

B6.Cg-Psen1^{tm3.1Shn}/J

Stock No: 027082

Protocol 27852: Sanger sequencing Assay - Psen1<sup>tm3.1Shn</sup>

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

Mut = GGT

WT= AGG

360bp

Sequence

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atcagccaaagctaccagagaaacccgtctcagaaaaagaaaaagtagacg
atggacatgggctagaaccagtaatgtagcagtgccagcgtgtaaaggctgggt
acccagcctcgacgaa
ggacaactagaaagcagactgtagcacatactgttctcatgttaaagctcctggtt
ctaaactgtggatctgttggttacattgagttgtctctctctagGTCATCCACGC
CTGGCTTATTATTTTC
ATCTCTGTTGTTGCTGTTCTTTTTTTTCGTTCACTTACTTA/GG
G/Tgtaagtaatacagggtttggtctctctctccattaactctgacatccaccactcct
  
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
23290		ATC AGC CAA AGC TAC CCA GA		Forward	A	
23291		AGG GAG TGG TGG ATG TCA GA		Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTPS-kapa	0.26 mM
23290	0.50 uM
23291	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

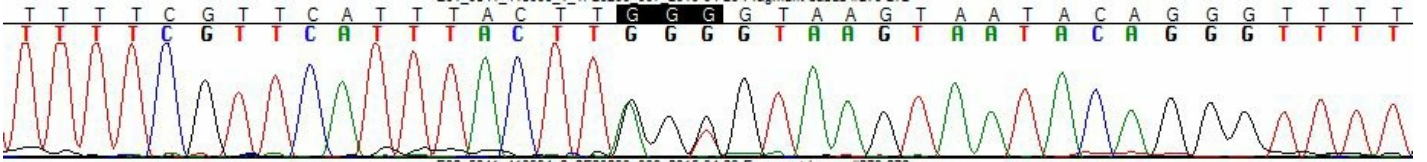
Cycling

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

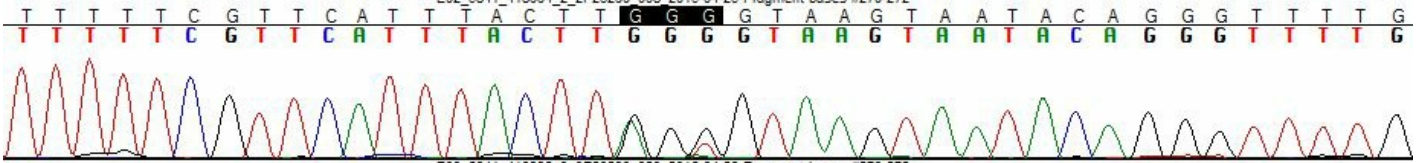
JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

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

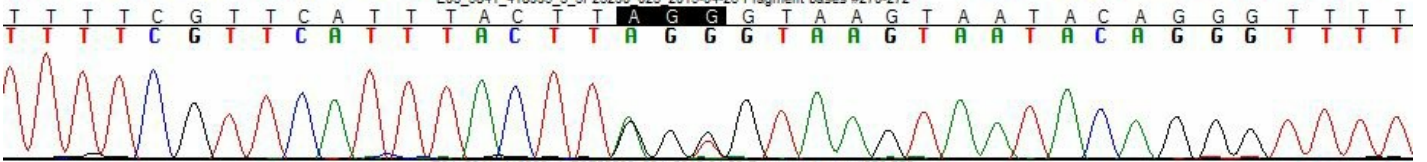
E01_5841_418993_1_1F23290_007 2015-04-26 Fragment bases #270-272



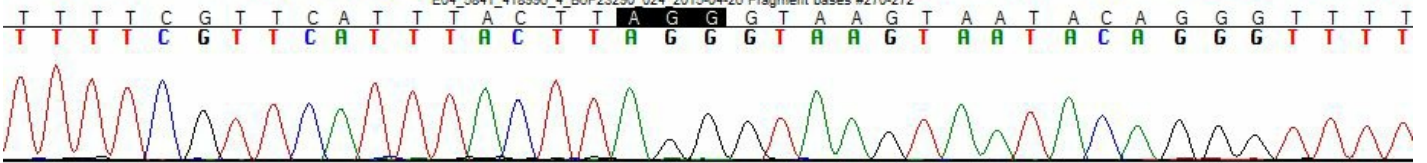
E02_5841_418994_2_2F23290_008 2015-04-26 Fragment bases #270-272



E03_5841_418995_3_3F23290_023 2015-04-26 Fragment bases #270-272



E04_5841_418996_4_B8F23290_024 2015-04-26 Fragment bases #270-272



E05_5841_418997_5_B8F23290_039 2015-04-26 Fragment bases #269-271

