

B6(129S4)-Et(icre/ERT2)14163Rdav/J

Stock No: 012689

Protocol 33818: Probe Assay - Generic iCre Probe

Version 5.0

Notes

This assay will NOT distinguish hemizygous from homozygous

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

Transgene = 89 bp

Internal positive control = 74 bp

Sequence

Tg Sequence:

```
ATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGC
AAGCTGAACAACAGGAAATGGTCCCTGCTGAACCTGAG
GATGTGAGGGA
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
22926		TCC CTC ACA TCC TCA GGT TC		Internal Positive Control Reverse	A	
41665		ATG CTC CTG TCT GTG TGC AG		Transgene Forward	A	
41666	Fluorophore- 1	TGC CTG GTG CAA GCT GA	Quencher- 1	Tg Probe		
oIMR1544		CAC GTG GGC TCC AGC ATT		Internal Positive Control Forward	A	
oIMR3580		TCA CCA GTC ATT TCT GCC TTT G		Internal Positive Control Reverse	A	
TmoIMR0105	Fluorophore- 2	CCA ATG GTC GGG CAC TGC TCA A	Quencher- 2	IC Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
22926	0.40 uM
41665	0.40 uM
oIMR1544	0.40 uM
oIMR3580	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

