GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: **B6N;129P2-*Pde4a*^{tm1Mct}/Mmcd** **MMRRC #** **034792-UCD**

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

Reagent/ Constituent	Volume (µL)
Water	13.0
10x Buffer	2.0
dNTPs (stock concentration is 10mM)	0.3
DMSO (4%)	0.325
Primer 1 (stock concentration is 10µM) 4A-5F	1.0
Primer 2 (stock concentration is 10µM) 4A-8R	1.0
Primer 3 (stock concentration is 10µM) KO-2F	0.8
Taq Polymerase 0.5U	0.1
Additives / Other (if applicable): DMSO	0.8
DNA sample extracted ☐ NaOH ☒ Proteinase K ☐ Other:	
TOTAL VOLUME OF REACTION:	**19µL**

Comments on protocol:

DNA extracted by high salt method and diluted 1:5 in TE. Use Invitrogen Taq (recombinant). 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	95	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	64	0:30	} **35**
4. Elongation	68	1:15	
5. Amplification	68	10:00	**1**
6. Finish	4	n/a	n/a

Primers:

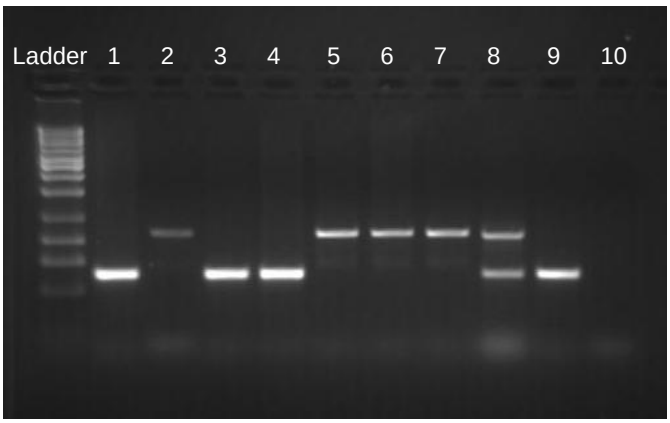
Name	Nucleotide Sequence (5' - 3')
1: 4A-5F	GATTCTCAGACTGTGGCACC
2: 4A-8R	GCAGGTGCAGGCCTTTACAC
3: KO-2F	CTAAAGCGCATGCTCCAGACTG

Electrophoresis Protocol:

Agarose: 1.5%in TAE **V:** 65

Estimated Running Time: 40 **min**

Primer Combination	Band	Genotype
5F/8R	780 bp	+/+
5F/8R, KO2F/8R	780, 420 bp	+/-
KO2F/8R	420 bp	-/-



Lanes
1kb ladder (GeneRuler)
7. WT control
8. het control
9. KO control
10. neg control