

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
 2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
 530-754-MMRRC

NAME OF PCR: C.129P2-Pde4d^{tm1Mct}/Mmcd MMRRC # 034589-UCD

Protocol: (PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (μL)
Water	12.675
10x Buffer (15mM MgCl ₂)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM) 4D-3F	1.25
Primer 2 (stock concentration is 10μM) 4D-4R	1.25
Primer 3 (stock concentration is 10μM) 4DKO-2F	0.625
Taq Polymerase 1U	0.2
Additives / Other (if applicable): 5X Q solution	5
DNA sample extracted ☐ NaOH ☒ Proteinase K ☐ Other:	
TOTAL VOLUME OF REACTION:	24μL

Comments on protocol:

DNA extracted by high salt method and diluted 1:10 in TE. Use Qiagen Taq, buffer and Q solution. Keep all reagents on ice

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	3:00	1
2. Denaturation	94	0:30	} 35
3. Annealing	59	0:30	
4. Elongation	68	1:00	
5. Amplification	68	5:00	1
6. Finish	4	n/a	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: -3F	GTGCTCACACAATAGTTTCTG
2: 4D-4R	CTATCGTGTCAAATCCCAAG
3: 4DKO-2F	CTAAAGCGCATGCTCCAGACTG

Electrophoresis Protocol:

Agarose: 1.5%in TAE V: 65 Estimated Running Time: 40 min

Primer Combination	Band	Genotype
3F/4R	832 bp	WT +/+
3F/4R, KO2F/4R	832, 500 bp	Het +/-
KO2F/4R	500 bp	KO -/-

GeneRuler 1kb ladder

