

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-II17rbtm1Lex/Mmucd **MMRRC: 032383-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:

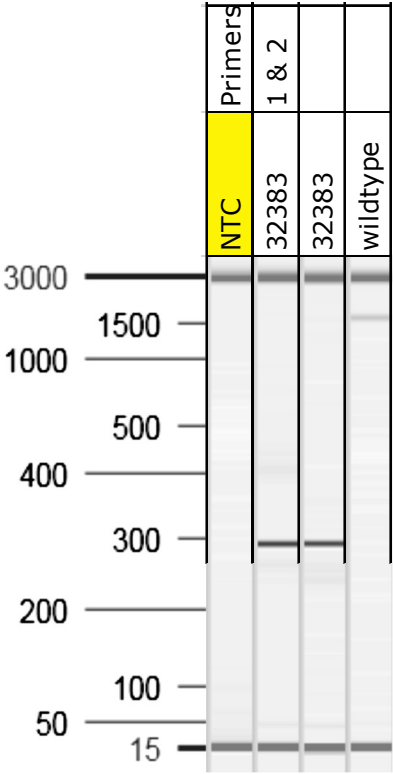
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. MEM665N1-6	ATTTGATGGAGCACCGCTAT	Estimated Running Time: 90 min.		
2. Neo3a	GCAGCGCATCGCCTTCTATC	Primer Combination	Band (bp)	Genotype
3. MEM665N1-15	GCCATGTTGCTAGTGTTGCT	1 & 2	299	Mutant
4. MEM665N1-16	GTCTTGTTGTCATCATTAACCCA	3 & 4	426	wildtype

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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ2501	Date of Submission:	5.13. 04
Lexicon Contract Name:	DNA044	Mutation Type:	x Standard Knock out
LexVision Name:	MEM665N1		☐ Conditional
Reference accessions:	ENSMUST00000016110, NM_019583	Is this gene X-linked?	No

Required Materials: x pKOS clone DNA(s) __KOS38,KOS89_____
x Target Vector DNA __TV89-3_____
x Targeted ES Cell DNA __2G6_____
x Genomic Map

Southern Blot Analysis: External/Internal Probe Strategies

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	13+14	7+8
Restriction Enzyme for Genomic Digest:	BamHI	MscI
Predicted Wild-type Band (kb):	15.2 kb	9.7 kb
Predicted Mutant Band (kb):	12.7 kb	5.0 kb
Probe Size:	243 bp	363 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:	MEM665N1-15	5' Primer Name:	Neo3a
3' Primer Name:	MEM665N1-16	3' Primer Name:	MEM665N1-6
Predicted Wild-type Band (bp):	426	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	299bp

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**

MEM665N1-13	5' – ATTTCCCTCGGTTTACGG
MEM665N1-14	5' – CCTCAATATAGACGGGAGTCAG
MEM665N1-7	5' – GGGCGTTCTCTAAGTCACTC
MEM665N1-8	5' – TCATGTTTGAAAGGAGGTTCTA

PCR Genotyping

MEM665N1-15	5' – GCCATGTTGCTAGTGTTGCT
MEM665N1-16	5' – GTCTTGTTTGTCATCATTAACCCA
Neo3a	5' – GCAGCGCATCGCCTTCTATC
MEM665N1-6	5' – ATTTGATGGAGCACCGCTAT

[illegible]

ATAAAGGGGGCTGCTTGCCCCCTCTCCCTCTCTTGATCTCTTGCTTCTCTCTGCTCTCGCTCTCCCCTTCCCCATTCCCATTCCCTTCCCCCTCTCTCCA
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Selection Cassette: LacZ/Neo

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Targeted Locus:

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