

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6;129S5-Gpr3711tm1Lex/Mmucd **MMRRC:** 011709-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? <input type="checkbox"/>	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:

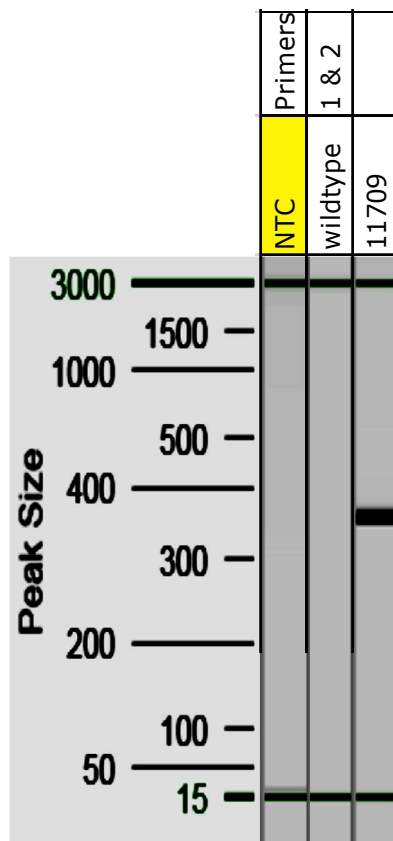
Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5%	V: 90		
1. 0579-24	ACACCTGCCTGTTTCATCTGG	Estimated Running Time: 90 min.			
2. neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype	
3. 0579-5	CACAGCTACTACTTGAAGAG	1 & 2	310	mutant	
		1 & 3	385	wildtype	

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NIH-0579 Genotyping Strategies

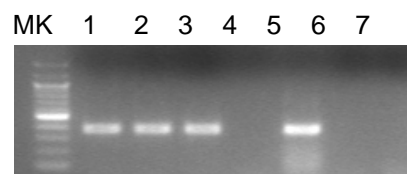
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

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

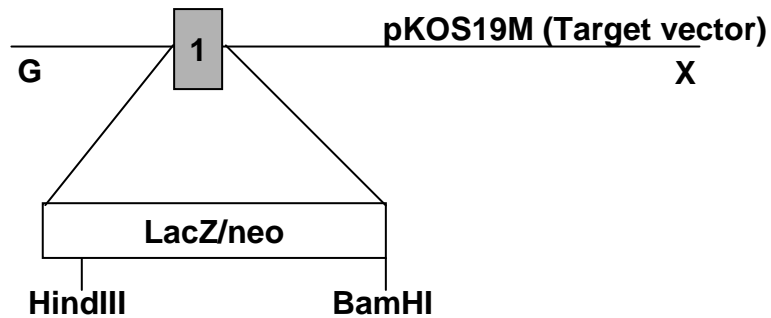
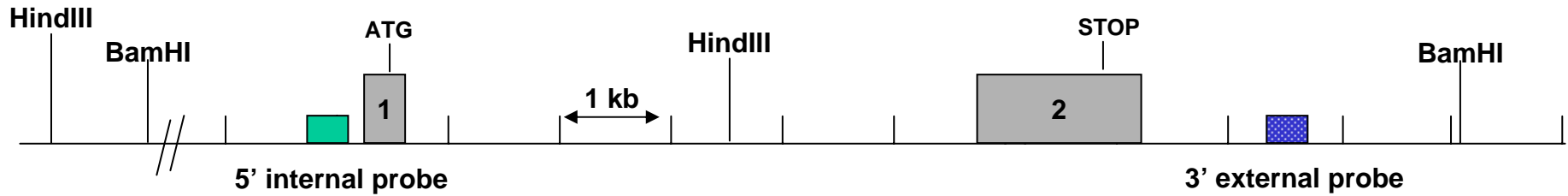
Primer Sequences (5' to 3')	
Mutant PCR: Primer Neo3a and Primer 0579-24, 366 bp	
Recommended Wt PCR: Primer 0579-5 and Primer 0579-24, 385 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0579-24	ACACCTGCCTGTTTCATCTGG
Primer 0579-5	CACAGCTACTACTTGAAGAG

Well	Sample	Genotype
1	22	het
2	23	het
3	24	het
4	25	wt
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



Mutant PCR



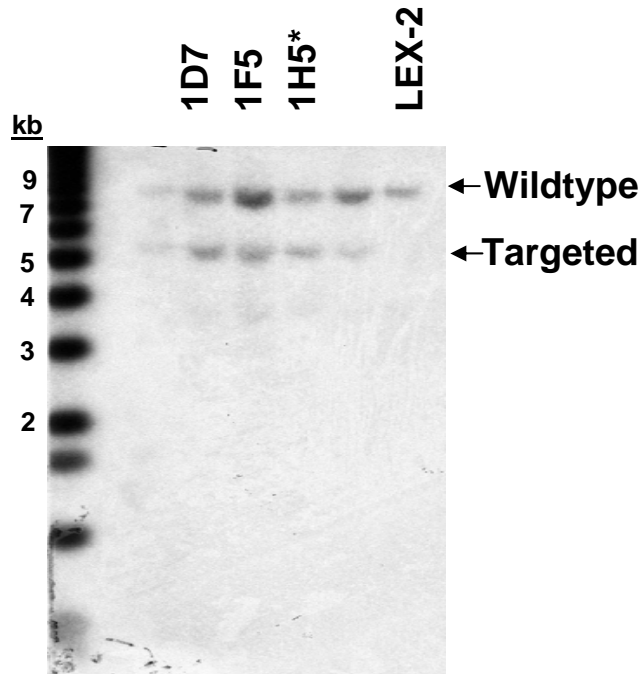
Targeting Strategy



Southern Strategies

Probe	5' internal 	3' external 
Enzyme	HindIII	BamHI
Wildtype	8.2 kb	11.0 kb
Targeted	5.5 kb	9.4 kb

