

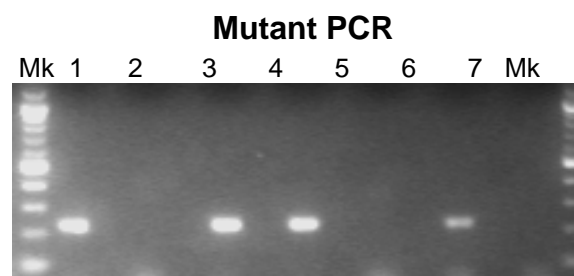
NIH-0394 Genotyping Strategies

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

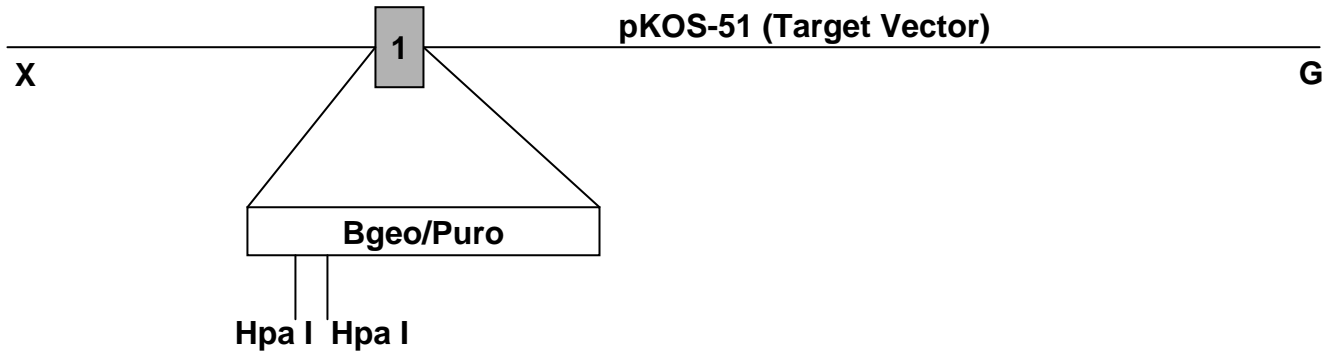
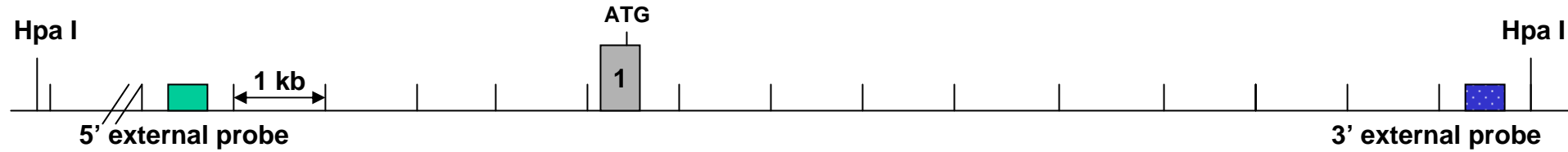
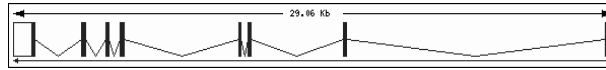
Step	Temp	Time	Note
1	96C	17"	
2	63C	15"	Decrease 1C/cycle
3	72C	15"	Go to 1, 6 cycles
4	96C	17"	
5	57C	15"	
6	72C	15"	Go to 4, 29 cycles

Primer Sequences (5' to 3')	
Mutant PCR: Primer 0394-22 and Primer 0394-23, 225 bp	
Recommended Wt PCR: Primer 0394-19 and Primer 0394-20, 108 bp	
Primer 0394-22	GATGCGGTGGGCTCTATG
Primer 0394-23	TGAACTAGCTAGCCGTCCGT
Primer 0394-19	AGACCCTCATCGCCGCCTA
Primer 0394-20	CAGACCCCTCGCGTGACAG



Well	Sample	Genotype
1	12	het
2	13	wt
3	14	het
4	30	het
5	wt lysate	wt
6	ES DNA	het
7	water	no amp



