GENOTYPING PROTOCOL
MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR10816 Fgfr1op2 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 (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_Fgfr1op2_comF	AAGACTCACTCCTAGTCATGCACCTACTC
2. CR_Fgfr1op2_wtR	GAGACTGTAAGCTCTGCCTGATGTGG
3. CR_Fgfr1op2_mutR	CCACAGAAGTGGAGTGTTGCGAT

Electrophoresis Protocol:

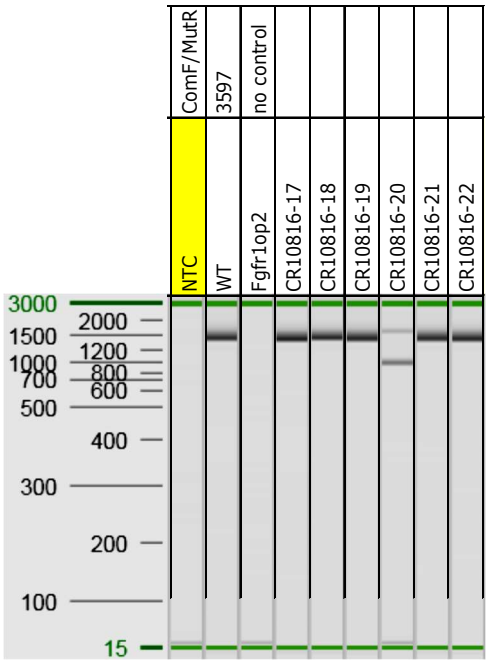
Agarose: **1.5%** **V:** **90**

Estimated Running **90** min.

Primer Combination	Band (bp)	Genotype
1 & 2,1 & 3	1089, 1666	wildtype
1 & 3	922	mutant

Allele Description: Exon 3 ENSMUSE00001273520 and flanking splicing regions were constitutively deleted from the Fgfr1op2 gene ENSMUST00000111663.8 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