PCR: Fzd5<flox>

Amplifluor: No

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
sj2 for	CCTTTAGCAAAGAGTCCTAAC
f5x rev WT	AGAGGAGGCCTTATAGAGCG
pn5b rev Flox	CTAAAGCGATGCTCCAGACT

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	Volume (µL)
DNA Sample			1.0
10x Buffer		15.0 mM MgCl2	2.5
dNTPs		10.0 mM	0.5
Primer: **sj2 for**		10.0 µM	
Primer: **f5x rev WT**		10.0 µM	
Primer: **pn5b rev Flox**		10.0 µM	
Water			13.5
Taq Polymerase			0.125
Reagent: QIAGEN x5 Q BUFFER			5.0

Comments on protocol: Primer 1 (1.25l) with Primer 2 OR Primer 3 (1.25l).

QIAGEN polymerase kit (cat. # 201205)

Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **No**		
Initiation/Melting	95.0	300
Denaturation	95.0	60
Annealing	55.0	60
Elongation	72.0	60
Number of cycles (repeat denature, anneal & elgonate): **35**		
Strand completion (i.e. 72°C, 10 min)	72.0	600
Finish (i.e. 4°C, indefinite)	4.0	Hold

Electrophoresis

Running buffer:	
% Agarose:	1.5
Volt:	80.0
Estimated running time (min):	30.0

Bands

Number	Band (bp)	Genotype
1.	250	WT
2.	700	MUTANT