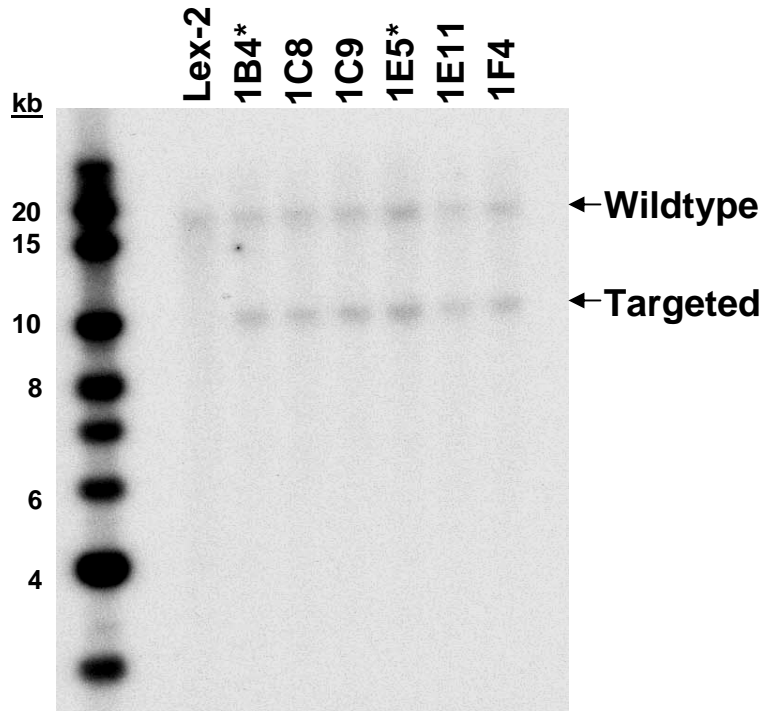
Targeting Strategy



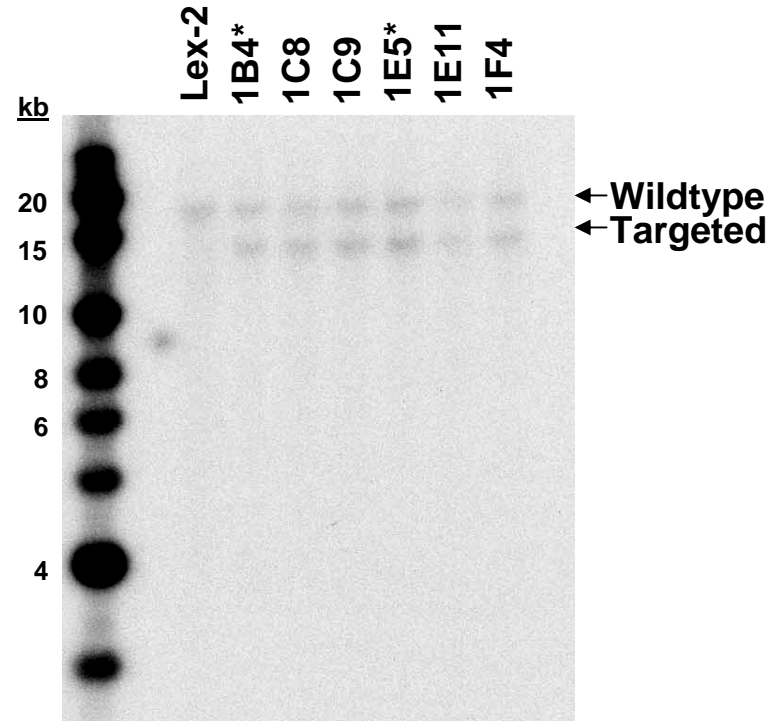
Southern Strategies

Probe	5' external 	3' external 
Enzyme	Hpa I	Hpa I
Wildtype	18.6 kb	18.6 kb
Targeted	10.2 kb	14.0 kb

Southern Data



5' external probe
Hpa I digests
Wildtype 18.6 kb
Targeted 10.2 kb

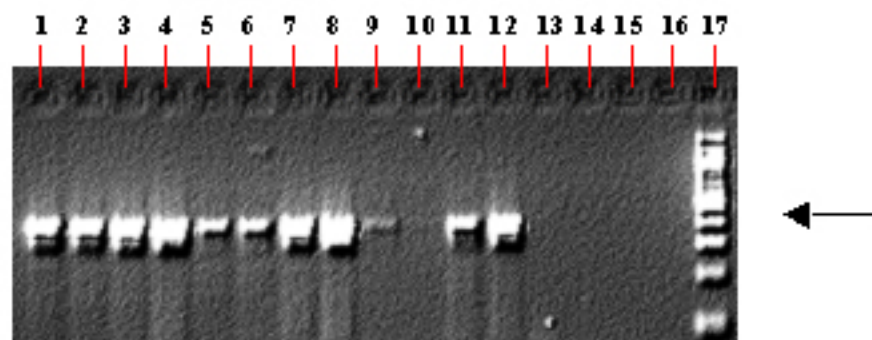


3' external probe
Hpa I digests
Wildtype 18.6 kb
Targeted 14.0 kb

* clones achieving germline transmission

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



06/21/2004

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-0394 (LEXKO-1018)

Reference accession(s): NM_026384

Standard KO or Conditional: Standard

Materials Submitted: x Target Vector pKOS-51TVpuro
x KOS clone(s) pKOS-51

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	15+16	11+12
Restriction Enzyme for Genomic Digest:	HpaI	HpaI
Predicted Wild-type Band (kb):	18.6	18.6
Predicted Mutant Band (kb):	10.2	14.0
Probe Size:	589 bp	469 bp

Primer sequences:

Southern probes

0394-15 5' – GTGTCATGTAGGGATGTCTG
0394-16 5' – GCAACTGTGTACATAGCCTTG
0394-11 5' – ACAGTGTAGGCGGAACA
0394-12 5' – TGGTTGTCTATAAGCGCAGTC

Genomic Sequence Deleted):

TGAAGACCTCATCGCCGCTACTCCGGGGTCTGCGGGGTGAGCGTCGGGCGGAAGCTGCCCCGAGCGAAAACAAGA
ATAAAGGATCTGCCCTGTCACGCGAGGGTCTGGGCGATGGGGTGAGTGCA

KOS clone sequence: (note: pKOS-##51 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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