

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

NAME OF PCR: STOCK *Mfn1*^{tm2Dcc}/Mmucd MMRRC # 029901-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) <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
Taq Polymerase 5Units/μL	0.2
DNA (50-200ng/ μL) 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 MgCl₂ to increase reaction or decrease non-specific amplifications.

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:

Name	Nucleotide Sequence (5' - 3')
1. 29901-F	TTGGTAATCTTTAGCGGTGCTC
2. 29901-R	AGCAGTTGGTTGTGTGACCA
3. 29901-exc-R	TTAAAGACACGGCTAATGGCAG

Electrophoresis Protocol:

Agarose: 1.5% V: 90

Estimated Running Time: 90 min.

Primer Combination	Band	Genotype
1 and 2	~350 bp	Wildtype
1 and 2	~450 bp	Floxed
1 and 3	691 bp	Wild-type
1 and 3	~325 bp	Cre excised
1 and 3	793 bp	Un-excised

For primers 1 and 3 (not shown), since the excised product is smaller it has a competitive edge, and tends to give more product; therefore product strength does not reflect quantitatively.

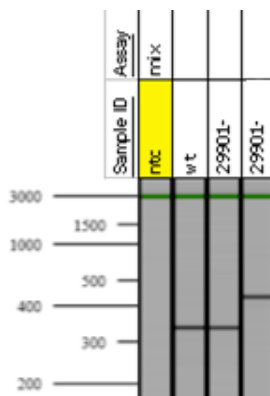


Image shows products from primers 1 and 2:
 Lane 1 – non sample control
 Lane 2 – Wild-type control
 Lane 3 – Wild-type 29901
 Lane 4 – Homozygous floxed