

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S5-Defb3^{tm1Lex}/Mmucd

MMRRC: 011694-UCD

Protocol:

| Reagent/Constituent | Volume (µL) |
|-------------------------------------------------------------------------------------|------------------|
| Water | 10.775 |
| 10x Buffer | 2.5 |
| MgCl ₂ (stock concentration is 25mM) | 1.7 |
| Betaine (stock concentration is 5M) <i>Optional</i> | 6.5 |
| dNTPs (stock concentration is 10mM) | 0.5 |
| DMSO <i>Optional</i> | 0.325 |
| Primer 1. (stock concentration is 20µM) | 0.5 |
| Primer 2. (stock concentration is 20µM) | 0.5 |
| Primer 3. (stock concentration is 20µM) | 0.5 |
| Taq Polymerase 5Units/µL | 0.2 |
| DNA (example) extracted w/ Qiagen DNeasy columns or other similar silica based kits | 1.0 |
| 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 MgCl₂ to increase reaction or decrease non-specific amplifications.

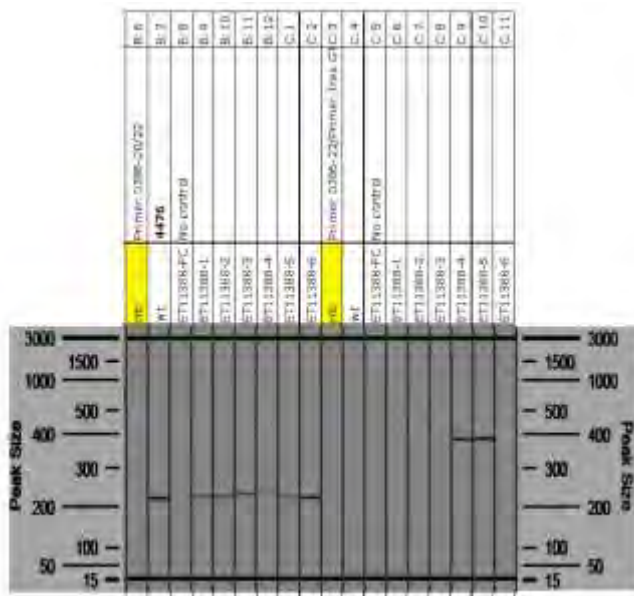
Strategy:

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

Primers:

Electrophoresis Protocol:

| Name | Nucleotide Sequence (5' - 3') | Argarose: 1.5% V: 90 | | |
|----------------|-------------------------------|---------------------------------|-----------|-----------|
| 1. 0386-22 | GGTGCTCTTGTTTCATAATTGT | Estimated Running Time: 90 min. | | |
| 2. Ires GT ext | GCTAGACTAGTCTAGCTAGAGCGG | Primer Combination | Band (bp) | Genotype |
| 3. 0386-20 | CTCATATCAGTGTGGGATGAC | 1 and 2 | 206 | mutant |
| | | 1 and 3 | 231 | wild-type |



PCR protocol developed by MMRRC at University of California, Davis

NIH-0386 Genotyping Strategies

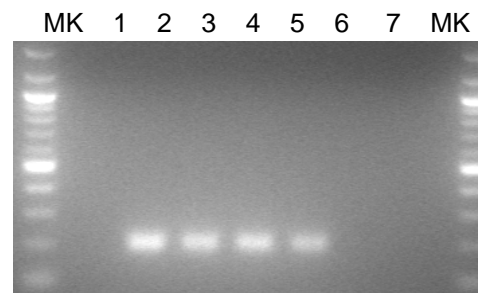
| Reaction Components | Vol (ul) |
|-----------------------------|----------|
| 5x Phusion buffer | 8 |
| 25mM MgCl ₂ | 3.2 |
| 10mM dNTPs | 1 |
| Primer 20 uM | 1 |
| Primer 20 uM | 1 |
| Phusion Enzyme | 0.1 |
| Water | 20.7 |
| Total mix volume | 35 |
| Tail lysate (1:20 dilution) | 5 |
| Total reaction volume | 40 |

| Step | Temp | Time | Note |
|------|------|------|--------------------|
| 1 | 96C | 17" | |
| 2 | 63C | 15" | Decrease 1C/cycle |
| 3 | 72C | 15" | Go to 1, 6 cycles |
| 4 | 96C | 17" | |
| 5 | 57C | 15" | |
| 6 | 72C | 15" | Go to 4, 29 cycles |

