GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Il23atm1Lex/Mmucd **MMRRC:** 011725-UCD

Protocol:

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM)	0.45
Primer 2. (stock concentration is 20µM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	TOTAL VOLUME
	15

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65°C decreasing in temperature by 1.0°C; next 30 cycles anneal at 55°C.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	**10x**
4. Elongation		68	2:00	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**25x**
7. Elongation		68	2:00 (↑20sec/cycle)	

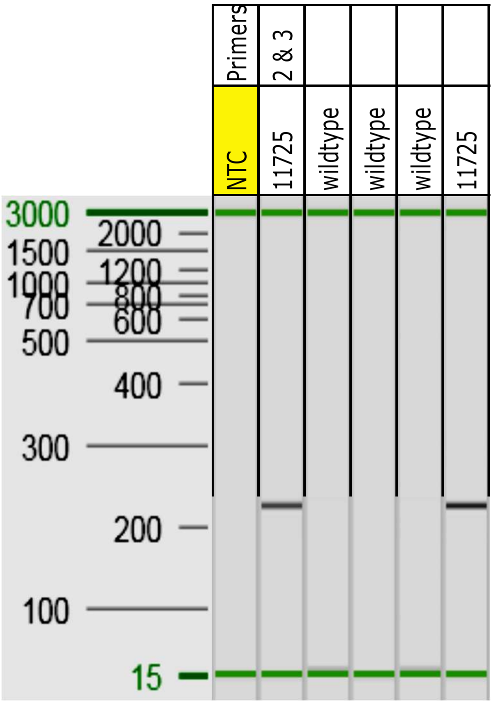
Primers:

Name	Nucleotide Sequence (5' - 3')
1. Primer 0769-1	TGCAGATCACAGAGCCAGC
2. Neo3a	GCAGCGCATCGCCTTCTATC
3. Primer 0769-3	CTTCCAACCCTCCAGATCC

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**
Estimated Running:Time: **90** min.

Primer Combination	Band (bp)	Genotype
2 & 3	250	mutant
1 & 3	307	wildtype



PCR protocol developed by MMRRC at University of California, Davis

NIH-0769 Genotyping Strategies

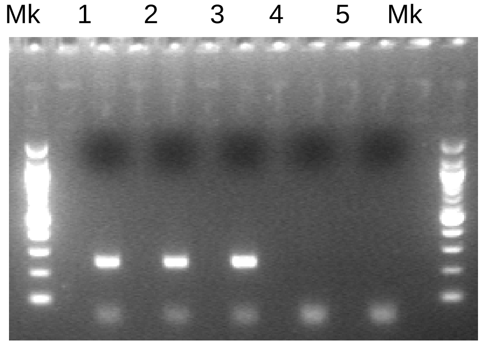
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl2	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

