

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: STOCK Gt(ROSA)26Sortm1(Prdm1)Brjo/Mmucd **MMRRC: 067389-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:

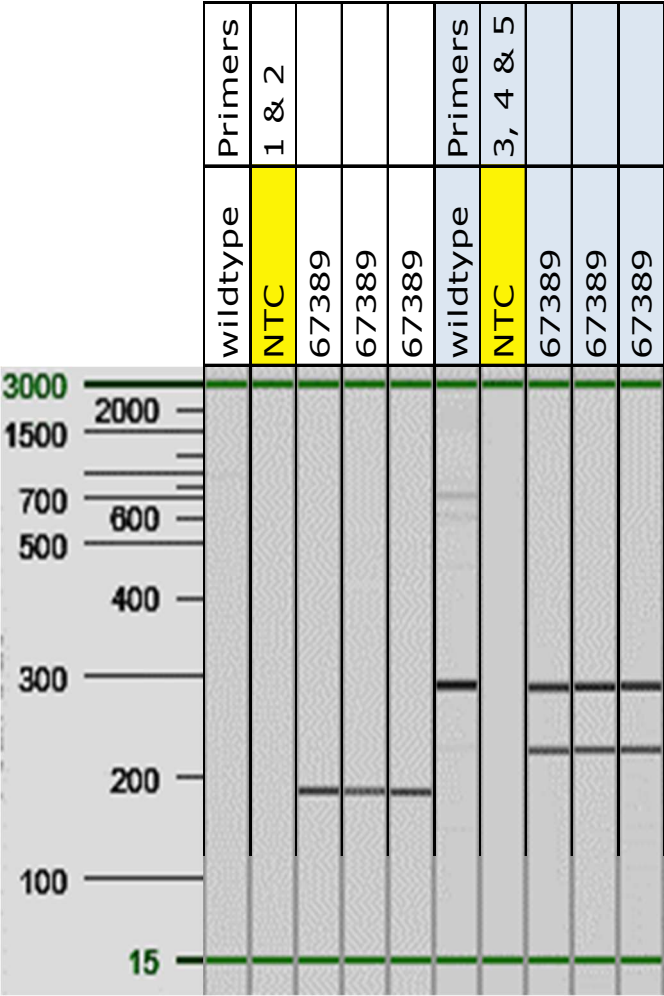
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00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

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 67389-F	GCTGATCCGGAACCCTTAAT	Estimated Running Time: 90 min.		
2. 67389-R	GATCCCAGTCTCTGCCAGTC	Primer Combination	Band (bp)	Genotype
3. 67389-ROSA-F	CTCTGCTGCCTCCTGGCTTCT	1 & 2	202	Prdm1 mut
4. 67389-ROSA-Mut-R	TCAATGGGCGGGGGTCGTT	3 & 4	249	Rosa mut
5. 67389-ROSA-WT-R	CGAGGCGGATCACAAGCAATA	3 & 5	326	wildtype

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