NIH-0489 Genotyping Strategies

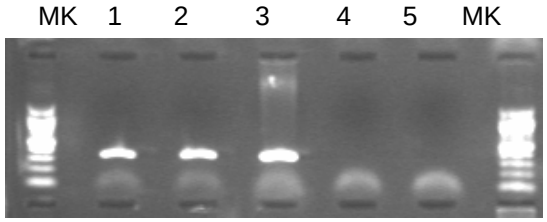
Reaction Components	Vol (ul)
5X GoTaq Buffer	10
25mM MgCl2	3.5
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
5 U/ul Taq polymerase	0.5
Water	28
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

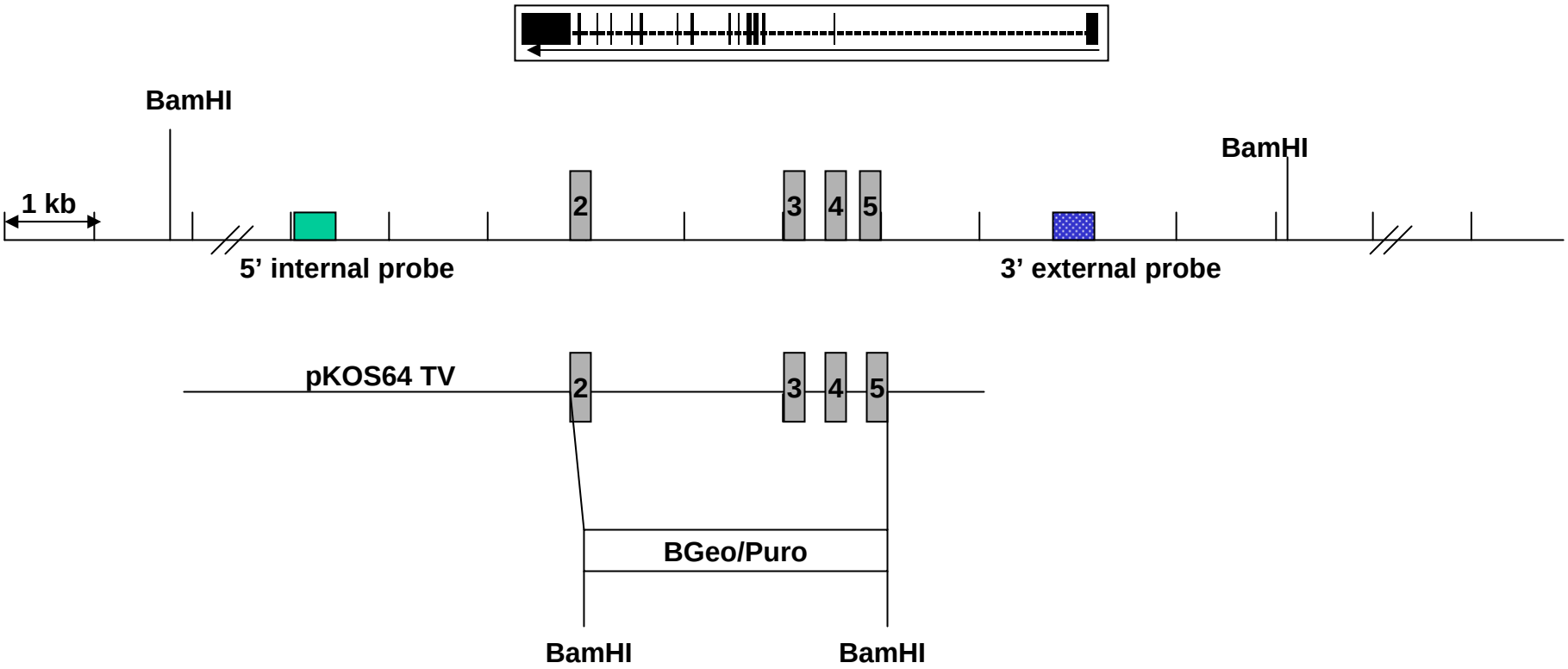
Primer Sequences (5' to 3'):	
Mutant PCR: Primer 0489-5' and Primer 0489-3', 311 bp	
Recommended Wt PCR: Primer 0489-19 and Primer 0489-25, 735 bp	
Primer 0489-5'	GGGTGAGATTGGGATGTAAAG
Primer 0489-3'	CCCTAGGAATGCTCGTCAAGA
Primer 0489-19	GTGGCATCTGTGGCCTCAAG
Primer 0489-25	CACCTGAGTTCTAGAGTTC

