NIH-0171 Genotyping Strategies

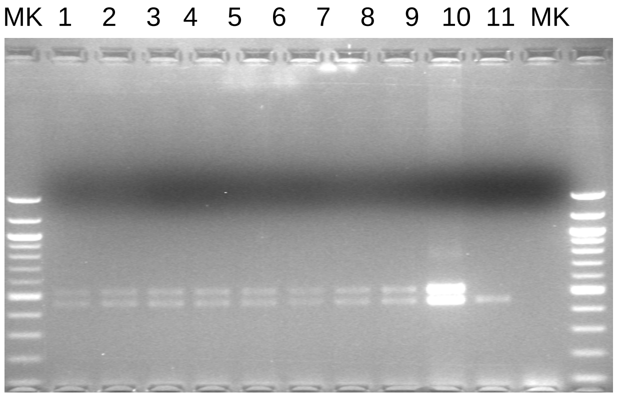
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl2	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

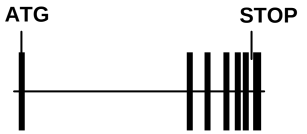
Primer Sequences (5’ to 3’):	
Cre Excision PCR: Primer 0171-34 and Primer 0171-35, 335 bp cre excised	
LoxP mutant PCR: Primer 0171-34 and Primer 0171-14, 529 bp mutant, 458 bp wt	
Primer 0171-34	TCCATGATCCCTCTAACTCG
Primer 0171-35	TTACACACAGCATGCTCACC
Primer 0171-14	GCTGATGACAGCAGCCATTG

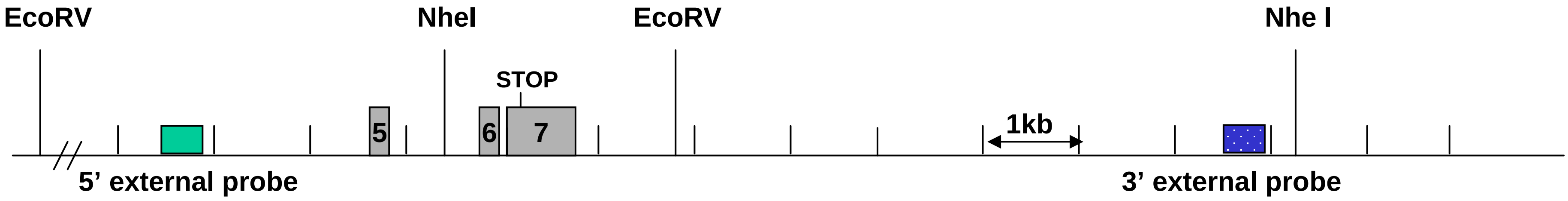
Well	Sample	Genotype
1	2	het
2	3	het
3	5	het
4	8	het
5	10	het
6	15	het
7	22	het
8	39	het
9	**ES DNA**	het
10	**wt lysate**	wt
11	**water**	no amp

