

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: CR10440 Cdc2 iDex

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:

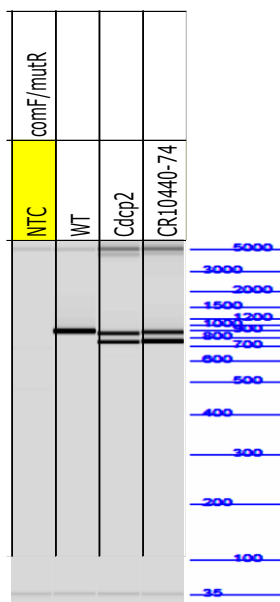
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Cdc2_comF	TGCCCAGTGTTCAATGTTTGTTC	Estimated Running Time: 90 min.	
2. CR_Cdc2_wtR*	GATCTCCAGGAAGTCAAAGCCACAG	Primer Combination	Band (bp)
3. CR_Cdc2_mutR	GCTCTGTCTGCTATCAGTTCTTCCTGT	1 & 2, 1 & 3	386,887
		1 & 3	754
			Genotype
			wildtype
			mutant

Allele Description: Exon 2 [ENSMUSE00000413682](#) had 133bp deleted from the 190th coding nucleotide through the 322nd coding nucleotide from the Cdc2 gene [ENSMUSG0000047636](#) 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)