

NIH-0008 Genotyping Strategies

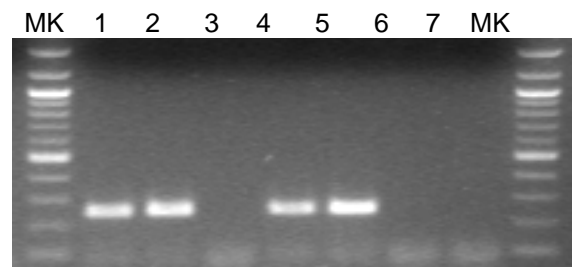
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

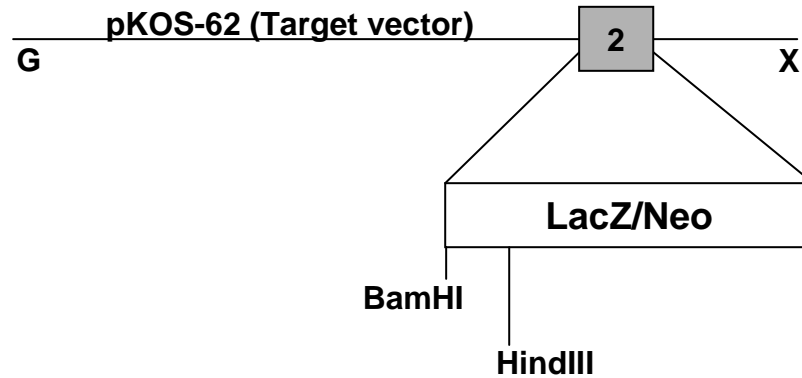
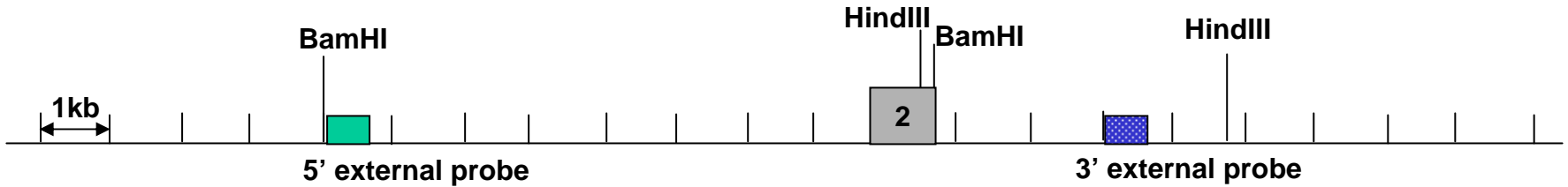
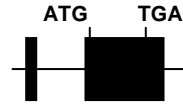
Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0008-10, 243 bp	
Recommended Wt PCR: Primer 0008-1 and Primer 0008-4, 350 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0008-10	AACCCGGGTCTCAGAGAAATGGCA
Primer 0008-1	CTGCCAAACCAGTCCCTAGAAG
Primer 0008-4	GCAGCACGTGATGTCAGAAGAC

Well	Sample	Genotype
1	20	het
2	28	het
3	29	wt
4	34	het
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



