

## NIH-0996 Genotyping Strategies

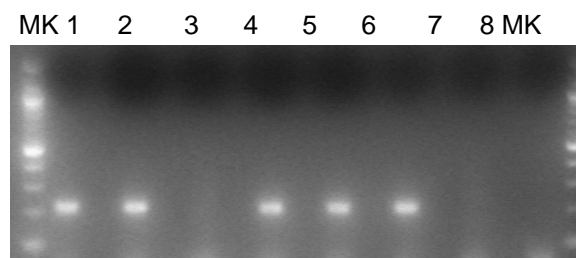
Reaction Components	Vol (ul)
5x Phusion buffer	8
25mM MgCl <sub>2</sub>	3.2
10mM dNTPs	1
Primer 20 uM	1
Primer 20 uM	1
Phusion Enzyme	0.1
Water	20.7
Total mix volume	35
Tail lysate (1:20 dilution)	5
Total reaction volume	40

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 0996-35, 217 bp	
Recommended Wt PCR: Primer 0996-34 and Primer 0996-35, 363 bp	
Primer Neo3a	GCAGCGCATCGCCTTCTATC
Primer 0996-35	CTGAGCTAAGCCTGGCATTCTG
Primer 0996-34	GTGAAAGGTCATGAAGCATGACTG

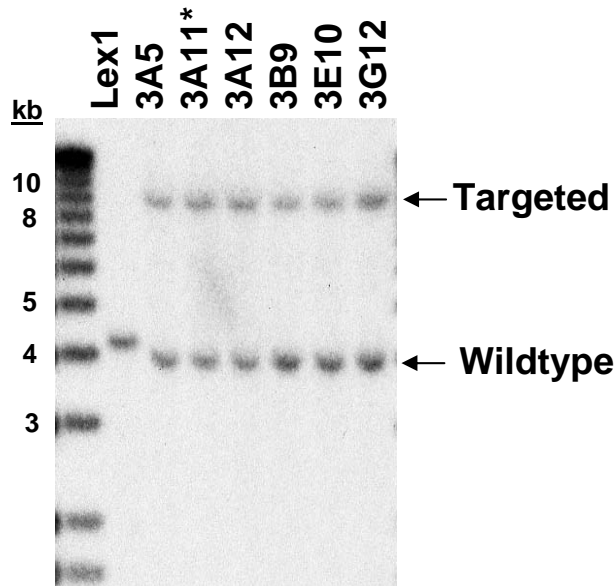
Well	Sample	Genotype
1	161	het
2	171	het
3	172	wt
4	180	het
5	183	het
6	<b>ES DNA</b>	het
7	<b>wt lysate</b>	wt
8	<b>water</b>	no amp

### Mutant PCR

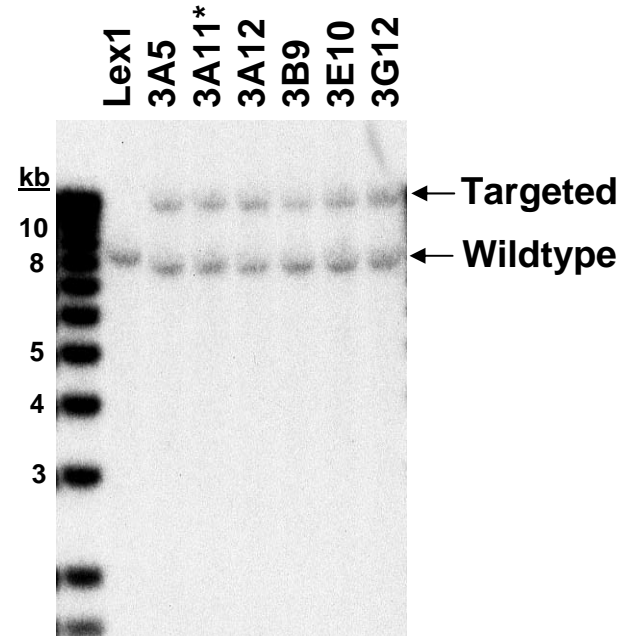




# Southern Data



**5' External Probe**  
**Scal digests**  
 Wildtype: 4.2 kb  
 Targeted: 9.7 kb



**3' External Probe**  
**HindIII digests**  
 Wildtype: 8.6 kb  
 Targeted: 13.0 kb

**\*Clone achieving germline transmission**



**Lexicon Genetics Incorporated  
Molecular Genetics Project Materials**

Catalog Number: NIH-0996 (LEXKO-378)

Reference accession(s): NM\_138648

Standard KO or Conditional: \_ standard

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Materials Submitted: x Target Vector \_ pKOS-20TVneo  
x KOS clone(s) \_ pKOS-20

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**Southern Blot Genotyping Strategies:**

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	61 + 65	71 + 75
Restriction Enzyme for Genomic Digest:	ScaI	HindIII
Predicted Wild-type Band (kb):	4.1	8.6
Predicted Mutant Band (kb):	9.7	13
Probe Size:	456 bp	423

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**Primer sequences:**

**Southern probes**

0996-61 5' – ATCCCATTCATTAAGCTTGTC  
0996-65 5' – GTTCCATGTCTCTGAGGTTGG  
0996-71 5' – CCATTGCTTCCCATATGTCTA  
0996-75 5' – GTCTACTGCCAGACTTCCT

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**Genomic Sequence Deleted:**

CTGTTTCAGTTTTTCACTCTTAGCAGGAATTTGGAGATGACTTTTTGATGACAAGATGAAGCCTGCGAATGACGAGCCTG  
ATCAGAAGTCATGTGGCAAGAAGCCTAAAG

**KOS clone sequence:** *(note: pKOS-20 was used to generate the TV and that is the sequence included here)*

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

GGCGCGCCGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCC  
TCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCAT  
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