Protocol Name: B6;129S5-Prkd3<sup>Gt(OST191038)Lex</sup>/Mmucd

**Protocol:**

<table>
<thead>
<tr>
<th>Reagent/Constituent</th>
<th>Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>10.775</td>
</tr>
<tr>
<td>10x Buffer</td>
<td>2.5</td>
</tr>
<tr>
<td>MgCl₂ (stock conc. is 25mM)</td>
<td>1.7</td>
</tr>
<tr>
<td>Betaine (stock conc. is 5M)</td>
<td>Optional</td>
</tr>
<tr>
<td>dNTPs (stock conc. is 10mM)</td>
<td>0.5</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.325</td>
</tr>
<tr>
<td>Primer 1. (stock conc. is 20μM)</td>
<td>0.5</td>
</tr>
<tr>
<td>Primer 2. (stock conc. is 20μM)</td>
<td>0.5</td>
</tr>
<tr>
<td>Taq Polymerase 5Units/μL</td>
<td>0.2</td>
</tr>
<tr>
<td>DNA (example) extracted w/ “Qiagen DNeasy columns or other similar silica based kits”</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**TOTAL VOLUME:**

<table>
<thead>
<tr>
<th>Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.5</td>
</tr>
</tbody>
</table>

**Comments on protocol:**

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

**Strategy:**

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temp (°C)</th>
<th>Time (m:ss)</th>
<th># of Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Initiation/Melting</td>
<td>HOT START?</td>
<td>94</td>
<td>5:00</td>
</tr>
<tr>
<td>2. Denaturation</td>
<td>94</td>
<td>0:15</td>
<td></td>
</tr>
<tr>
<td>3. Annealing</td>
<td>steps 2-3-4 cycle in sequence</td>
<td>65 to 55 (↓1°C/cycle)</td>
<td>0:30</td>
</tr>
<tr>
<td>4. Elongation</td>
<td>72</td>
<td>0:40</td>
<td></td>
</tr>
<tr>
<td>5. Amplification</td>
<td>72</td>
<td>5:00</td>
<td>1</td>
</tr>
<tr>
<td>6. Finish</td>
<td>15</td>
<td>∞</td>
<td>n/a</td>
</tr>
</tbody>
</table>

**Primers:**

<table>
<thead>
<tr>
<th>Name</th>
<th>Nucleotide Sequence (5′ - 3′)</th>
<th>Aragose: 1.5%</th>
<th>V: 90 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Primer 1194-lower</td>
<td>TATTCAGATGTGTTAGGAATGCCAAA</td>
<td>Estimated</td>
<td>9</td>
</tr>
<tr>
<td>2. Primer LTR-2</td>
<td>AAATGGCGTTACCTAAGCTAGCTTGGC</td>
<td>Primer</td>
<td>Band (bp)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Primer</th>
<th>Band (bp)</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 &amp; 2</td>
<td></td>
<td>390</td>
<td>mutant</td>
</tr>
</tbody>
</table>