

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11785 Pym1 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? ☐	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. CR_Pym1_comF	CTCTGTGTACTTCTTGCCCTGG	Estimated Running Time: 90 min.	
2. CR_Pym1_wtR*	GCTAGAGTTAGTCTCTCATTCTCAAGC	Primer Combination	Band (bp)
3. CR_Pym1_mutR	ACTGTGAACAGGAAGTAAAGACTCC	1 & 2, 1 & 3	365, 1690
		1 & 3	342
			Genotype
			wildtype
			mutant

Allele Description: Exon 2-3 (ENSMUSE00000460812, ENSMUSE00002175698) and flanking splicing regions were constitutively deleted from the Pym1 Gene ENSMUSG00000064030 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1348bp deletion from Chr10:128600853-128602200 GRCm39 was sequence verified. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified)

