

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Il4i1tm1Lex/Mmucd **MMRRC: 011726-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.

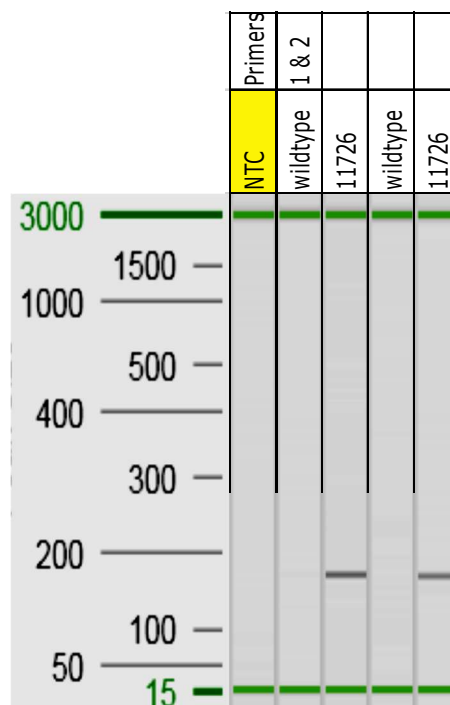
Strategy:

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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 0770-29	GTGCTCACTTCCTCTTTGCGACT	Estimated Running Time: 90 min.		
2. Neo3a	GCAGCGCATCGCTTCTATC	Primer Combination	Band (bp)	Genotype
3. 0770-31	TTGAGACCTTTCTTTCCGAGCAG	1 & 2	187	mutant
4. 0770-32	ACTGGGCTCTGGCTAAACCTTG	3 & 4	325	wildtype



NIH-0770 Genotyping Strategies

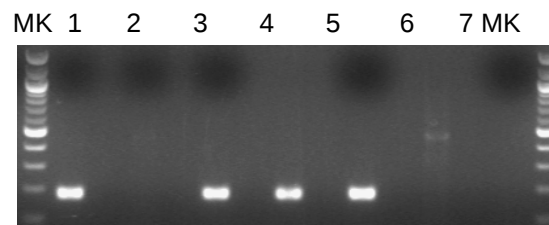
Reaction Components	Vol (ul)
5x Phusion buffer	8
25mM MgCl ₂	3.2
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
Phusion Enzyme	0.1
Water	20.7
Total mix volume	35
Tail lysate (1:20 dilution)	5
Total reaction volume	40

Step	Temp	Time	Note
1	96C	17"	
2	63C	15"	Decrease 1C/cycle
3	72C	15"	Go to 1, 6 cycles
4	96C	17"	
5	57C	15"	
6	72C	15"	Go to 4, 29 cycles

