

NIH-0129 Genotyping Strategies

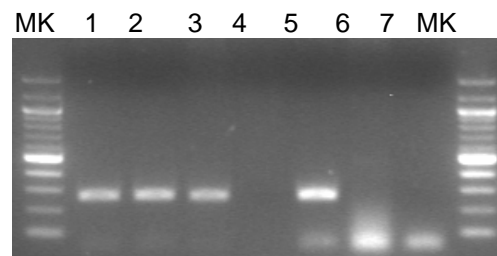
Reaction Components	Vol (ul)
10X Sigma Buffer	5
25mM MgCl ₂	3.5
10mM dNTPs	2
Primer 20 uM	1.5
Primer 20 uM	1.5
5 U/ul Taq polymerase	0.5
Water	31
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Step	Temp	Time	Note
1	95C	30"	
2	58C	30"	
3	72C	45"	Go to 1, 39 cycles

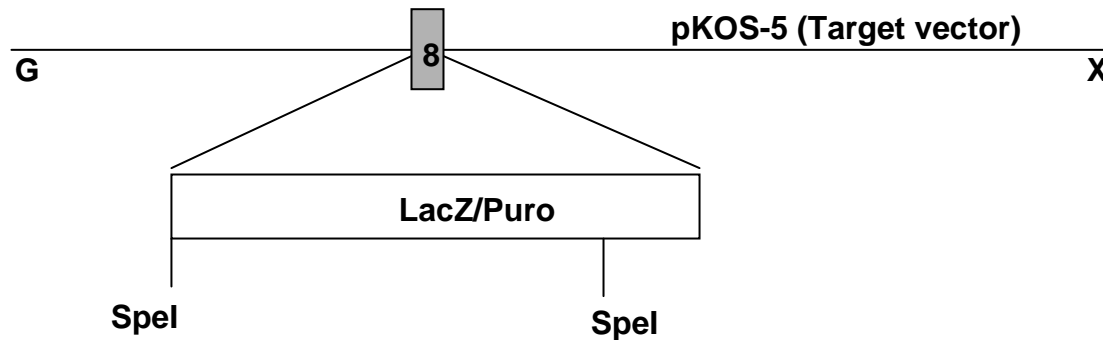
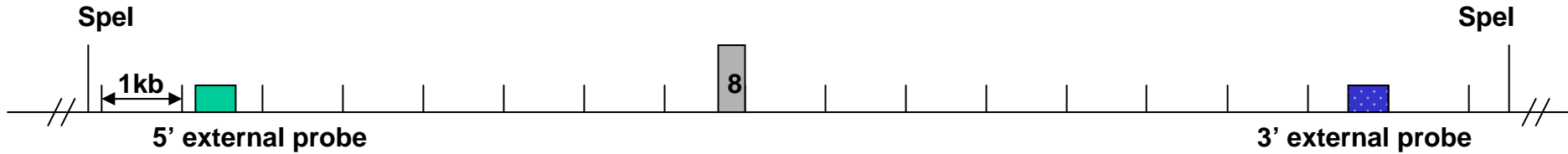
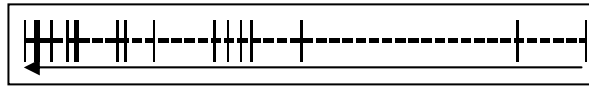
Primer Sequences (5' to 3')	
Mutant PCR: Primer puro out and Primer 0129-7, 272 bp	
Recommended Wt PCR: Primer 0129-3 and Primer 0129-2, 507 bp	
Primer puro out	GGATTGGGAAGACAATAGCAGG
Primer 0129-7	CTTGGCAGAGTACATCTCAG
Primer 0129-3	CAGCTGCATGCGGCGATCAC
Primer 0129-2	CATGCGTGCGTTGAAT

Well	Sample	Genotype
1	11	het
2	12	het
3	13	het
4	14	wt
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



