# NIH-1841 Genotyping Strategies

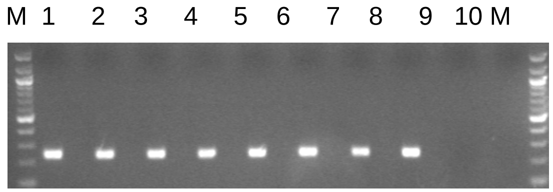
| Reaction Components | Vol (ul) |
|---|---|
| 5X GoTaq Buffer | 10.0 |
| 25mM MgCl2 | 3.5 |
| 10mM dNTPs | 1.0 |
| Primer 20 uM | 1.0 |
| Primer 20 uM | 1.0 |
| 5 U/ul Taq polymerase | 0.5 |
| Water | 28.0 |
| Tail lysate (1:20 dilution) | 5.0 |
| Total reaction volume | 50.0 |

| Step | Temp | Time | Note |
|---|---|---|---|
| 1 | 94C | 4' | |
| 2 | 94C | 15" | |
| 3 | 65C | 30" | Decrease 1C/cycle |
| 4 | 72C | 40" | Go to 2, 10 cycles |
| 5 | 94C | 15" | |
| 6 | 55C | 30" | |
| 7 | 72C | 40" | Go to 5, 30 cycles |

| **Primer Sequences (5’ to 3’):** | |
|---|---|
| Mutant PCR: Primer Neo3a and Primer 1841-11, 247 bp | |
| Recommended Wt PCR: Primer 1841-10 and Primer 1841-11, 399 bp | |
| Neo3a | GCAGCGCATCGCCTTCTATC |
| 1841-11 | TCCAACATTAAAGCGATAACC |
| 1841-10 | CCGAATCTCTCCGATCACTAC |

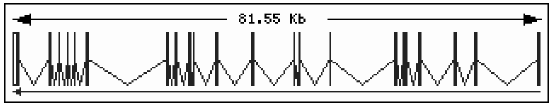
| Well | Sample | Genotype |
|---|---|---|
| 1 | 166 | het |
| 2 | 175 | het |
| 3 | 176 | het |
| 4 | 178 | het |
| 5 | 179 | het |
| 6 | 184 | het |
| 7 | 187 | het |
| 8 | es DNA | het |
| 9 | wt lysate | wt |
| 10 | water | no amp |