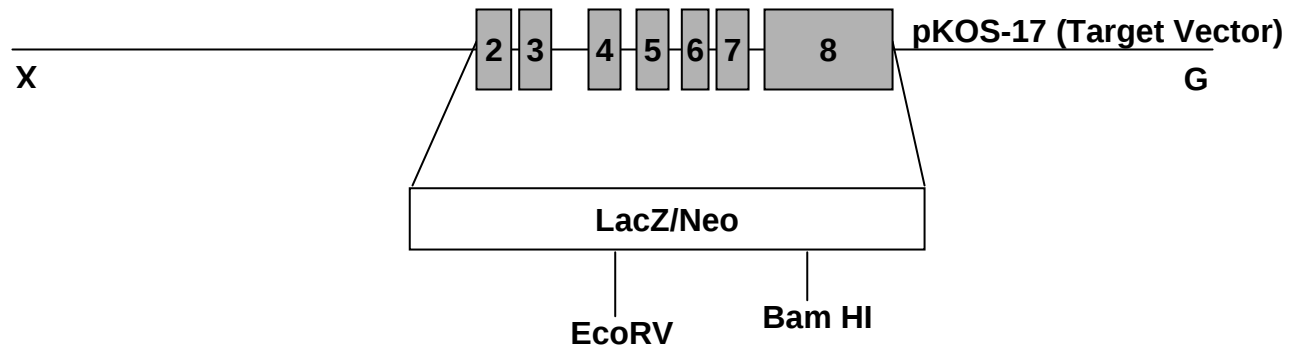
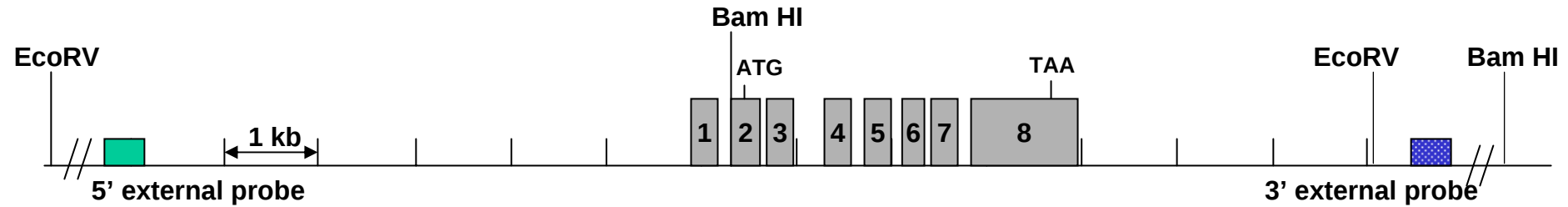
Primer Sequences (5' to 3'):	
Mutant PCR: Primer Neo3a and Primer 0770-29, 187bp	
Recommended Wt PCR: Primer 0770-31 and Primer 0770-32, 325 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0770-29	GTGCTCACTTCCTCTTTGCGACT
Primer 0770-31	TTGAGACCTTTCTTTCCGAGCAG
Primer 0770-32	ACTGGGCTCTGGCTAAACCTTG

Well	Sample	Genotype
1	65	het
2	66	wt
3	67	het
4	69	het
5	ES DNA	het
6	wt lysate	wt
7	water	no amp

