

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

**Protocol Name:** C57BL/6NTac-Gckrem1(IMPC)H/Mmucd **MMRRC: 050950-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
<b>TOTAL VOLUME</b>	
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? <input type="checkbox"/>	94	2:00	<b>1x</b>
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	<b>10x</b>
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	<b>25x</b>
7. Elongation	68	2:00 (↑20sec/cycle)	

**Primers:**

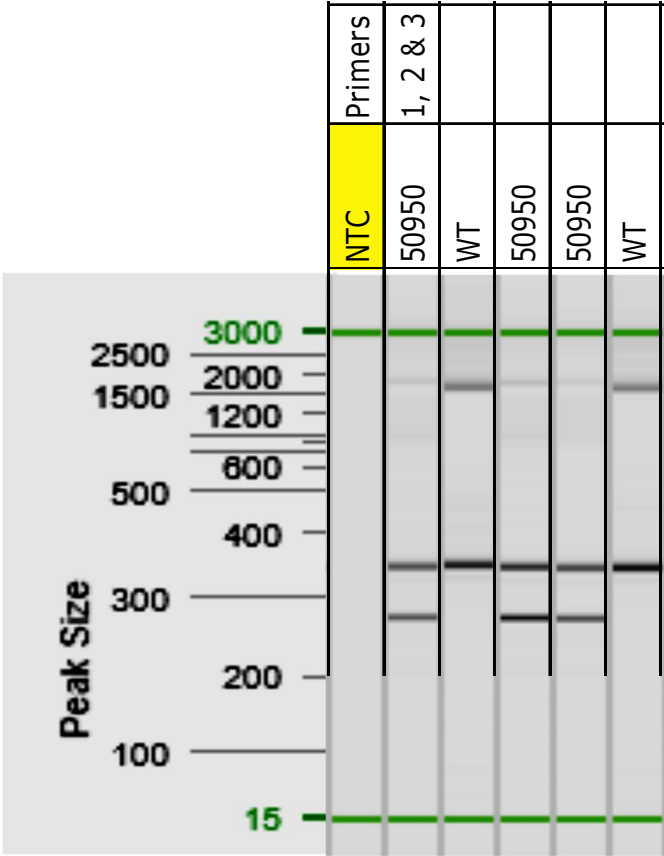
**Electrophoresis Protocol:**

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 50950-comF	GCTACCAGATGGATACTACTTTATCAGC	Estimated Running Time: 90 min.		
2. 50950-mutR	GATGGAGGAAAGCTATCATTGAACAGC	<b>Primer Combination</b>	<b>Band (bp)</b>	<b>Genotype</b>
3. 50950-wtR	ACATCCAAGCAAGTGACTACAATCAGC	1 & 2	280,1542	Mutant.WT
		1 & 3	355	wildtype

Please note, these primers are auto-designed and may not have been verified by the repository, and as such may require optimization or redesign by your facility.

We recommend running primers singleplex. For screening of pups prior to any Flp or Cre recombination, the Floxed or PreCre primers may be used to identify the mutant mice. The Floxed primers test for the distal LoxP site. The PostCre primers should be used if mutant mice were crossed with a Cre recombinase line (without any FLP recombination). The PostFlp primers should be used if mutant mice were crossed with a Flp recombinase line. The PostFlp & Cre primers should be used if mutant mice were crossed with a Flp recombinase line and then a Cre recombinase line. The wildtype primers should be used for zygosity testing of all mutant mice (pre or post recombination).

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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