

B6(C)-H2-Ab1^{bm12}/KhEgJ

Stock No: 001162

Protocol 28929: Sanger sequencing Assay - H2-Ab1<bm12>-SEQ

Version 1.2

Notes

Mut = T A A

WT= A G C

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

```
AGTGCTACTTCACCAACGGGACGCAGCGCATACGATATG
TGACCAGATACATCTACAACCGGGAGGAGTA
CGTGCGCTACGACAGCGACGTGGGCGAGCACCGCGCG
GTGACCGAGCTGGGGCGGCCAGACGCCGAGTAC
TGGAACAGCCAGCCGGAG(a/t)TCCTGGAGC(g/a)AA(c/a)G
CGGGCCGAGCTGGACACGGTGTGCAGACACAACACTACG
AGGGGCCGGAGACCCACACCTCCCTGCGGCGGCTTGGT
GAGCGCGGCGGGTCCC GCGGGAGCGCGGCGGG
CCGTGAGGGGACGCGGAGCAGAGTTCCCGCCTGAGGAG
CTGCATGGCCTCCTTCCCTCCCGTCTGCCCTGC
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
24439		GAG TAC GTG CGC TAC GAC AG		Forward	A	
oIMR5239		AGG GAG GTG TGG GTC TCC		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
24439	0.50 uM
oIMR5239	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

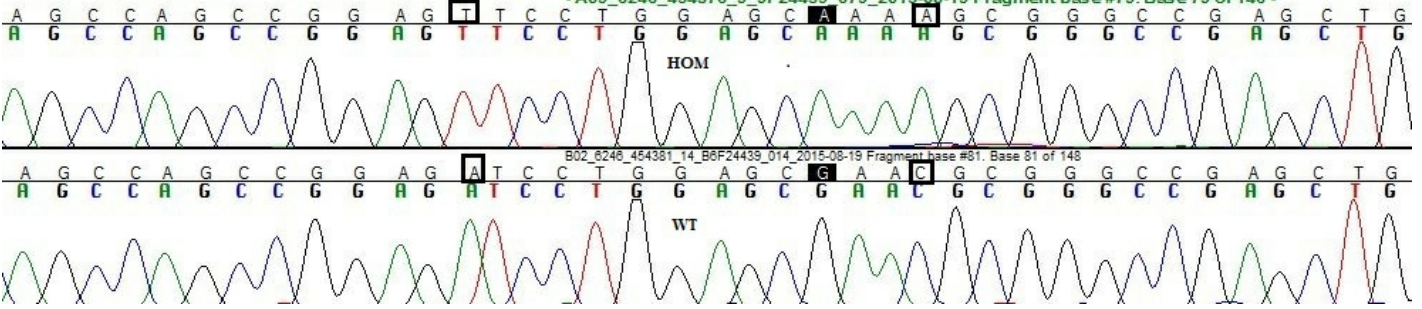
Cycling

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.

• A09_6246_454376_9_9F24439_079_2015-08-19 Fragment base #79. Base 79 of 146 •



B02_6246_454381_14_B0F24439_014_2015-08-19 Fragment base #81. Base 81 of 148