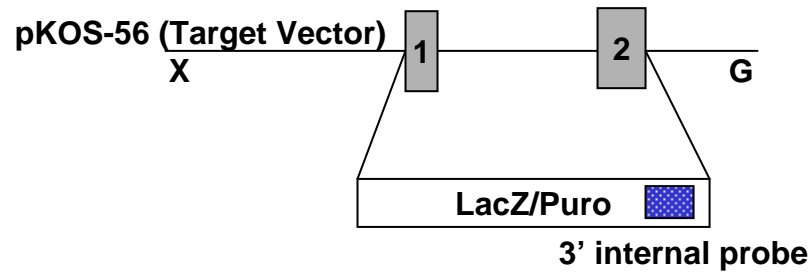
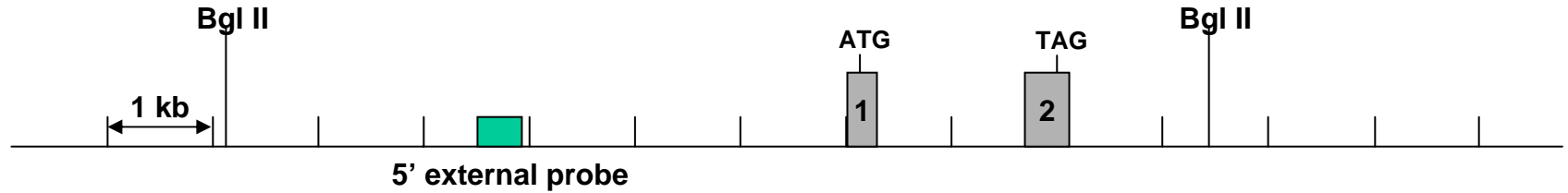
| Primer Sequences (5' to 3') | |
|---------------------------------------------------------------|--------------------------|
| Mutant PCR: Primer 0386-22 and Primer Ires GT ext, 206 bp | |
| Recommended Wt PCR: Primer 0386-22 and Primer 0386-20, 231 bp | |
| Primer 0386-22 | GGTGCTCTTGTTTCATAATTGT |
| Primer Ires GT ext | GCTAGACTAGTCTAGCTAGAGCGG |
| Primer 0386-20 | CTCATATCAGTGTGGGATGAC |

| Well | Sample | Genotype |
|------|------------------|----------|
| 1 | 11 | wt |
| 2 | 12 | het |
| 3 | 46 | het |
| 4 | 60 | het |
| 5 | ES DNA | het |
| 6 | wt lysate | wt |
| 7 | water | no amp |