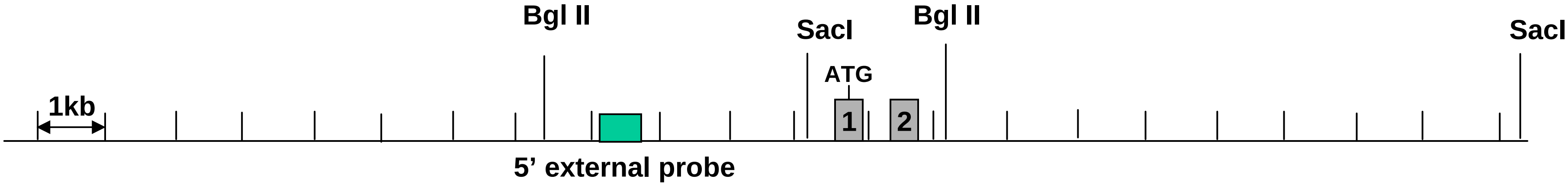
**Mutant PCR**

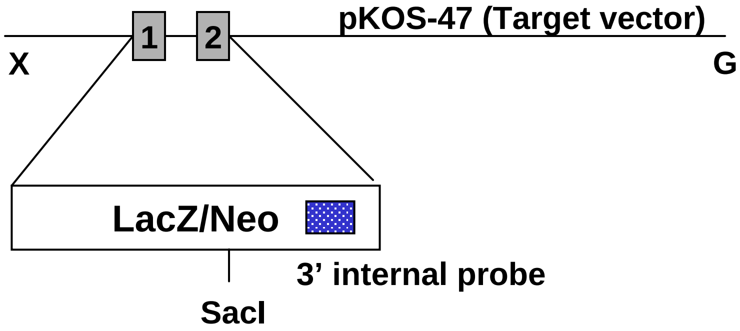


*2% Agarose, NEB 100 bp ladder

# Targeting Strategy



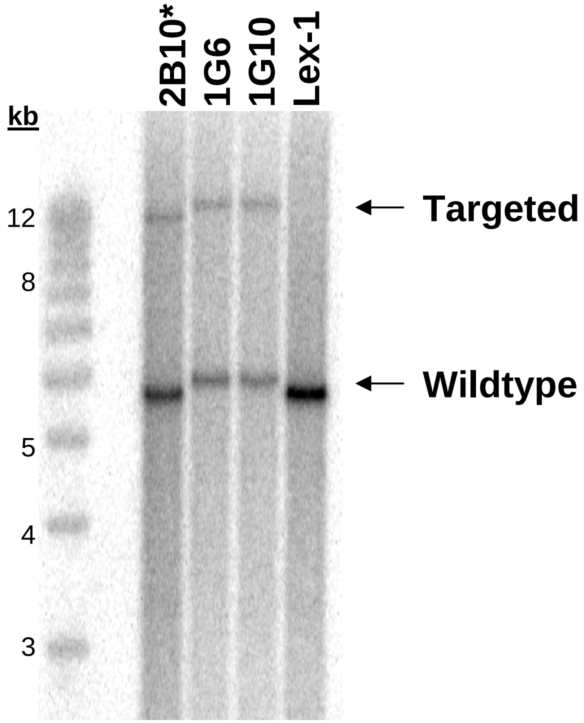




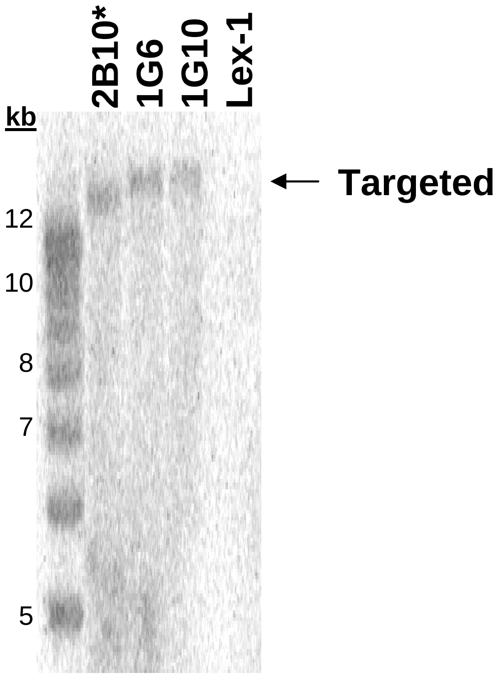
**Southern Strategies**

| Probe | 5’ external 🟩 | 3’ internal ▨ |
|---|---|---|
| Enzyme | Bgl II | SacI |
| Wildtype | 5.4 kb | ------- |
| Targeted | 10.3 kb | 12.2 kb |

# Southern Data



**5’ external probe**
**Bgl II digests**
**Wildtype 5.4 kb**
**Targeted 10.3 kb**

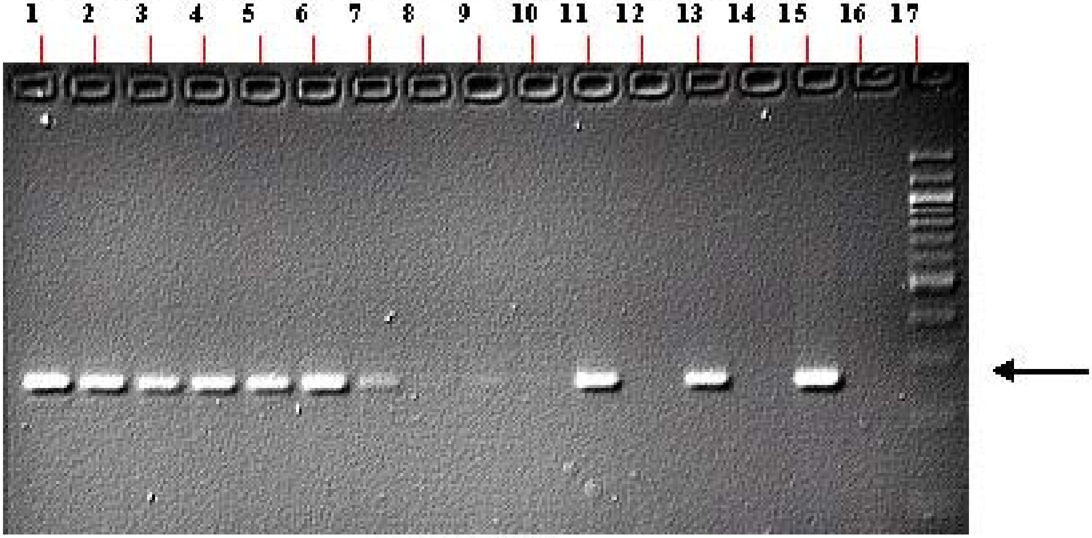


**3’ internal probe**
**SacI digests**
**Targeted 12.2 kb**

*** Clone achieving germline transmission**

# RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



02/05/2003

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

**Mouse cDNA Tissues**

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

LEXICON

# Lexicon Genetics Incorporated
# Molecular Genetics Project Materials

**Catalog Number:** **NIH-1841** (LEXKO-184)

**Reference accession(s):** NM_015744

**Standard KO or Conditional:** Standard KO

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**Materials Submitted:** X Target Vector pKOS-47TVneo
X KOS clone(s) pKOS-47

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## Southern Blot Genotyping Strategies:

| | **5' External** | **3' Internal** |
|---|---|---|
| Name of Probe: | 14+15 | Neo 2+5 |
| Restriction Enzyme for Genomic Digest: | Bgl II | SacI |
| Predicted Wild-type Band (kb): | 5.4 | ------- |
| Predicted Mutant Band (kb): | 10.3 | 12.2 |
| Probe Size (bp): | 549 | 609 |

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## Primer sequences:

### Southern probes

| | |
|---|---|
| 1841-14 | 5' – CTGATGCACAGTGATCTGGG |
| 1841-15 | 5' – GCCAAGCTAAACTGATCTGG |
| Neo-2 | 5' – CCTCAGAAGAACTCGTCAAG |
| Neo-5 | 5' – GGCAGCGCGGCTATCGTG |

**Genomic Sequence Deleted :**

GGCAAGACAAGGCTGTTTCGGGTCATACCAGGTAGGAGGATGGTACGGTGACTAGCGAGGTGCCGAATCTCTCCGATCACTACCATTTGAATTCGATT
TTTTTTCTTTTTTCTTTTTCCTTGTTCAAATTCAGACATCATCTGAGAATGCCTTTCCGAATTGGCAGGATTCGGGTTGTTAGGGTCATCTGAACTGCTTC
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**KOS clone sequence:** *(note: pKOS-47 was used to generate the TV and that is the sequence included here)*

GATCCTAGAAGAGCCACTGAGTGTCTGGCAGTGTCTCTTCTTCTTTTCAATGAGCTGTTTACTAAATTCTAGAGTTTCTAATGAAACCCAGGCTTACTCT
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ATC

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

### LacZ/Neo

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCCTAACGTTACT
GGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGC
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