

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

**Protocol Name:** C57BL/6N-Atm1Brd Kcng3tm1e(KOMP)Wtsi/BayMmucd **MMRRC:** 065551-UCD

**Protocol:**

Reagent/Constituent	Volume (μL)
Water	11.275
10x Buffer	2.5
MgCl <sub>2</sub> (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	0.325
Primer 1. (stock concentration is 20μM)	0.5
Primer 2. (stock concentration is 20μM)	0.5
Taq Polymerase 5Units/μL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25.000 μL</b>

**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.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust MgCl<sub>2</sub> to increase reaction or decrease non-specific amplifications.

**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	40x
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

**Primers:**

**Electrophoresis Protocol:**

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. 65551-lacF	GCTACCATTACCAGTTGGTCTGGTGTC	Estimated Running Time: 90 min.		
2. 65551-neoF	GGGATCTCATGCTGGAGTTCTTCG	Primer Combination	Band (bp)	Genotype
4. 65551-TTR	AGGTGGTAACTGAATCCCCAAAGGG	2 & 4	655	PreCre
		1 & 4	606	PostCre
6. 65551-F	AGGGAAGTGGGCCTGATACTCC	6 & 4	351	Wildtype
		6 & 4	485	PostFlp

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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