Mutant PCR



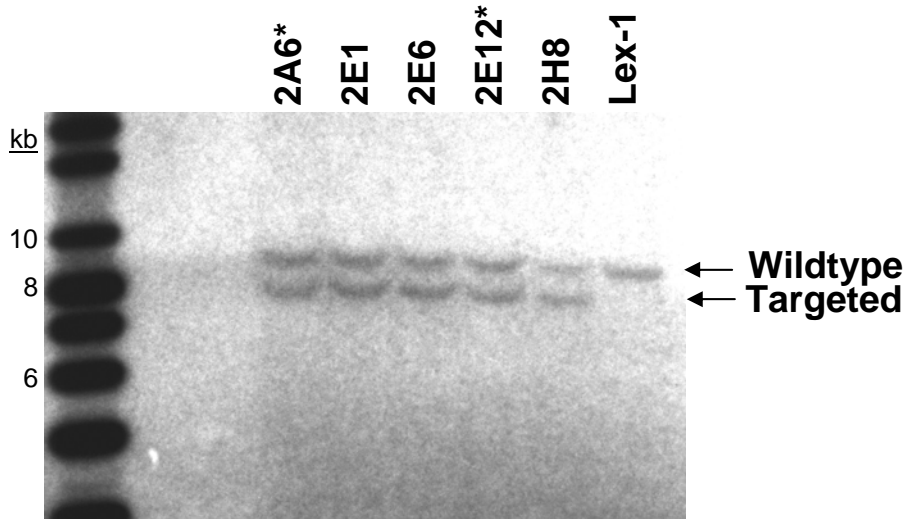
Targeting Strategy



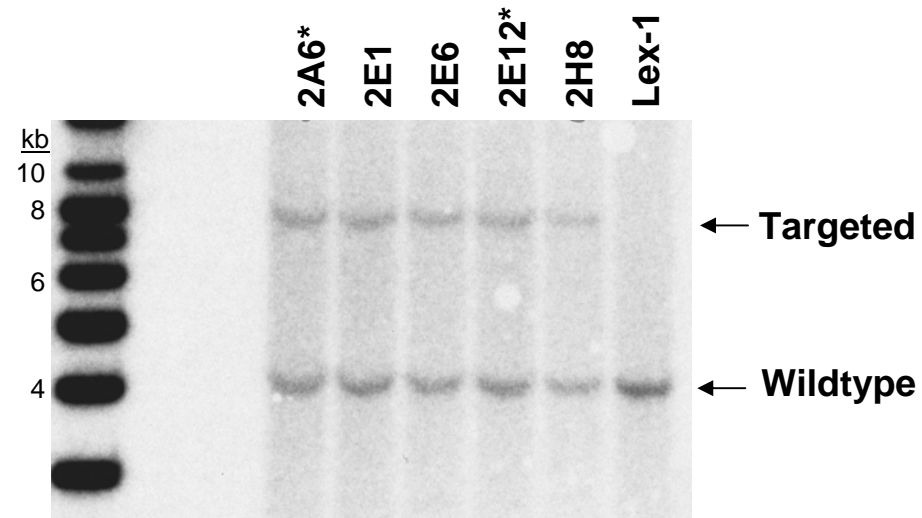
Southern Strategies

Probe	5' external 	3' external 
Enzyme	BamHI	HindIII
Wildtype	8.7 kb	4.3 kb
Targeted	7.8 kb	8.6 kb

Southern Data



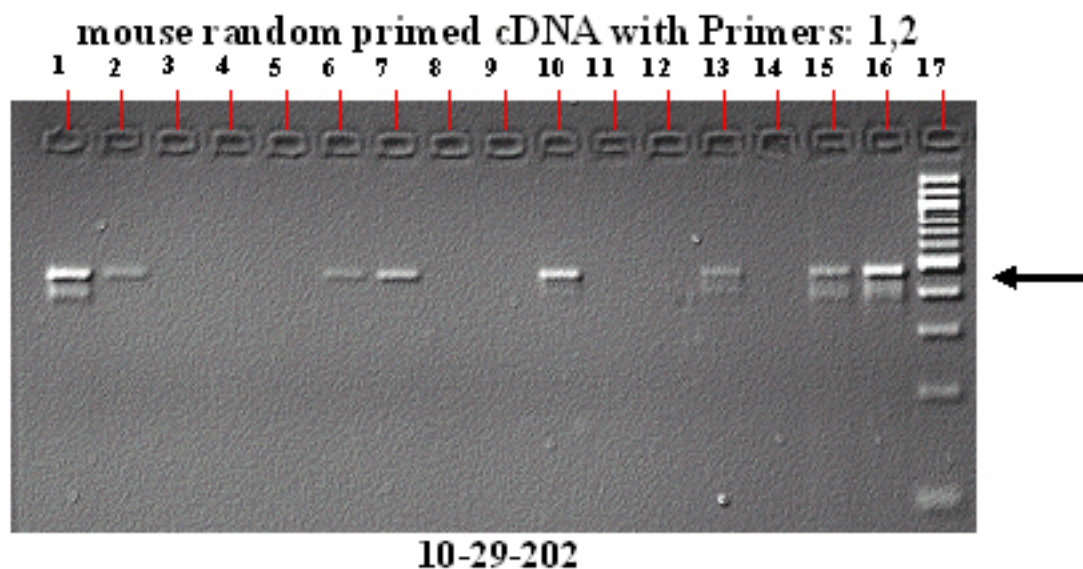
5' external probe
BamHI digests
Wildtype 8.7kb
Targeted 7.8kb



3' external probe
HindIII digests
Wildtype 4.3kb
Targeted 8.6kb

* Clones achieving germline transmission

RT-PCR WT Expression Analysis



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 Genetics Incorporated
Molecular Genetics Project Materials**

Catalog Number: NIH-0008 (LEXKO-295)

Reference accession(s): NM_008309

Standard KO or Conditional: Standard

Materials Submitted: Target Vector pKOS-62TVneo
 KOS clone(s) pKOS-62

Southern Blot Genotyping Strategies:

	<u>5' External/ Internal</u>	<u>3' External/ Internal</u>
Name of Probe:	22/23	24/15
Restriction Enzyme for Genomic Digest:	BamHI	HindIII
Predicted Wild-type Band (kb):	8.7	4.3
Predicted Mutant Band (kb):	7.8	8.6
Probe Size:	182 bp	492 bp

Primer sequences:

Southern probes

0008-22 5' – GAAGAAACTGGACATAGATGTTC
0008-23 5' – GAGCTGTAAAAATTCACCAGGCC
0008-24 5' – CCCTACAATTTGGAGATCCAAG
0008-15 5' – GGAGTCCAGGAACTGAACTG

Genomic Sequence Deleted:

AAACCCAGTCCCTAGAAAGGCCTTCTCAGGAGGCCTCCAACAGATCCCTGAATGTGACAGGGGGCTTGGGACCCAGAGGCTCCTGCAGGCTCTCAGAATCT
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ATTTCCGGAAGATCTCATAGTCGGATTGGG

KOS clone sequence: (note: pKOS-62 was used to generate the TV and that is the sequence included here)

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

LacZ/Neo

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