

NIH-0932 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

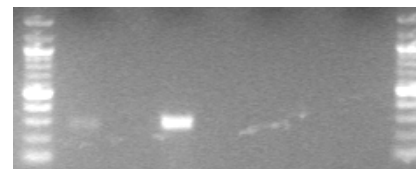
Step	Temp	Time	Note
1	96C	17"	
2	63C	15"	Decrease 1C/cycle
3	72C	15"	Go to 1, 6 cycles
4	96C	17"	
5	57C	15"	
6	72C	15"	Go to 4, 29 cycles

Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0932-10, 285 bp	
Recommended Wt PCR: Primer 0932-8 and Primer 0932-10, 346 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0932-8	GTCATGAAGCATGCCAGCTG
Primer 0932-10	GTATTACTCAGTCGGAAC

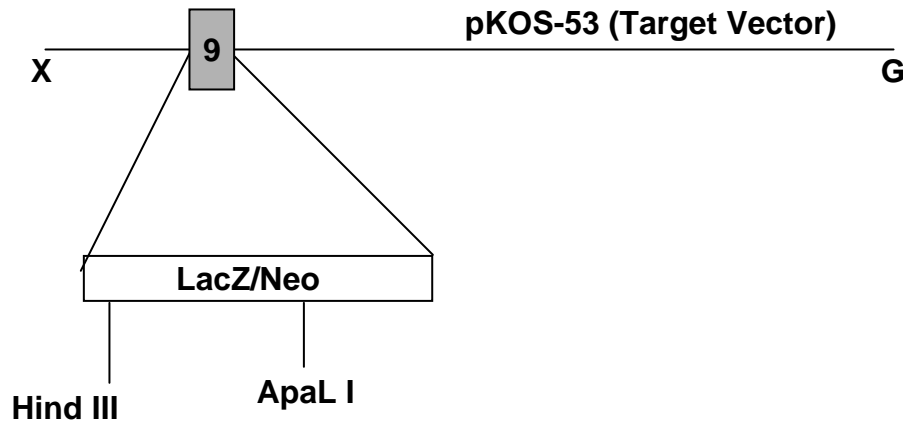
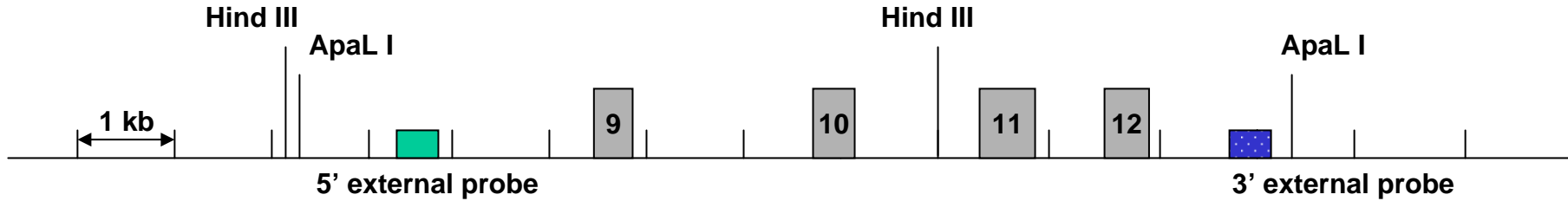
Well	Sample	Genotype
1	219	het
6	ES DNA	het
7	wt lysate	wt
8	water	no amp

