NIH-0739 Genotyping Strategies

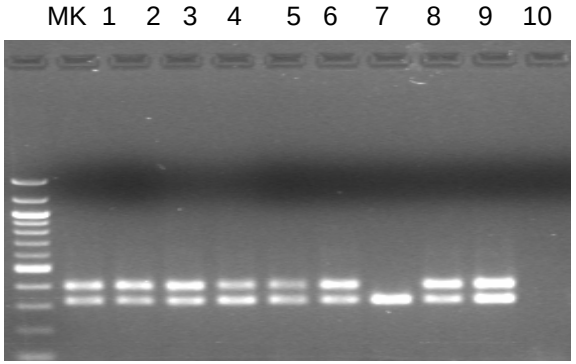
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl2	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

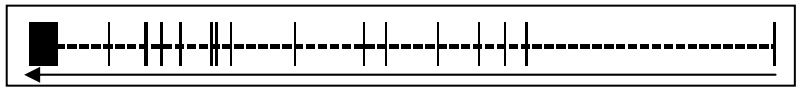
Primer Sequences (5' to 3'):	
Cre excision PCR: Primer 0739-lox5 and Primer 0739-3'cre, 426 bp	
LoxP Mutant PCR: Primer 0739-lox5 and Primer 0739-lox3, mutant 415 bp, wt 325 bp	
Primer 0739-lox5	CCCAAGAGAAGCCACCTAGC
Primer 0739-lox3	AGCCCAGTCCAGGATCAAG
Primer 0739-3'cre	TCAAATTTTACTGTCTACCAGTCCC

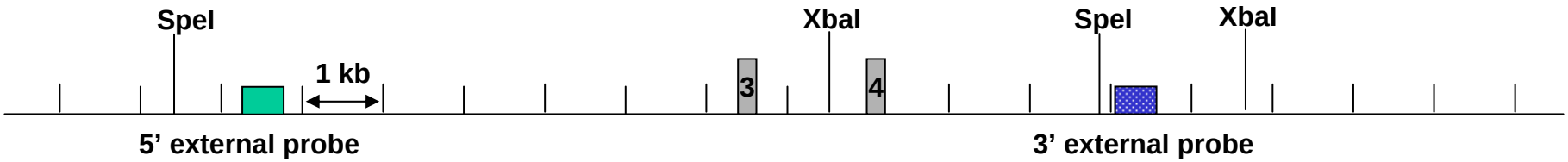
Well	Sample	Genotype
1	5	het
2	6	het
3	10	het
4	16	het
5	18	het
6	37	het
7	22	wt
8	23	het
9	**es DNA**	het
10	**water**	no amp

