

C57BL/6-Efna3^{tm1Ebp}/J

Stock No: 019108

Protocol 22182: QPCR Assay - Generic GFP/EGFP qPCR

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 Δ Ct 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

JAX Protocol

Protocol Primers

| PRIMER | 5' LABEL | SEQUENCE 5' → 3' | 3' LABEL | PRIMER TYPE | REACTION | NOTE |
|------------|---------------|----------------------------------|------------|-----------------------------------|----------|------|
| 14977 | | AAG GGC ATC GAC TTC AAG G | | Transgene Forward | A | |
| 14978 | | TGC TTG TCG GCC ATG ATA TAG | | Transgene Reverse | A | |
| 14979 | Fluorophore-1 | CTT GTG CCC CAG GAT GTT GCC | 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 | |
| 14977 | 0.40 uM |
| 14978 | 0.40 uM |
| oIMR1544 | 0.40 uM |
| oIMR3580 | 0.40 uM |
| Tg Probe | 0.15 uM |
| IC 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.