GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR1751 Zfp503 iDex**

Protocol: ***GoTaq® Long PCR Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® Long PCR 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-Zfp503-comF	CACCCAGGATTATTCGCTTCTGG
2. CR-Zfp503-wtR*	CGGAGCAATTTCCAGAAAGCG
3. CR-Zfp503-mutR	GCTCTCTCGATCTCTGTGGTTGGG

Electrophoresis Protocol:

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

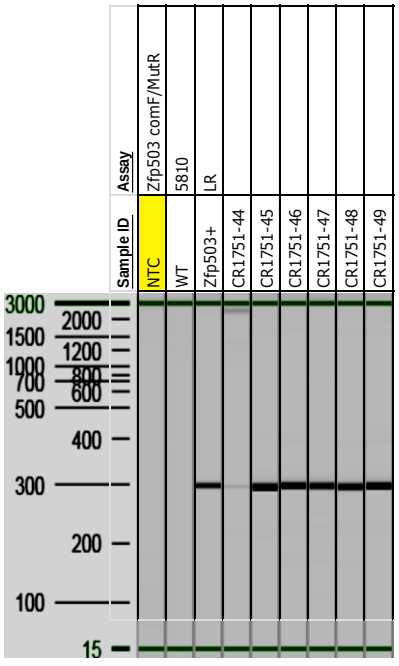
Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genoty**
1 & 2,1 & 3	315,2995**	wildtype
1 & 3	328	mutant

Allele Description: Exon 2-3 (ENSMUSE00000563914, ENSMUSE00000563894) were constitutively deleted from the 56th coding nucleotide through the 3' UTR from the Zfp503 gene ENSMUSG00000039081 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**)**

****** May not see larger wildtype band



PCR protocol developed by MMRRC at University of California, Davis