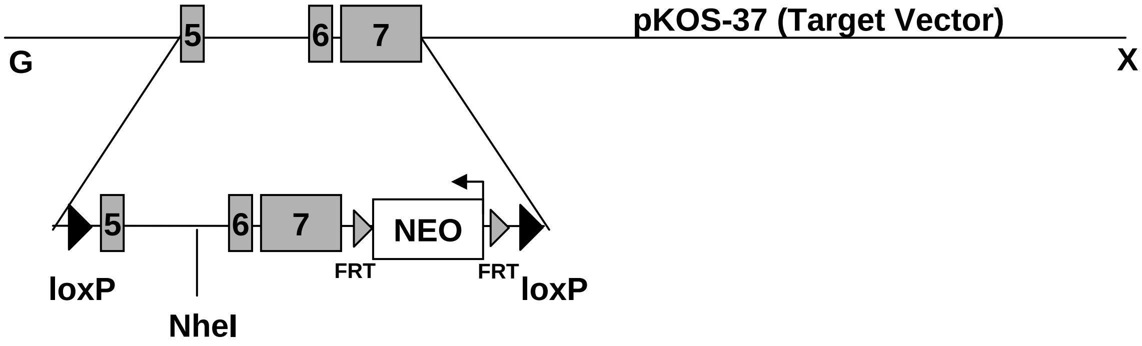
Mutant PCR



Targeting Strategy



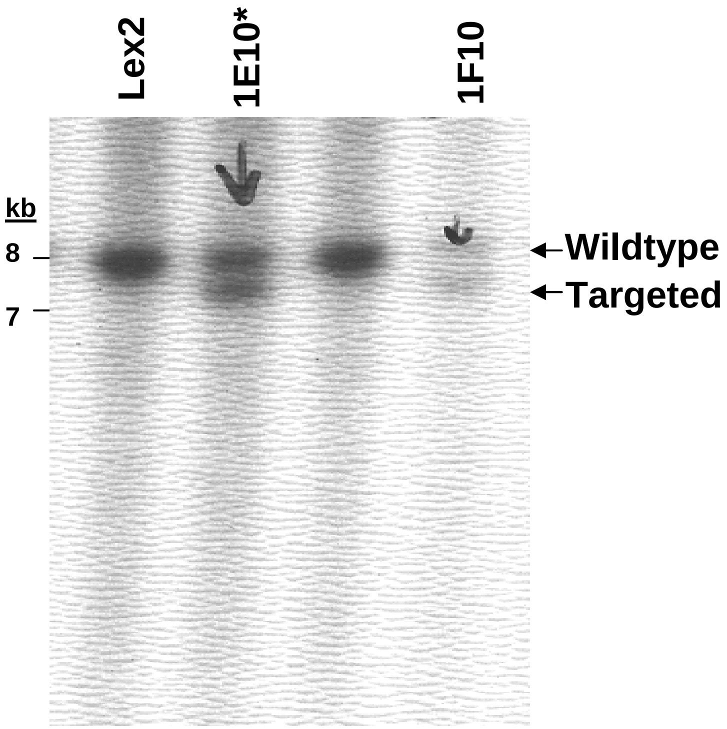




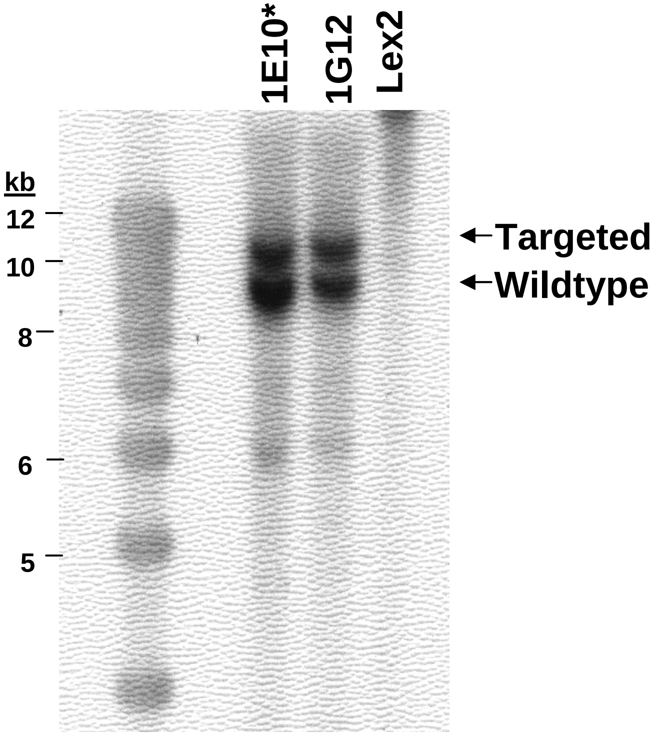
Southern Strategies

Probe	5’ external 🟩	3’ external 🟦
Enzyme	EcoRV	NheI
Wildtype	8.2 kb	9.0 kb
Targeted	7.6 kb	10.9 kb