Mutant PCR



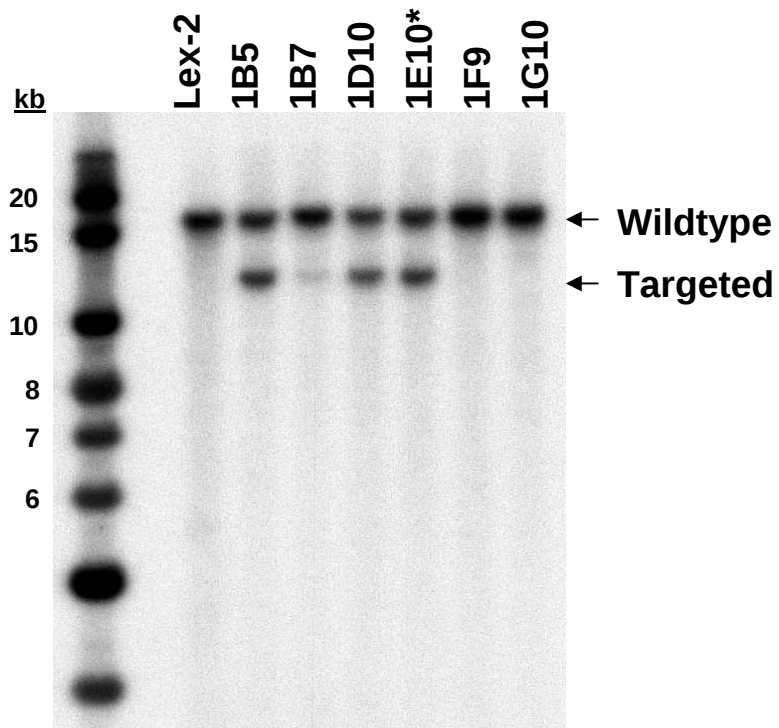
Targeting Strategy



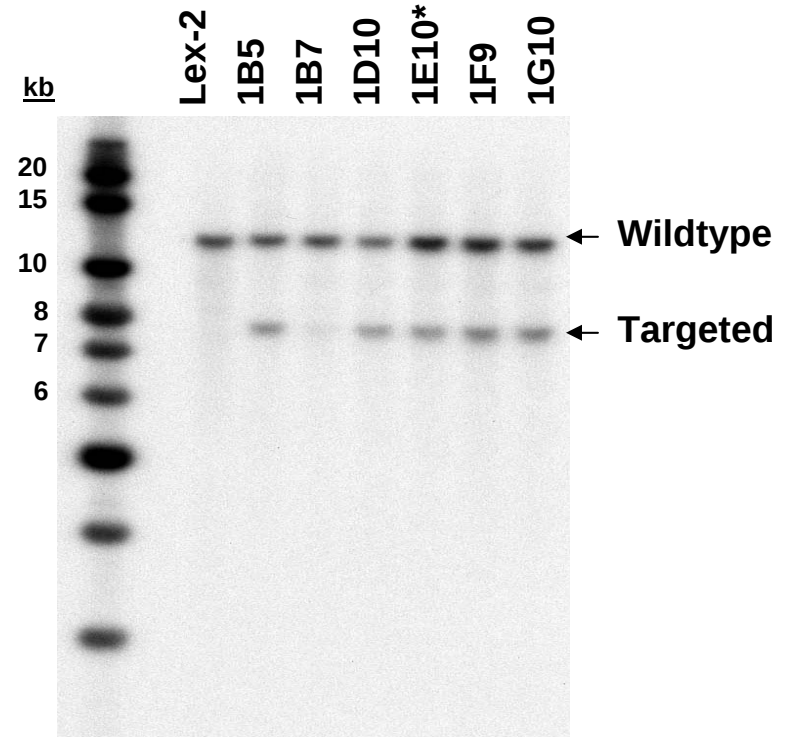
Southern Strategies

Probe	5' external 	3' external 
Enzyme	EcoRV	Bam HI
Wildtype	16.7 kb	12.0 kb
Targeted	12.0 kb	7.8 kb

Southern Data



5' external probe
Eco RV digests
Wildtype 16.7 kb
Targeted 12.0 kb

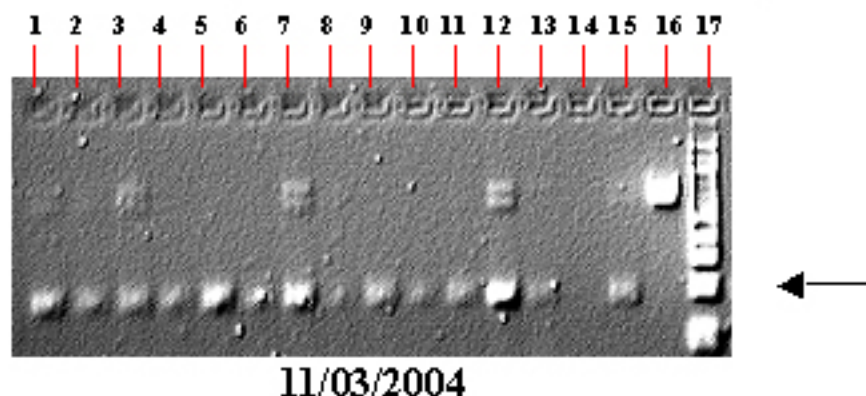


3' external probe
Bam HI digests
Wildtype 12.0 kb
Targeted 7.8 kb

* 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/Lex1 DNA
- 17) 100 bp ladder/marker



Lexicon Genetics Incorporated
Molecular Genetics Project Materials

Catalog Number: NIH-0770 (LEXKO-975)

Reference accession(s): NM_010215

Standard KO or Conditional: Standard

Materials Submitted: x Target Vector pKOS-17TVneo
x KOS clone(s) pKOS-17

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	21+22	23+24
Restriction Enzyme for Genomic Digest:	EcoRV	BamHI
Predicted Wild-type Band (kb):	16.7	12.0
Predicted Mutant Band (kb):	12.0	7.8
Probe Size:	595 bp	618 bp

Primer sequences:

Southern probes

0770-21 5' – CAGGTCCAGCGATGACCTATG
0770-22 5' – TCAAAGGCAATGCGCAGACTG
0770-23 5' – GCTTGCCCAGGGACACTAAG
0770-24 5' – GAGGAAGAGTGGAACCGCTAC

Genomic Sequence Deleted:

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KOS clone sequence: (note: pKOS-17 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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