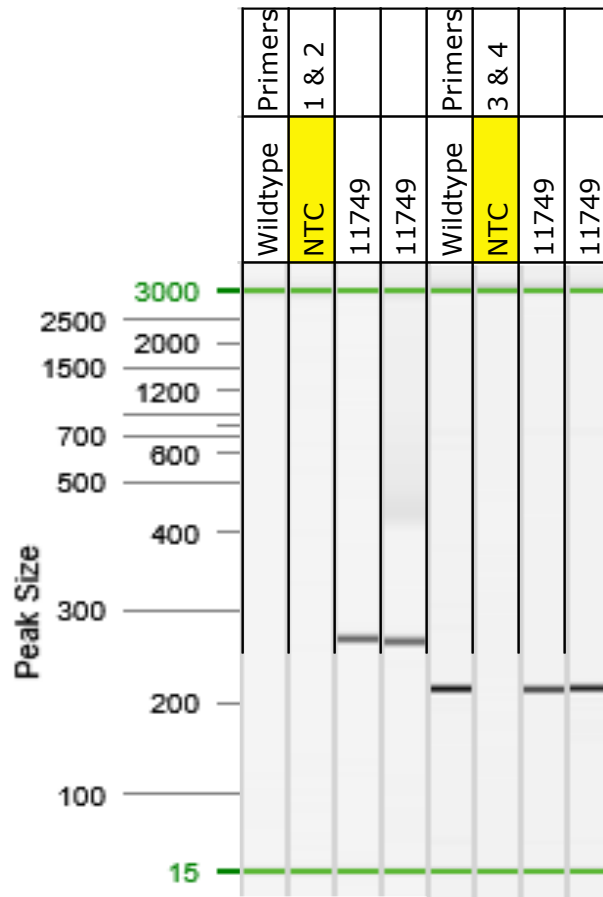


GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC



NIH-0994 Genotyping Strategies

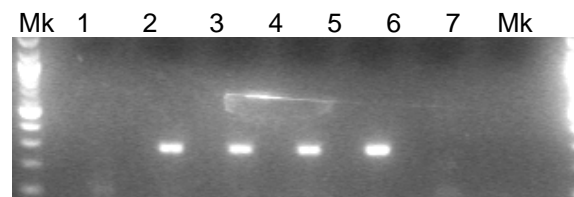
Reaction Components	Vol (ul)
5x Phusion buffer	8
25mM MgCl ₂	3.2
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
Phusion Enzyme	0.1
Water	20.7
Total mix volume	35
Tail lysate (1:20 dilution)	5
Total reaction volume	40

Step	Temp	Time	Note
1	98C	25"	
2	65C	15"	Decrease 1C/cycle
3	72C	15"	Go to 1, 6 cycles
4	98C	25"	
5	60C	15"	
6	72C	15"	Go to 4, 30 cycles

