

B6.129S1-Tlr1^{tm1Flv}/J

Stock No: 007020

Protocol 22989: Standard PCR Assay - Tlr1<tm1Flv>

Version 1.3

Notes

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Expected Results

Mutant = 282 bp

Heterozygote = 282 bp and 537 bp

Wild type = 537 bp

Separated by gel electrophoresis on a 1.5% agarose gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR6966		GCC AAA CGC AAA CCT TAC CAG AGT G		Wild type Forward	A	
oIMR6967		ACG GAC ACA TCC AGA AGA AAA CGG		Wild type Reverse	A	
oIMR6968		TTC GGC TAT GAC TGG GCA CAA CAG		Mutant Forward	A	Neo
oIMR6969		TAC TTT CTC GGC AGG AGC AAG GTG		Mutant Reverse	A	Neo

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
oIMR6966	0.50 uM
oIMR6967	0.50 uM
oIMR6968	0.50 uM
oIMR6969	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