Southern Data



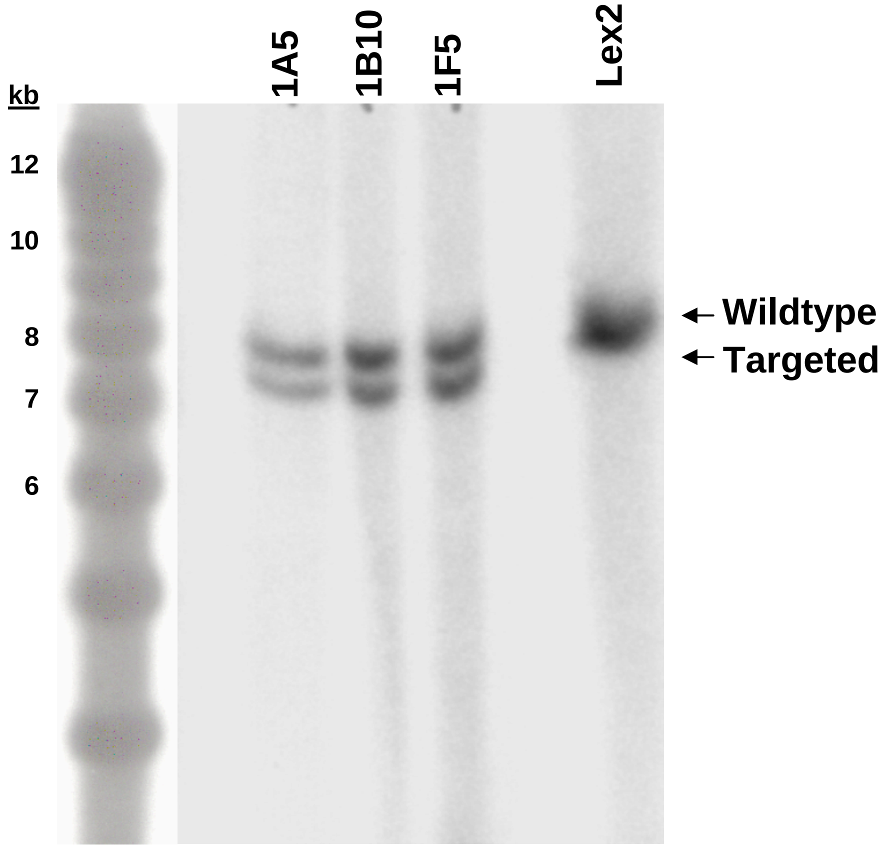
5’ external probe
EcoRV digests
Wildtype 8.1 kb
Targeted 7.6 kb



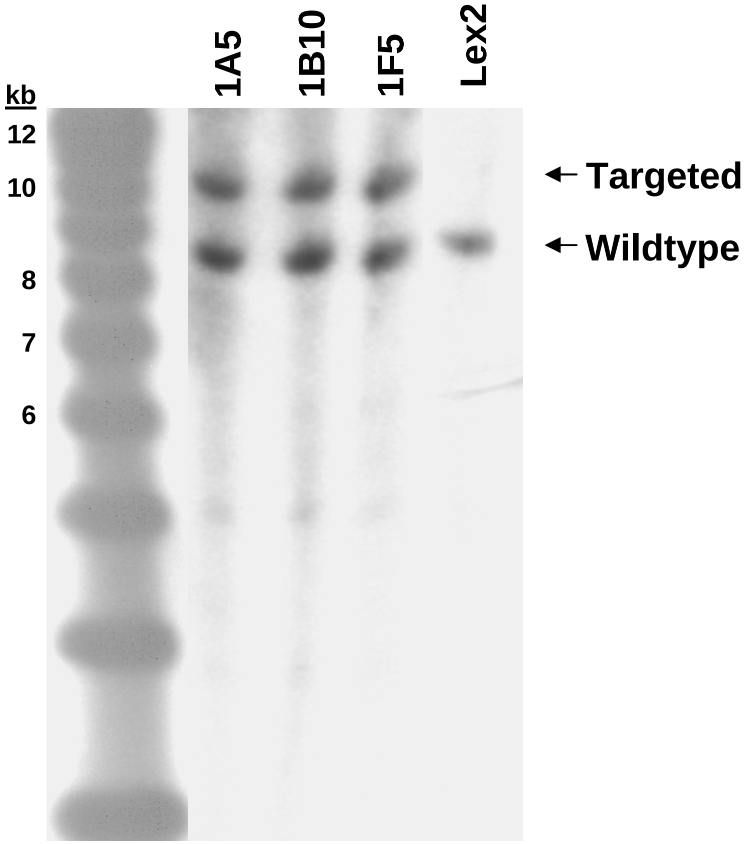
3’ external probe
NheI digests
Wildtype 8.9 kb
Targeted 10.9 kb

