

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-C1qtnf1tm1Lex/Mmucd **MMRRC: 032164-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.
- The mutant PCR is a general LacZ PCR. The wild type is specific for this strain.

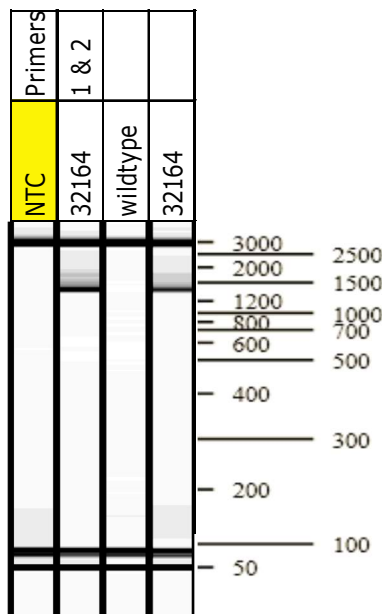
Strategy:

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. DNA063-31	GGAAGTCCCTCTCACGTGTC	Estimated Running Time: 90 min.	
2. Neo 3a	GCAGCGCATCGCTTCTATCG	Primer Combination	Band (bp)
3. DNA063-1	GGTTCTACAGGTCCCAGGG	1 & 2	1100
4. DNA063-2	GTGATGTAGGTGTCTGAAGTCG	3 & 4	458
			Genotype
			mutant
			wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ310 DNA41234	Date of Submission:	9/30/03
Lexicon Contract Name:	DNA063	Mutation Type:	☒ Standard Knock out
LexVision Name:	ZSIG37M_1		☐ Conditional
Reference accessions:	NM_019959, ENSMUST00000017590	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS-54, pKOS-29_____
X Target Vector DNA __pKOS-29TV_____
X Targeted ES Cell DNA __A10_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	40/41	38/39
Restriction Enzyme for Genomic Digest:	Apa I	Eco RI
Predicted Wild-type Band (kb):	6.2	20
Predicted Mutant Band (kb):	2.7	9
Probe Size:	412 bp	413 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:	DNA063-1	5' Primer Name:	Neo 3a
3' Primer Name:	DNA063-2	3' Primer Name:	DNA063-31
Predicted Wild-type Band (bp):	458	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	1100

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**

DNA063-40	5' – GCTGGTGGAGAGACAAGCTC
DNA063-41	5' – GTCTACCCCCAGACCATCTG
DNA063-38	5' – CACACTGACAGGTGCCAGAG
DNA063-39	5' – GAGTGTACACGTGCCACAG

PCR Genotyping

DNA063-1	5' – GGTCTCTACAGGTCCCAGGG
DNA063-2	5' – GTGATGTAGGTGTCGAACTCG
Neo 3a	5' – GCAGCGCATCGCCTTCTATCG
DNA063-31	5' – GGAAGTCCCTCTCACGTGTC

Genomic Sequence Deleted:

GGGGAAGTACGGCAAAAATAGGTTCTACAGGTCCCAGGGGCCATGTTGGCCCCAAAGGGCAGAAGGGGATCC
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TGACCTATCCCTGCATTCTACAGACACTGGAGTCCTGCCCGGGCTGACCCCATTTTCTCTCTGCTCCATCCT
GGCTTCCTTGGCCTTGGCTTCCAAAGTTTTGGCTTTT

Genomic Locus: (the deleted sequence represents nt-2080-2758 -in the sequence below)

[illegible]

[illegible]

Selection Cassette:

GGCCGCTCTAGAGGCCATAGCGGCCATTTAATGGCGCGCCGGATCCCGGGCCGCTCTAGCTAGACTAGTCTAGCTAG
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Targeted Locus:

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