GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **B6.129S1-*Syngap1*$^{tm1.2Mabk}$/Mmcd** **MMRRC #** **031831-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	10.775
10x Buffer (contains / without 15mM $MgCl_2$)	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 extracted with "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0 ° C; next 30 cycles anneal at 55° C.
- 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 (°C)	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 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 031831-mutR	GAAGAGGAGTTTACGTCCAGCCAAGCT
2: 031831-wtR	CGGATGCTATGTGCAGTGGTGGA
3: 031831-comF2	GAAGTACCAATGGCATCCTTGAGG

Electrophoresis Protocol:

Agarose: 1.3% **V:** 90 **Estimated Running Time:** 90 **min.**

Primer Combination	Expected Bands	Genotype
1 and 3	243 bp	mutant -/-
2 and 3	380 bp	wild-type +/+