NIH-0013 Genotyping Strategies

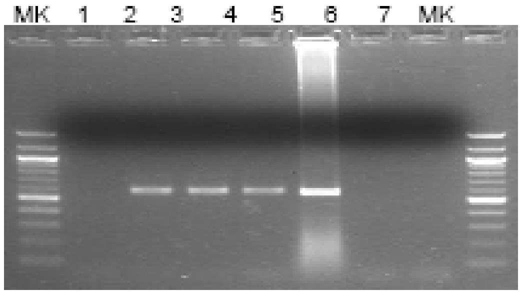
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl2	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

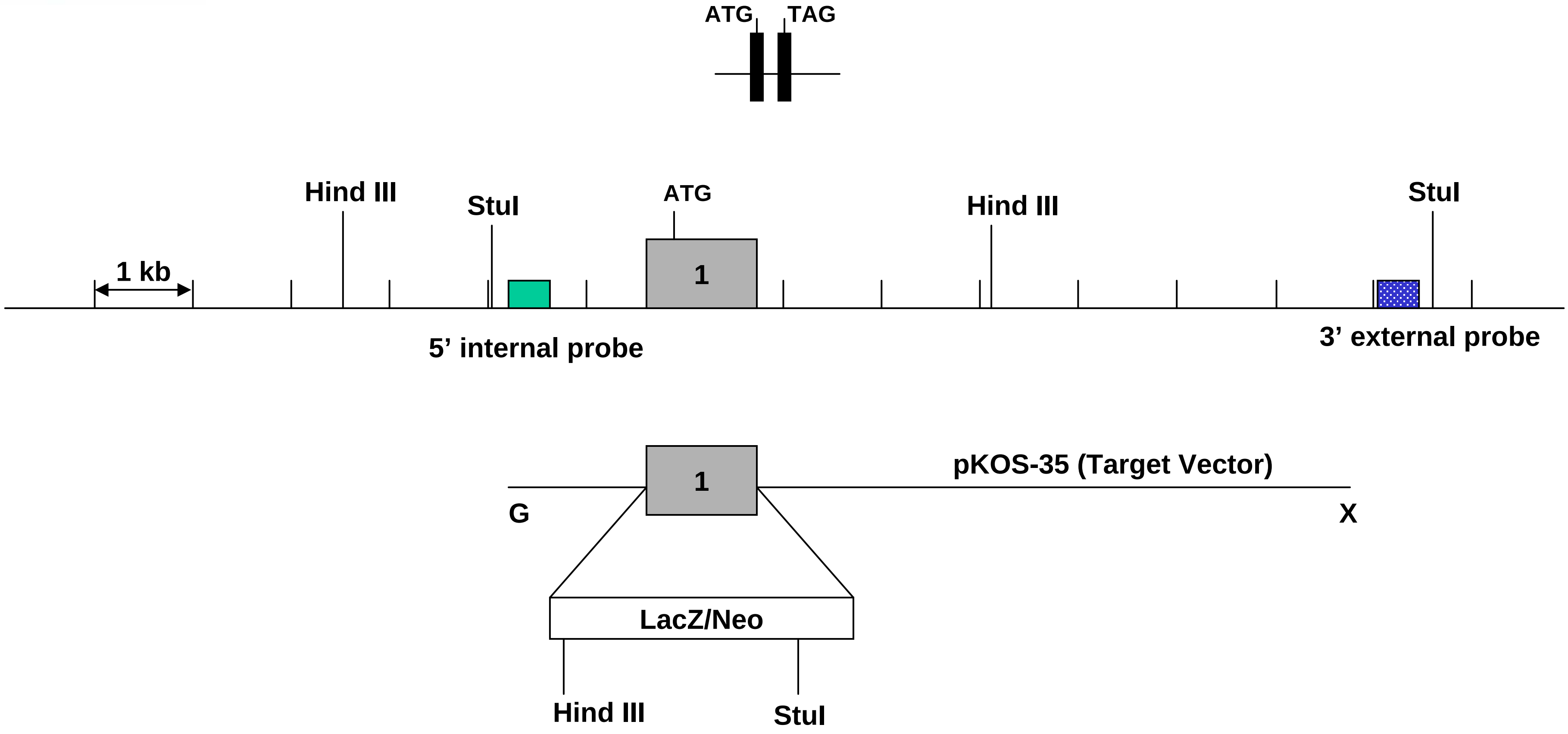
Primer Sequences (5' to 3'):	
Mutant PCR: Primer neo3a and Primer 0013-12, 570 bp	
Recommended Wt PCR: Primer 0013-1 and Primer 0013-6, 600 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0013-12	GTGCTGGGATTAGAAGTCC
Primer 0013-1	CTCTGCAGTCGGTTTGATG
Primer 0013-6	GAGCATCATAGGCTTCGCC

