

## B6.129-Per1<sup>tm1Drw</sup>/J

Stock No: 010491

Protocol 23574: Standard PCR Assay - Per1&lt;tm1Drw&gt;

Version 1.2

### 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 = ~540bp

Heterozygote = ~540 bp and ~774 bp

Wild type = ~774bp

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
9207		CAG TAC TTC TCT TTC TAC ATC		Wild type Forward	A	
9208		CAT TGC TAT CAC TGG AGG AG		Common	A	
9209		TGC CCC AAA GGC CTA CCC GC		Mutant Forward	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
9207	0.50 uM
9208	0.50 uM
9209	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.

