**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 \( \Delta Ct \) 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**

\( Tg = 72 \text{ bp} \)

\( IPC = 74 \text{ bp} \)

**JAX Protocol**

**Protocol Primers**

<table>
<thead>
<tr>
<th>PRIMER</th>
<th>5' LABEL</th>
<th>SEQUENCE 5' → 3'</th>
<th>3' LABEL</th>
<th>PRIMER TYPE</th>
<th>REACTION NOTE</th>
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</thead>
<tbody>
<tr>
<td>36944</td>
<td></td>
<td>GGT GGC CTG ACC TAA GGA AG</td>
<td></td>
<td>Transgene Forward</td>
<td>A</td>
</tr>
<tr>
<td>36945</td>
<td></td>
<td>GTG CCA TCG AGA CTT CCA CT</td>
<td></td>
<td>Transgene Reverse</td>
<td>A</td>
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<tr>
<td>36946</td>
<td>Fluorophore-1</td>
<td>TGC CAT AGC AGG TAA TAC CAG CTT</td>
<td>Quencher-1</td>
<td>Tg Probe</td>
<td></td>
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<tr>
<td>oIMR1544</td>
<td></td>
<td>CAC GTG GGC TCC AGC ATT</td>
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<td>Internal Positive Control Forward</td>
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</tr>
<tr>
<td>oIMR3580</td>
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<td>TCA CCA GTC ATT TCT GCC TTT G</td>
<td></td>
<td>Internal Positive Control Reverse</td>
<td>A</td>
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<tr>
<td>TmoIMR0105</td>
<td>Fluorophore-2</td>
<td>CCA ATG GTC GGG CAC TGC TCA A</td>
<td>Quencher-2</td>
<td>IC Probe</td>
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**Reaction A**

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>FINAL CONCENTRATION</th>
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<tbody>
<tr>
<td>ddH2O</td>
<td>1.00 X</td>
</tr>
<tr>
<td>Kapa Probe Fast QPCR</td>
<td>0.40 uM</td>
</tr>
<tr>
<td>36944</td>
<td>0.40 uM</td>
</tr>
<tr>
<td>36945</td>
<td>0.40 uM</td>
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<tr>
<td>oIMR1544</td>
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<tr>
<td>oIMR3580</td>
<td>0.40 uM</td>
</tr>
<tr>
<td>Tg Probe</td>
<td>0.15 uM</td>
</tr>
<tr>
<td>IC Probe</td>
<td>0.15 uM</td>
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<tr>
<td>DNA</td>
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</table>

**Cycling**

<table>
<thead>
<tr>
<th>STEP</th>
<th>TEMP °C</th>
<th>TIME</th>
<th>NOTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>95.0</td>
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<tr>
<td>3</td>
<td>60.0</td>
<td>--</td>
<td>repeat steps 2-3 for 40 cycles</td>
</tr>
</tbody>
</table>

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