

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Kcna6^{tm1Lex}/Mmucd **MMRRC:** 011729-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.275
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Primer 3. (stock concentration is 20μM)	0.5
Primer 4. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90		
1. Primer 1119-7	GCTTCCACCGCGCCCATCTG	Estimated Running:Time: 90 min.		
2. Primer 1119-2	CGGCGGCGGCTGCTGTAGTAGT	Primer Combination	Band (bp)	Genotype
3. Primer 1119-20	AACCTCGAATGCTGCGAGGATC	1 & 2	523	Wild-type
4. Primer Neo3a	GCAGCGCATCGCCTTCTATC	3 & 4	301	Mutant



NIH-1119 Genotyping Strategies

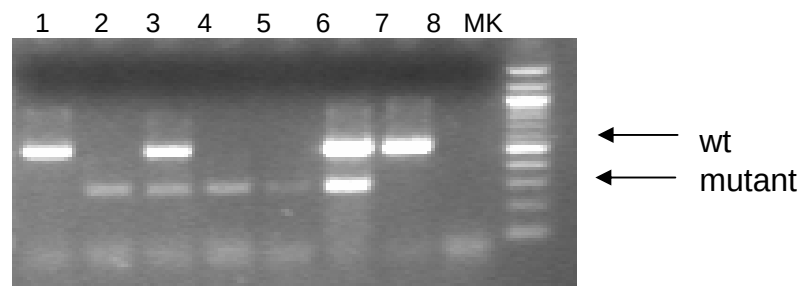
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

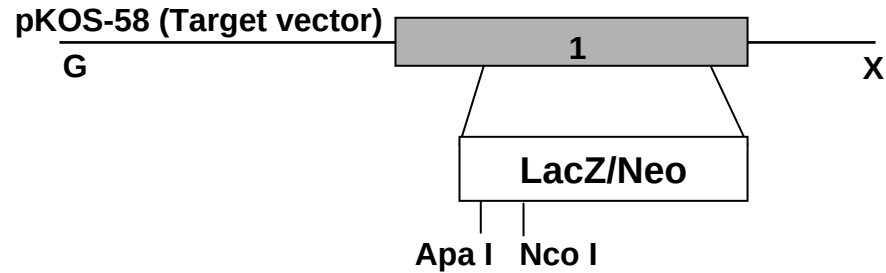
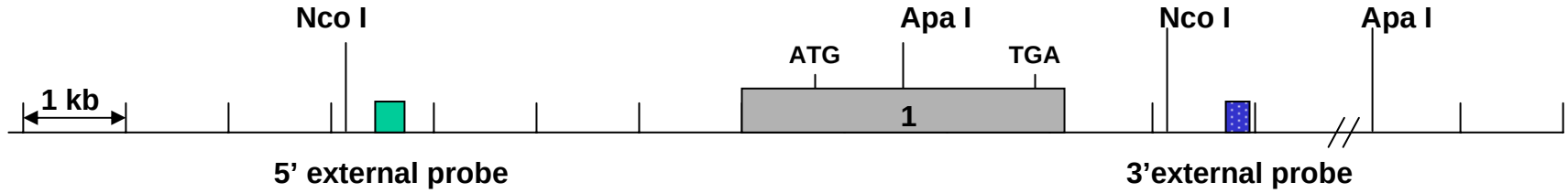
Primer Sequences (5' to 3'):	
Mutant PCR: Primer Neo3a and Primer 1119-20, 301 bp	
Wt PCR: Primer 1119-2 and Primer 1119-7, 523 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 1119-20	AACCTCGAATGCTGCGAGGATC
Primer 1119-2	CGGCGGCGGCTGCTGTAGTAGT
Primer 1119-7	GCTTCCACCGCGCCCATCTG

Well	Sample	Genotype
1	98	wt
2	100	hom
3	104	het
4	106	hom
5	123	hom
6	ES DNA	het
7	wt lysate	wt
8	water	no amp

