

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: C57BL/6NCrl-Rab39bem1(IMPC)Tcp/TcpMmucd **MMRRC: 066531-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)	

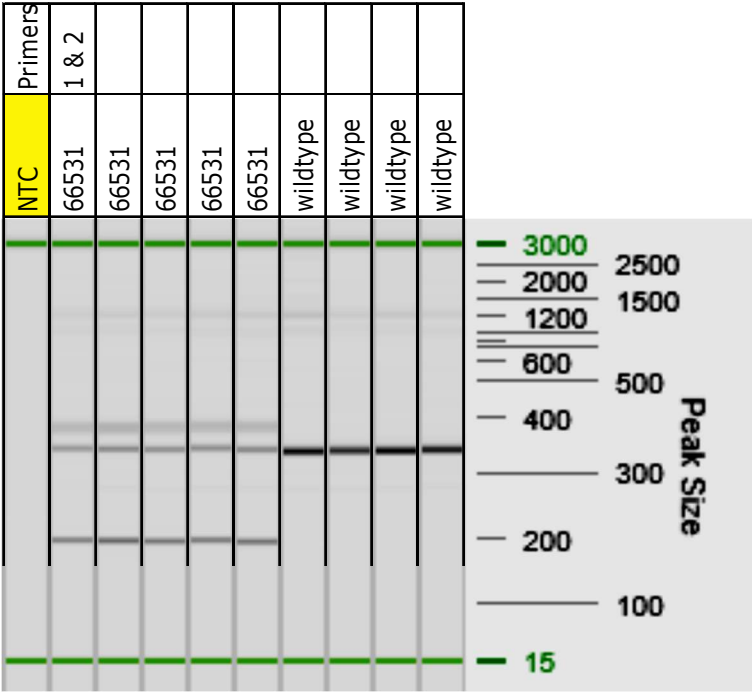
Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 66531-F	GAAACGGGTTGATCTGTGCTAAGG	Estimated Running Time: 90 min.		
2. 66531-R	CCCAAAGACCCTCCCACTGC	Primer Combination	Band (bp)	Genotype
		1 & 2	218	mutant
		1 & 2	351	wildtype

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Sample ID	Assay	Well
ntc		A1
wt		B1
10466		A2
2		B2
3		A3
4		B3
5		A4
6		B4
7		A5
8		B5
9		A6
ntc		B6
wt		A7
32358		B7
3		A8
4		B8
5		A9
6		B9
7		A10
8		B10
9		A11
10		B11
11		A12
12		B12
13		C1
14		D1
15		C2
ntc		D2
wt		C3
31665		D3
3		C4
4		D4
5		C5
6		D5
7		C6
ntc		D6
wt		C7
13807-REG		D7
15		C8
16		D8
17		C9
18		D9
19		C10
20		D10
21		C11
22		D11
23		C12
ntc		D12