

NIH-0769 Genotyping Strategies

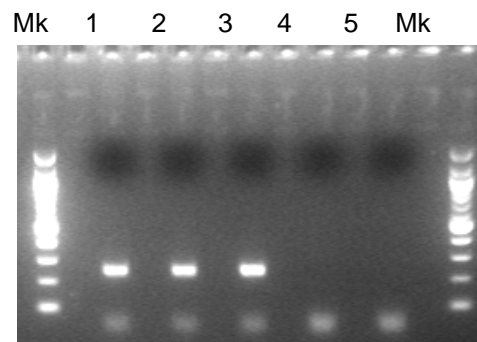
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

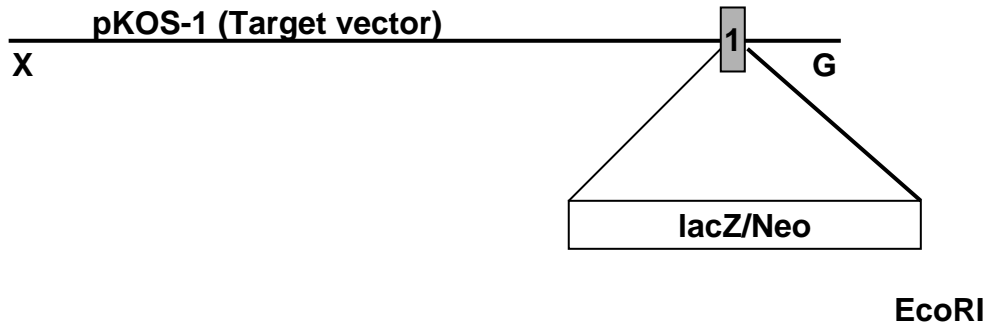
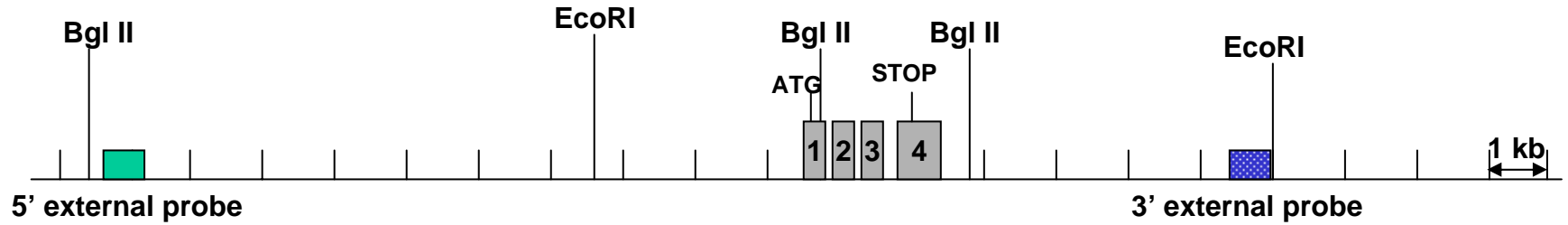
Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0769-3, 250 bp	
Recommended Wt PCR: Primer 0769-1 and Primer 0769-3, 307 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0769-3	CTTCCAACCCTCCAGATCC
Primer 0769-1	TGCAGATCACAGAGCCAGC

Well	Sample	Genotype
1	111	het
2	119	het
3	ES DNA	het
4	wt lysate	wt
5	water	no amp