Mutant PCR



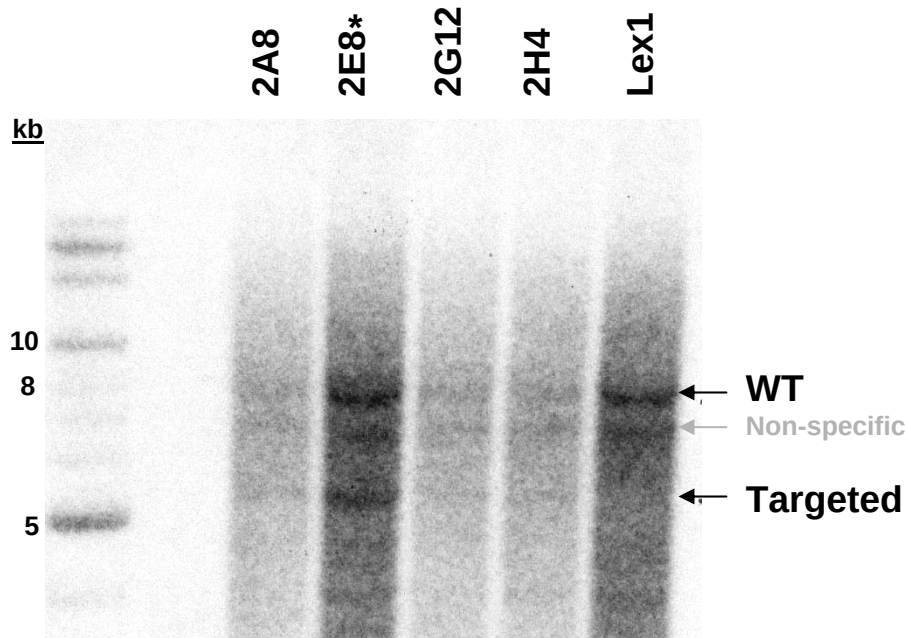
Targeting Strategy



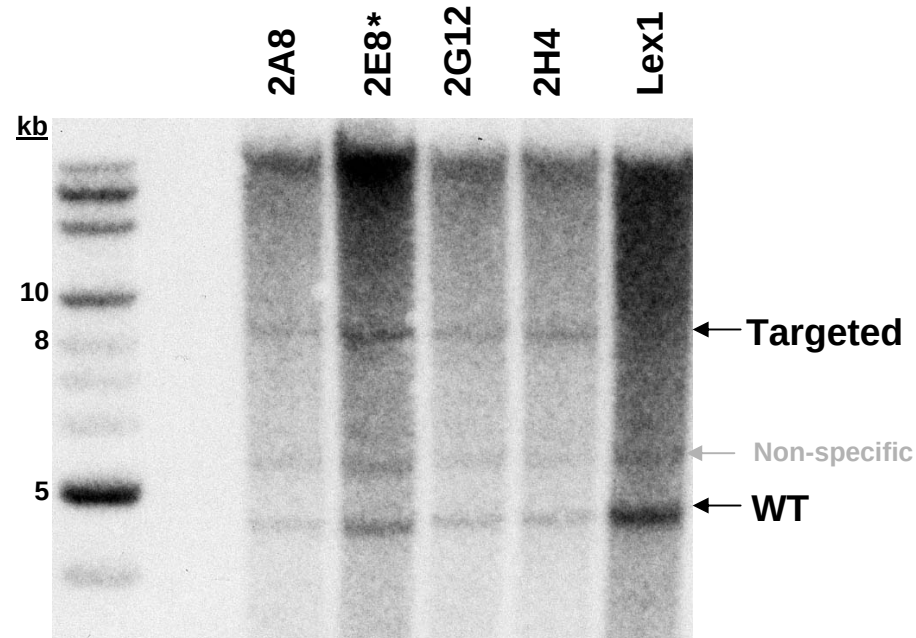
Southern Strategies

Probe	5' external 	3' external 
Enzyme	Nco I	Apa I
Wildtype	8 kb	4.5 kb
Targeted	5.4 kb	9 kb