Mutant PCR



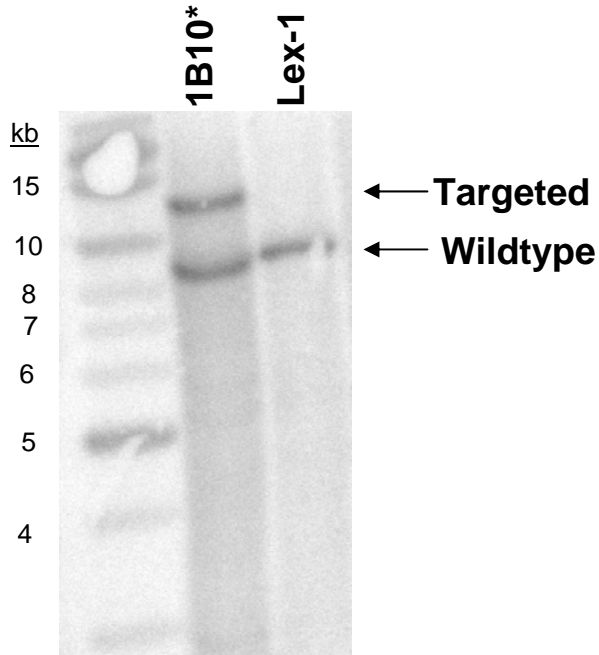
Targeting Strategy



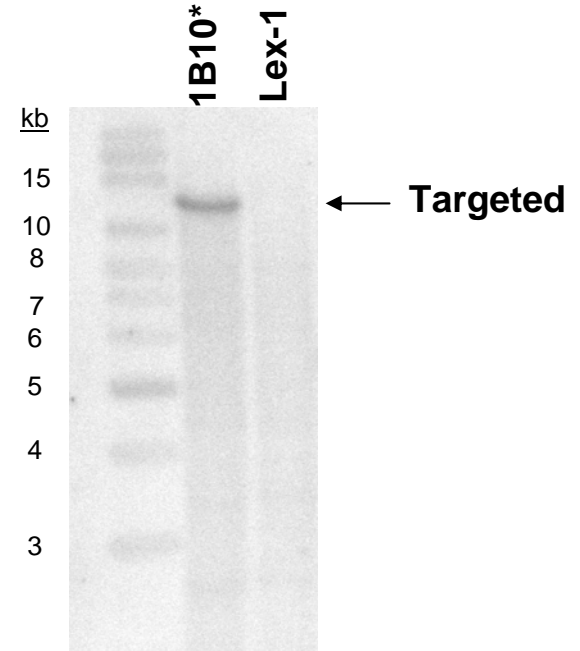
Southern Strategies

| Probe | 5' external  | 3' internal  |
|----------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Enzyme | Bgl II | Bgl II |
| Wildtype | 9.3 kb | ----- |
| Targeted | 12.8 kb | 12.8 kb |

Southern Data



5' external probe
Bgl II digests
Wildtype 9.3 kb
Targeted 12.8 kb

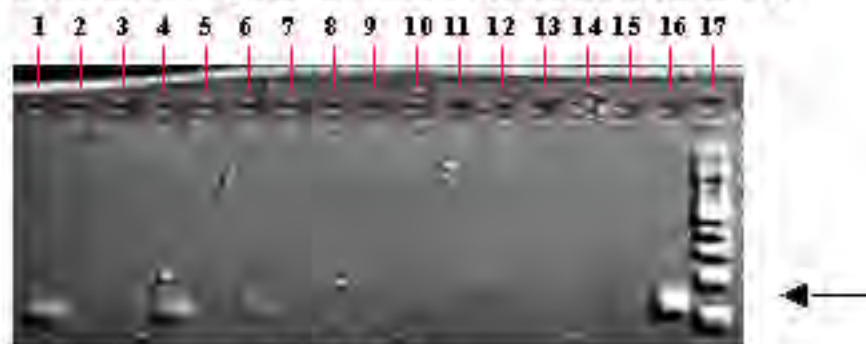


3' internal probe
Bgl II digests
Targeted 12.8 kb

*** Clone achieving germline transmission**

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 3,4



09/25/2004

Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

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/NotI DNA
- 17) 100 bp ladder/marker



**Lexicon Genetics Incorporated
Molecular Genetics Project Materials**

Catalog Number: NIH-0386 (~~LEXKO-159~~)

Reference accession(s): NM_013756

Standard KO or Conditional: Standard

Materials Submitted: Target Vector pKOS-56TVpuro
 KOS clone(s) pKOS-56

Southern Blot Genotyping Strategies:

| | <u>5' External</u> | <u>3' Internal</u> |
|----------------------------------------|---------------------------|---------------------------|
| Name of Probe: | 10/11 | PuroA/B |
| Restriction Enzyme for Genomic Digest: | BglII | BglII |
| Predicted Wild-type Band (kb): | 9.3 | ---- |
| Predicted Mutant Band (kb): | 12.8 | 12.8 |
| Probe Size: | 599 | 308 |

Primer sequences:

Southern probes

0386-10 5' – TGCCCTTCCTTAAACCT
0386-11 5' – TGAATAAATCATGCCAACGTG
PuroA 5' – CCCGCAGCGCCCGACCGAAAG
PuroB 5' – CGGTGAGTTATATGCGTACTC

ATGGTTAGTAAAGCAAAAAGTTCAGAGTCTGTCTAGTCTGACCTTCCCACTGGGGCTTTTACAGTATGATGGTCTCCCCATCTGGCTTGGACGGGGCT
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CATACCATAATACAGTATATCTTTAAATTTAAAGTCGGAGTCTGAAGCCAGGGCACTGTCCATAAGTGTGTGCTGTGCAAGCTTACAGATC

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

LacZ/Puro

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCTAACGTTACT
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