

NIH-0719 Genotyping Strategies

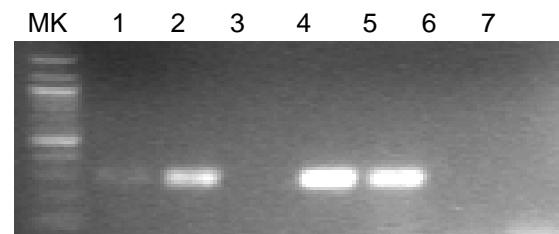
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

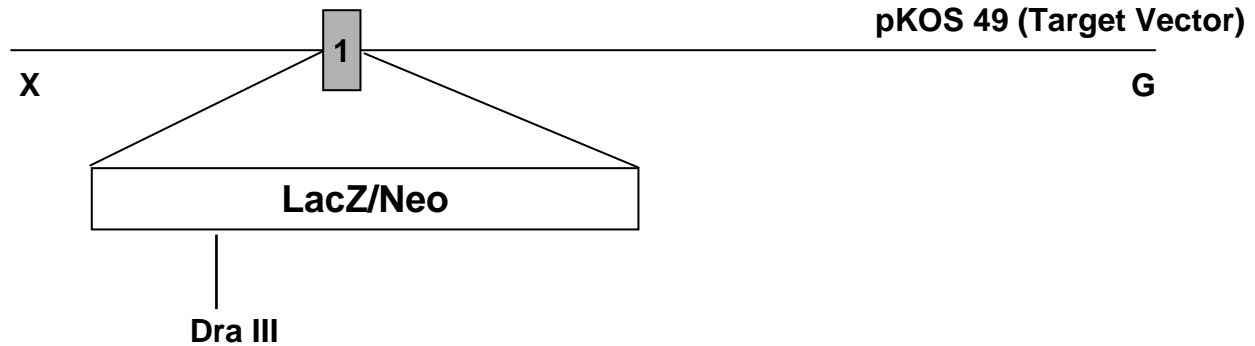
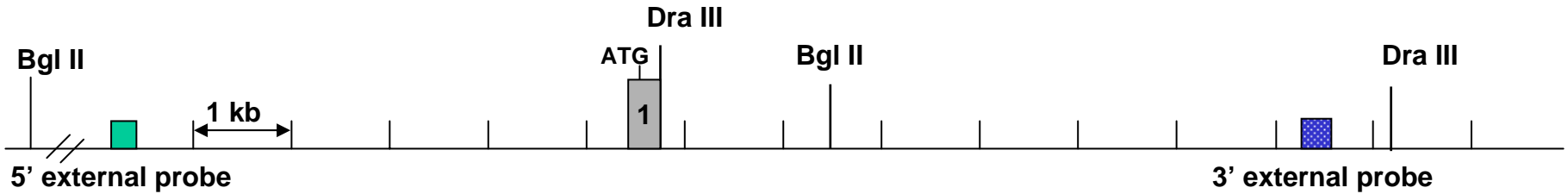
Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0719-7, 296 bp	
Recommended Wt PCR: Primer 0719-34 and Primer 0719-35, 298 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0719-7	CTAAGATAGGCTCTAGTG
Primer 0719-34	GTTCGGCCAGGTTTGTACC
Primer 0719-35	CAGCTGGTGAGCTCGGTC

Well	Sample	Genotype
1	35	het
2	36	het
3	37	wt
4	38	het
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