Southern Data



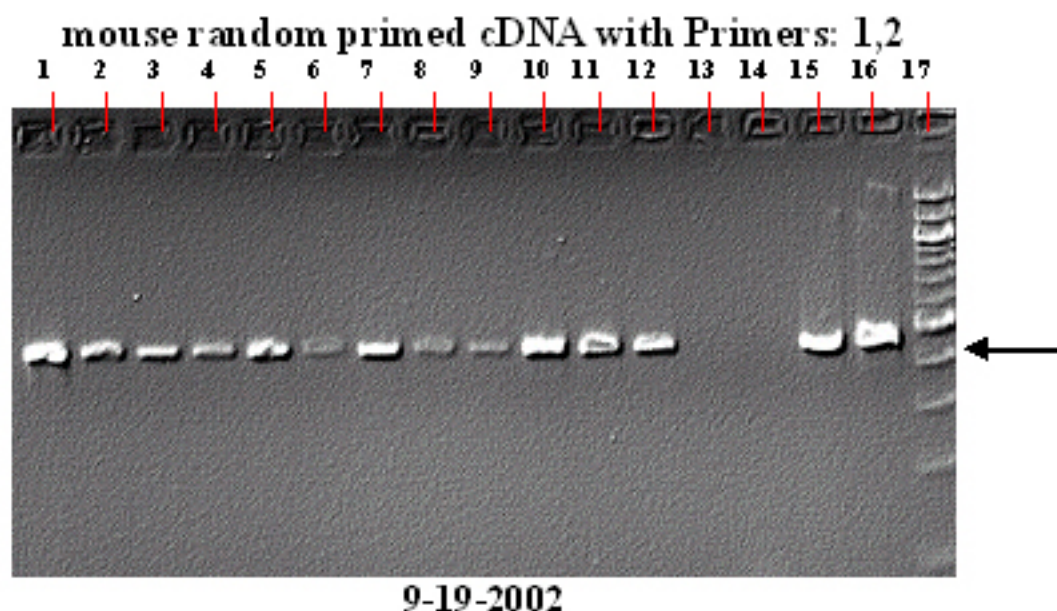
5' External Probe
Nco I digests
 WT 8 kb
 Targeted 5.4 kb



3' External Probe
Apa I digests
 WT 4.5 kb
 Targeted 9 kb

* Clone achieving germline transmission

RT-PCR WT Expression Analysis



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) 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-1119 (LEXKO-339)

Reference accession(s): NM_013568

Standard KO or Conditional: Standard

Materials Submitted: x Target Vector pKOS-58TVneo
x KOS clone(s) pKOS-58

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	21 + 22	27 + 28
Restriction Enzyme for Genomic Digest:	NcoI	Apal
Predicted Wild-type Band (kb):	8.0	4.5
Predicted Mutant Band (kb):	5.4	9.0
Probe Size:	461 bp	532 bp

Primer sequences:

Southern probes

1119-21 5' – TGGAAGTGTGTTGGGAAGGATTAGGAG
1119-22 5' – CATGAAGACCGTGCAAGAACAACAGT
1119-27 5' – TTGTCACCCTCATCACTGTCCCTGTC
1119-28 5' – GAACCTGCGCCTGAGATGAGAATACC

Genomic Sequence Deleted:

ATGAGATCGGAGAAATCCCTGACGCTGGCGGCGCCGGGGGAGGTCCGTGGGCCGGAGGGGGAGCAACAGGATGCGGG
TGAGTTCCAGGAGGCCGAGGGCGGCGGCGGCTGCTGTAGTAGTGAGAGGTTGGTGATCAACATCTCCGGGCTGCGCTT
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ACCGAGGTTTGATGGATGCGGGCAGGCCTGCAGGAAAACAGGAGCTCTGAGCAAGTCATCTC

KOS clone sequence: *(note: pKOS-58 was used to generate the TV and that is the sequence included here)*

GATCACTGAACCTTGTTATCACTGGGACAAAACACCGGACAAAAGAGAGGAAGGGTGTGTGTTTTGTCTTACCTGTTT
GTCTCACATGATGGCAGGGTAGGCAGGAATATAGGCTGGTCACACTGTGTCTGCACTCAGGGAAGAGAGAGAGGCGGT
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[illegible]

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

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCC
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