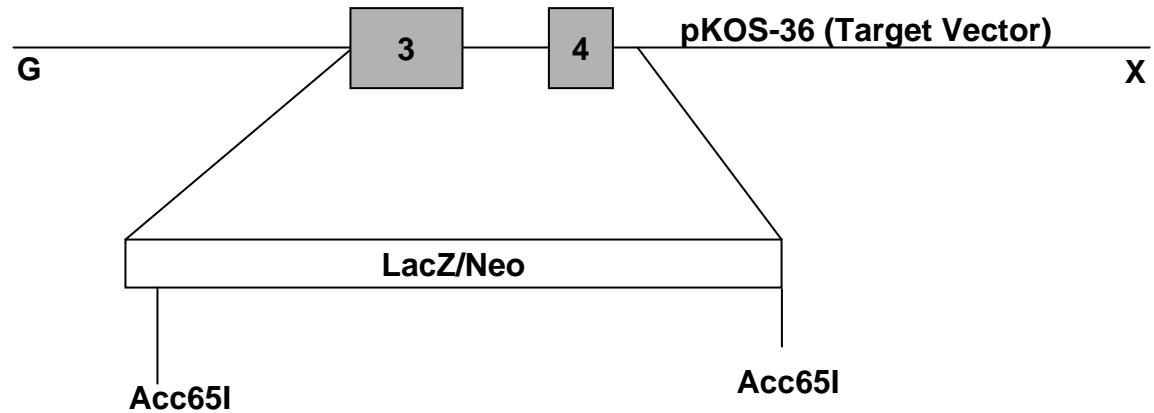
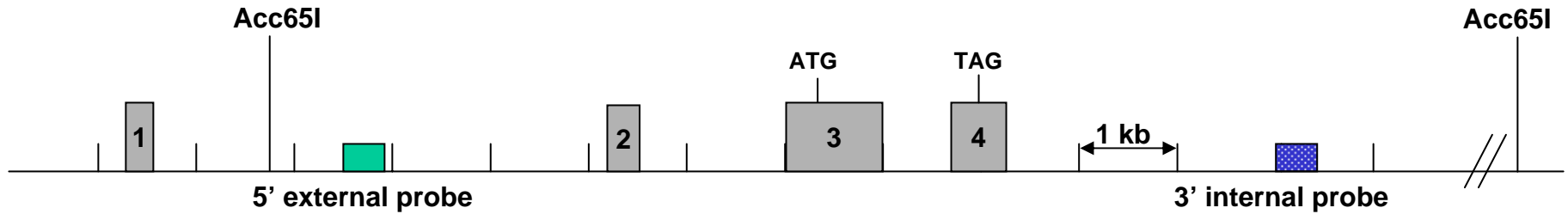
Primer Sequences (5' to 3'):	
Mutant PCR: Primer Neo3a and Primer 0994-lower, 286 bp	
Recommended Wt PCR: Primer 0994-16 and Primer 0994-17, 218 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0994-lower	TCCCCATTGTCTCATGTCC
Primer 0994-16	GATGCCCTGGAAGTTACTATGC
Primer 0994-17	GTACTCCCTCGGCCACTATCTGC

Well	Sample	Genotype
1	65	wt
2	66	het
3	77	het
4	101	het
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



