

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6;129S5-Colec11tm1Lex/Mmucd **MMRRC: 032220-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:

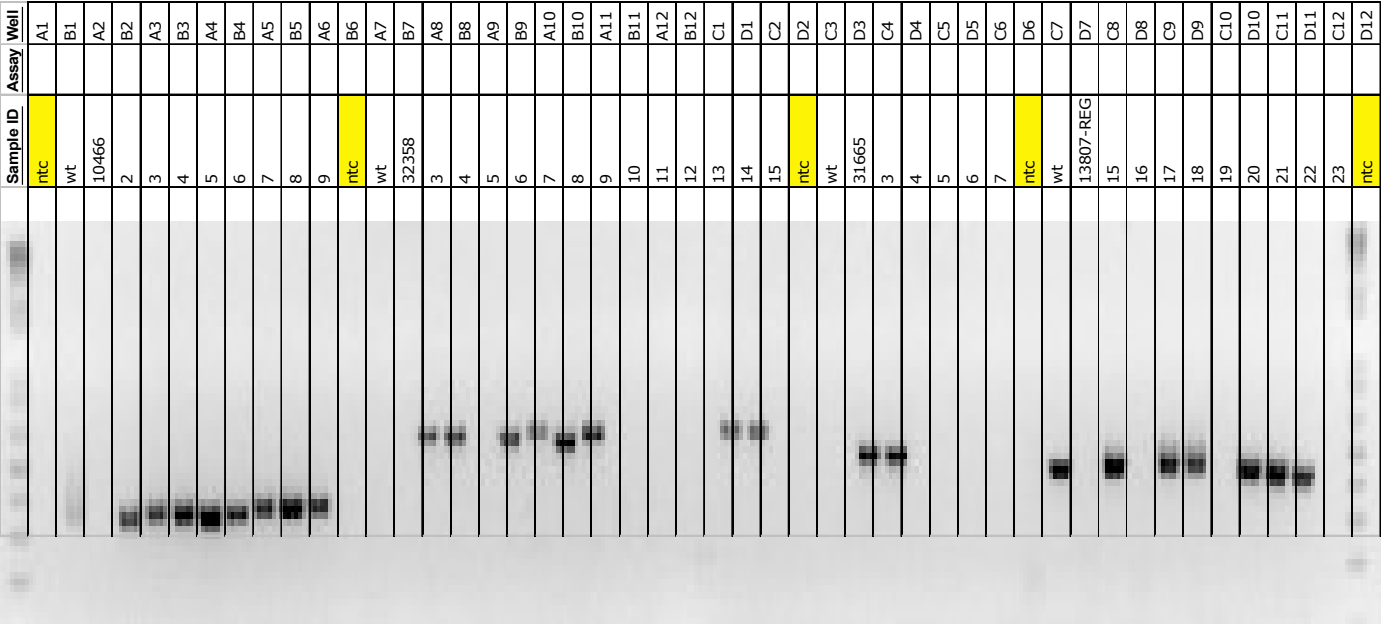
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00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

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. MEM637-6	TTGGATATACTCAGTGTCAGC	Estimated Running Time: 90 min.		
2. GT-IRES	CCCTAGGAATGCTCGTCAAGA	Primer Combination	Band (bp)	Genotype
3. MEM637-25	CTCTGTGCAGATTCTTGTC	1 & 2	611	mutant
		1 & 3	476	wildtype

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Lexicon Genetics Incorporated – Genentech Project Materials

Genentech ID:	UNQ596	Date of Submission:	07/19/04
Lexicon Contract Name:	DNA094	Mutation Type:	☒ Standard Knock out
LexVision Name:	MEM637N1		☐ Conditional
Reference accessions:	XM_283054	Is this gene X-linked?	No

Required Materials:

- ✓ pKOS clone DNA(s) pKOS-MEM637-52
- ✓ Target Vector DNA pKOS-MEM637-TV52
- ✓ Targeted ES Cell DNA 2A10
- ✓ Genomic Map

Southern Blot Analysis: *External/Internal Probe Strategies*

	<u>5' External</u>	<u>3' External</u>
Name of Probe:	MEM637-21/22	MEM637-23/24
Restriction Enzyme for Genomic Digest:	SpeI	SpeI
Predicted Wild-type Band (kb):	15.7	15.7
Predicted Mutant Band (kb):	8.4	12.1
Probe Size:	252 bp	239 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:	MEM637-25	5' Primer Name:	Neo3a
3' Primer Name:	MEM637-6	3' Primer Name:	MEM637-6
Predicted Wild-type Band (bp):	495	Predicted Wild-type Band (bp):	none
Predicted mutant band (bp)	none	Predicted mutant band (bp)	611

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**

MEM637-21 5' – GCAGATAGTTCAGAGTCTGC
MEM637-22 5' – CGGAGACTATGCAGCAAGC
MEM637-23 5' – CACTCAGGAACTAGATGACC
MEM637-24 5' – TACACGGACACATACATATGC

PCR Genotyping

MEM637-25 5' –CTCTGTGCAGATTCTTGTC
MEM637-6 5' –TTGGATATACTCAGTGTCAGC
Neo3a 5' –GCAGCGCATCGCCTTCTATC

Genomic Sequence Deleted:

GCCTGCACTCATGATGATGAGGGACCTGGCTCTTGCAGGCATGCTGATTAGCCTGGCTTTCCTGTCCCTGCTGCCATCTGGATGTCTCAGCAGACCAC
AGAGGACGCCTGCTCTGTGCAGATTCTGTCCCCGGCTCAAAGGTAATGGCTT

Genomic Locus: (the deleted sequence represents nt12645 to 12797 in the sequence below)

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[illegible]

Selection Cassette:

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Targeted Locus:

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