Mutant PCR



MK 1 2 3 4 MK



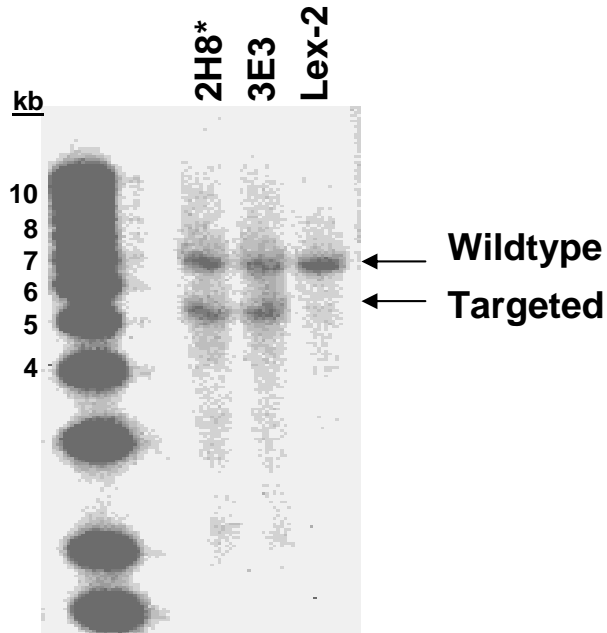
Targeting Strategy



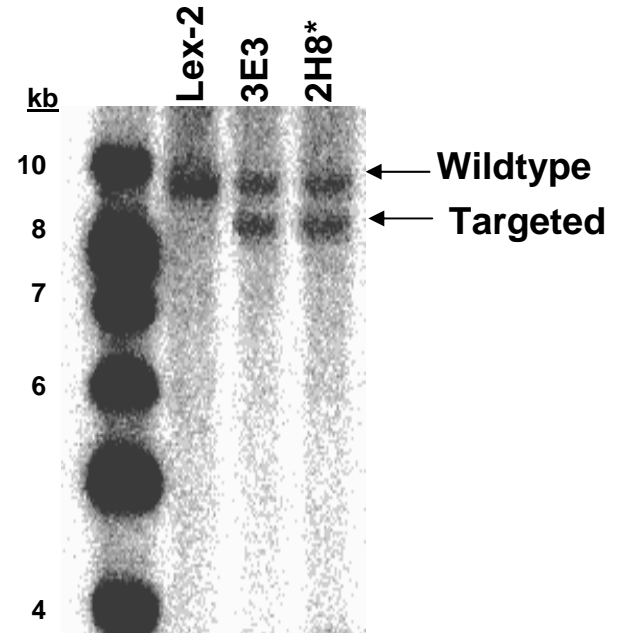
Southern Strategies

Probe	5' external 	3' external 
Enzyme	Hind III	ApaL I
Wildtype	6.9 kb	9.8 kb
Targeted	5.4 kb	8.7 kb

Southern Data



5' external probe
Hind III digests
Wildtype 6.9 kb
Targeted 5.4 kb

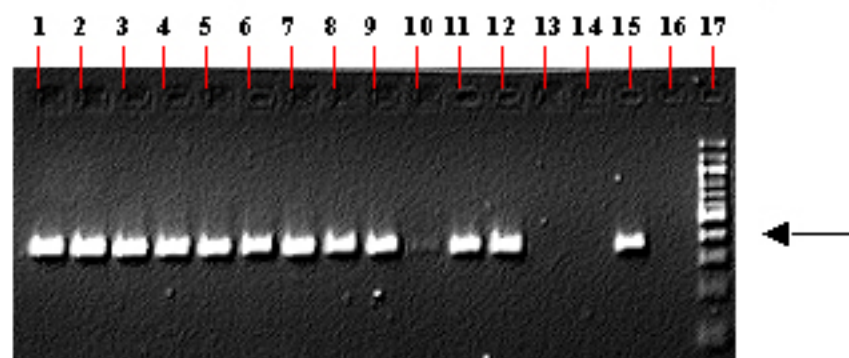


3' external probe
ApaL I digests
Wildtype 9.8 kb
Targeted 8.7 kb

* Clone achieving germline transmission

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



07/14/2004

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-0932 (LEXKO-1007)

Reference accession(s): XM 486093, AY648976

Standard KO or Conditional: Standard

Materials Submitted: Target Vector pKOS53NTV
 KOS clone(s) pKOS53

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	18/19	16/17
Restriction Enzyme for Genomic Digest:	HindIII	ApaLI
Predicted Wild-type Band (kb):	6.9 kb	9.8 kb
Predicted Mutant Band (kb):	5.4 kb	8.7 kb
Probe Size:	312 bp	178 bp

Primer sequences:

Southern probes

0932-16 5' – GAAGGTCTAATTTTCGTTACCC
0932-17 5' – CTCTGAACTCAAGCTAATGG
0932-18 5' – CTCAGCCTTGGAAGTTATACC
0932-19 5' – TGACCTGTTAACCGGGACTTG

Genomic Sequence Deleted :

CTCTACCAGAAGAGGGTACTGACAATTACTGGCATCTGTATCGCCCTGTTGGTGGTCGGCATCATGTGTGTGGTGGCCT
ACTGCAAAACCAAGTAAAGCTCCCCCTCGTCTCTGTCTCTCCCCCCCCCACA

KOS clone sequence: *(note: pKOS-53 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)

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