

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6;129S5-Tpbgtm1Lex/Mmucd **MMRRC: 031630-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.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	1x
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	72	0:40	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	30X
7. Elongation	72	0:40	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA321-20	ACAGAACACACCGGGATT	Estimated Running Time: 90 min.		
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. DNA321-19	AGGCTTACCTGCGCATTC	1 & 2	304	mutant
		1 & 3	454	wildtype

No gel image available



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2257	Date of Submission:	1-19-05
Lexicon Contract Name:	DNA321	Mutation Type:	X Standard Knock out
LexVision Name:	SEC560N1		☐ Conditional
Reference accessions:	NM_011627	Is this gene X-linked?	No

Required Materials:

- ☐ pKOS clone DNA(s) __pKOS27__
- ☐ Target Vector DNA __KOS27TV__
- ☐ Targeted ES Cell DNA __1C4__
- ☐ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	14+15	7+8
Restriction Enzyme for Genomic Digest:	Bgl II	BamHI
Predicted Wild-type Band (kb):	6.7 kb	11.8 kb
Predicted Mutant Band (kb):	10.6 kb	10.0 kb
Probe Size:	342 bp	347 bp

PCR Strategies:*For standard knockouts, give wildtype and mutant-specific strategies**For conditionals, give 5' loxP and cre-excision strategies*

Wild type-specific (absent in targeted allele)		Mutation-specific product (absent in wt)	
5' Primer Name:	DNA321-19	5' Primer Name:	Neo3A
3' Primer Name:	DNA321-20	3' Primer Name:	DNA321-20
Predicted Wild-type Band (bp):	454 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	304 bp

5' loxP strategy		Distinguish Cre-excised and wt	
5' Primer Name:		5' Primer Name:	
3' Primer Name:		3' Primer Name:	
Predicted Wild-type Band (bp):		Predicted Wild-type Band (bp):	
Predicted mutant band (bp)		Predicted mutant band (bp)	

Primer sequences:**Southern probes**

DNA321-14 5' – AATCTCTTCATCTGGCCTAGAC
 DNA321-15 5' – ATACCTCAGAGGCAAGATACAC
 DNA321-7 5' – GTCTCGTCTCTATCCCGCCACC
 DNA321-8 5' – GCCCACTATTCTTACTAGACC

PCR Genotyping

DNA321-19 5' – AGGCTTACCTGCGCATTC
 DNA321-20 5' –ACAGAACACACCGGGATT
 Neo3A 5' –GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

TCCGGGGAACCGCGAGCCGCGATGCCTGGGGCGGGCTCCCGGGGCCCTCCGCCGGGGACGGACGGCTGAGGTTGGCA
AGGCTGGCGCTAGTGCTGCTGGGTGCTCCGCGTCGGCCCCCAGCTCTTCGGTACCCTCGTCTTCCACCTCCCCGGC
AGCCTTCCTGGCCTCGGGGTCTGCGCAGCCTCCGCCAGCCGAGAGATGCCCCGCGGCGTGCGAGTGCTCCGAGGCGGC
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ACCCAGATTAACAAATCTTAGTTCCAACCTCGGATGTCTGAGAAACAGGAC

Genomic Locus: *(The deleted sequence represents nt995-11306-in the sequence below. KOS27 used to generate the TV represents nt 7370-18466 in the sequence below.)*

GTAAATATCACTGCATCAATAGCTCTTGGCCAAAGCCTTCTGGGGTCATGGGTCCCTCCAGCAGCTATGATTGGAGTTC
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[illegible]

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Selection Cassette:

GGCCATAGCGGCCATTTAAATGGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAGAGAATTCCGCCCC
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