

GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: C57BL/6N-Tg(CAG-C330027C09Rik)1Btk/Mmucd MMRRC # 036741-UCD

Protocol: *(PCR protocol provided by Donating Investigator)*

Reagent/ Constituent	Volume (μL)
Water	13.0
10x Buffer (contains 20 mM MgCl ₂) see comments	2.5
dNTPs (stock concentration is 10 mM)	1.0
Primer 1 (stock concentration is 10 μM)	1.0
Primer 2 (stock concentration is 10 μM)	1.0
Taq Polymerase 1 U/μl	1.0
Additives / Other (if applicable): Cresol red	5.0
DNA sample extracted ☐ NaOH ☒ Proteinase K ☐ Other:	0.5
TOTAL VOLUME OF REACTION:	25.00 μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	96	3:00	1
2. Denaturation	96	1:10	} 34
3. Annealing	59	1:30	
4. Elongation	72	1:30	
5. Amplification	72	10:00	1
6. Finish	10	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. UTU167for	TATGGTAATCGTGCGAGAGGG
2. UTU167rev	CGTGTGAGCTTTTGTCCAGA

Electrophoresis Protocol:

Agarose: 2% V: 100

Estimated Running Time: 45 min.

The ladder is Promega 1 kb DNA Ladder (G5711). Diagnostic samples are either CIP2A transgenic (+) or non-transgenic (-). One sample is unclear (not marked).

Primer Combination	Expected Bands	Genotype
1 and 2	550 bp	hemi

