GENOTYPING BY PCR PROTOCOL

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Protocol Name: B6J;129S6-Rr69tm1Axvi/Mmucd **MMRRC:** 065565-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 OF REACTION:	**25.000 µL**

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:

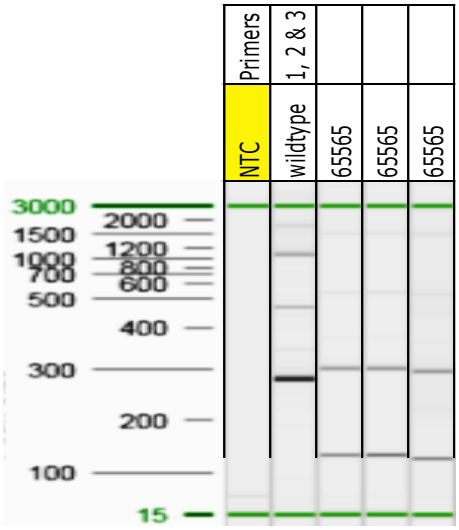
Name	Nucleotide Sequence (5' - 3')
1. 65565-ComR	GACTAGTTAAAGCTGAGGGTGTGCTG
2. 65565-MutF	GTTCTCACTTTGGAGCCTAGACATGGC
3. 65565-WtF	GTGTCTTCTCTGTCCTCCTGGAGTCA

Electrophoresis Protocol:

Argarose: **1.5%** V: **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	Genotype
1 & 2	140	mutant
1 & 3	319	wildtype



PCR protocol developed by MMRRC at University of California, Davis