

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6N.129S5-Fcrl5tm1Lex/Mmucd **MMRRC: 032303-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	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. DNA082-2	AAACTCTAGTGCCATTGCTACCGT	Estimated Running Time: 90 min.		
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. DNA082-9	CACAGATGTGGCTGACTCTACTGG	1 & 2	302	Mutant
		1 & 3	266	wildtype



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ503	Date of Submission:	7/13/05
Lexicon Contract Name:	DNA082	Mutation Type:	☒ Standard Knock out
LexVision Name:	MAS240N1		☐ Conditional
Reference accessions:	NM_183222.2, AY158090	Is this gene X-linked?	No

Required Materials: ☒ pKOS clone DNA(s) __pKOS-77_____
☒ Target Vector DNA __pKOS-77TV Neo_____
☒ Targeted ES Cell DNA __2C1_____
☒ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	54+55	48+49
Restriction Enzyme for Genomic Digest:	BamHI	BamHI
Predicted Wild-type Band (kb):	~12.0	~12.0
Predicted Mutant Band (kb):	6.5	~5.4
Probe Size:	305	598

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:	DNA082-9	5' Primer Name:	Neo3a
3' Primer Name:	DNA082-2	3' Primer Name:	DNA082-2
Predicted Wild-type Band (bp):	266	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	302

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**

DNA082-48 5' – GGATGGCACAGACAGATAACC
DNA082-49 5' – CCCTAAGCATAAGAACGA
DNA082-54 5' – ACAAAAAGGACATGTCGAATC
DNA082-55 5' – AGCAAGATTTCCTAAATATTC

PCR Genotyping

DNA082-2 5' – AAACCTCTAGTGCCATTGCTACCGT
DNA082-9 5' –CACAGATGTGGCTGACTCTACTGG
Neo3a 5' –GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

GTGAGTGACTACCATTGCGAGCAAGAGCAAGAGGAAAGCACTACCTGTGAGCAGATGTCTGGTTCATTCTCACCCTGT
GTGGTGTTACACAGATGTGGCTGACTCTACTGGTTGTGAGTGAGTAGATGGCTCAATATGGGGATAGAGTGGGTGCG
ACAGCATCTCTCTCCACTACAGGCCTGGTACTCTCTTAAGTTTTAGCTCCTCAGTTTCCCCG

**Genomic Locus: (The deleted sequence represents nt9760-9977-in the sequence below. KOS77 used to generate the TV
represents nt4170-11720- in the sequence below.)**

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[illegible]

[illegible]

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Selection Cassette:

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Targeted Locus:

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[illegible]