

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11384 Pdia2 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:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Pdia2_comF	TTGTCAGTTGCAGAGACCTGC	Estimated Running Time: 90 min.	
2. CR_Pdia2_wtR*	GCACTTACAAAACCTCCACCATCAG	Primer Combination	Band (bp)
3. CR_Pdia2_mutR	GTCAGTGCCTTCAACATAG	1 & 2, 1 & 3	482, 3770
		1 & 3	481
			wildtype
			mutant

Electrophoresis Protocol:

Allele Description: Exon 1-11 (ENSMUSE00000701389, ENSMUSE00001227964, ENSMUSE00001258017, ENSMUSE00001278381, ENSMUSE00001248486, ENSMUSE00001294547, ENSMUSE00001261718, ENSMUSE00001312068, ENSMUSE00001231281, ENSMUSE00000399961, ENSMUSE00000139232) and flanking splicing regions were constitutively deleted from the Pdia2 gene ENSMUSG00000024184 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)

*Run in Simplex

