

C57BL/6J

Stock No: 000664

Protocol 36835: Sanger sequencing Assay - Gabra2<C57BL/6J>-SEQ

Version 1.0

Notes

Mut = -/-

WT= T/T

Assay detect a deletion at Chr5:71014638 (GRCm38/mm10)

[>chr5:71014434-71014803](#) 370bp AGAGGCAACCTTCTTGATAACT GTTCATCATCCTGGATTCCG

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

Sequence

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TTCTGTAAACTATTTGGAATACTCTGTCTCCTGAAATTTATA
GGCTTACTACTTCTAAAACATGTACTGTTTTCAAAGGAATT
GTAATTTATATTT(t-
)AGGAGTATACAATAGATGTTTTCTTTCCGCCAAAAATGGAA
AGATGAGCGTTTTAAAATTTAAAGGTCCTATGAATATCCTTC
GACTTAACAATTTAATGGCCAGCAAAATCTGGAC
  
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
49841		AGA GGC AAC CTT TCT TGA TAA CT		Forward	A	
49842		GTT CCA TCA TCC TGG ATT CG		Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTPS-kapa	0.26 mM
49841	0.50 uM
49842	0.50 uM
Glycerol	6.50 %
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.

