GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: **B6.129P2-*Rdh9*^{tm1Jln}/Mmucd** **MMRRC #** **036649-UCD**

Protocol:

(PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (µL)
Water	14.8
10x Buffer	2.0
dNTPs (stock concentration is 10mM)	0.4
Primer 1 (stock concentration is 20µM)	0.4
Primer 2 (stock concentration is 20µM)	0.4
Taq Polymerase	0.2
Additives / Other (if applicable): 50 mM MgCl2	0.6
DNA sample extracted ☒ NaOH ☐ Proteinase K ☐ Other:	1.2
TOTAL VOLUME OF REACTION:	**20.000 µL**

Comments on protocol:

Strategy:

Steps	Temp (^{o}C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☒	93	3:00	**1**
2. Denaturation	93	0:30	
3. Annealing } steps 2-3-4 will cycle in sequence	55	0:30	} **35**
4. Elongation	72	1:30	
5. Amplification	72	15:00	**1**
6. Finish	4	n/a	n/a

Primers:

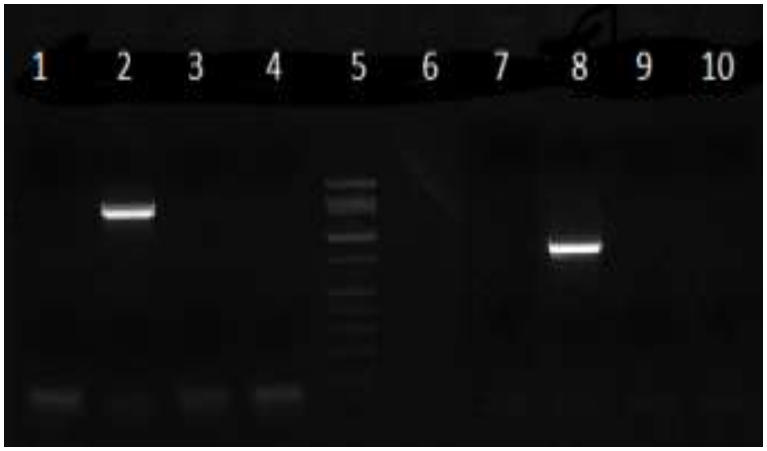
Name	Nucleotide Sequence (5' - 3')
1: CRAD3-F	CCC TCT TGA AGT AAG AAG GTA
2: CRAD3-WTR	ACC AGG ACA TTG TGA GCA TA
3: CRAD3-KOR	GAA TCG ATA AAT GGG ACC TC

Electrophoresis Protocol:

Agarose: 1% **V:** 120

Estimated Running Time: 25 **min.**

Primer Combination	Band	Genotype
1+2	1472 bp	WT +/+
1+3	700 bp	KO -/-



Lanes 1-4 use primers 1+2.

Lane 1: No template
Lane 2: WT sample
Lane 3: KO sample
Lane 4: Water control
Lane 5: 100 bp Ladder

Lanes 6-9 use primers 1+3.

Lane 6: No template
Lane 7: WT sample
Lane 8: KO sample
Lane 9: Water control
Lane 10: empty