NIH-0778 Genotyping Strategies

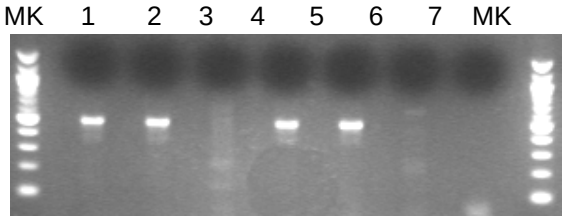
Reaction Components	Vol (ul)
5x Phusion buffer	8
25mM MgCl2	3.2
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
Phusion Enzyme	0.1
Water	20.7
Total mix volume	35
Tail lysate (1:20 dilution)	5
Total reaction volume	40

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

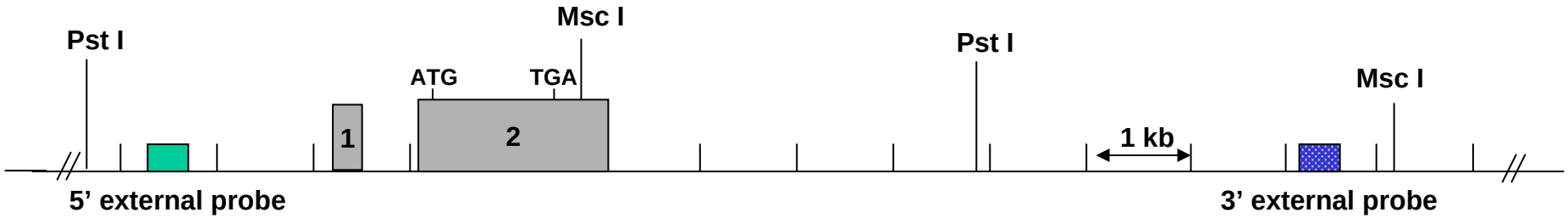
Primer Sequences (5' to 3'):	
Mutant PCR: Primer Neo3a and Primer 0778-9, 499 bp	
Recommended Wt PCR: Primer 0778-3 and Primer 0778-4, 603 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0778-9	CCCAGGTGCCCCTTCATAATG
Primer 0778-3	GCCGGCGCTCACATAGATG
Primer 0778-4	GCCACAGCTGCCTGCGGAAGT

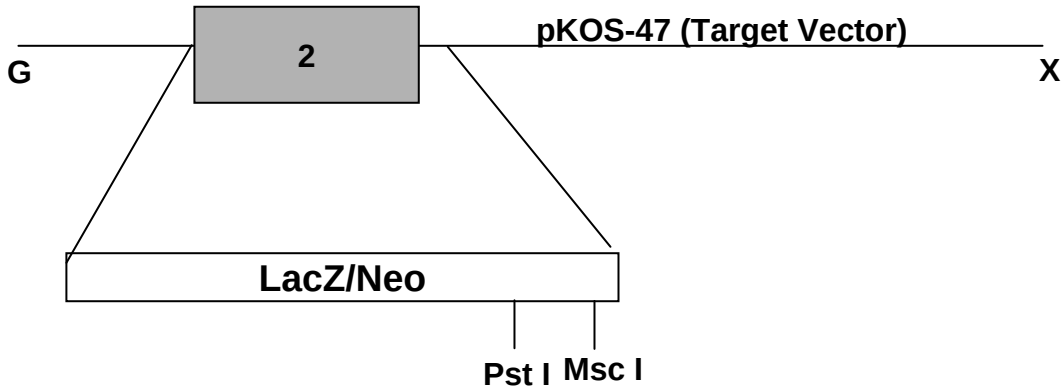
Well	Sample	Genotype
1	116	het
2	117	het
3	118	wt
4	119	het
5	**ES DNA**	het
6	**wt lysate**	wt
7	**water**	no amp

