**GENOTYPING BY PCR PROTOCOL**  
**MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS**  
mmrrc@ucdavis.edu  
530-754-MMRRC  

**Protocol Name:** B6;129S5-Il23atm1Lex/Mmucd  
**MMRRC:** 011725-UCD  

### Protocol:

<table>
<thead>
<tr>
<th>Reagent/Constituent</th>
<th>Volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>5.6</td>
</tr>
<tr>
<td>GoTaq® G2 Colorless Master Mix, 2X</td>
<td>7.5</td>
</tr>
<tr>
<td>Primer 1. (stock concentration is 20μM)</td>
<td>0.45</td>
</tr>
<tr>
<td>Primer 2. (stock concentration is 20μM)</td>
<td>0.45</td>
</tr>
<tr>
<td>DNA (example) extracted w/ &quot;Qiagen DNeasy columns or other similar silica based kits&quot;</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>TOTAL VOLUME</strong></td>
<td>15</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.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

### 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>2:00</td>
</tr>
<tr>
<td>2. Denaturation</td>
<td>94</td>
<td>0:10</td>
<td></td>
</tr>
<tr>
<td>3. Annealing</td>
<td>65 (↓1°C/cycle)</td>
<td>0:30</td>
<td>10x</td>
</tr>
<tr>
<td>4. Elongation</td>
<td>68</td>
<td>2:00</td>
<td></td>
</tr>
<tr>
<td>5. Denaturation</td>
<td>94</td>
<td>0:15</td>
<td></td>
</tr>
<tr>
<td>6. Annealing</td>
<td>55</td>
<td>0:30</td>
<td>25x</td>
</tr>
<tr>
<td>7. Elongation</td>
<td>68</td>
<td>2:00 (↑20sec/cycle)</td>
<td></td>
</tr>
</tbody>
</table>

### Primers:

<table>
<thead>
<tr>
<th>Name</th>
<th>Nucleotide Sequence (5’ - 3’)</th>
<th>Electrophoresis Protocol:</th>
<th>Argarose: 1.5%</th>
<th>V: 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Primer 0769-1</td>
<td>TGCAGATCACAGAGCCAGC</td>
<td>Estimated Running; Time: 90 min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Neo3a</td>
<td>GCAGCGCAGTCCGGCTATAC</td>
<td>Primer Combination</td>
<td>Band (bp)</td>
<td>Genotype</td>
</tr>
<tr>
<td>3. Primer 0769-3</td>
<td>CTTCCAACCCTCCAGATCC</td>
<td></td>
<td>2 &amp; 3</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 &amp; 3</td>
<td>307</td>
</tr>
</tbody>
</table>

**Electrophoresis Protocol:**

- Primer combination
- Band (bp)
- Genotype
### NIH-0769 Genotyping Strategies

<table>
<thead>
<tr>
<th>Reaction Components</th>
<th>Vol (ul)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Sigma Buffer</td>
<td>5</td>
</tr>
<tr>
<td>25mM MgCl2</td>
<td>3.5</td>
</tr>
<tr>
<td>10mM dNTPs</td>
<td>2</td>
</tr>
<tr>
<td>Primer 20 uM</td>
<td>1.5</td>
</tr>
<tr>
<td>Primer 20 uM</td>
<td>1.5</td>
</tr>
<tr>
<td>5 U/ul Taq polymerase</td>
<td>0.5</td>
</tr>
<tr>
<td>Water</td>
<td>31</td>
</tr>
<tr>
<td>Total mix volume</td>
<td>45</td>
</tr>
<tr>
<td>Tail lysate (1:20 dilution)</td>
<td>5</td>
</tr>
<tr>
<td>Total reaction volume</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp</th>
<th>Time</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94C</td>
<td>15&quot;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>65C</td>
<td>30&quot;</td>
<td>Decrease 1C/cycle</td>
</tr>
<tr>
<td>3</td>
<td>72C</td>
<td>40&quot;</td>
<td>Go to 1, 10 cycles</td>
</tr>
<tr>
<td>4</td>
<td>94C</td>
<td>15&quot;</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>55C</td>
<td>30&quot;</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>72C</td>
<td>40&quot;</td>
<td>Go to 4, 30 cycles</td>
</tr>
</tbody>
</table>

### Primer Sequences (5’ to 3’):

- Mutant PCR: Primer Neo3a and Primer 0769-3, 250 bp
- Recommended Wt PCR: Primer 0769-1 and Primer 0769-3, 307 bp

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer Neo3a</td>
<td>GCAGCGCATCGCCTTCTATC</td>
</tr>
<tr>
<td>Primer 0769-3</td>
<td>CTTCCAACCCTCCAGATCC</td>
</tr>
<tr>
<td>Primer 0769-1</td>
<td>TGCAGATCACAGAGCCAGC</td>
</tr>
</tbody>
</table>

### Mutant PCR

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>111</td>
<td>het</td>
</tr>
<tr>
<td>2</td>
<td>119</td>
<td>het</td>
</tr>
<tr>
<td>3</td>
<td>ES DNA</td>
<td>het</td>
</tr>
<tr>
<td>4</td>
<td>wt lysate</td>
<td>wt</td>
</tr>
<tr>
<td>5</td>
<td>water</td>
<td>no amp</td>
</tr>
</tbody>
</table>