Mutant PCR



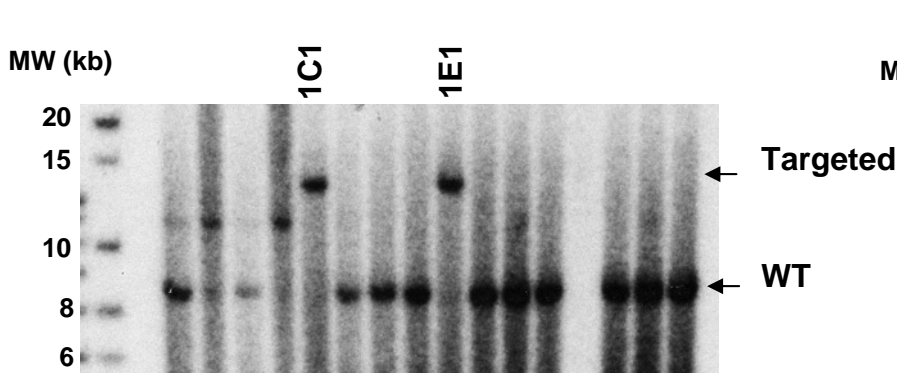
Targeting Strategy



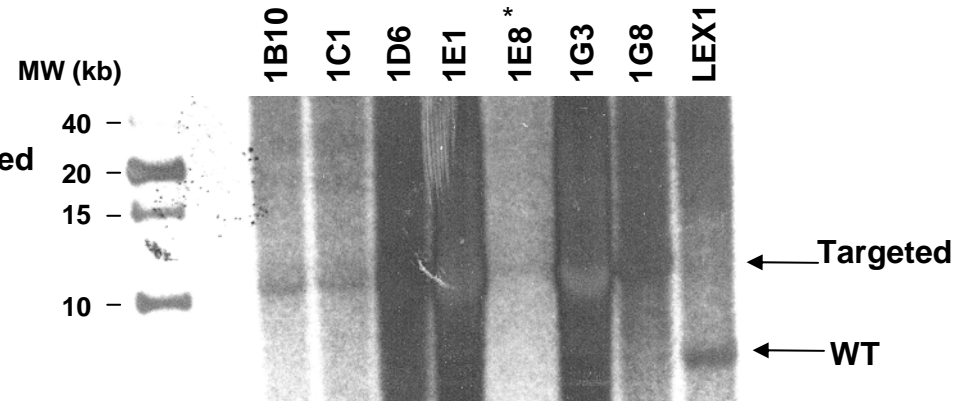
Southern Strategies: X-linked gene

Probe	5' external 	3' external 
Enzyme	Bgl II	Dra III
Wildtype	9 kb	7.5 kb
Targeted	13.5 kb	10.7 kb

Southern Data



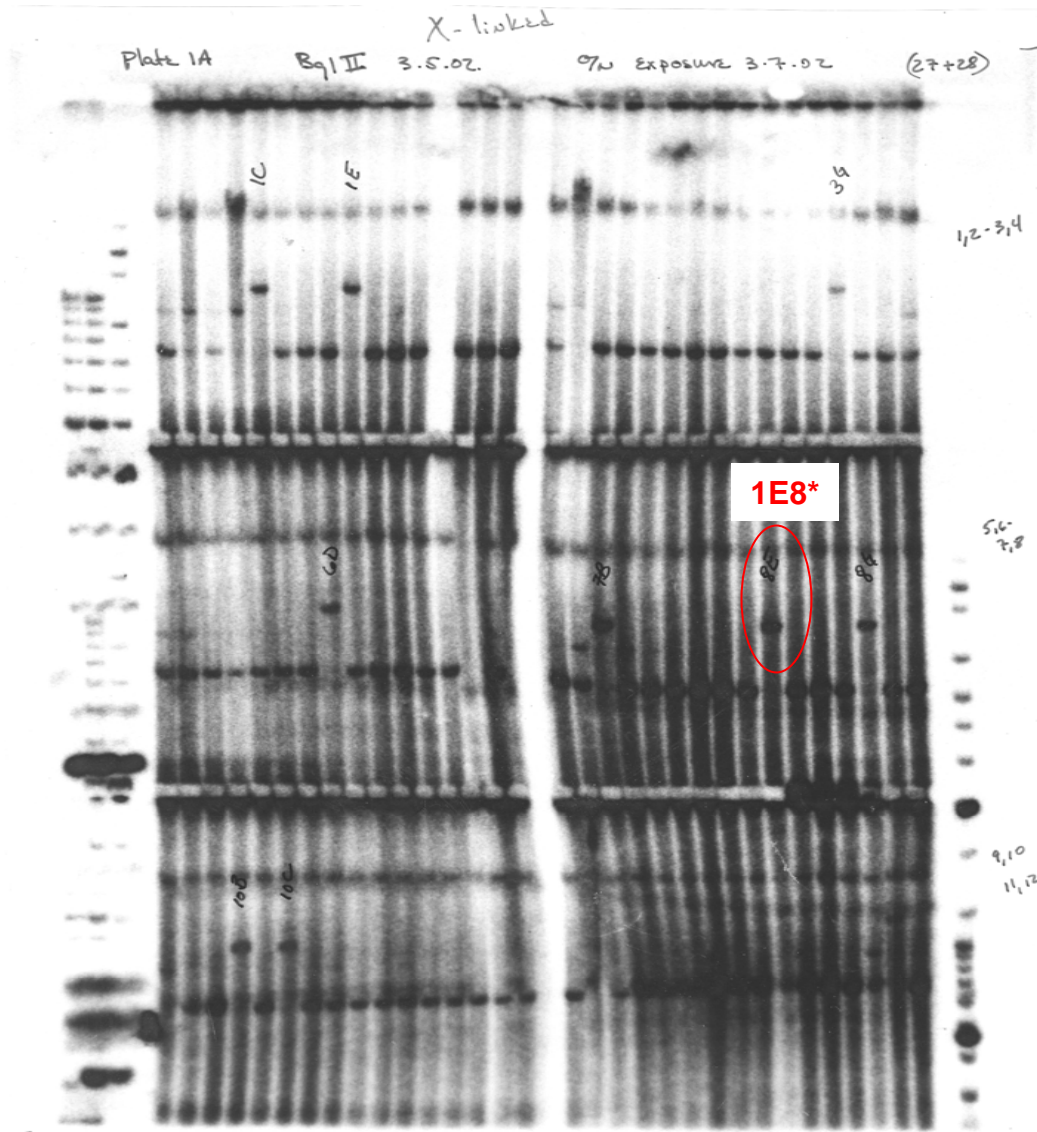
5' external probe
 Bgl II digests
 Wildtpe 9 kb
 Targeted 13.5 kb



