NIH-0600 Genotyping Strategies

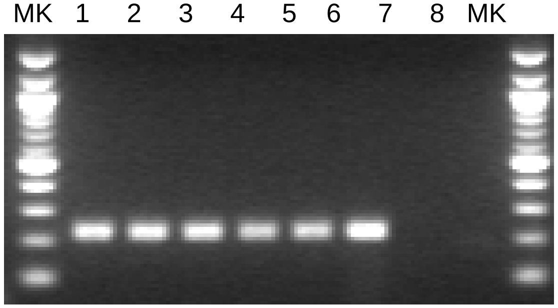
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

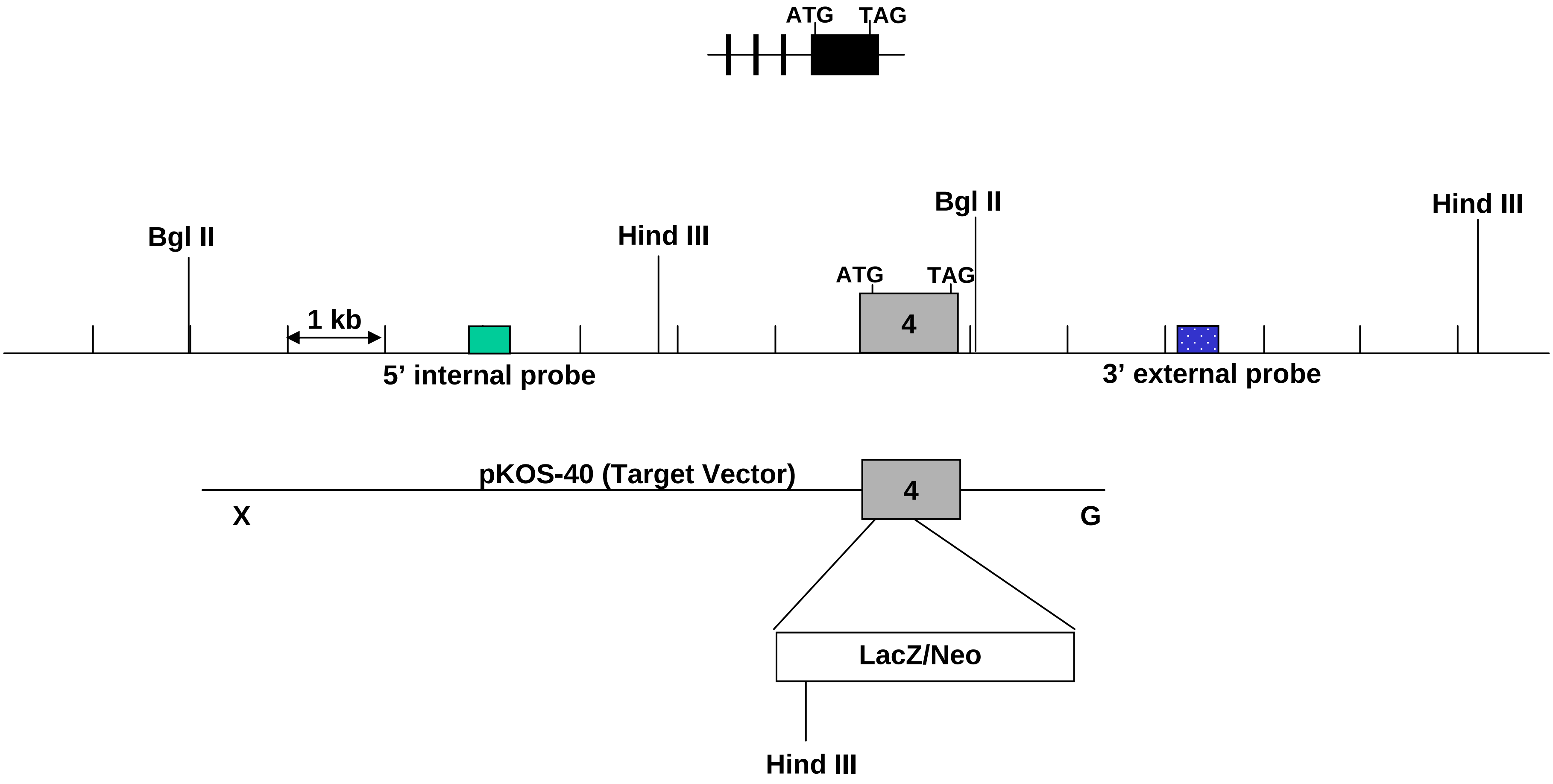
Primer Sequences (5’ to 3’):	
Mutant PCR: Primer Neo3a and Primer 0600-39, 224 bp	
Recommended Wt PCR: Primer 0600-3 and Primer 0600-6, 322 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0600-39	TTCTCGGTAATGGAGGTACAG
Primer 0600-3	TATATAATTCCATTCTAGCTCC
Primer 0600-6	CAGTCTGCATTGTACAGATTG

