

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: _____ **B6;129S5-Pgap3tm1Lex/Mmucd** _____ **MMRRC: 032518-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. DNA275-7	AACATGTGTCCTCCTTCCCTC	Estimated Running:Time: 90 min.		
2. NEO3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. DNA275-18	CGTCATGGAAAGGTGGACCAG	1 & 2	360	Mutant
		1 & 3	432	wildtype



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ546	Date of Submission:	10/11/2004
Lexicon Contract Name:	DNA275	Mutation Type:	X Standard Knock out
LexVision Name:	MEM692N1		☐ Conditional
Reference accessions:	AK052486, AK078974	Is this gene X-linked?	NO

Required Materials: X pKOS clone DNA(s) __pKOS 33_____
X Target Vector DNA __DNA275 33TV_____
X Targeted ES Cell DNA 2B4
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	11 + 12	13 + 14
Restriction Enzyme for Genomic Digest:	EcoRV	EcoRV
Predicted Wild-type Band (kb):	17.4 kb	17.4 kb
Predicted Mutant Band (kb):	12.1 kb	8.0 kb
Probe Size:	283 bp	341 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:	DNA275-18	5' Primer Name:	Neo3a
3' Primer Name:	DNA275-7	3' Primer Name:	DNA275-7
Predicted Wild-type Band (bp):	432	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	360

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

DNA275-11 5' - GGATTGAGAGCTTAAACTCC
 DNA275-12 5' - GAGCAGACACAACAAGGTGG
 DNA275-13 5' - GCGTGCAATGAGTGTTCTG
 DNA275-14 5' - CTGTCATTGACACCATCACG

PCR Genotyping

DNA275-18 5' - CGTCATGGAAAGGTGGACCAG
 DNA275-7 5' - AACATGTGTCCTCCTTCCCTC
 NEO3a 5' - GCAGCGCATCGCCTTCTATC

[illegible][illegible]

[illegible]

[illegible]

Selection Cassette:

Targeted Locus:

GGAACCAGAGTTACAGACAACTGTGAGCTGCCATGTGGGTGCTGGGAATTGAACCTGGGACCTCTGGAAGAGCAGCCAATGCTCTTAACCACTGAGCC

[illegible]

AAACGCAGGTGCGCAGCGGCACCGCGCCTTTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTACGTTCTGAACGTCGAA
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