Mutant PCR



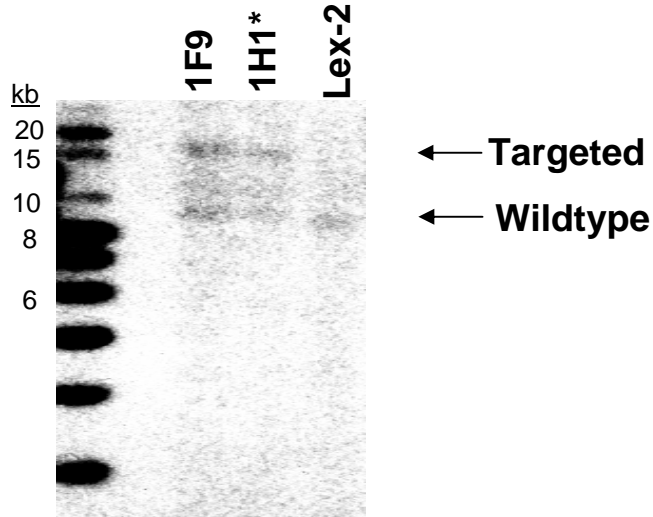
Targeting Strategy



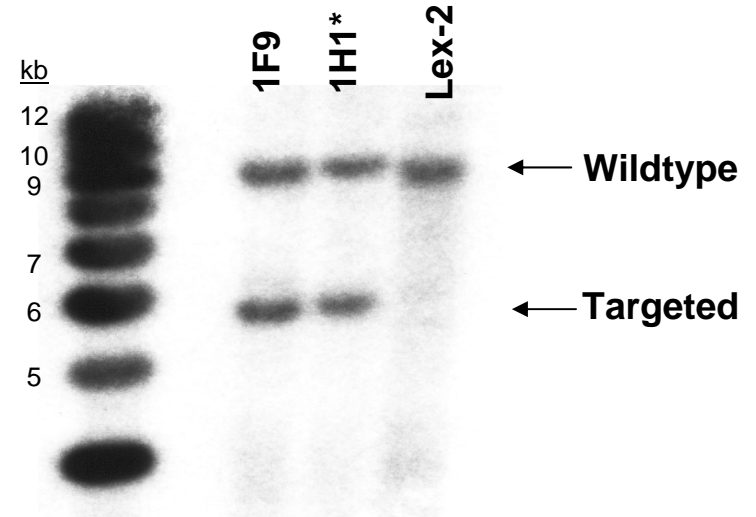
Southern Strategies

Probe	5' external 	3' external 
Enzyme	Bgl II	EcoRI
Wildtype	9.5 kb	9.3 kb
Targeted	16.8 kb	5.9 kb

Southern Data



5' external probe
 Bgl II digests
 Wildtype: 9.5 kb
 Targeted: 16.8 kb

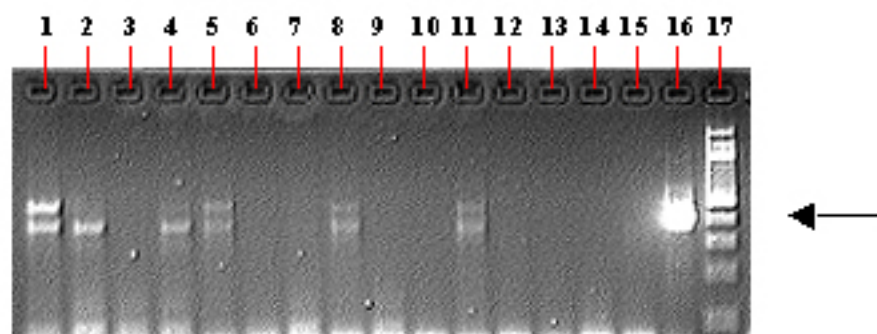


3' external probe
 EcoRI digests
 Wildtype: 9.3 kb
 Targeted: 5.9 kb

* 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-0769 (LEXKO-1052)

Reference accession(s): NM_031252

Standard KO or Conditional: Standard

Materials Submitted: X Target Vector: pKOS-1.TVneo
X KOS clone(s): pKOS-1

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	30-31	27-28
Restriction Enzyme for Genomic Digest:	Bgl II	EcoRI
Predicted Wild-type Band (kb):	9.5 kb	9.3 kb
Predicted Mutant Band (kb):	16.8 kb	5.9 kb
Probe Size:	270 bp	577 bp

Primer sequences:

Southern probes

0769-30 5' – CAATCCATACGGAGTCAATGG
0769-31 5' – AACCTTTTGAGAGCAAATGAGG
0769-27 5' – GATGACCTAATGCCTGTACC
0769-28 5' – GAACCCAGGAGGCAATTGC

Genomic Sequence Deleted:

AGAGTTAGACTCAGAACCAAAGGAGGTGGATAGGGGGTCCACAGGCCTGGTGCAGATCACAGAGCCAGCCAGATCTG
AGAAGCAGGGAACAAGATGCTGGATTGCAGAGCAGTAATAATGCTATGGCTGTTGCCCTGGGTCCTCAGGGCCTGGC
TGTGCCTAGGAGTAGCAGTCTGACTGGGCTCAGTGCCAGCAGCTCTCTCGGAATCTCTGCATGCTAGCCTGGAACGCA
CATGCACCAGCGGGACATATGGTAAGTGTCA

KOS clone sequence: (note: pKOS-1 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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