

## B6.129P2-Acvrl1<sup>tm1Dgen</sup>/J

Stock No: 005772

Protocol 23302: Standard PCR Assay - Acvrl1&lt;tm1Dgen&gt;

Version 2.3

### 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 = 385 bp

Heterozygote = 273 bp and 385 bp

Wild type = 273 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
moIMR0012		GGG TGG GAT TAG ATA AAT GCC TGC TCT			A	
oIMR5029		TCT GAC TGT AAG TAG TCT GGC TCA G		Wild type	A	
oIMR5030		CCT GTT CAG ATG CCT TCA GGA TGA G		Common	A	

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
Kapa 2G HS buffer	1.30 X
MgCl <sub>2</sub>	2.60 mM
dNTP KAPA	0.26 mM
moIMR0012	0.50 uM
oIMR5029	0.50 uM
oIMR5030	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.