

GENOTYPING BY PCR PROTOCOL

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530-754-MMRRC

Protocol Name: B6.129S2-Rbp1^{tm1pc}/Mmucd

MMRRC: 041471-UCD

Protocol:

GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	4
GoTaq® G2 Colorless Master Mix,2X	7.5
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
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	15.00 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.

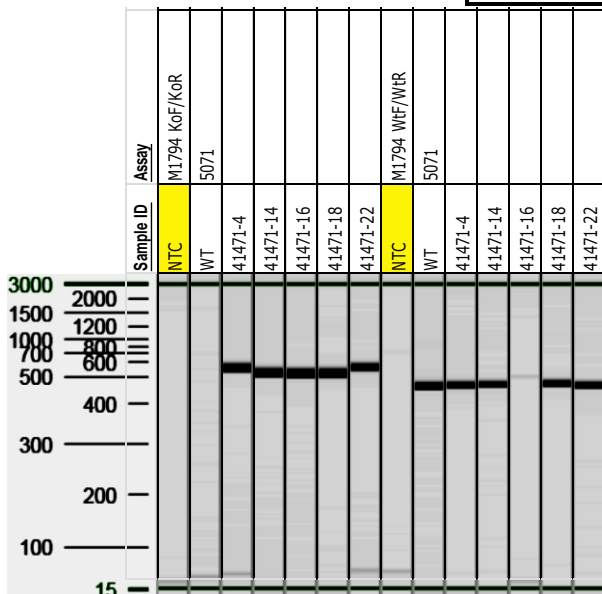
Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	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')	Agarose: 1.5%	V: 90
1.M1794-WtF	ATTGGTGGCAAGTGTCCGAT	Estimated Running Time: 90 min.	
2.M1794-WtR	CAGGTGACGCTAAGGAGTCG	Primer Combination	Band (bp)
3.M1794-KoF	GCCTTCTATCGCCTTCTTGACGAGTTC	1 & 2	503
4.M1794-KoR	GCACTTGCGGTCGTCTATGC	3 & 4	593
			Genotype
			wildtype
			KO



PCR protocol developed by MMRRC at University of California, Davis