NIH-0694 Genotyping Strategies

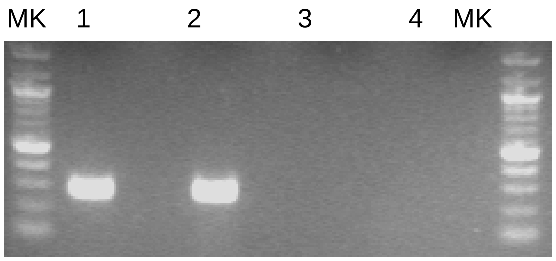
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl2	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

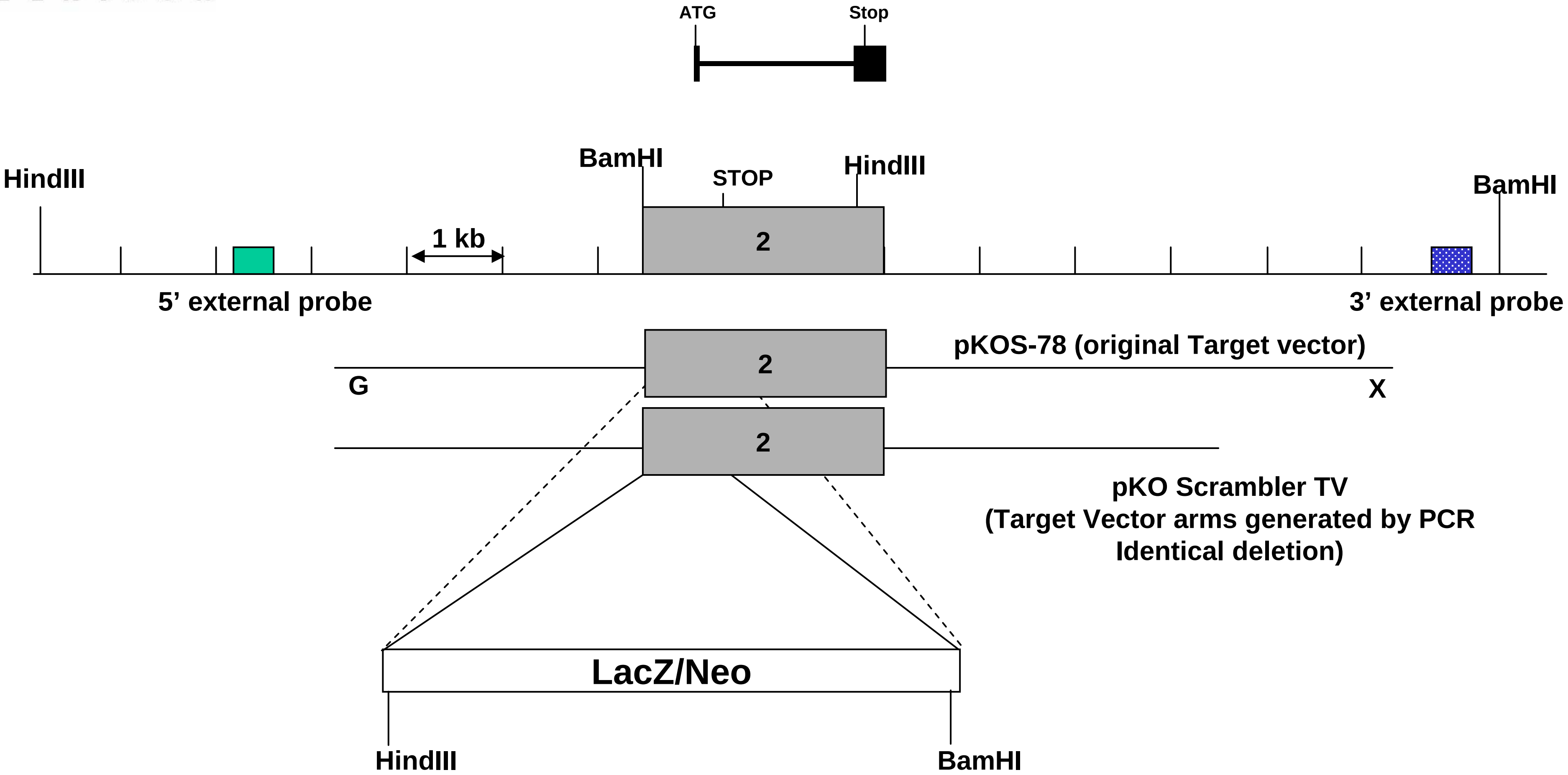
Primer Sequences (5' to 3'):	
Mutant PCR: Primer Neo3a and Primer 0694-13, 268 bp	
Recommended Wt PCR: Primer 0694-17 and Primer 0694-13, 647 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0694-17	GTGTTCACCAGATCGCAGC
Primer 0694-13	CTGCTTCTTACTGTGCTCC

