

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Ace2tm1Lex/Mmucd **MMRRC:** 031665-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.

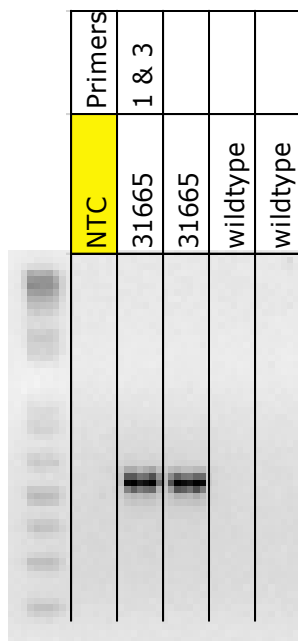
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	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. 31665-Neo3a	GCAGCGCATCGCCTTCTATC	Estimated Running Time: 90 min.		
2. 31665-3	GATGTCCAGCTCCTCCTGG	Primer Combination	Band (bp)	Genotype
3. 31665-37	GCTCAATAACGACTTAGAACAT	1 & 3	468	mutant
		2 & 3	500	wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	DNA128	Date of Submission:	8 September 2003
Lexicon Contract Name:	UNQ868	Mutation Type:	☒ Standard Knock out
LexVision Name:	ENZ211N1		☐ Conditional
Reference accessions:	ENSMUST00000015549, AB053181	Is this gene X-linked?	YES

Required Materials: X pKOS clone DNA(s) __pKOS66_____
 X Target Vector DNA __DNA128 pKOS-66-FTV1_____
 X Targeted ES Cell DNA __2C1_____
 X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	28/27	29/30
Restriction Enzyme for Genomic Digest:	KpnI	HindIII
Predicted Wild-type Band (kb):	14.4	10.3
Predicted Mutant Band (kb):	7.9	11
Probe Size:	335 bp	488 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:	3	5' Primer Name:	Neo3A
3' Primer Name:	37	3' Primer Name:	37
Predicted Wild-type Band (bp):	500	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	468

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**

27 5' – GCTACACATTGATCACATACAG
 28 5' – ACGAATCCTGTTTCATGTAGG
 29 5' – ACATTTACCACCAAAAGTTGTC
 30 5' – CTCTGCTGTGCTTTTGGCTG

PCR Genotyping

3 5' – GATGTCCAGCTCCTCCTGG
 37 5' – GCTCAATAACGACTTAGAACAT
 Neo3A 5' – GCAGCGCATCGCCTTCTATC

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[illegible]

[illegible]

[illegible]

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Selection Cassette:

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