* Clone achieving germline transmission

Additional Southern Data



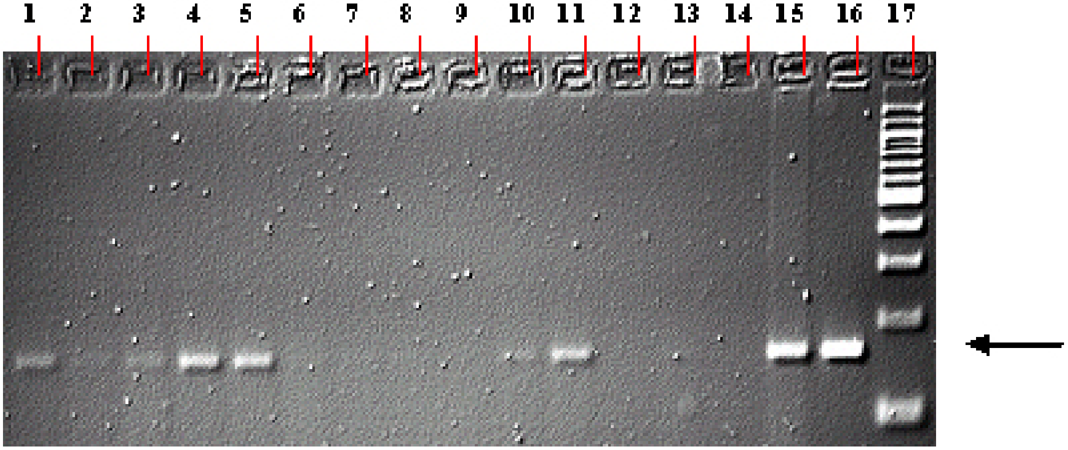
5’ external probe
EcoRV digests
Wildtype 8.1 kb
Targeted 7.6 kb



3’ external probe
NheI digests
Wildtype 8.9 kb
Targeted 10.9 kb

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 3,4



01/23/2003

Note: Expected band size denoted by arrow adjacent to 100bp ladder/marker.

Mouse cDNA Tissues

1) Brain
2) Thymus
3) Spleen
4) Lung
5) Kidney
6) Liver
7) Testis
8) Bone
9) Small Intestine & Colon
10) Skin Fibroblast
11) Heart
12) Adipose
13) Blood
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-0171** (LEXKO-878)

Reference accession(s): _ NM_007556

Standard KO or Conditional: _ Conditional

Materials Submitted: ☐ Target Vector _ pKOS-37 TVneo _________
☐ KOS clone(s)__ pKOS-37_________

Southern Blot Genotyping Strategies:

	5' External	**3' External**
Name of Probe:	38/39	46/47
Restriction Enzyme for Genomic Digest:	EcoRV	NheI
Predicted Wild-type Band (kb):	8.2 kb	9.0 kb
Predicted Mutant Band (kb):	7.6 kb	10.9 kb
Probe Size:	313 bp	352 bp

Primer sequences:

Southern probes

0171-38	5' – TCTACACTGCTGCTATCTGC
0171-39	5' – ATAGGCCTTCCTCACTTTCC
0171-46	5' – CTTTCCTGATACCCAACAGC
0171-47	5' – AAGATGCTTGGTGTCAAGCC

