

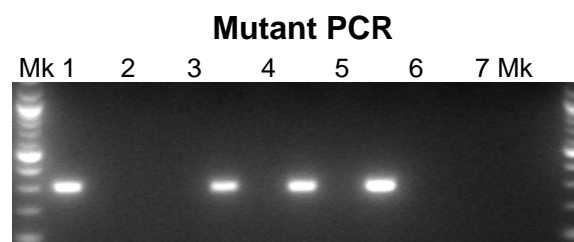
NIH-0101 Genotyping Strategies

Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl ₂	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

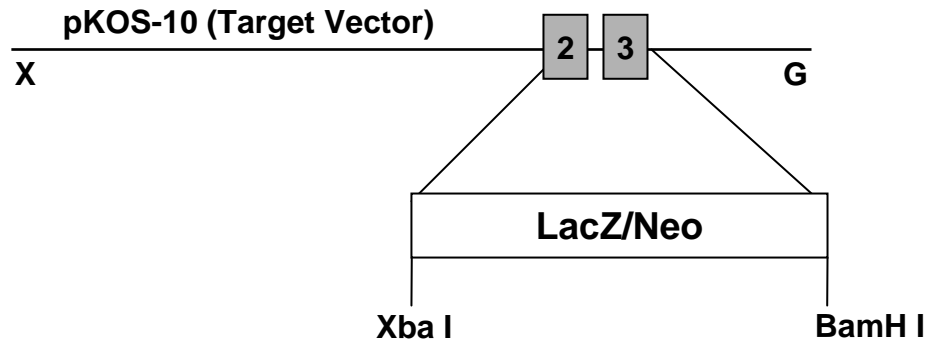
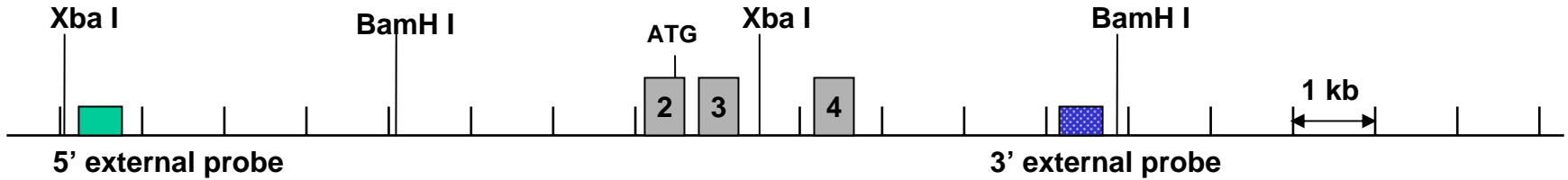
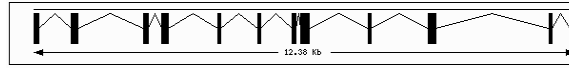
Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0101-9, 310 bp	
Recommended Wt PCR: Primer 0101-1 and Primer 0101-9, 485 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0101-9	GAGGGGTCACACAGCAGGTAG
Primer 0101-1	AAGTGGGGCGAGTGGGAGGAGTC



Well	Sample	Genotype
1	28	het
2	29	wt
3	33	het
4	55	het
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