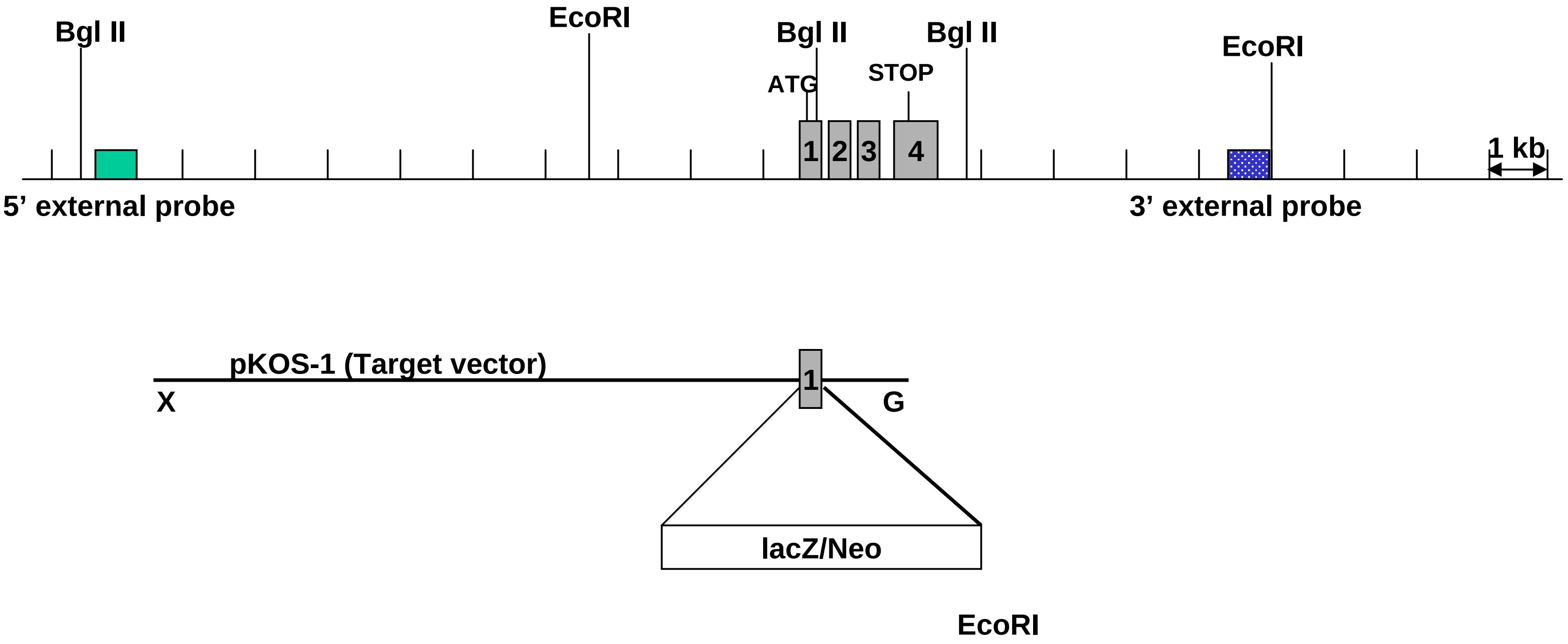
Primer Sequences (5’ to 3’):	
Mutant PCR: Primer Neo3a and Primer 0769-3, 250 bp	
Recommended Wt PCR: Primer 0769-1 and Primer 0769-3, 307 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0769-3	CTTCCAACCCTCCAGATCC
Primer 0769-1	TGCAGATCACAGAGCCAGC

Well	Sample	Genotype
1	111	het
2	119	het
3	**ES DNA**	het
4	**wt lysate**	wt
5	**water**	no amp