Mutant PCR



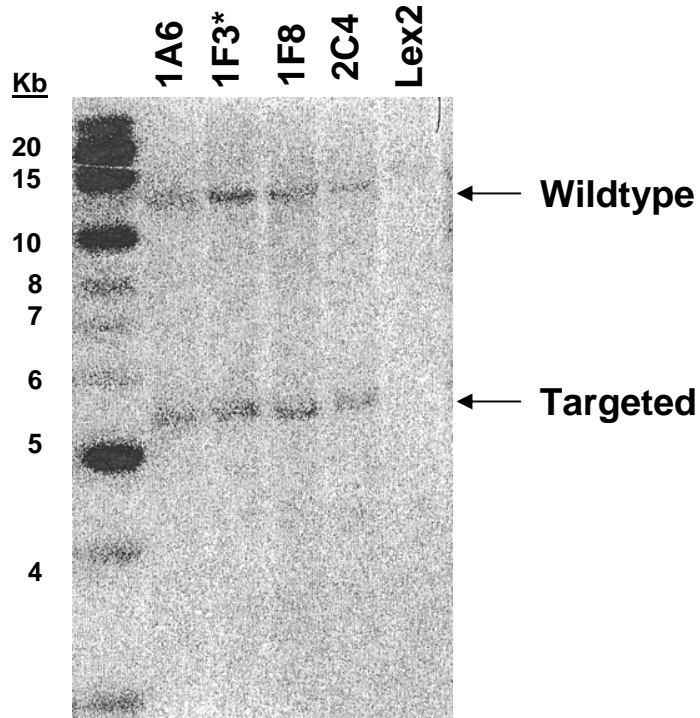
Targeting Strategy



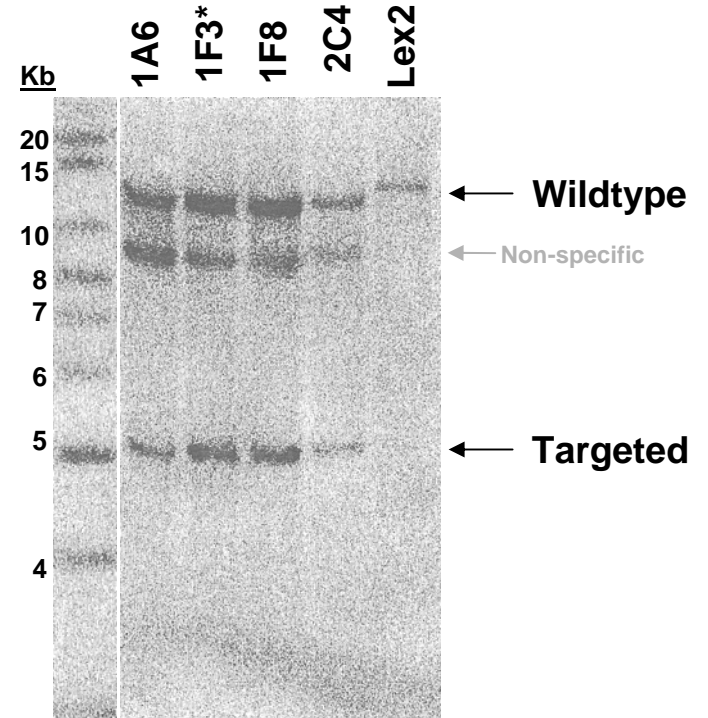
Southern Strategies

Probe	5' external 	3' internal 
Enzyme	Acc65I	Acc65I
Wildtype	13.9 kb	13.9 kb
Targeted	5.8 kb	5.3 kb

Southern Data



5' external probe
 Acc65I digests
 Wildtype: 13.9 kb
 Targeted: 5.8 kb

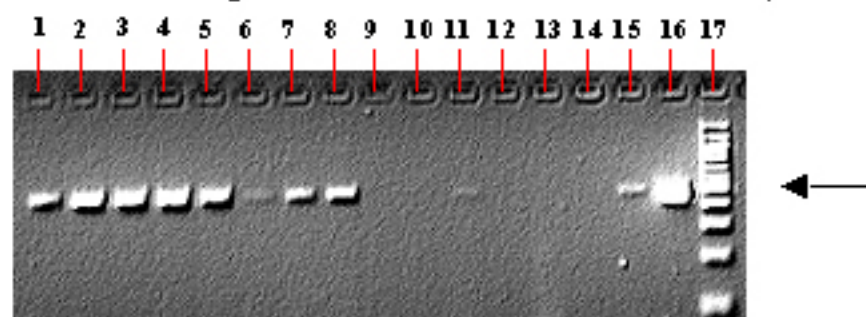


3' internal probe
 Acc65I digests
 Wildtype: 13.9 kb
 Targeted: 5.3 kb

* Clone achieving germline transmission

RT-PCR WT Expression Analysis

mouse random primed cDNA with Primers: 1,2



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/NotI DNA
- 17) 100 bp ladder/marker



**Lexicon Genetics Incorporated
Molecular Genetics Project Materials**

Catalog Number: NIH-0994 (LEXKO-1055)

Reference accession(s): NM_012050

Standard KO or Conditional: Standard KO

Materials Submitted: X Target Vector pKOS-36TVneo
X KOS clone(s) pKOS-36

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	13+14	7+8
Restriction Enzyme for Genomic Digest:	Acc65I	Acc65I
Predicted Wild-type Band (kb):	13.9	13.9
Predicted Mutant Band (kb):	5.8	5.3
Probe Size:	182 bp	364 bp

Primer sequences:

Southern probes

0994-7 5' – CAGAAGGGAATGCTTTGACTTT
0994-8 5' – TTTTATTCTCTTTATTGGCATAAC
0994-13 5' – CCCAAAGCAGACTCTACTAAGA
0994-14 5' – AAGTAACACCATGACCTAGGAA

Genomic Sequence Deleted:

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AAA

KOS clone sequence: (note: pKOS-36 was used to generate the TV and that is the sequence included here)

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CATACCATCTGCAAAAAGTGATATTTTGACTTCCTCTTTTCCAATTTGTATTCCTTGATC

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

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCGCCCCCCCCCCCCCCCCCTCTCCC
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CTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAG
ATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCT
CAAGCGTATTCAACAAGGGGCTGAAGGATGCCAGAAGGTACCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCA
CATGCTTTACATGTGTTTGTGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAA
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CCAACTTAATCGCCTTGACGACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTCC
CAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCTGGTTTTCCGGCACAGAAGCGGTGCCGGAAGGTGGCTG
GAGTGCATCTTCTGAGGCCGATACTGTGTCGTCCTCCCTCAAACCTGGCAGATGCACGGTTACGATGCGCCATCTACA
CCAACGTGACCTATCCATTACGGTCAATCCGCGTTTTGTTCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTT
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GCAACGGGCGCTGGGTGCGTTACGGCCAGGACAGTCGTTTGGCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGG
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CGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCGACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATG
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CGCATTATCCGAACCATCCGCTGTGGTACACGCTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGA
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