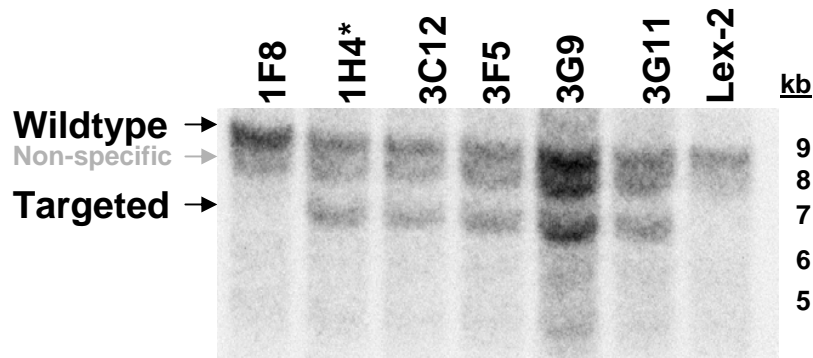
Targeting Strategy



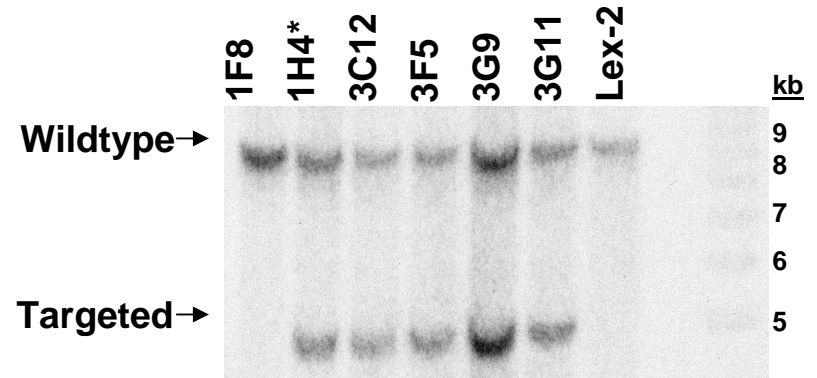
Southern Strategies

Probe	5' external 	3' external 
Enzyme	Xba I	BamH I
Wildtype	9.0 kb	8.9 kb
Targeted	6.7 kb	5.1 kb

Southern Data



5' external probe
Xba I digests
Wildtype 9.0 kb
Targeted 6.7 kb

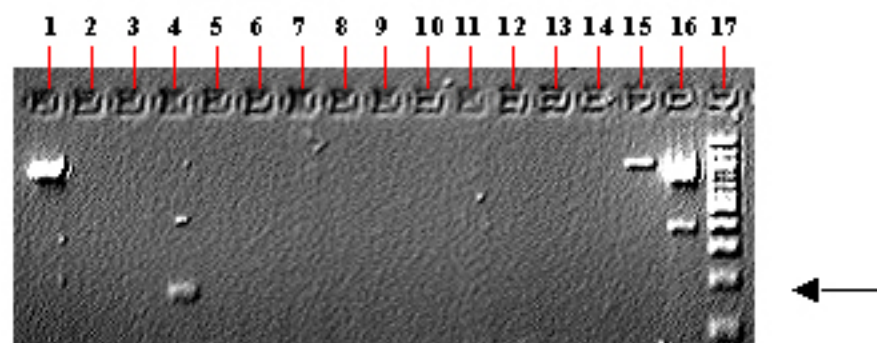


3' external probe
Hind III digests
Wildtype 8.9 kb
Targeted 5.1 kb

* Clone achieving germline transmission

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



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-0101 (LEXKO-1057)

Reference accession(s): NM_194357

Standard KO or Conditional: _ Standard KO

Materials Submitted: Target Vector: pKOS-10 TVneo
 KOS clone(s): pKOS-10

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	14-15	20-21
Restriction Enzyme for Genomic Digest:	Xba I	Bam HI
Predicted Wild-type Band (kb):	9.0 kb	8.9 kb
Predicted Mutant Band (kb):	6.7 kb	5.1 kb
Probe Size:	420 bp	516 bp

Primer sequences:

Southern probes

0101-14 5' – GTGGGCACATCAGGGCATACTA
0101-15 5' – CACACGGCAAAGAGGCAGAAC
0101-20 5' – CGGGGCCAAGGCTTAAACACAGG
0101-21 5' – GGCAGCCAGGCAGGAAAACACTACA

Genomic Sequence Deleted:

GCTGGGTGTGTATACCCTGCTTCTGCTCTGGGGCCTGGCCACTCCATGCCTGGGGCTGCTTGAGACAGTGGGCACGCTT
GCTCGGATTGACAAGGATGAACTGGGCAAAGGTGAGGCAGAGGCTCGTGGGCAGGCCGGAAGGGACCCAGCTGCAAC
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CTCGGAGGTGTGGCCTGCTGAGTTACGGTGGCATCTTTAGTCTTGTGGAAGAACTCTCTGGGTGAGGCCCCAGGGCTC
TGAAGGGCTC

KOS clone sequence: (note: pKOS-10 was used to generate the TV and that is the sequence included here)

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

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCC
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