Well	Sample	Genotype
1	288	het
2	289	het
3	**ES DNA**	het
4	**wt lysate**	wt
5	**water**	no amp

Mutant PCR



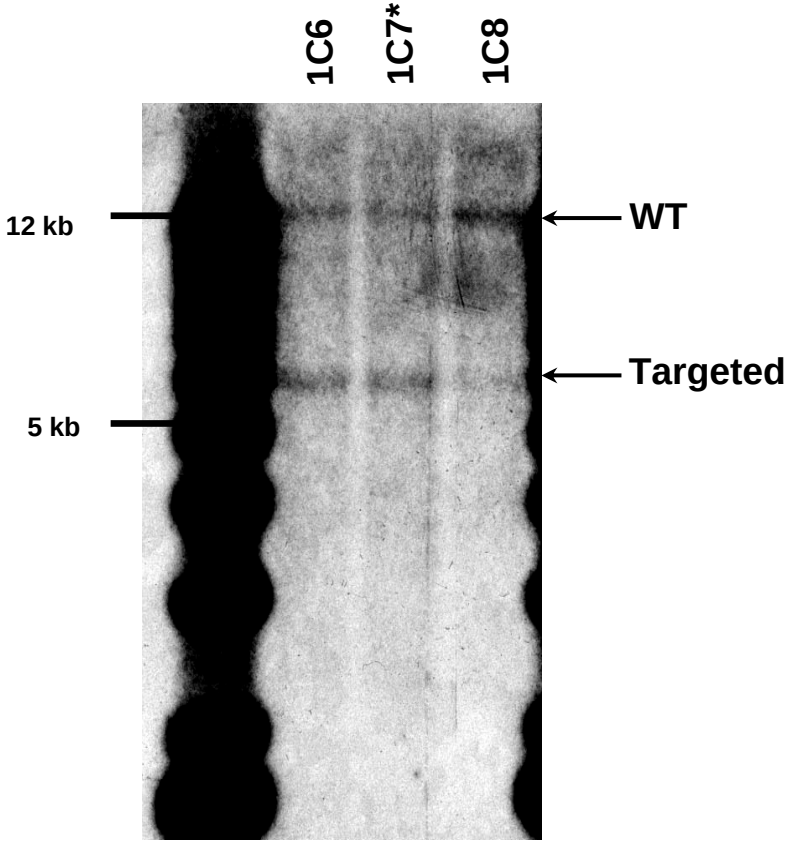
Targeting Strategy



Southern Strategies

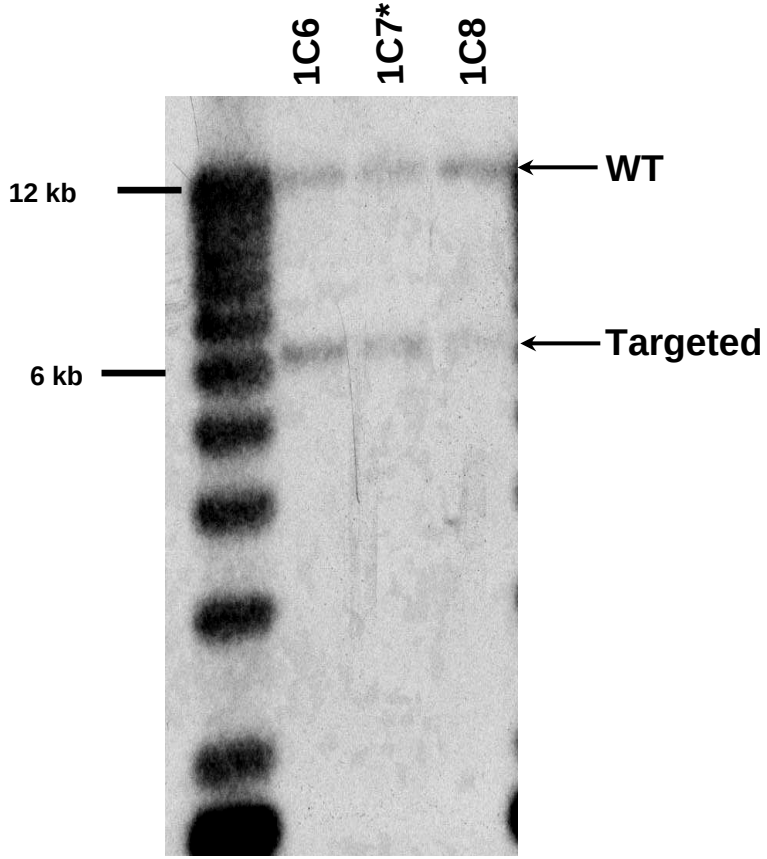
Probe	5’ external	3’ external
Enzyme	BamHI	BamHI
Wildtype	13 kb	13 kb
Targeted	5.5 kb	6.5 kb