Southern Data

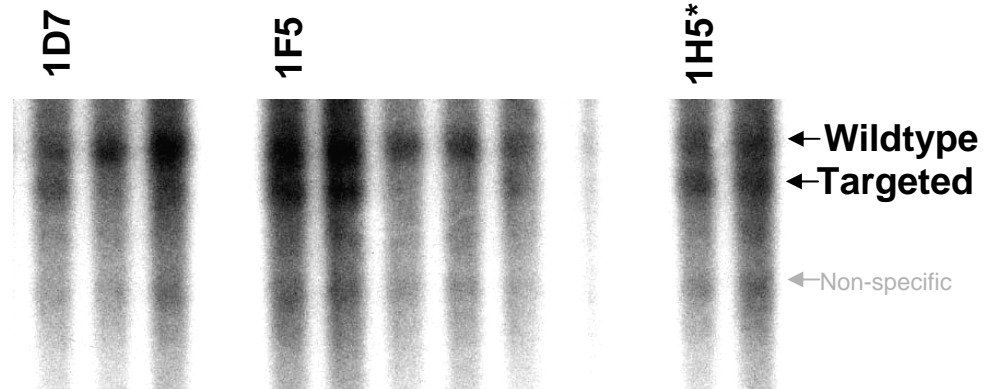


5' internal probe

HindIII digests

Wildtype 8.2 kb

Targeted 5.5 kb



3' external probe

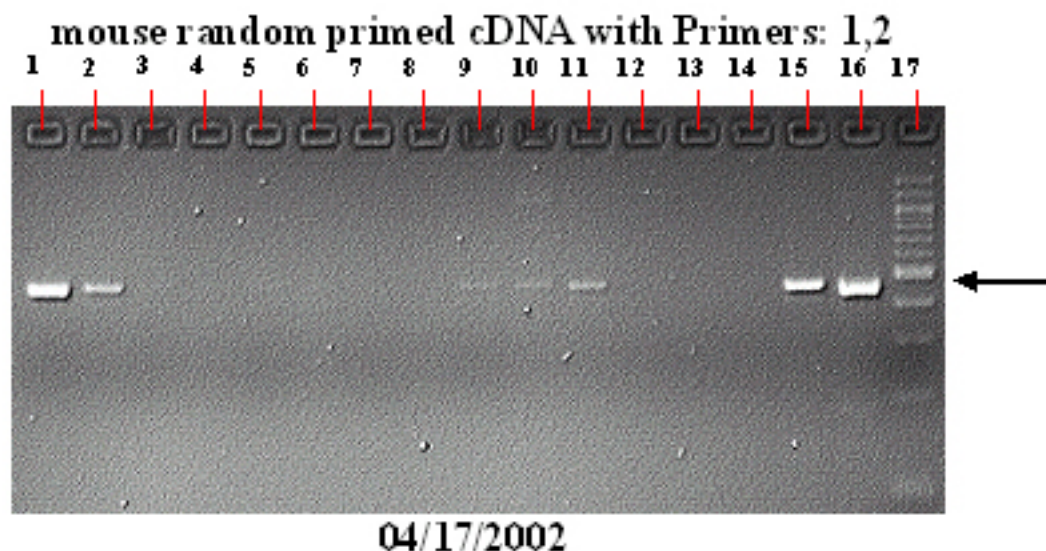
BamHI digests

Wildtype 11.0 kb

Targeted 9.4 kb

* clones 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) Stomach
- 9) Small Intestine & Colon
- 10) Skin Fibroblast
- 11) Heart
- 12) Adipose
- 13) Tail
- 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-0579 (LEXKO-227)

Reference accession(s): AB016602, NM_134438

Standard KO or Conditional: Standard

Materials Submitted: x Target Vector pKOS-19mTVneo
x KOS clone(s) pKOS19m

Southern Blot Genotyping Strategies:

	<u>5' Internal</u>	<u>3' External</u>
Name of Probe:	25+26	10+6
Restriction Enzyme for Genomic Digest:	HindIII	BamHI
Predicted Wild-type Band (kb):	8.2 kb	11.0 kb
Predicted Mutant Band (kb):	5.5 kb	9.4 kb
Probe Size:	233 bp	527 bp

Primer sequences:

Southern probes

0579-25 5' – AACGGATGTGGTCGGGAATG
0579-26 5' – ACCGCATGGCCAGGAAAGC
0579-10 5' – CCATGTCTCTGAACAGAACC
0579-6 5' – GAGGACTGTGGATGTCATC

Genomic Sequence Deleted:

ATGCTCCTGGCTCTGGTGGTGTGGTGTGGTATCGTTGGCAATCTGTCTGTCATGTGCATTGTGTGGCACAGCTACTA
CTTGAAGAGTGCCTGGAACCTCTATCCTTGCCAGCCTGGCTCTCTGGGATTTCTTGGTCTCTTCTTCTGCCTCCCAATTGT
CATCTTCAATGAGATTACCAAGCAAAGGCTACTCGGGGATGTTTCTTGGCCGGGCAGTGCCTTTCATGGAGGTGAGTATG
TCTTTC

KOS clone sequence: *(note: pKOS-19m was used to generate the TV and that is the sequence included here)*

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

GGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCC
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