

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: B6;129S5-Lrp10tm1Lex/Mmucd **MMRRC: 032438-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.

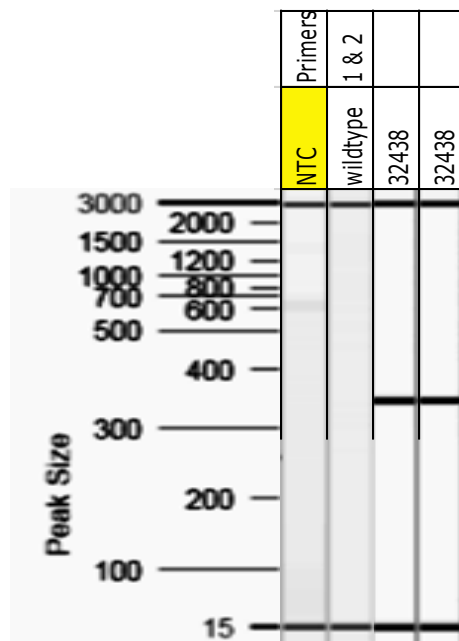
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	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. MEM660-10	CCAGTTGGCTGTAAGTGGTG	Estimated Running Time: 90 min.		
2. GT-IRES	CCCTAGGAATGCTCGTCAAGA	Primer Combination	Band (bp)	Genotype
3. MEM660-1	GACTGTTGACCCGTGGTAC	1 & 2	358	mutant
4. MEM660-2	CCAGATGCAGCTTCTGGAAC	3 & 4	456	wildtype





Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ389	Date of Submission:	4.16.04
Lexicon Contract Name:	DNA072	Mutation Type:	X Standard Knock out
LexVision Name:	MEM660N1		☐ Conditional
Reference accessions:	ENSMUST00000022782, NM_022993	Is this gene X-linked?	No

Required Materials: X pKOS clone DNA(s) __pKOS 66_____
X Target Vector DNA __TV 66 Bgeo_____
X Targeted ES Cell DNA __1E3_____
X Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' Internal</u>
Name of Probe:	32/34	35/36
Restriction Enzyme for Genomic Digest:	HindIII	BglII
Predicted Wild-type Band (kb):	8.6	5
Predicted Mutant Band (kb):	7.4	15
Probe Size:	132bps	384bps

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:	MEM660-1	5' Primer Name:	MEM660-10
3' Primer Name:	MEM660-2	3' Primer Name:	GT-IRES
Predicted Wild-type Band (bp):	456bps	Predicted Wild-type Band (bp):	None
Predicted mutant band (bp)	None	Predicted mutant band (bp)	358bps

Primer sequences:**Southern probes**

MEM660-32 5' – GACACGTAGCAGTTACGGC
MEM660-34 5' – CCTGCGCTCAGCTCCTTTC
MEM660-35 5' – CAGTCTGAACTCAGTGCAGAC
MEM660-36 5' – CCAGCGCTTCAGCTCCAACCTC

PCR Genotyping

GT-IRES 5' – CCCTAGGAATGCTCGTCAAGA
MEM660-1 5' – GACTGTTGACCCGTGGTAC
MEM660-2 5' – CCAGATGCAGCTTCTGGAAC
MEM660-10 5' – CCAGTTGGCTGTAAGTGGTG

Genomic Sequence Deleted:

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Genomic Locus: (the deleted sequence represents nt 3498-7775 in the sequence below)

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Selection Cassette: Bgeo/Puro

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ACTTGCAAGAAG