Mutant PCR



Targeting Strategy

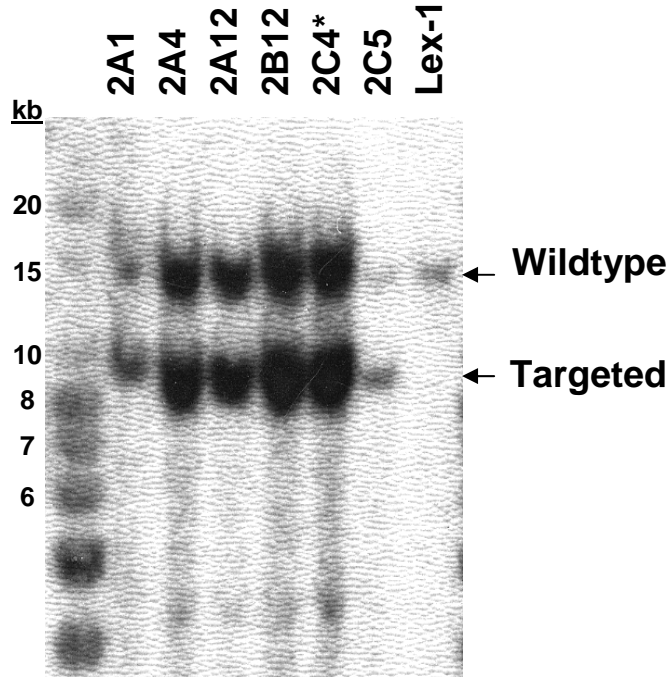


Southern Strategies

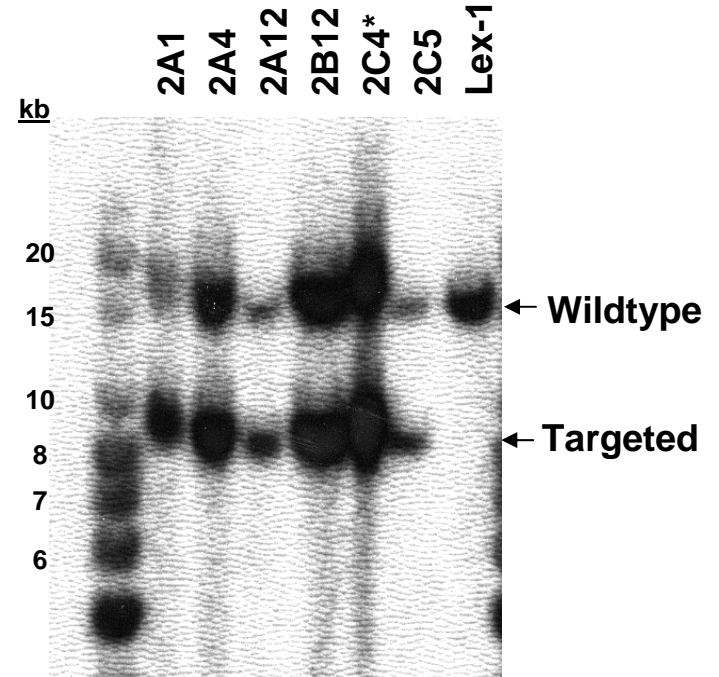
Probe	5' external 	3' external 
Enzyme	SpeI	SpeI
Wildtype	15.7 kb	15.7 kb
Targeted	8.0 kb	8.7 kb

Note: Target vector design will not allow for LacZ expression from this locus.

Southern Data



5' external probe
Spel digests
Wildtype 15.7 kb
Targeted 8.0 kb

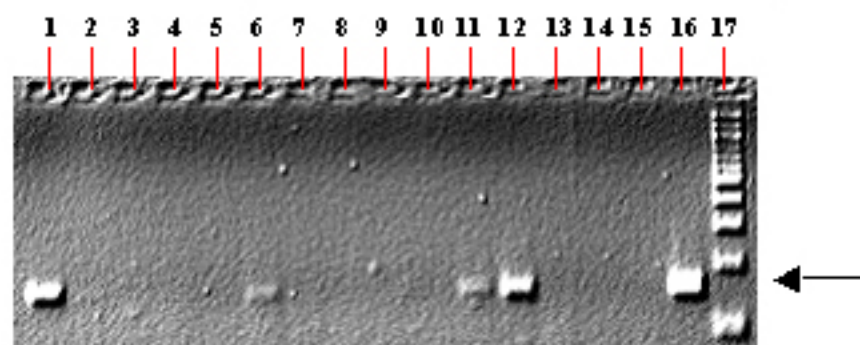


3' external probe
Spel digests
Wildtype 15.7 kb
Targeted 8.7 kb

* 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-0129 (LEXKO-005)

Reference accession(s): _ XM 129867, NM 175210.2

Standard KO or Conditional: _ Standard KO

Materials Submitted: Target Vector _ pKOS5TVpuro
 KOS clone(s) _ pKOS5

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' External/ Internal</u>
Name of Probe:	8/9	10/11
Restriction Enzyme for Genomic Digest:	SpeI	SpeI
Predicted Wild-type Band (kb):	15.7 kb	15.7 kb
Predicted Mutant Band (kb):	8.0 kb	8.7 kb
Probe Size:	217 bp	254 bp

Primer sequences:

Southern probes

0129-8 5' – TTTGTGAGATTGCGACA
0129-9 5' – CATTGGCTTTCTGTGAGTTAT
0129-10 5' – CTTAGTGCTCTTGGGTTGTTG
0129-11 5' – TCCAGGCTTAGATATGTGTCG

Genomic Sequence Deleted :

TTTCCAAAAAAGGAAAATGAATAGCATTGTTTAAAAAATGCATCTTTTCTTTCCACTATTAATTTGATTTATGGCTT
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GTGACAATTCACGCACGCATGGAGTGATGATGATGAGCAGACACTGTCCCGAGCAGTCTGGCTGCACAGTAAGTAG
TGTTCC

KOS clone sequence: (note: pKOS5 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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