Mutant PCR



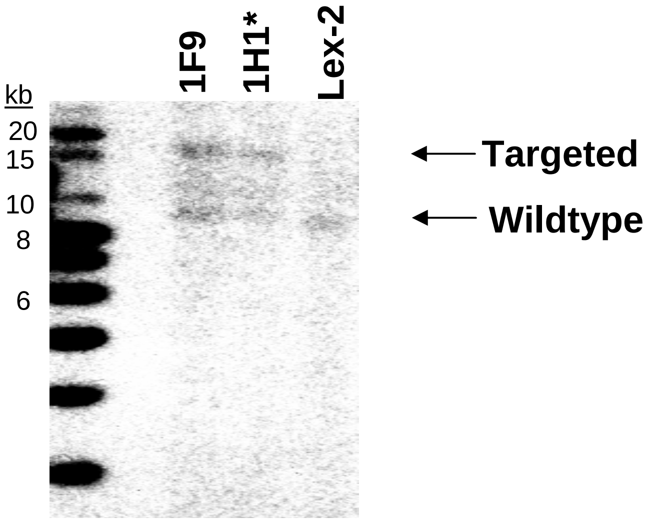
Targeting Strategy



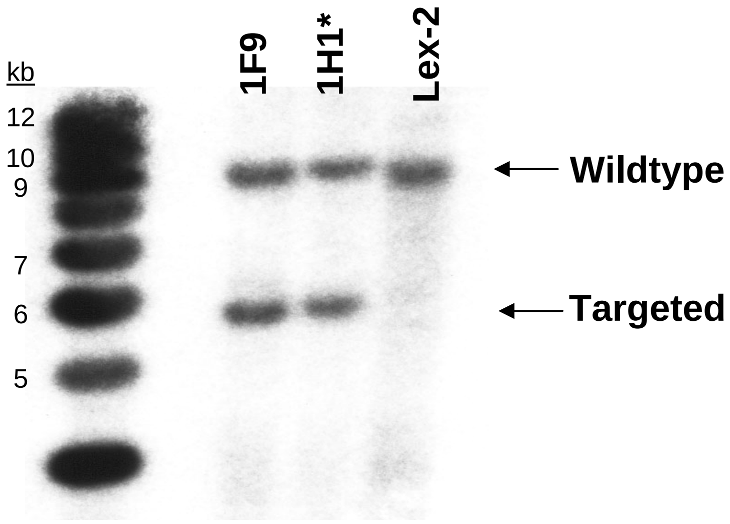
Southern Strategies

Probe	5’ external 🟩	3’ external 🟦
Enzyme	Bgl II	EcoRI
Wildtype	9.5 kb	9.3 kb
Targeted	16.8 kb	5.9 kb

Southern Data



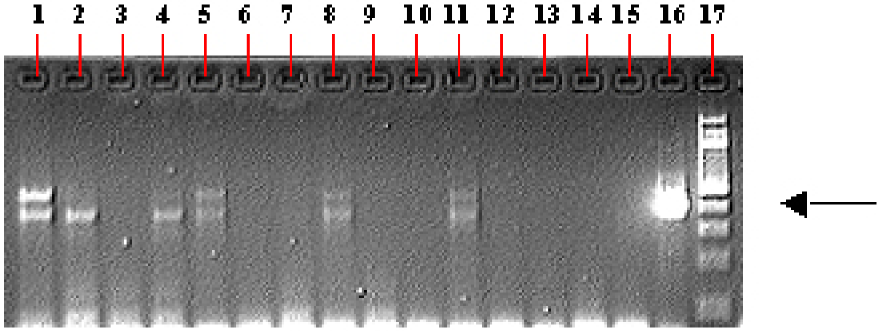
5’ external probe
Bgl II digests
Wildtype: 9.5 kb
Targeted: 16.8 kb



3’ external probe
EcoRI digests
Wildtype: 9.3 kb
Targeted: 5.9 kb

*** Clone achieving germline transmission**

RT-PCR WT Expression Analysis



mouse random primed cDNA with Primers: 1,2

06/08/2004

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/Lex1 DNA
17) 100 bp ladder/marker

Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: NIH-0769 (LEXKO-1052)

Reference accession(s): _NM_031252

Standard KO or Conditional: Standard

Materials Submitted: X Target Vector: pKOS-1.TVneo
X KOS clone(s): pKOS-1

Southern Blot Genotyping Strategies:

	5' External	**3' External**
Name of Probe:	30-31	27-28
Restriction Enzyme for Genomic Digest:	Bgl II	EcoRI
Predicted Wild-type Band (kb):	9.5 kb	9.3 kb
Predicted Mutant Band (kb):	16.8 kb	5.9 kb
Probe Size:	270 bp	577 bp

Primer sequences:

Southern probes

0769-30	5' – CAATCCATACGGAGTCAATGG
0769-31	5' – AACCTTTTGAGAGCAAATGAGG
0769-27	5' – GATGACCTAATGCCTGTACC
0769-28	5' – GAACCCAGGAGGCAATTGC

Genomic Sequence Deleted:
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CATGCACCAGCGGGACATATGGTAAGTGTCA

KOS clone sequence: *(note: pKOS-1 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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