTPs		2.0 mM	0.1
Primer: **F**		10.0 µM	
Primer: **WR**		10.0 µM	
Primer: **MF**		10.0 µM	
Primer: **R**		10.0 µM	
Water			38.95
Taq Polymerase			0.2

Comments on protocol: MgCl2 concentration: 1.5mM. Bands expected: F/R 257bp (common), MF/R 180bp (SCID mutation specific), F/WR 101bp (wt SCID specific). For more information refer to Maruyama et al., (2002) Exp. Anim. 51(4): 391-393.

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	94.0	180
Denaturation	94.0	30
Annealing	60.0	30
Elongation	72.0	30
Number of cycles (repeat denature, anneal & elgonate): **30**		
Strand completion (i.e. 72°C, 10 min)	72.0	60
Finish (i.e. 4°C, indefinite)	4.0	Hold

Electrophoresis

Running buffer:	
% Agarose:	3.0
Volt:	90.0
Estimated running time (min):	50.0

Bands

Number	Band (bp)	Genotype
1.	257	Common Band
2.	180	SCID Mutation
3.	101	WT SCID Specific