

## B6.129X1-Snap25<sup>tm1Mcw</sup>/J

Stock No: 004863

Protocol 28550: Standard PCR Assay - Snap25&lt;tm1Mcw&gt;

Version 2.2

### 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 = ~340 bp

Heterozygote = ~340 bp and 548 bp

Wild type = 548 bp

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR2060		CAC GAG ACT AGT GAG ACG TG		Mutant Reverse	A	
oIMR3223		CAT TTC CTT CCA CCC ATG TC		Common	A	
oIMR3224		ATC CCC ACA GAA TGA AGC AG		Wild type Reverse	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
oIMR2060	0.50 uM
oIMR3223	0.50 uM
oIMR3224	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.