![Mutant PCR gel image]
Targeting Strategy

Southern Strategies

<table>
<thead>
<tr>
<th>Probe</th>
<th>5' external</th>
<th>3' external</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>Bgl II</td>
<td>EcoRI</td>
</tr>
<tr>
<td>Wildtype</td>
<td>9.5 kb</td>
<td>9.3 kb</td>
</tr>
<tr>
<td>Targeted</td>
<td>16.8 kb</td>
<td>5.9 kb</td>
</tr>
</tbody>
</table>
**Southern Data**

5' external probe
Bgl II digests
Wildtype: 9.5 kb
Targeted: 16.8 kb

1F9  1H1*  Lex-2

3' external probe
EcoRI digests
Wildtype: 9.3 kb
Targeted: 5.9 kb

* Clone achieving germline transmission
Mouse cDNA Tissues

1) Brain
2) Spinal Cord
3) Eye
4) Thymus
5) Spleen
6) Lung
7) Kidney
8) Liver
9) Skeletal Muscle
10) Bone
11) Stomach, Small Intestine & Colon
12) Heart
13) Adipose
14) (-) Control
15) (+) Control - ES cell cDNA
16) (+) Control - Genomic/Lex1 DNA
17) 100 bp ladder/marker

Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.
Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: NIH-0769 (LEXKO-1052)
Reference accession(s): NM_031252
Standard KO or Conditional: Standard

Materials Submitted:
X Target Vector: pKOS-1.TVneo
X KOS clone(s): pKOS-1

Southern Blot Genotyping Strategies:

<table>
<thead>
<tr>
<th></th>
<th>5' External</th>
<th>3' External</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of Probe:</td>
<td>30-31</td>
<td>27-28</td>
</tr>
<tr>
<td>Restriction Enzyme for Genomic Digest:</td>
<td>Bgl II</td>
<td>EcoRI</td>
</tr>
<tr>
<td>Predicted Wild-type Band (kb):</td>
<td>9.5 kb</td>
<td>9.3 kb</td>
</tr>
<tr>
<td>Predicted Mutant Band (kb):</td>
<td>16.8 kb</td>
<td>5.9 kb</td>
</tr>
<tr>
<td>Probe Size:</td>
<td>270 bp</td>
<td>577 bp</td>
</tr>
</tbody>
</table>

Primer sequences:

Southern probes
0769-30  5’ – CAATCCATACGGAGTCAATGG
0769-31  5’ – AACCTTTTGAGGCAATGAGG
0769-27  5’ – GATGACCTAATGCCTGTACC
0769-28  5’ – GAACCCAGGAGGCAATGAGG
Genomic Sequence Deleted:
AGAGTTAGACTGAACCAAGGAGGTGAGATGGGCTAGCAGATACAGAGCCAGCAGCTGAGAGCAGGGAACAAGATATGCTG
GTTCCTAGGATAGCTCCAGTCGAGGCTCCAGTCCAGGCTCGTTAACTCTAGCTACGTGAACGCGGACTGAGTGAAC
CATGACCAAGCGGGAACATATGGTAGAAAGC

KOS clone sequence: (note: pKOS-1 was used to generate the TV and that is the sequence included here)
GTCCCCAGCCTTTGAAATTTTGAGATAGGTTCTTGCTACATAGTACTGGCTGGCCTGGGCCTTATGCAATCTGCCTTGGT
TTCTCACCCCATATATATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
CCAAAGGAGGTGGATAGGGGGTCCACAGGCCTGGTGCAGATCACAGAGCCAGCCAGATCTGAGAAGCAGGGAACAAGATGCTGGATTGCAGAGCAGTAATAATGCTATGGCTGTTGCCCTGGGTCACTCAGGGCCTGGCTGTGCCTAGGAGTAGCA
GTCCTGACTGGGCTCAGTGCCAGCAGCTCTCTCGGAATCTCTGCATGCTAGCCTGGAACGCACATGCACCAGCGGGACA
TATGGTAAGTGTCAGCTCCTGGGACCGCGCAGAAAACCTTCCCAGTCCTCCAAGTGTGTAGGTTTAATGGAAGCTGTGG
CCCCGGGTGGATCTGGAGGGTTGGAAGCCATCGTGGAATGAGATAGGACAGAAGACTGGGGCTTCTGGAAGAGTTGTG
GGCCGGCGGTTGAGCGCGAATGCAGCTCACTCGCCTACTCTCCTAGTTAAAATGTGAAAAGGAAAGAGGACCTGCTGGCT
TTTATAAGACCTCTGTTAGCTCAGAATCTTAAAAAGGGGACCTGCTCTTACCTCCTCCTAGTATAGAAGCTAG
CCTCCCTACTAGGACTCTCAGATCCACTCTCCTCAGATGACTAGGA

Selection cassette sequence:

(note: linker sequences may vary and are not provided)

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCCCCCCTCTCCC
TCCCGAAAAGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGT
CATGAGGACCTCTTTAGGAAAAATGCGTTGCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCG
GTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCGGTTTCCGCGAGGTGCGGATT
AAAATGGTCTGCTGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATG
GTCAGGTCATCTGCGGATCTATTGCTGAGACCCGCAATGTCGCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCG
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