

SWR/J-Clcn1^{adr-mto}/J

Stock No: 000939

Protocol 39657: Sanger sequencing Assay - Clcn1<adr-mto>

Version 1.0

Notes

Mut = T

WT= 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

```
SEQ
CAGTCCCAGCGGCATGGAGGGGAACAGAGCTGGTGGGG
CAGTGCCCCCAGTACCAGTACATGCCCTTTGAACATTG
TACCAGCTACGGACTGCCCTcAGAGAATGGGGGCCTTCA
GCACCGGCCCC(c/t)GAAAGGACATGGGTCCCAGGCACAAT
GCCCACCCAACACAGgtaatgtcctggggaggggaaggggagcagcgg
ttgtctggggccaaggattagtgaaaatggattgtatgtgagaaggggtacagaat
ttatftgaaaattaaatgtaa
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
54320		TGC CCT TTG AAC ATT GTA CC		Forward	A	
54321		AGT GTG GCT TTG CTG GTT C		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
54320	0.50 uM
54321	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.

