

B6J.129S6(FVB)-*Slc32a1*^{tm2(cre)Lowl}/MwarJ

Stock No: 028862

 Protocol 36020: Probe Assay - *Slc32a1*<tm2(cre)Lowl> Probe-Alternate 3

Version 1.0

Notes

Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination.

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= 96 bp

Wild Type = 82 bp

>[chr2:158614981+158615062](#) 82bp TACCGAACCAACGCAGAGG GGAATGACGGGGAGTGG

Sequence

Wt Sequence:

```
CTACCGAACCAACGCAGAGGACTAGGGGGTGGGGACCC
TGCCCCAGCTCCATCCCCGCCACCCCCACTCCCCGT
CATTCCCGCCCCACCCCCACTCCCAGCCCTCTGCGCC
GTCGCGCTAGGGAGGCCGAGCTTTAA
```

Mutant Sequence:

```
CTACCGAACCAACGCAGAGGACTAGGGGaatTCGGCCCC
TCTCCCTCCCCCCCCCTAACGTTACTGGCCGAAGCCG
CTTGGAATAAGGCCGGTGT
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
13007		ACA CCG GCC TTA TTC CAA G		Mutant Reverse	A	
45508	Fluorophore-1	TAG GGG AAT TCC GCC	Quencher-1	MUT Probe		
47594		TAC CGA ACC AAC GCA GAG G		Common	A	
47595		GGG AAT GAC GGG GAG TGG		Wild type Reverse	A	
47596	Fluorophore-2	CTG CCC CCA GCT CCA TCC	Quencher-2	WT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
13007	0.40 uM
47594	0.40 uM
47595	0.40 uM
Wt Probe	0.15 uM
Mutant Probe	0.15 uM
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	
4		--	repeat steps 2-3 for 40 cycles
5	4.0	--	Forever

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

Endpoint Fluorescence Scatter Plot

