

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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STRAIN NAME: C.Cg-Zfp423^{nur12} Amzn2^{129S1/Svlm/J}/HamlMmucd

MMRRC: 041443-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 (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 MgCl₂ to increase reaction or decrease non-specific amplifications.
- Sequence PCR from primers 9 & 10 using primer 10. Mutant sequence GAGCTACTTGAAGAGGCATGAAT. Wild-type sequence GAGCTACTTGAAGAGGCATGAAC.

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

Name	Nucleotide Sequence (5' - 3')	Argarose: 1.5% V: 90		
1. D17Mit113-F	TCTGTCTCCTCCGTACTGGG	Estimated Running Time: 90 min.		
2. D17Mit113-R	GTCAATAAGTTCAATCACTGAACACA	Primer Combination	Band (bp)	Genotype
3. D17Mit139-F	AGACATGTGAGTACTGCACAGACA	1 & 2	~120	Wild-type
4. D17Mit139-R	ATGATGACATACCTCCTAGTAGTCCC	3 & 4	~170	Wild-type
5. D17Mit39-F	CCTCTGAGGAGTAACCAAGCC	5 & 6	~120	Wild-type
6. D17Mit39-R	CACAGAGTTCTACCTCCAACCC	7 & 8	~120	Wild-type
7. D17Mit221-F	CCTCTGAGGAGTAACCAAGCC	9 & 10	436	Wild-type
8. D17Mit221-R	CACAGAGTTCTACCTCCAACCC			
9. nur12-3	GAGCTGGTGGAGGAGAAGC			
10 nur12-5end	CTGCAGATGGTGATGACGAC			

