

GENOTYPING BY PCR PROTOCOL

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530-754-MMRRC

Protocol Name: STOCK Egfrtm1Rdk/Mmucd **MMRRC:** 037646-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Water	4.7
GoTaq® G2 Colorless Master Mix, 2X	7.5
Primer 1. (stock concentration is 20μM)	0.45
Primer 2. (stock concentration is 20μM)	0.45
Primer 3. (stock concentration is 20μM)	0.45
Primer 4. (stock concentration is 20μM)	0.45
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.0
TOTAL VOLUME	
15	

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.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. Neo-F	GACGTTGTCACCTGAAGCGGGAAG	Estimated Running Time: 90 min.		
2. Neo-R	AATCGGGAGCGGCGATACCGTAA	Primer Combination	Band (bp)	Genotype
3. 37646-wtF	AGTCTGCCAAGGCACAAGTAACA	1 & 2	508	Mutant
4. 37646-R	GTGCTGGGATTAAAGGCATGC	3 & 4	282	wildtype

The mutant PCR uses generic Neo primers and is not strain specific.
The wildtype PCR is strain specific.

