GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR10517 Champ1 iDex** Stock # 065591

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_Champ1_XD_S181Cfs_comF	TGGAGTGAGCAGCCGAAAGAGC
2. CR_Champ1_XD_S181Cfs_wtR*	AGCAGGAAGACAAGGGGCTTGG
3. CR_Champ1_XD_S181Cfs_mutR	GGAGATGCACTGAGGGATGGTCC

Electrophoresis Protocol:

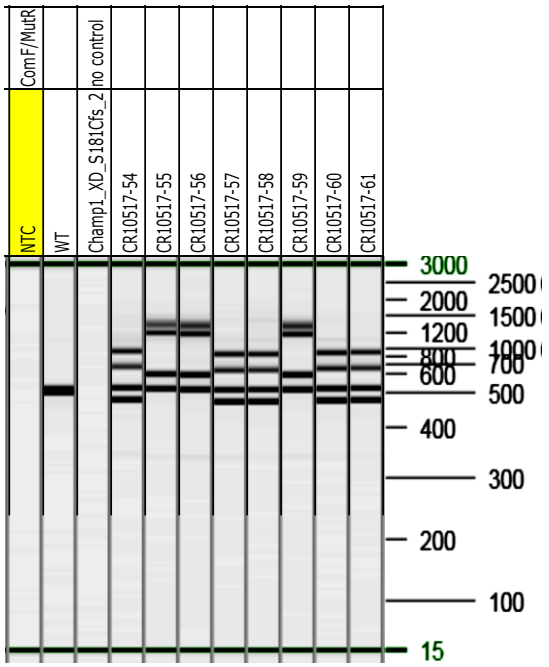
Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	249,494	wildtype
1 & 3	459	mutant

Allele Description: Exon 2 ENSMUSE00000357801 had 35bp deleted from the 485th coding nucleotide through the 519th coding nucleotide from the Champ1 gene ENSMUSG00000047710.8 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