

NIH-0014 Genotyping Strategies

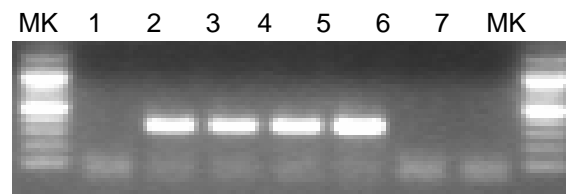
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

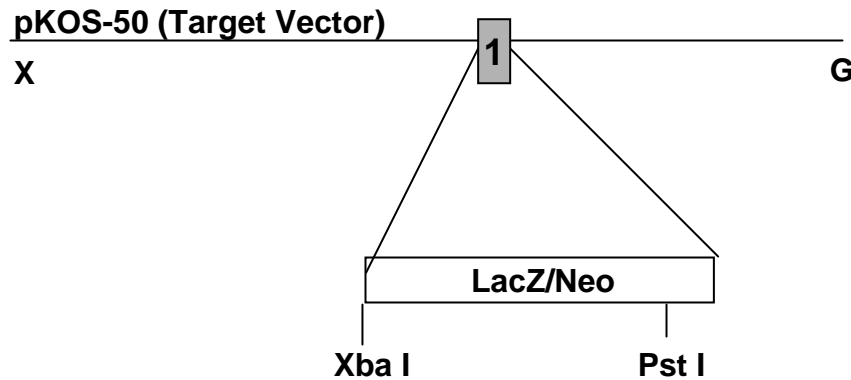
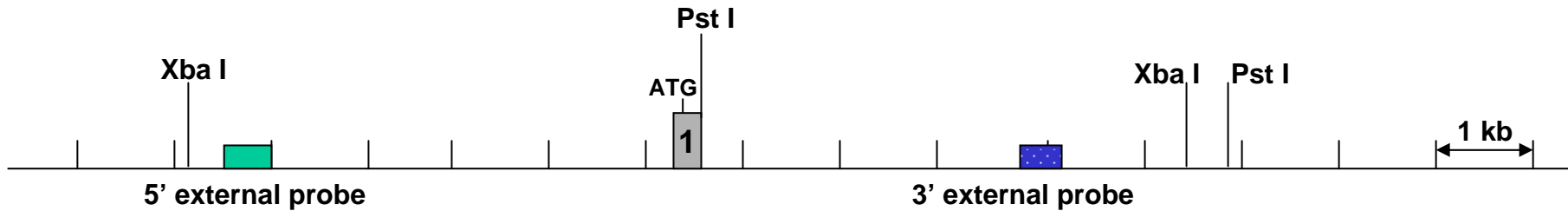
Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0014-12, 330 bp	
Recommended Wt PCR: Primer 0014-5 and Primer 0014-8, 423 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0014-12	CCCGTCACAGAGAAGCATGCCAGC
Primer 0014-5	AGAGCCCGGCCCTGTCAAC
Primer 0014-8	AGGGCACGCGGGGCTGTCAT

Well	Sample	Genotype
1	21	wt
2	25	het
3	39	het
4	41	het
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