Southern Data



5’ internal probe

BamHI digests
WT = 13.0 kb
TARGETED = 5.5 kb

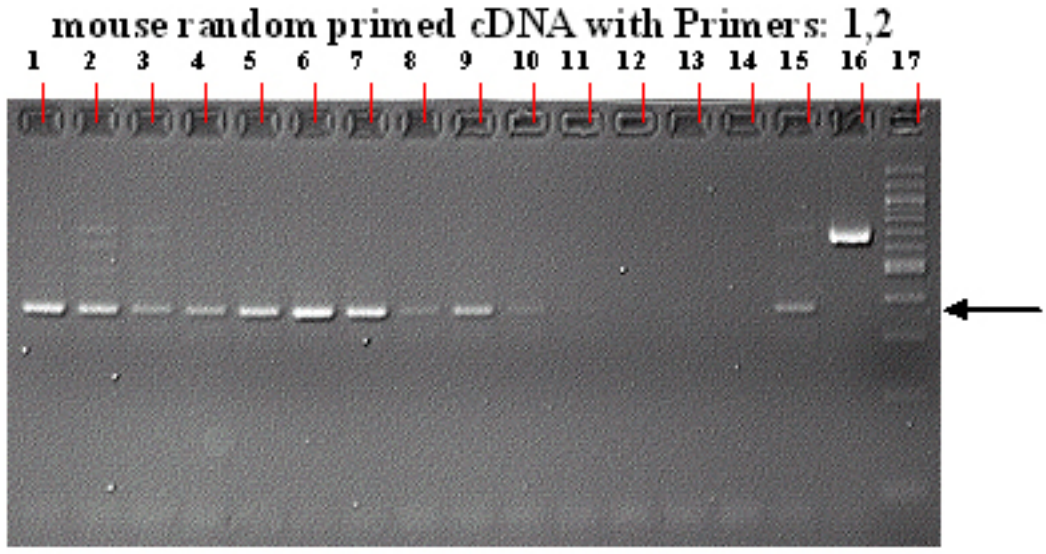


3’ external probe

BamHI digests
WT = 13.0 kb
TARGETED = 6.5 kb

*** Clone achieving germline transmission**

RT-PCR WT Expression Analysis



04/08/2002

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) Stomach
9) Small Intestine & Colon
10) Skin Fibroblast
11) Heart
12) Adipose
13) Tail
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-0489** (LEXKO-205)

Reference accession(s): _ NM_010173

Standard KO or Conditional: _ Standard KO

Materials Submitted: ☐ Target Vector _ pKOS-64TVneo_____
☐ KOS clone(s)__ pKOS64_____

Southern Blot Genotyping Strategies:

	5' External/ Internal	**3' External/ Internal**
Name of Probe:	47/31	45/46
Restriction Enzyme for Genomic Digest:	BamHI	BamHI
Predicted Wild-type Band (kb):	13 kb	13 kb
Predicted Mutant Band (kb):	5.5 kb	6.5 kb
Probe Size:	225 bp	416 bp

Primer sequences:

Southern probes

0489-47	5' – GCTGTGGCTGTTACAGTAGTG
0489-31	5' – GCTACATAATGCATTGAGATC
0489-45	5' – GGTTCAGAGTCATGGTGAGC
0489-46	5' – CAGACCTCCTCACCTCTC

Genomic Sequence Deleted :

TGGATTCAGAGGCCTTGCTGGCTCTGCCCCTGCTCCAACTGGTACAGAAGTTACAGAGTGGGGAACTGTCCCCAGAAGC
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TACTGG

KOS clone sequence: *(note: pKOS-64 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)*

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