

GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: STOCK *Pirb^{tm1Stz}*/Mmucd MMRRC: 030668-UCD

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

Reagent/Constituent	Volume (μL)
Water	10.275
10x Buffer	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) <i>Optional</i>	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO <i>Optional</i>	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
Primer 4. (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 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

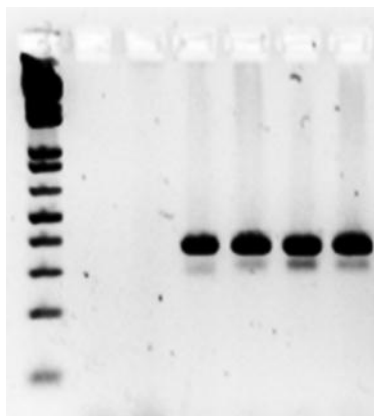
Strategy:

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 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	15	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5 V: 90		
1. 8602	GAGAATCACCAGACACATGC	Estimated Running Time: 90 min.		
2. 6926	CTGCCCTCATGTCTTAACCTT	Primer Combination	Band	Genotype
3. 8443	GCTCTTCTTTCTTCTGCCTC	1 and 2	1500 bp	Wildtype
4. 8846	TGCTGTTGCAGAGGACATG	1 and 2	350 bp	Knockout
		3 and 4	400 bp	Wildtype or Het



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