

## B6(129S4)-Et(cre/ERT2)296Rdav/J

Stock No: 009577

Protocol 20626: Probe Assay - Generic Cre Probe

Version 3.0

### Notes

This assay will NOT distinguish hemizygous from homozygous transgenic animals.

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 T<sub>m</sub> = 84°C +/- 1.0°C

Internal positive control T<sub>m</sub> = 79.4°C +/- 1.0°C

Transgene = 102 bp

Internal Positive Control = 74 bp

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
13593	Fluorophore-1	AAA CAT GCT TCA TCG TCG GTC CGG	Quencher-1	Tg Probe		Cre
oIMR1084		GCG GTC TGG CAG TAA AAA CTA TC		Transgene Forward	A	Cre
oIMR1085		GTG AAA CAG CAT TGC TGT CAC TT		Transgene Reverse	A	Cre
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
ddH <sub>2</sub> O	
oIMR1084	0.40 uM
oIMR1085	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	--	repeat steps 2-3 for 40 cycles

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

Endpoint Fluorescence Scatter Plot