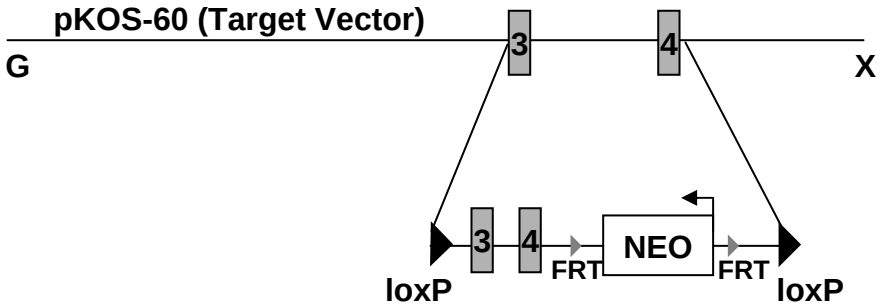
Mutant PCR



Targeting Strategy



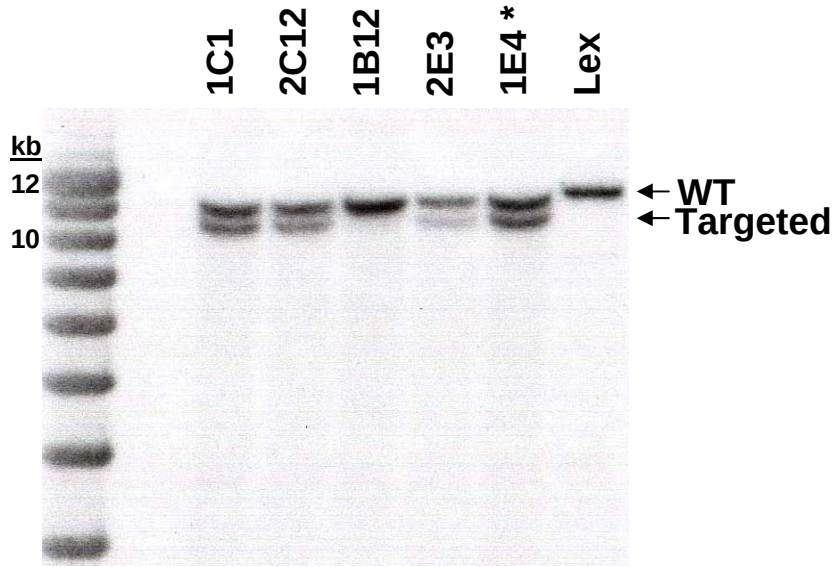




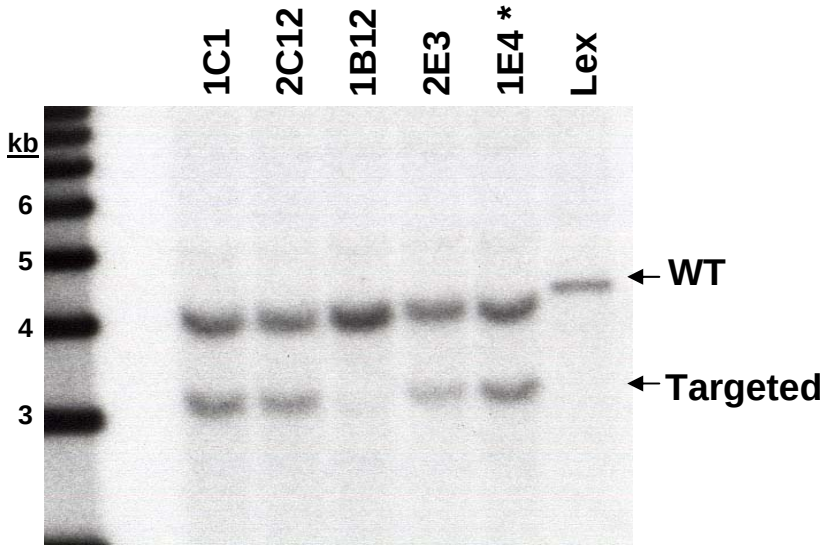
Southern Strategies:

Probe	5’ external	3’ external
Enzyme	SpeI	XbaI
Wildtype	11.0 kb	4.4 kb
Targeted	10.0 kb	3.3 kb

Southern Data



5' external probe
SpeI digests
Wildtype 11.0 kb
Targeted 10.0 kb

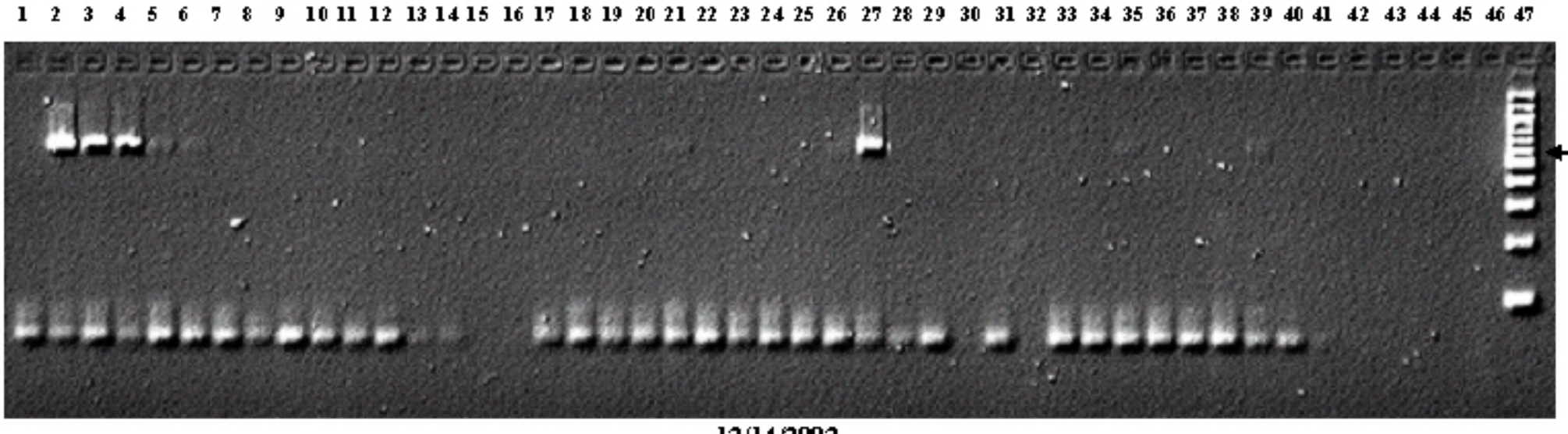


3' external probe
XbaI digests
Wildtype 4.4 kb
Targeted 3.3 kb

***Clone achieving germline transmission**

RT-PCR Analysis

mouse random primed cDNA with Primers: 1,2



12/14/2002

Note: mouse ES cDNA used as positive (+) control. Expected band size denoted by arrow adjacent to 100bp ladder/marker.

Mouse cDNA Tissues

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

Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: NIH-0739 (LEXKO-331)

Reference accession(s): NM_010583

Standard KO or Conditional: conditional

Materials Submitted: x Target Vector pKOS-60TVpLFneo
KOS clone(s) not available

Southern Blot Genotyping Strategies:

	5' External	**3' External**
Name of Probe:	73 + 74	67 + 68
Restriction Enzyme for Genomic Digest:	SpeI	XbaI
Predicted Wild-type Band (kb):	11.0	4.4
Predicted Mutant Band (kb):	10.0	3.3
Probe Size:	597 bp	262 bp

Primer sequences:

Southern probes

0739-73 5' – CATTAGCTAACCTAGCCC
0739-74 5' – CCTGGCTCGTTCTTCACATC
0739-67 5' – TCCCAACCCAGAAGCCAG
0739-68 5' – AGGCATGGGGTATGTGTGCT

Genomic Sequence Floxed:

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KOS clone sequence: *(note: pKOS-60 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)*

GGCCATAGCGGCCGGCC**ATAACTTCGTATAGCATACATTATACGAAGTTAT**GGCGCGGA*GTCGACGATCAAGCTTTCG*
*AAGATCTACGTGGCGCGCC*CTCGAGCTTTCG***GAAGTTCCTATTCGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC***TC
GAGATCCGATATCGAATTCCCGCGCCCCCAGCTGGTTCTTTCCGCCTCAGAAGCCATAGAGCCCACCGCATCCCCAGCA
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AGTGCCCAGCGGGGCTGCTAAAGCGCATGCTCCAGACTGCCTTGGGAAAAGCGCCTCCCCTACCCGGTAGGGCGCGGG
AATTCGATATCGAATTCGAGCTCGGTACCCGGGGATC***GAAGTTCCTATTCGGAAGTTCCTATTCTCTAGAAAGTATAGGA***
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GGCCAGCGAGGCC