

NIH-0699 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

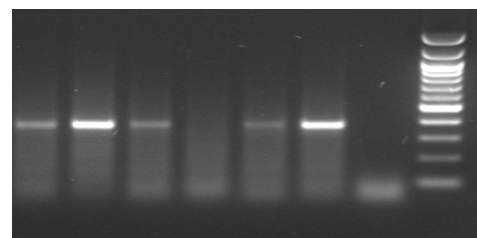
Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

Primer Sequences (5' to 3')	
Cre Excision PCR: Primer 0699-11 and Primer 0699-47, 371 bp	
LoxP mutant PCR: Primer 0699-11 and Primer 0699-8, wt 351 bp, mutant 419 bp	
Primer 0699-47	AGGAAGATCCCGGCATTAC
Primer 0699-11	CCTGGGCCTTGGTGGTCACAG
Primer 0699-8	CAATGCGCAGCTGCTCCACC

Well	Sample	Genotype
1	619	het
2	620	hom
3	621	het
4	wt	wt
5	het	het
6	hom	hom
7	water	no amp

Cre Excision PCR

1 2 3 4 5 6 7 MK



P#10442 LEXKO-1081 PCR - FLOXED mice

	LoxP
5' primer	51
3' primer	50
wt band	319
mut band	376
primer storage	P152 E3-6
denature temp	TD

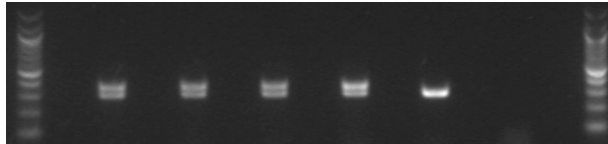
Reagents	1 rxn(μL)
H2O	8.5
5X GoTaq Buffer	4
10mM dNPT	0.4
25mM MgCl ₂	1.4
20uM 5'	0.3
20uM 3'	0.3
Taq	0.1
1:30 DNA:H2O	5
	20

Primer 51 5'- GATGGGCTACTGTGAGCAACATG

Primer 50 5'- CATCTCAGTTAGGCCAGGCGACAG

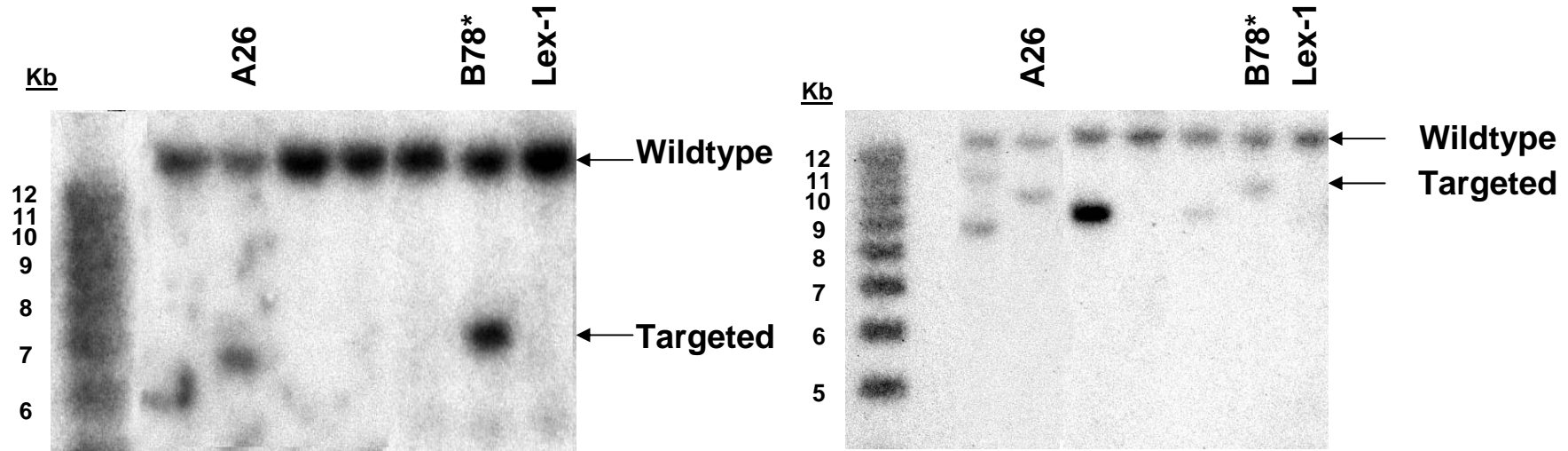
Step	Temp	Time	Note
1	94C	15"	
2	65C	30"	Decrease 1C/cycle
3	72C	40"	Go to 1, 10 cycles
4	94C	15"	
5	55C	30"	
6	72C	40"	Go to 4, 30 cycles

PCR#	Name	tail #
1	11707	649
2	11707	650
3	11707	652
4	es DNA	1C4
5	wt lysate	
6	water	