Mutant PCR



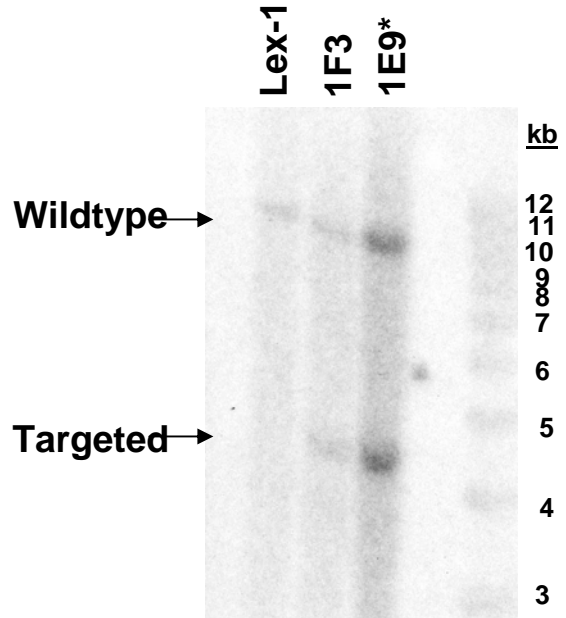
Targeting Strategy



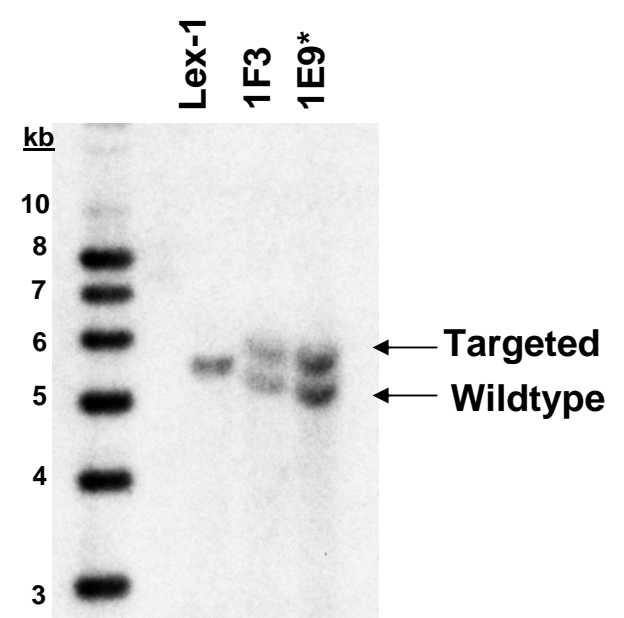
Southern Strategies

Probe	5' external 	3' external 
Enzyme	Xba I	Pst I
Wildtype	10.4 kb	5.6 kb
Targeted	4.7 kb	6.2 kb

Southern Data



5' external probe
Xba I digests
WT: 10.4 kb
Targeted: 4.7 kb

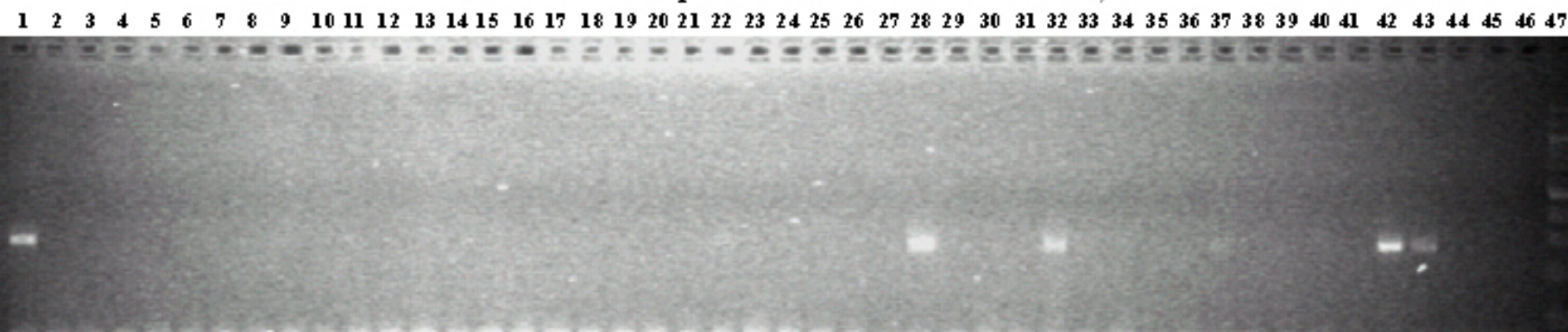


3' external probe
Pst I digests
WT: 5.6 kb
Targeted: 6.2 kb

*** Clone achieving germline transmission**

RT-PCR Analysis

mouse random primed cDNA with Primers: 3,4



07/01/2003

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

Mouse cDNA Tissues

1	Brain	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-0014 (LEXKO-721)

Reference accession(s): NM_021358

Standard KO or Conditional: Standard

Materials Submitted: Target Vector pKOS-50TVNeo
 KOS clone(s) pKOS-50

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	24/25	30/31
Restriction Enzyme for Genomic Digest:	XbaI	PstI
Predicted Wild-type Band (kb):	10.4	5.5
Predicted Mutant Band (kb):	4.7	6.2
Probe Size:	284	220

Primer sequences:

Southern probes

0014-24 5' – GTCTCCCAGCACCTTCAGC
0014-25 5' – GGCCTCCCTAGCGACCAC
0014-30 5' – AGGTATGGCTGGACGACACTTG
0014-31 5' – GTACTAGGGCAGGGCTTGACC

Genomic Sequence Deleted:

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KOS clone sequence: (note: pKOS-50 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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