

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10781 Pof1b 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:

Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. CR Pof1b_comF	GAGAAACAGAAAGCATGTGGATTCC	Agarose: 1.5%	V: 90	
2. CR Pof1b_wtR	GACCATGTAGTCGAGGTCAATGAAGG	Estimated Running Time: 90 min.		
3. CR Pof1b_mutR	GCATGTCAGATCCACAATGGATACATT	Primer Combination	Band (bp)	Genotype
		1 & 2, 1 & 3	1670	wildtype
		1 & 3	627	mutant

Allele Description: Exon 7 ENSMUSE00000295310 and flanking splicing regions were constitutively deleted from the Pof1b gene ENSMUST00000039887.3 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)

