

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: CR10755 Chd6 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

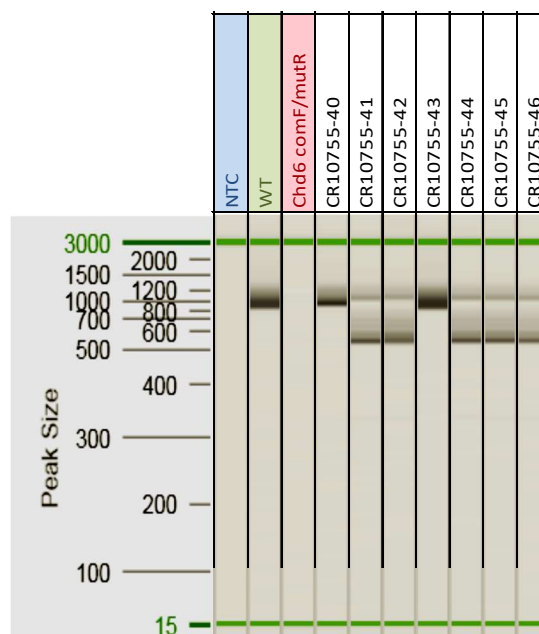
Primers:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5% V: 90
1. CR Chd6 comF	GACGAGGATGACAGATTGCACTGC	Estimated Running Time: 90 min.
2. CR Chd6 wtR	GCAGACAGAGCAGTACTTGATGAACGT	Primer Combination Band (bp) Genotype
3. CR Chd6 mutR	CGAGAAACTCAAGGGGACCTACCA	1 & 2, 1 & 3 588, 1214 wildtype
		1 & 3 623 mutant

Electrophoresis Protocol:

Allele Description: Exon 4 ENSMUSE00001227344 and flanking splicing regions were constitutively deleted from the Chd6 gene [ENSMUST00000039782.13](#) 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