

GENOTYPING BY PCR PROTOCOL

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530-754-MMRRC

Protocol Name: C57BL/6N-Coq8atm1(KOMP)Vlbg/MbpMmucd **MMRRC: 047178-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.

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. REG-Neo-F	GCAGCCTCTGTTCCACATACACTTCA	Estimated Running Time: 90 min.		
2. Reg-14522-R3	CATTCCCATGGCCAACTAGACC	Primer Combination	Band (bp)	Genotype
3. 47178-wtF	GCTCTGTGCTCATGATGTAATCTCC	1 & 2	335	PreCre
4. 47178-wtR	TGAGGTTTCCTGGACCTTTGCC	1 & 5	535	PostCre
5. Reg-LacF	ACTTGCTTTAAAAAACCTCCCACAC	3 & 4	276	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).