**B6.Cg-Rag1^tm1Mom Fcgrt^tm1Dcr Tg(CAG-FCGRT)276Dcr/DcrJ**

Stock No: 016919  
Protocol 22455: Standard PCR Assay - Fcgrt<sup>tm1Dcr</sup>

**Version 1.3**

**Notes**

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**

- **Mutant** = 378 bp
- **Heterozygote** = 248 bp and 378 bp
- **Wild type** = 248 bp

Separated by gel electrophoresis on a 1.5% agarose gel.

**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>
</tr>
</thead>
<tbody>
<tr>
<td>oIMR7720</td>
<td></td>
<td>GGG ATG CCA CTG CCC TG</td>
<td></td>
<td></td>
<td>Wild type</td>
<td>Forward</td>
</tr>
<tr>
<td>oIMR7721</td>
<td></td>
<td>CGA GCC TGA GAT TGT CAA GTG TAT T</td>
<td></td>
<td></td>
<td>Common</td>
<td></td>
</tr>
<tr>
<td>oIMR7722</td>
<td></td>
<td>GGA ATT CCC AGT GAA GGG C</td>
<td></td>
<td></td>
<td>Mutant Forward</td>
<td></td>
</tr>
</tbody>
</table>

**Reaction A**

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>FINAL CONCENTRATION</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ddH2O</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kapa 2G HS buffer</td>
<td>1.30 X</td>
<td></td>
</tr>
<tr>
<td>MgCl2</td>
<td>2.60 mM</td>
<td></td>
</tr>
<tr>
<td>dNTP KAPA</td>
<td>0.26 mM</td>
<td></td>
</tr>
<tr>
<td>oIMR7720</td>
<td>0.50 uM</td>
<td></td>
</tr>
<tr>
<td>oIMR7721</td>
<td>0.50 uM</td>
<td></td>
</tr>
<tr>
<td>oIMR7722</td>
<td>0.50 uM</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>6.50 %</td>
<td></td>
</tr>
<tr>
<td>Dye</td>
<td>1.00 X</td>
<td></td>
</tr>
<tr>
<td>Kapa 2G HS taq polym</td>
<td>0.03 U/ul</td>
<td></td>
</tr>
</tbody>
</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>94.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>94.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>65.0</td>
<td>--</td>
<td>-0.5 °C per cycle decrease</td>
</tr>
<tr>
<td>4</td>
<td>68.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>--</td>
<td>repeat steps 2-4 for 10 cycles (Touchdown)</td>
</tr>
<tr>
<td>6</td>
<td>94.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>60.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>72.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>--</td>
<td>--</td>
<td>repeat steps 6-8 for 28 cycles</td>
</tr>
<tr>
<td>10</td>
<td>72.0</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>10.0</td>
<td>--</td>
<td>hold</td>
</tr>
</tbody>
</table>

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.