

B6(129S4)-Et(cre/ERT2)13866Rdav/J

Stock No: 012688

Protocol 22106: Probe Assay - Gt(rosa)26sor<Tm1Sor> Probe

Version 3.0

Notes

Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing ΔCt values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references.

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= 195 bp

Wt= 193 bp

Sequence

Wt Sequence:

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CTGGCTTCTGAGGACCGCCCTGGGCCTGGGAGAATCCCT
TCCCCCTCTTCCCTCGTGATCTGCAACTCCAGtcttctagaaG
ATGGGCGGG
AGTCTTCTGGGCAGGCTTAAAGGCTAACCTGGTGTGTGGG
CGTTGTCCGTCAGGGGAATTGAACAGGTGTAATAATTGGAG
GGACAAGACTTCCCACAGATT
```

Mutant Sequence:

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CTGGCTTCTGAGGACCGCCCTGGGCCTGGGAGAATCCCTTCCCCCTTCCCTC
GTGATCTGCAACTCCAGTCTTTCTAGtgGATCCCCCg
ggcTGCAGATctgTAGGGCGCAGTAGTCCAGGGTTTCCTTGATGATGTCATACTT
ATCCTGTCCCTTTTTTTCCACAGCTCGCGTTGAGGACAAACTCTTCG
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JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
21306		CTG GCT TCT GAG GAC CG		Common	A	Gt(ROSA)26Sor
21307		CGA AGA GTT TGT CCT CAA CCG		Mutant Reverse	A	SA site
21308	Fluorophore-1	ACC CTG GAC TAC TGC GCC C	Quencher-1	MUT Probe		SA site
21309		AAT CTG TGG GAA GTC TTG TCC		Wild type Reverse	A	Gt(ROSA)26Sor
21310	Fluorophore-2	TAA CCT GGT GTG TGG GCG TTG T	Quencher-2	WT Probe		Gt(ROSA)26Sor

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
21306	0.40 uM
21307	0.40 uM
21309	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