Well	Sample	Genotype
1	524	het
2	**ES DNA**	het
3	**wt lysate**	wt
4	**water**	no amp

Mutant PCR



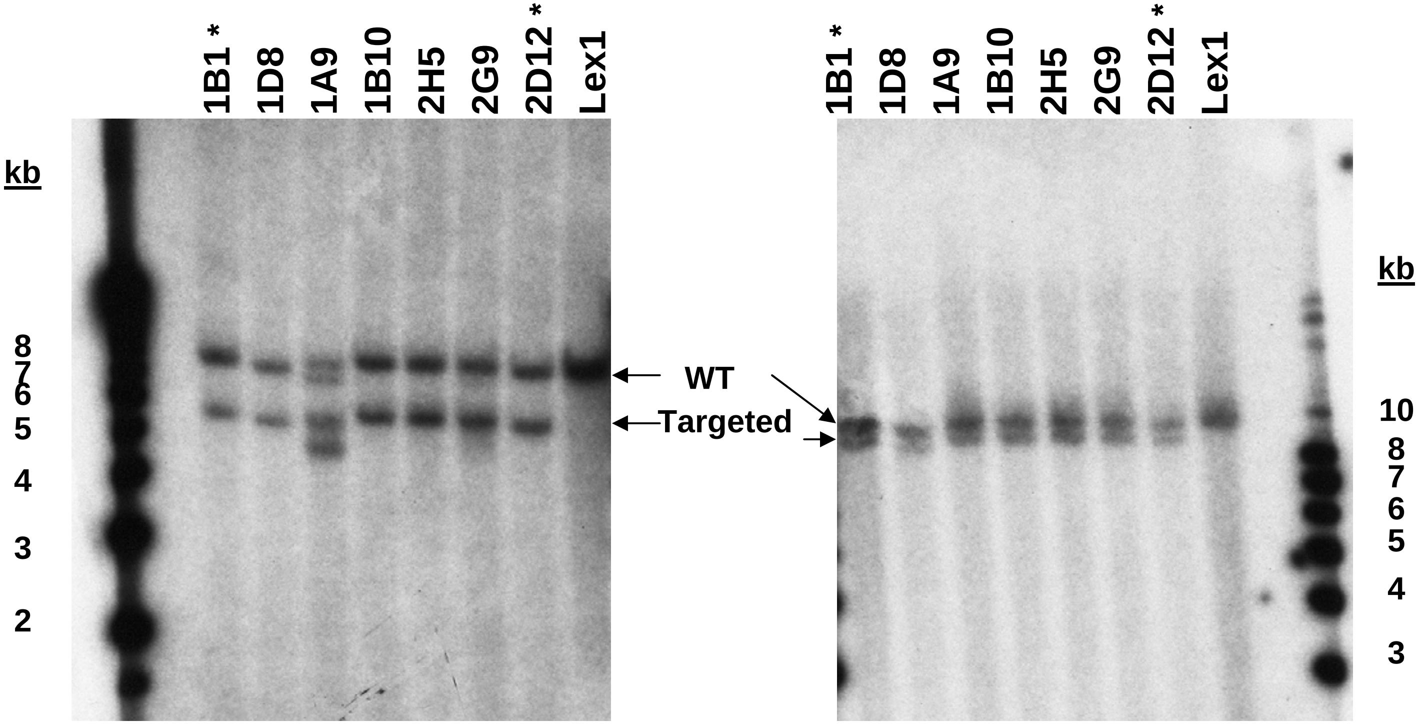
Targeting Strategy



Southern Strategies

Probe	5’ external 🟩	3’ external 🟦
Enzyme	Hind III	BamHI
Wildtype	7.4 kb	9.0 kb
Targeted	5.3 kb	8.3 kb

