

C57BL/6-Tg(Hspa2-cre)1Eddy/J

Stock No: 008870

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_m = 84°C +/- 1.0°C

Internal positive control T_m = 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 | |
| TmIMR0105 | 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 ₂ 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