Mutant PCR



Targeting Strategy

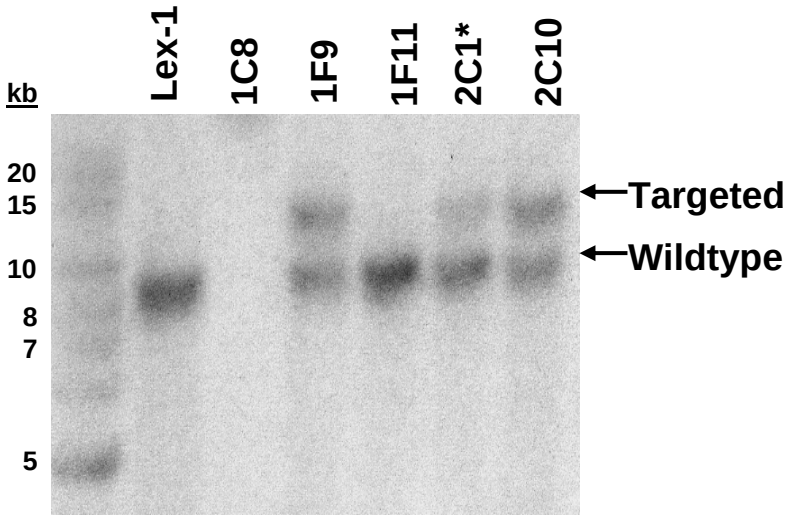




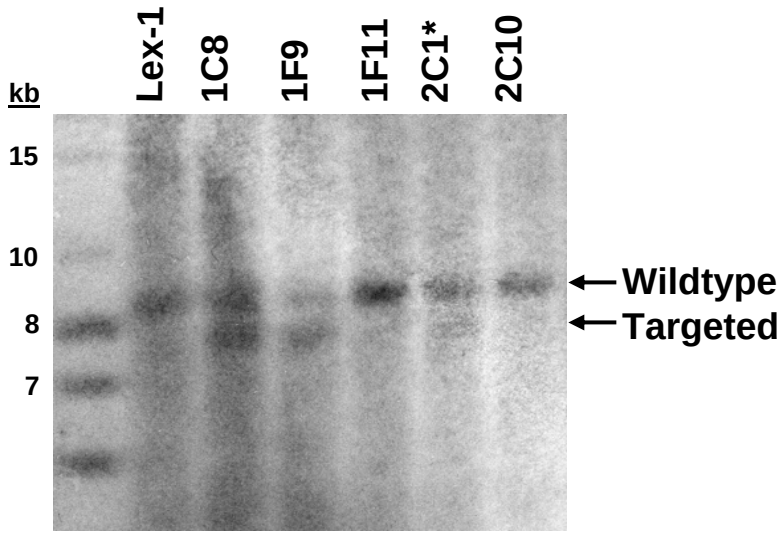
Southern Strategies

Probe	5' external	3' external
Enzyme	Pst I	Msc I
Wildtype	9.4 kb	8.8 kb
Targeted	13.3 kb	8.0 kb

Southern Data



5' external probe
Pst I digests
Wildtype 9.4 kb
Targeted 13.3 kb

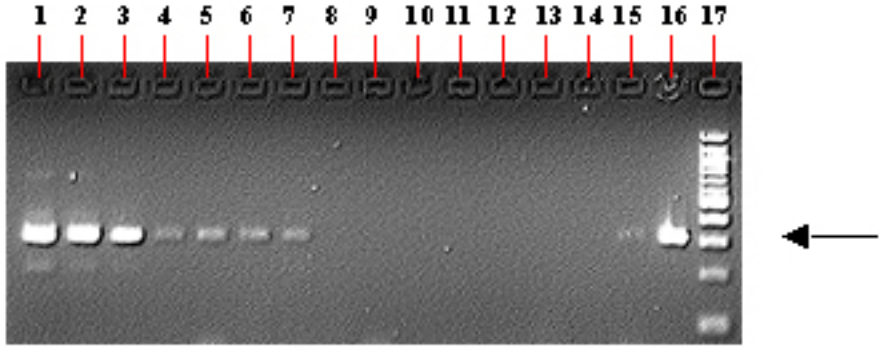


3' external probe
Msc I digests
Wildtype 8.8 kb
Targeted 8.0 kb

***Clone achieving germline transmission**

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



08/09/2004

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/Lex1 DNA
17) 100 bp ladder/marker

Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: **NIH-0778** (LEXKO-951)

Reference accession(s): NM_008436

Standard KO or Conditional: Standard

Materials Submitted: ☐ Target Vector pKOS47NTV
☐ KOS clone(s) pKOS47

Southern Blot Genotyping Strategies:

	5' External	**3' External**
Name of Probe:	22/23	26/27
Restriction Enzyme for Genomic Digest:	PstI	XbaI
Predicted Wild-type Band (kb):	9 kb	9.7 kb
Predicted Mutant Band (kb):	12 kb	14.1 kb
Probe Size:	690 bp	433 bp

Primer sequences:

Southern probes

0778-22	5' – AAGCTGCCATGATTGTAGGTA
0778-23	5' – TCCGAGTCAATGCACTAAAG
0778-26	5' – AACGGACTTGCCACTAGACCC
0778-27	5' – AAGATGTCAGTGCGGTGAAG

Genomic Sequence Deleted:

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KOS clone sequence: *(note: pKOS-47 was used to generate the TV and that is the sequence included here)*

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C

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

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