

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: B6;129S5-Gpx8^{tm1Lex}/Mmucd MMRRC: 032346-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	10.275
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Primer 3. (stock concentration is 20μM)	0.5
Primer 4. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	25.000 μL

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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl₂ to increase reaction or decrease non-specific amplifications.

Strategy:

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

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. DNA432-5	GAAGCCGAGAACCAACAGCTT	Estimated Running Time: 90 min.		
2. DNA432-6	CCATAACCCAAATGAATGGTCG	Primer Combination	Band (bp)	Genotype
3. DNA432-4	CGATATTACTTTGAAGACTAC	1 and 2	408	Wild-type
4. Neo3a	GCAGCGCATCGCCTTCTATC	3 and 4	218	Knockout



Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ487	Date of Submission:	5-7-04
Lexicon Contract Name:	DNA292	Mutation Type:	X Standard Knock out
LexVision Name:	SEC308N1		☐ Conditional
Reference accessions:	NM_032541 ENSMUST00000014202	Is this gene X-linked?	no

Required Materials: X pKOS clone DNA(s) DNA292-kos88
X Target Vector DNA DNA292-88 FTV
X Targeted ES Cell DNA 1E7
X Genomic Map

Southern Blot Analysis: External/Internal Probe Strategies

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	DNA292-14/15	DNA292-17/19
Restriction Enzyme for Genomic Digest:	Nhe I	DraI
Predicted Wild-type Band (kb):	11.3	10.6
Predicted Mutant Band (kb):	15.3	2.9
Probe Size:	268 bp	255 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:	DNA292-9	5' Primer Name:	Neo3a
3' Primer Name:	DNA292-10	3' Primer Name:	DNA292-10
Predicted Wild-type Band (bp):	429 bp	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	543 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**

DNA292-14 5' – TTCAGAGCTCTACCCTTTCC
 DNA292-15 5' – AGCAGAAAGAGCTGTCCAGC
 DNA292-17 5' – TTTGTAATGTGGATGCTAGC
 DNA292-19 5' – GAAGTCCTTCTTTGCATGCC

PCR Genotyping

DNA292-9 5' – TTGCACGGGGAAGAAAGCAG
 DNA292-10 5' – GACCTGTAAACCCAGCTCAG
 Neo3a 5' – GCAGCGCATCGCCTTCTATC

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Genomic Sequence Deleted:

GGCATGATGGCACTCAGCACTCGGACCCAGGCTGCCTGTCTCCTGCTTCTCCTCCTTGCCAGCCTGAGCAGCACCACCTATCTCCATCAACAGGTGAGC
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Genomic Locus: (the deleted sequence represents nt 14031-15637 in the sequence below)

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[illegible]

Selection Cassette: LacZ/Neo

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Targeted Locus:

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[illegible]

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