Well	Sample	Genotype
1	259	het
2	268	het
3	287	het
4	297	het
5	307	het
6	**es DNA**	het
7	**wt lysate**	wt
8	**water**	no amp

Mutant PCR



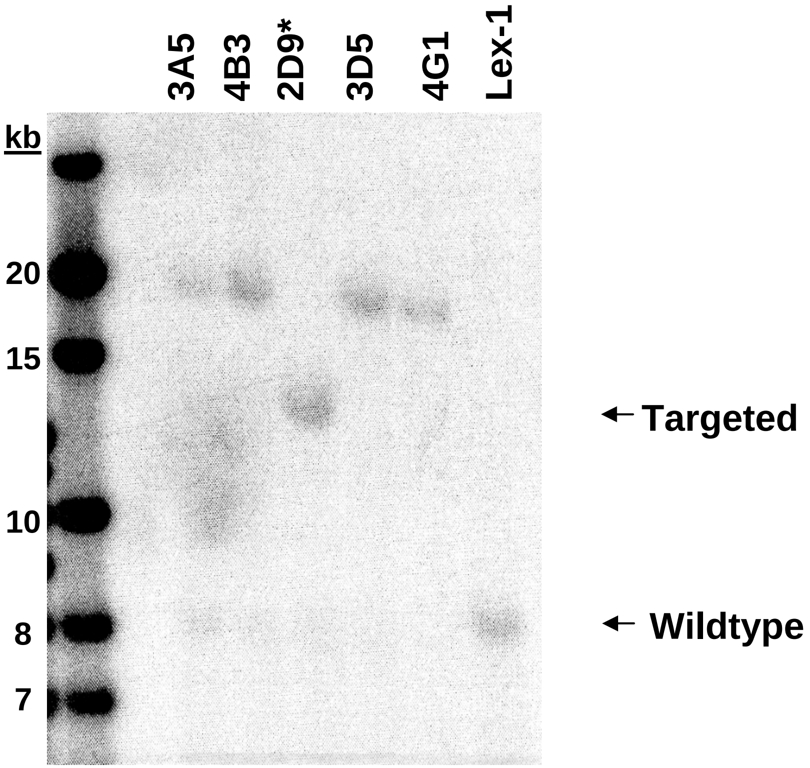
Targeting Strategy



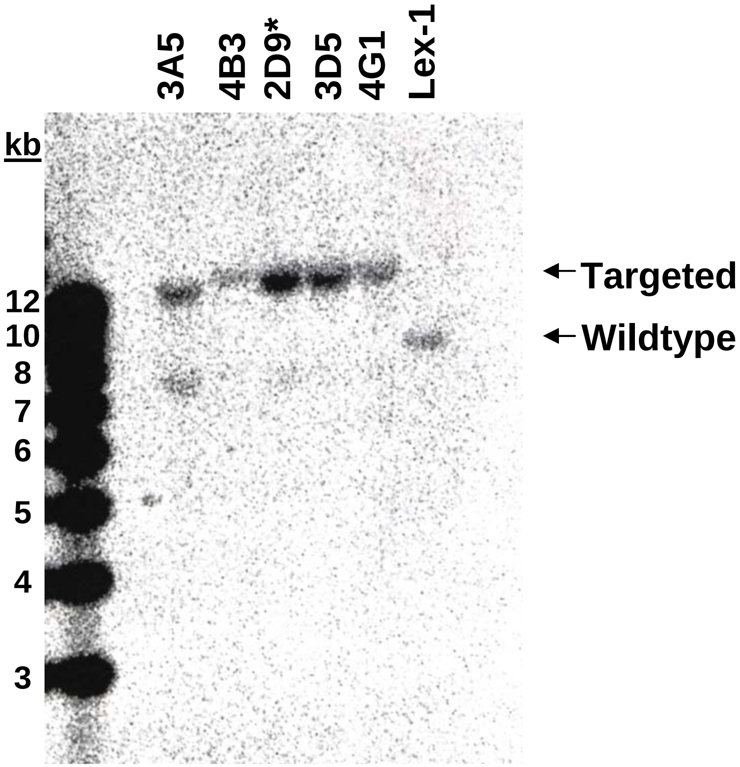
Southern Strategies: X linked gene

Probe	5’ internal 🟩	3’ external 🟦
Enzyme	Bgl II	Hind III
Wildtype	8.0 kb	8.5 kb
Targeted	12.9 kb	12.0 kb

Southern Data



5’ internal probe
Bgl II digests
Wildtype 8.0 kb
Targeted 12.9 kb



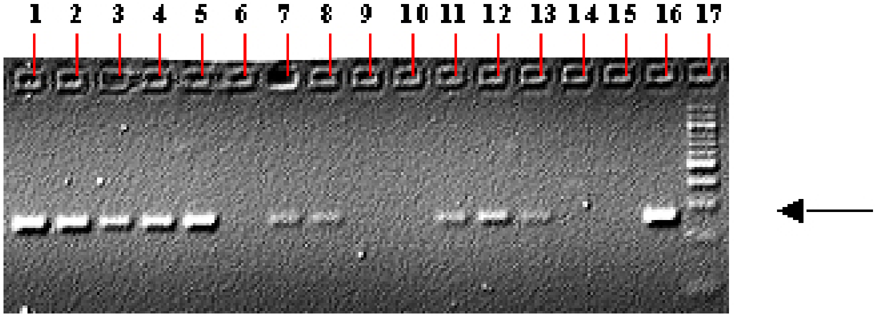
3’ external probe
Hind III digests
Wildtype 8.5 kb
Targeted 12.0 kb

- **Clone achieving germline transmission**

X-linked gene

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



02/16/2004

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

Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: **NIH-0600** (LEXKO-915)

Reference accession(s): NM_175669

Standard KO or Conditional: Standard

Materials Submitted:
☒ Target Vector pKOS-40 TV Neo
☒ KOS clone(s) pKOS-40

Southern Blot Genotyping Strategies:

	5' Internal	**3' External**
Name of Probe:	33/34	37/38
Restriction Enzyme for Genomic Digest:	BglII	HindIII
Predicted Wild-type Band (kb):	8.0	8.5
Predicted Mutant Band (kb):	12.9	12.0
Probe Size:	604	361

Primer sequences:

Southern probes

0600-33	5' – TTTTATTTCTCAGACACATCA
0600-34	5' – TGGCTAGAACTGTAATCAACC
0600-37	5' – TGGGAAAGAACTGGGAGTATG
0600-38	5' – TGGTTTCCTTTGTAGCCTTTG

Genomic Sequence Deleted:

ATGCCTTTCATGGGTATATATTTCTTGAGAGGTTTTTATTGGAAATATCAATCTGTACAATGCAGACTGGTCAATTTTTTGGGAACTCTTTCTATGCATGT
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KOS clone sequence: *(note: pKOS-40 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)*

LacZ/Neo

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