Well	Sample	Genotype
1	10	wt
2	11	het
3	53	het
4	92	het
5	**ES DNA**	het
6	wt lysate	wt
7	water	no amp

Mutant PCR



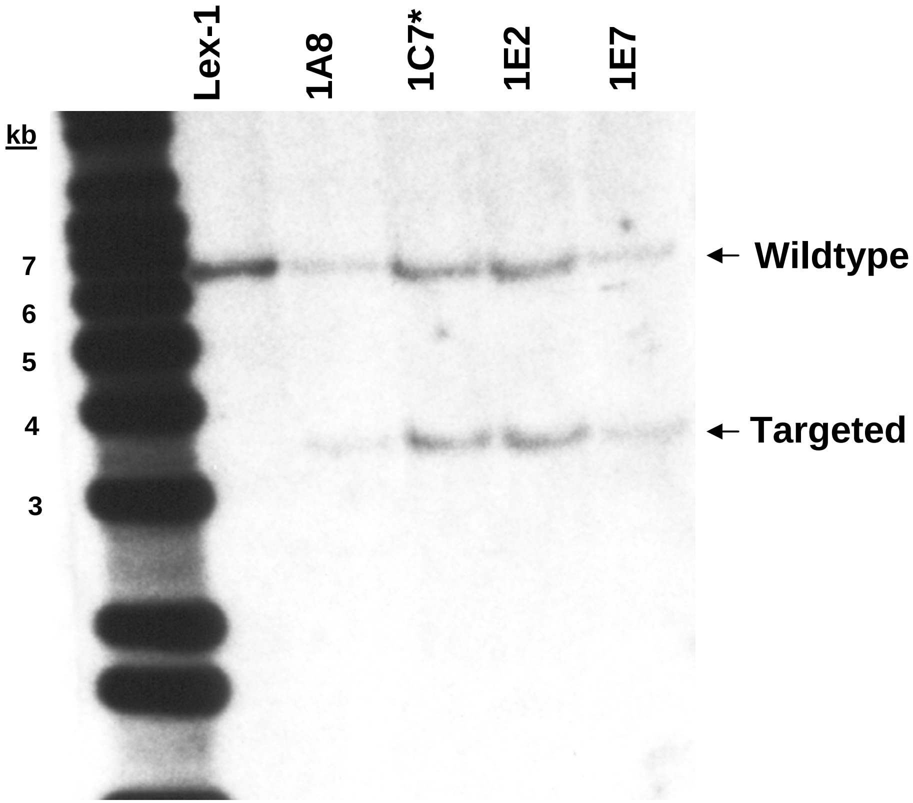
Targeting Strategy



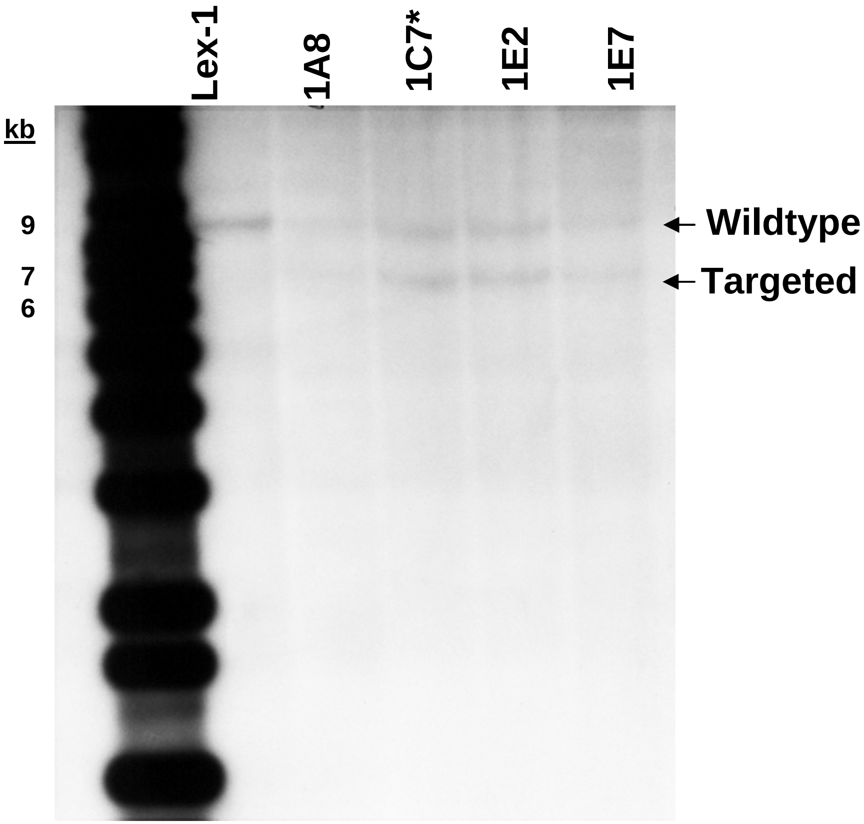
Southern Strategies

Probe	5' internal 🟩	3' external 🟦
Enzyme	Hind III	StuI
Wildtype	6.8 kb	9.2 kb
Targeted	3.6 kb	7.1 kb

Southern Data



5’ Internal probe
HindIII digests
Wildtype : 6.8 kb
Targeted: 3.6 kb

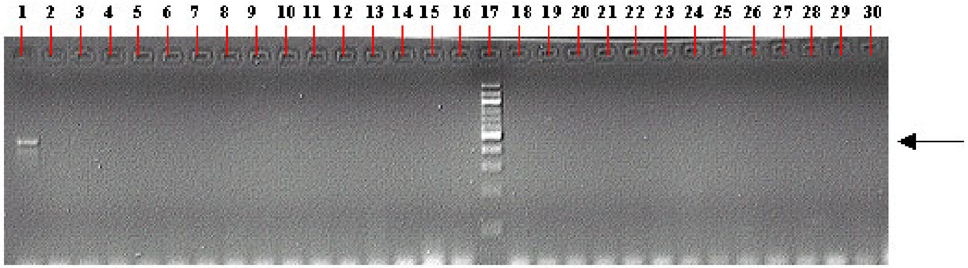


3’ External probe
StuI digests
Wildtype: 9.2 kb
Targeted: 7.1 kb

* Clone achieving germline transmission

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: mGPR64ST1-L2



11/06/2003

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/Lex1 DNA
17) 100 bp ladder/marker
18) Asthmatic Lung
19) LPS Liver
20) Blood
21) Banded Heart
22) Aortic Tree
23) Skin Fibroblast
24) Prostate
25) MG 5 week virgin
26) MG mature virgin
27) MG 12 DPC
28) MG 3 day post-partum (lactating)
29) BLANK
30) MG 7 day post-wearing (late involution)

LEXICON

Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: _ NIH-0013 (LEXKO-003)

Reference accession(s): _ NM_010483

Standard KO or Conditional: _ Standard KO

Materials Submitted: X Target Vector _ pKOS-35TVneo_________
X KOS clone(s)__ pKOS-35_________

Southern Blot Genotyping Strategies:

	5' Internal	**3' External**
Name of Probe:	21+23	16+17
Restriction Enzyme for Genomic Digest:	Hind III	StuI
Predicted Wild-type Band (kb):	6.8	9.2
Predicted Mutant Band (kb):	3.6	7.1
Probe Size:	253 bp	332 bp

Primer sequences:

Southern probes

0013-21	5' – CAGCCTGGAGTTGTTAGC
0013-23	5' – GTTAGACACTGAGACATTGT
0013-16	5' – GGACAGACTGAAGTGCCTC
0013-17	5' – CCTGTACTCACTCCTTGGC

Genomic Sequence Deleted:

GGGCAAAGCTGGACTAAGGAGTCTCATCTGGAAAAGAGGGTCCACGAAAAAGGCCAAAAGAAGCGCCAAAAGGAGGC
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KOS clone sequence: *(note: pKOS-35 was used to generate the TV and that is the sequence included here)*

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

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