GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11251 Defb1 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 (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_Defb1_comF	GGGACTGCTTTGTCTGGTGTCTC
2. CR_Defb1_wtR*	AGTCCCAAACAAAGCCATCAACAA
3. CR_Defb1_mutR	GTGACTACCAGGGAATGTGAAAGGTC
4. attP_comKI-R	TGTACCGTACACCACTGAGACC

Electrophoresis Protocol:

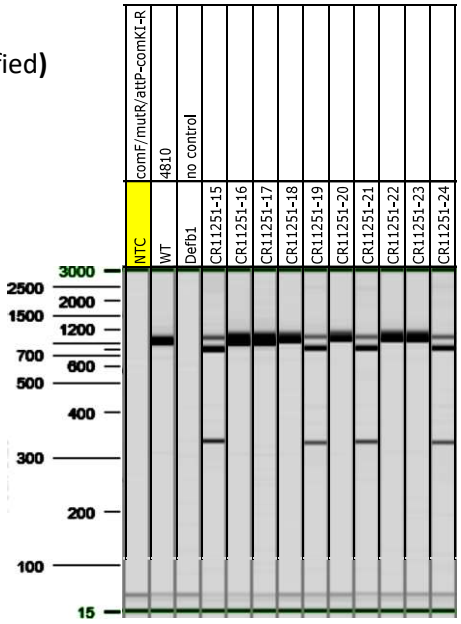
Agarose: **1.5%** **V:** **90**

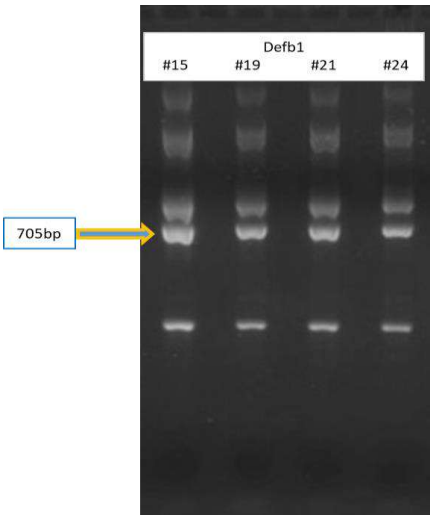
Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	436, 855	wildtype
1 & 3, 1 & 4	705, 335	mutant

Allele Description: Exon 1 (ENSMUSE00000342971) and flanking splicing regions were constitutively deleted from the Defb1 gene ENSMUSG00000044748 and replaced with Bxb1 attP KI (58bp sequence) 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**)**





PCR protocol developed by MMRRC at University of California, Davis