Genomic Sequence Floxed:

TTTGGGCAAACAACTGGAAGCTCAACTGAGCTGTTGTAGGGGCCACATGTGTCTAACAGCTGTCCTGAGCCTCATCCATTGCCTGGGATGTATGAGAGG
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KOS clone sequence: *note: pKOS-37 was used to generate the TV and that is the sequence included here)*

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ACAGTTATACCTTGGTGTCCACAAGATGCTGGTGCCAGGGGCCTCCCTAGTCCACAGTCCAAGGATTTGCACCCATGAAATGACTAGGCAATCTGCATG
GAATATACACAGATC

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

pLFneo
The 5’ and 3’ **Lox P sites** are in **bold**. The 5’ and 3’ ***FRT sites*** are in ***bold italics***. The floxed region was subcloned into the multi-enzyme cloning site (italics, underlined). The region between the loxP sites was excised upon exposure to Cre recombinase.

GGCCATAGCGGCCGGCC**ATAACTTCGTATAGCATACATTATACGAAGTTAT**GGCGCGGA*GTCGACGATCAAGCTTTCGAAGATCTACGTGGCGCGCC*CT
CGAGCTTTCG***GAAGTTCCTATTCGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC***TCGAGATCCGATATCGAATTCCCGCGCCCCCAGCTGGTTCTTT

CCGCCTCAGAAGCCATAGAGCCCACCGCATCCCCAGCATGCCTGCTATTGTCTTCCCAATCCTCCCCCTTGCTGTCCTGCCCCACCCCACCCCCCAGAAT
AGAATGACACCTACTCAGACAATGCGATGCAATTTCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGG
AGGGGCAAACAACAGATGGCTGGCAACTAGAAGGCACAGTCGAGGCTGATCAGCGAGCTCTAGAGAATTGATCCCCTCAGAAGAACTCGTCAAGAAG
GCGATAGAAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCA
CGGGTAGCCAACGCTATGTCCTGATAGCGATCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTCCACCATGATATTCGG
CAAGCAGGCATCGCCATGGGTCACGACGAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGCT
CTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCG
GATCAAGCGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCG
CCCAATAGCAGCCAGTCCCTTCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGCTGCCTC
GTCCTGCAGTTCATTCAGGGCACCGGACAGGTCGGTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGCATCAGAGCAG
CCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCCAAGGCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAATGGCCGATCCCATTAT
GACCTGCAGGTCGAAAGGCCCGGAGATGAGGAAGAGGAGAACAGCGCGGCAGACGTGCGCTTTTGAAGCGTGCAGAATGCCGGGCCCTCCGGAGGA
CCTTCGCGCCCGCCCCGCCCCTGAGCCCGCCCCTGAGCCCGCCCCCGGACCCACCCCTTCCCAGCCTCTGAGCCCAGAAAGCGAAGGAGCCAAGCTGC
TATTGGCCGCTGCCCCAAAGGCCTACCCGCTTCCATTGCTCAGCGGTGCTGTCCATCTGCACGAGACTAGTGAGACGTGCTACTTCCATTTGTCACGTCC
TGCACGACGCGAGCTGCGGGGCGGGGGGGAACTTCCTGACTAGGGGAGGAGTAGAAGGTGGCGCGAAGGGGCCACCAAAGAACGGAGCCGGTTGGC
GCCTACCGGTGGATGTGGAATGTGTGCGAGGCCAGAGGCCACTTGTGTAGCGCCAAGTGCCCAGCGGGGCTGCTAAAGCGCATGCTCCAGACTGCCTT
GGGAAAAGCGCCTCCCCTACCCGGTAGGGCGCGGGAATTCGATATCGAATTCGAGCTCGGTACCCGGGGATC***GAAGTTCCTATTCGGAAGTTCCTATTC***
TCTAGAAAGTATAGGAACTTCTCGACCTGCAGGCATGCAAGCTGATCCGGCGCGT**ATAACTTCGTATAGCATACATTATACGAAGTTAT**CCTCAGGCC
AGCGAGGCC