3' internal probe
 Dra III digests
 Wildtype 7.5 kb
 Targeted 10.7 kb

Note: X-linked gene
 *Clone achieving germline transmission

Additional Southern Data

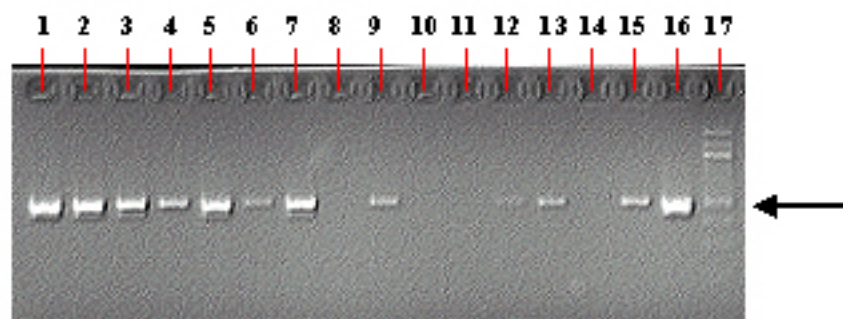


5' external probe
 Bgl II digests
 Wildtype 9 kb
 Targeted 13.5 kb

Note: X-linked gene
 *Clone achieving germline transmission

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 3,4



03/15/2004

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-0719 (LEXKO-290)

Reference accession(s): NM_015819

Standard KO or Conditional: Standard KO

Materials Submitted: Target Vector: pKOS-49-TVneo
 KOS clone(s): pKOS-49

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	28+27	26+24
Restriction Enzyme for Genomic Digest:	BglII	DraIII
Predicted Wild-type Band (kb):	9 kb	7.5 kb
Predicted Mutant Band (kb):	13.5 kb	10.7 kb
Probe Size:	532 bp	778 bp

Primer sequences:

Southern probes

0719-24 5' – CTTGTCAGTACTGGCTAC
0719-26 5' – GGACCCATGTGAGTATGC
0719-27 5' – GCATGCAAATGGTAGTGCC
0719-28 5' – GAAGGCTCACTTGAGATTGG

Genomic Sequence Deleted:

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KOS clone sequence:

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

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