GENOTYPING BY PCR PROTOCOL

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Protocol Name: C.Cg-*Ryr1*$^{tm2.1Alle}$/Mmucd **MMRRC: 042040-UCD**

Protocol:

Reagent/Constituent	Volume (µL)
Water	10.775
10x Buffer	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1. (stock concentration is 20µM)	0.5
Primer 2. (stock concentration is 20µM)	0.5
Primer 3. (stock concentration is 20µM)	0.5
Taq Polymerase 5Units/µL	0.2
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
	TOTAL VOLUME
	25

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65ºC decreasing in temperature by 1.0ºC; next 30 cycles anneal at 55ºC.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non-specific amplifications.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 cycle in sequence	65 to 55 (↓1ºC/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		15	∞	n/a

Primers:

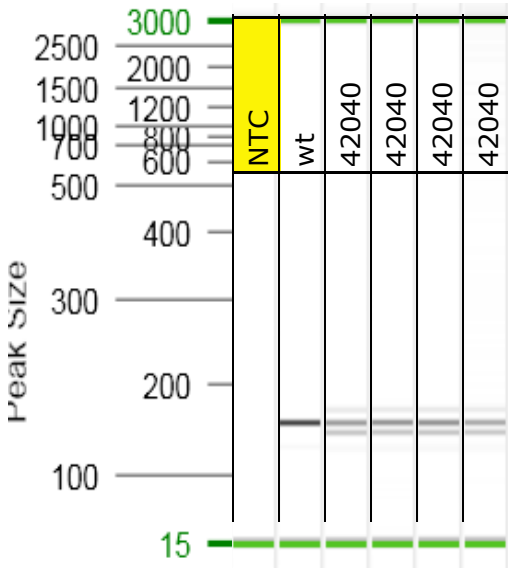
Name	Nucleotide Sequence (5' - 3')
1. 42039.40-mutF	GAGAGAAGGTTTGCGTTGGAGAC
2. 42039.40-wtF	AGAGGTCTGAAGGAGAGAAGGTTCGA
3. 42039.40-comR	CATGCCAATGATGAAAGATGTGG

Electrophoresis Protocol:

Agarose :1.5% V:90

Running Time: 90 min.

Primer	Band (bp)	Genotype
1 & 3	141	mutant
2 & 3	153	wildtype



PCR protocol developed by MMRRC at University of California, Davis