GENOTYPING PROTOCOL MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: CR11545 Zng1 (Cbwd1) EXDEL

Protocol: GoTag® 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.

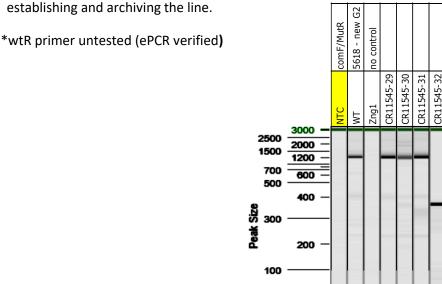
Strategy:

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:

mers:	Electrophoresis Protocol:			
Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V:	90	
1. CR_Cbwd1_comF	GTTGTGTTTCATAGACATCAGATTCTGC	Estimated Running Time:	90 min.	
2. CR_Cbwd1_wtR*	GTGTTTCAAACCCAAACTAAGACAAATTCC	Primer Combination	Band (bp)	Genotype
3. CR_Cbwd1_mutR	CAAACCTTAAAGACATCAGCAGTTAGC	1 & 2,1 & 3	301,1036	wildtype
		1 & 3	360	mutant

Allele Description: Exon 6 ENSMUSE00000145775 and flanking splicing regions were constitutively deleted from the Cbwd1 Gene ENSMUSG00000024878 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 676bp deletion from Chr 19: 24925173 - 24925848 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for



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