GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
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530-754-MMRRRC

NAME OF PCR: B6;129P2-Pde4d<sup>tm1Mct</sup>/Mmucd

MMRRC: 034404-UCD

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>MgCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.7</td>
</tr>
<tr>
<td>Betaine</td>
<td>0.5</td>
</tr>
<tr>
<td>dNTPs</td>
<td>Optional</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.325</td>
</tr>
<tr>
<td>Primer 1.</td>
<td>0.5</td>
</tr>
<tr>
<td>Primer 2.</td>
<td>0.5</td>
</tr>
<tr>
<td>Primer 3.</td>
<td>0.5</td>
</tr>
<tr>
<td>Taq Polymerase</td>
<td>0.2</td>
</tr>
<tr>
<td>DNA (example)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

TOTAL VOLUME OF REACTION: 25.000 μL

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

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>Electrophoresis Protocol:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 34404-comF</td>
<td>CTATCGTGTCAAATCCCAAG</td>
<td>Primer Combination</td>
</tr>
<tr>
<td>2. 34404-koR</td>
<td>CTTAAGCGCATGCTCCAGACTG</td>
<td>1 and 2</td>
</tr>
<tr>
<td>3. 34404-wtR</td>
<td>GTGCTCACACAATAGTTTCTG</td>
<td>1 and 3</td>
</tr>
</tbody>
</table>

Electrophoresis Protocol:

<table>
<thead>
<tr>
<th>Name</th>
<th>Nucleotide Sequence (5' - 3')</th>
<th>Agarose: 1.5% V: 90</th>
<th>Estimated Running: Time: 90 min.</th>
</tr>
</thead>
</table>