Southern Data



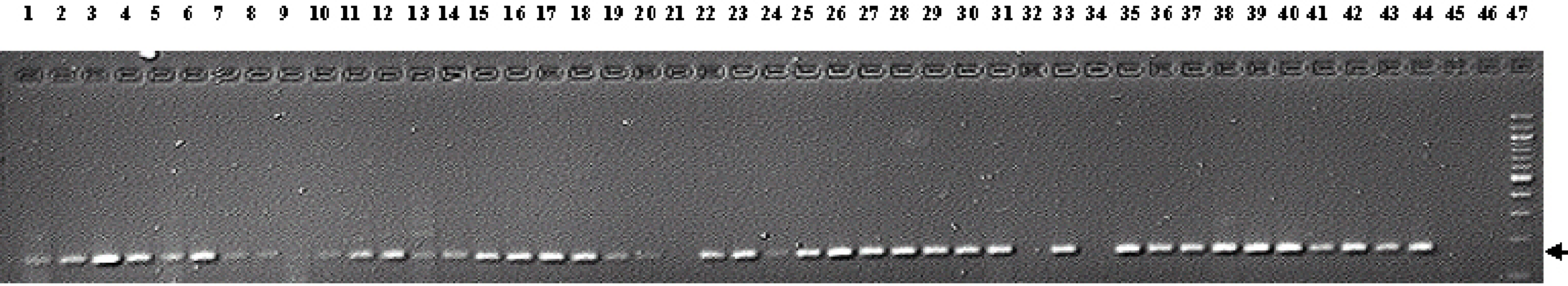
5’ external probe
Hind III digests
WT: 7.4 kb
Targeted: 5.3 kb

3’ internal probe
BamH I digests
WT: 9.0 kb
Targeted: 8.3 kb

*** Clones achieving germline transmission**

RT-PCR Analysis

mouse random primed cDNA with Primers: 3,4



10/18/2003

Note: mouse ES cDNA used as positive (+) control. 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	Adrenal Gland
9	Pancreas
10	Salivary Gland
11	Stomach
12	Small Intestine
13	Colon
14	Skeletal Muscle
15	Heart
16	Uterus
17	Adipose
18	Bladder
19	Ovary
20	Gall Bladder
21	Lymph node
22	Cerebellum
23	Esophagus
24	Prostate
25	Tongue
26	Thyroid
27	Bone Marrow
28	Spinal Cord
29	Trachea
30	Aorta
31	Whole Blood
32	Eye
33	Pituitary gland
34	Skin
35	Nasal Epithelium
36	Whole Bone (femur)
37	Mammary Gland
38	Placenta
39	9.5 day Embryo
40	12.5 day Embryo
41	Fetal Brain
42	Fetal Lung
43	Fetal Liver
44	Fetal Kidney
45	(-) Control
46	(+) Control
47	100 bp ladder/marker

Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: NIH-0694 (LEXKO-939)

Reference accession(s): NM_013526

Standard KO or Conditional: Standard

Materials Submitted: ☒ Target Vector pKOS Scrambler TV
☒ KOS clone(s) pKOS-78

Southern Blot Genotyping Strategies:

	5' External	**3' External**
Name of Probe:	6/6R	14/16
Restriction Enzyme for Genomic Digest:	HindIII	BamHI
Predicted Wild-type Band (kb):	7.4	9.0
Predicted Mutant Band (kb):	5.3	8.3
Probe Size:	427	690

Primer sequences:

Southern probes

0694-6	5' – CTGTAGAGAGTAACTACCTC
0694-6R	5' – CACACCATATACACCAAATAC
0694-14	5' – GTGGAGATGACAGGTCAAGG
0694-16	5' – GTGTGAAGGATGCTAAGAAGC

Genomic Sequence Deleted:

CACACTCCTCTCCGGAGACAGAAGTATTTGTTTGATGTGTCCACACTCTCAGACAAAGAAGAGCTGGTGGGCGCAGAGCTAAGGCTTTATCGCCAGGC
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ATGTAGTCTACAAGCAGTATGAGGACATGGTGGTGGAGTCCTGCGGCTGTAGGTAGCGGTG

KOS clone sequence: *(note: This is the genomic sequence spanned by pKO Scrambler TV)*

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

LacZ/Neo

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