Genotypes
het
het
het
het
wt

Southern Data

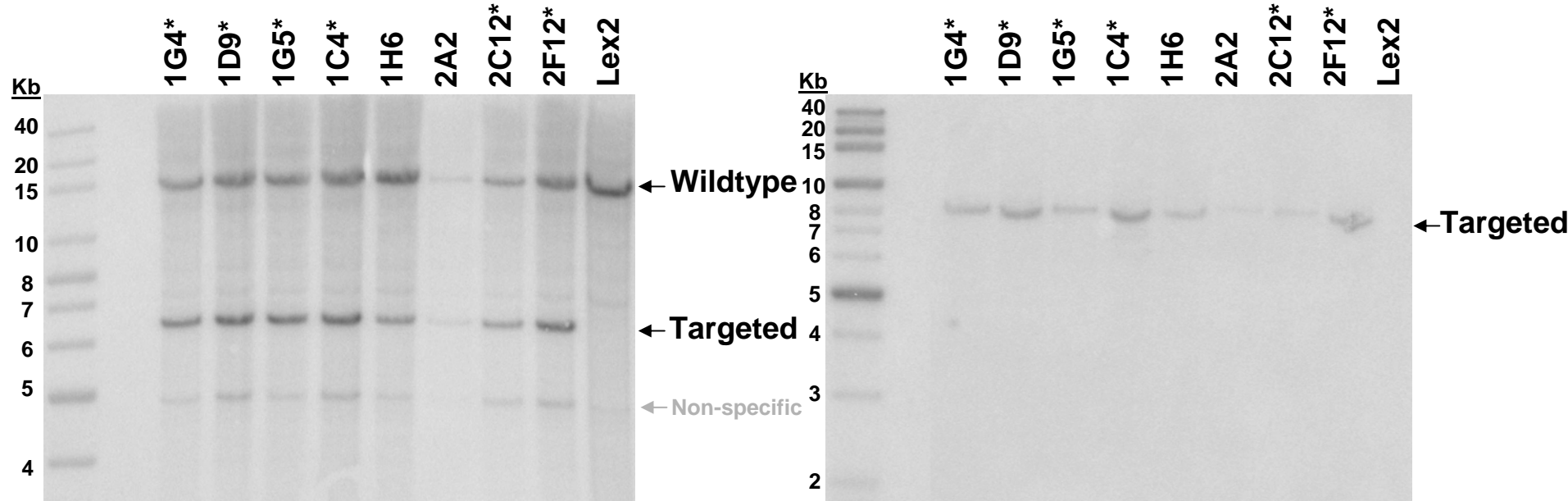


5' external probe
 KpnI digests
 Wildtype 13.9 kb
 Targeted 6.3 kb

3' Internal probe
 KpnI digests
 Wildtype 13.9 kb
 Targeted 9.6 kb

* Clone injected

Additional Southern Data



5' external probe
KpnI digests
Wildtype 13.9 kb
Targeted 6.3 kb

3' internal probe
KpnI digests
Targeted 9.6 kb

***Additional clones fully confirmed**



**Lexicon Genetics Incorporated
Molecular Genetics Project Materials**

Catalog Number: NIH-0699 (LEXKO-1081)

Reference accession(s): NM_010319

Standard KO or Conditional: Conditional

Materials Submitted: Target Vector pKOS-49TV
 KOS clone(s) pKOS-49

Southern Blot Genotyping Strategies:

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	14/28	31/42
Restriction Enzyme for Genomic Digest:	KpnI	KpnI
Predicted Wild-type Band (kb):	13.9 kb	13.9 kb
Predicted Mutant Band (kb):	6.3 kb	9.6 kb
Probe Size:	799 bp	436 bp

Primer sequences:

Southern probes

0699-14 5' – AGCCAGGTGACGTGAGACTC
0699-28 5' – AAGGACACGTGGCCACGTCAC
0699-31 5' – GAAGCCAGTCTAGTTGTCC
0699-42 5' – ACCTGGTTCCTAGCCTAATGC

Genomic Sequence Floxed:

CCTTACTCTGAGAGGCCAGCGGGGTTCCGGGTCAAGGTTCCAGGGTCTAAGCAGCAGGTTACGCCATGCCATCTGTGCCCTCCAGCTCTCTAGACGC
CAGGTCCACGCTCATGGCCGATGATCAGGTACTAACAACGTCGCCAGGCCCGGAAGCTGGGAGCAGCTGCCATTGAAGCTGGGATCGAACGC
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ATCAAAAGACAAAAAGCCTTGCAATAATCTCTAGTTCTCTCTCTCTCTCTCT

KOS clone sequence: (note: pKOS-49 was used to generate the TV and that is the sequence included here)

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ATCCAGGACAGCCCCAGCTCGGGACCCTCAGCTTGATCCGTCGAGCAGTGT

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

pLFneo (conditional projects only)

The 5' and 3' **Lox P sites** are in **bold**. The 5' and 3' **FRT sites** are in **bold italics**. The floxed region was subcloned into the multi-enzyme cloning site (*italics, underlined*). The region between the loxP sites was excised upon exposure to Cre recombinase.

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