

C57BL/6-Tg(Ins2-OVA)307Wehi/WehiJ

Stock No: 005432

Protocol 37749: Standard PCR Assay - Tg(Ins2-TFRC/OVA)296Wehi Alternate3

Version 1.0

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 = X bp

Internal positive control = 521 bp

JAX Protocol

Protocol Primers

| PRIMER | 5' LABEL | SEQUENCE 5' → 3' | 3' LABEL | PRIMER TYPE | REACTION | NOTE |
|--------|----------|----------------------------|----------|-----------------------------------|----------|------|
| 31704 | | AGT GGC CTC TTC CAG AAA TG | | Internal Positive Control Forward | A | |
| 31705 | | TGC GAC TGT GTC TGA TTT CC | | Internal Positive Control Reverse | A | |
| 42896 | | GAT CCA CCC TTA ATG GGA CA | | Transgene Forward | A | |
| 51829 | | CAG GCT GAA CCG GGT ATA TG | | Transgene Reverse | A | |

Reaction A

| COMPONENT | FINAL CONCENTRATION |
|----------------------|---------------------|
| ddH ₂ O | |
| Kapa 2G HS buffer | 1.30 X |
| MgCl ₂ | 2.60 mM |
| dNTP KAPA | 0.26 mM |
| 31704 | 0.50 uM |
| 31705 | 0.50 uM |
| 42896 | 0.50 uM |
| 51829 | 0.50 uM |
| Glycerol | 6.50 % |
| Dye | 1.00 X |
| Kapa 2G HS taq polym | 0.03 U/ul |
| DNA | |

Cycling

| STEP | TEMP °C | TIME | NOTE |
|------|---------|------|--|
| 1 | 94.0 | -- | |
| 2 | 94.0 | -- | |
| 3 | 65.0 | -- | -0.5 C per cycle decrease |
| 4 | 68.0 | -- | |
| 5 | | -- | repeat steps 2-4 for 10 cycles (Touchdown) |
| 6 | 94.0 | -- | |
| 7 | 60.0 | -- | |
| 8 | 72.0 | -- | |
| 9 | | -- | repeat steps 6-8 for 28 cycles |
| 10 | 72.0 | -- | |
| 11 | 10.0 | -- | hold |

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

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

