

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR10741 Malrd1 exdel

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.5
Primer 2. (stock concentration is 20µM) wtR	0.5
Primer 3. (stock concentration is 20µM) mutR	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.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? <input type="checkbox"/>	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)	
8. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. Malrd1-cond-IV5F	GTAAGGAATCATACTGGCACACCATG	Estimated Running Time: 90 min.	
2. Malrd1-cond-IV5R	AGGTTCCATCTTGACCTCTGGACC	Primer Combination	Band (bp)
3. Malrd1-cond-IV3R	TGGTGCCATACACAGTGTTGTATTTATATC	1 & 2, 1 & 3	527, 1909
		1 & 3	733
			Genotype
			wildtype
			mutant

Allele Description: Exon 4 ENSMUSE00001344262 and flanking splicing regions were constitutively deleted from the Malrd1 gene ENSMUST00000146205.2 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

*wtR primer untested (ePCR verified)

