**Ctg-Tg(DO11.10)10Dlo/J**

Stock No: 003303
Protocol 30013: QPCR Assay - Tg(DO11.10)10Dlo-Tcra qPCR
Version 7.0

**Notes**

Taqman qPCR protocols are run on an ABI 7500, 7700, 7900 or the Roche LightCycler 480. Use an appropriate instrument specific Fluorophore/Quencher combination.

The transgene genotype is determined by comparing ΔCt 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**

<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</th>
<th>NOTE</th>
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<tbody>
<tr>
<td>oIMR1544</td>
<td></td>
<td>CAC GTG GGC TCC AGC ATT</td>
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<td>Internal Positive Control Forward</td>
<td>A</td>
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<tr>
<td>oIMR1811</td>
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<td>AAC GCT TCT CCC TGC ACA TC</td>
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<td>Transgene Forward</td>
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<tr>
<td>oIMR1812</td>
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<td>AGG ACT TGC AGC ACA GAA GTA CAT</td>
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<tr>
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<td>TCA CCA GTC ATT TCT GCC TTT G</td>
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<tr>
<td>TmoIMR0028</td>
<td>Fluorophore-1</td>
<td>CAG ACA CCC AGC CTG GAG ACT CAG C</td>
<td>Quencher-1</td>
<td>Tg Probe</td>
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<td>TmoIMR0105</td>
<td>Fluorophore-2</td>
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<td>IC Probe</td>
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**Reaction A**

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<tr>
<th>COMPONENT</th>
<th>FINAL CONCENTRATION</th>
<th>STEP</th>
<th>TEMP °C</th>
<th>TIME</th>
<th>NOTE</th>
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<tbody>
<tr>
<td>Kapa Probe Fast QPCR</td>
<td>1.00 X</td>
<td>1</td>
<td>95.0</td>
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<tr>
<td>ddH2O</td>
<td>0.40 uM</td>
<td>2</td>
<td>95.0</td>
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<tr>
<td>oIMR1544</td>
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<td>3</td>
<td>60.0</td>
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<td>repeat steps 2-3 for 40 cycles</td>
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<tr>
<td>oIMR1811</td>
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<td>Tg Probe</td>
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</tr>
<tr>
<td>IC Probe</td>
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<td>DNA</td>
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JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.