GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR1071 Bpifa2 exdel**

Protocol: ***GoTaq® Long PCR Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	5.6
GoTaq® Long PCR Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.6
Primer 2. (stock concentration is 20µM) wtR	1.2
Primer 3. (stock concentration is 20µM) mutR	0.3
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 (ºC)	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 (↓1ºC/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')
1. CR_Bpifa2-comF	CTGGGATGTTACCCTAAGAGACTTGC
2. CR_Bpifa2-wtR	TCAGATAGTCCCTTCCCCAGAAGC
3. CR_Bpifa2-mutR	GGGACTGGAGGCTATGAATTCTAGC

Electrophoresis Protocol:

Argarose: **1.5%** **V:** **90**

Estimated Running:Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	522,829	wildtype
1 & 3	234	mutant

Allele Description: Exon 3 ENSMUSE00000274952 and flanking splicing regions were constitutively deleted from the Bpifa2 gene ENSMUST00000048103.8 using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.