

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

Protocol Name: CR10820 Il1f5 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:

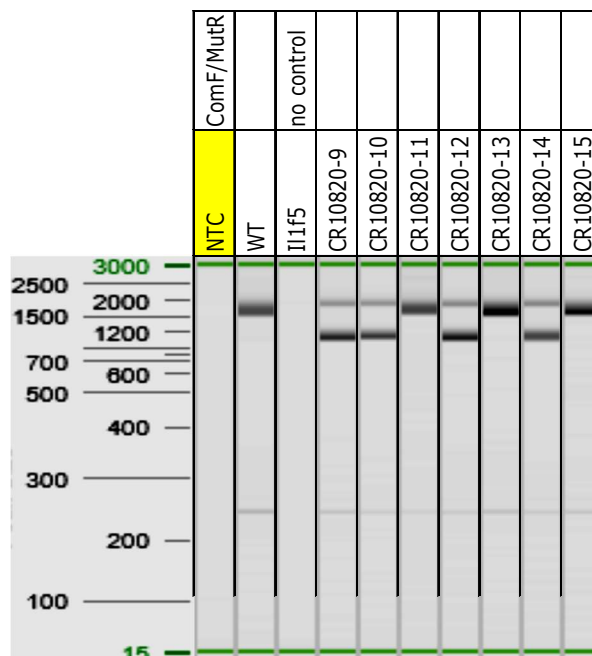
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1x
2. Denaturation	94	0:10	
3. Annealing steps 2-3-4 cycle in sequence	65 (↓1°C/cycle)	0:30	10x
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing steps 5-6-7 cycle in sequence	55	0:30	25x
7. Elongation	68	2:00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

Primers:

Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. CR Il1f5 comF	GGTAGAGTGGGTGGAAGTATGAGTGTG	Agarose: 1.5%	V: 90	
2. CR Il1f5 wtR	CCTTCAAGGCTGAATCCTTCATTCT	Estimated Running Time: 90 min.		
3. CR Il1f5 mutR	CTCCTACTACTCGCTTGGTCCCAG	Primer Combination	Band (bp)	Genotype
		1 & 2, 1 & 3	703, 1597	wildtype
		1 & 3	958	mutant

Allele Description: Exon 4 ENSMUSE00001342031 and flanking splicing regions were constitutively deleted from the Il1f5 gene ENSMUST00000028360.7 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 MMRRRC at University of California, Davis