

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1540 Hs3st5 iDex

Protocol: GoTaq® G2 Colorless Master Mix(Promega)

Reagent/Constituent	Volume (µL)
Water	3.9
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.6
Primer 2. (stock concentration is 20µM) wtR	1.2
Primer 3. (stock concentration is 20µM) mutR	0.3
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? <input type="checkbox"/>	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_Hs3st5-comF	AGGAGCACTAATAAAAAAATCCCATC	Estimated Running Time: 90 min.	
2. CR_Hs3st5-wtR	GGCTCTTTTCAATCGTGATTTGC	Primer Combination	Band (bp)
3. CR_Hs3st5-mutR	CAATCCAGTGTGTAATTAAGAGCC	1 & 2, 1 & 3	571,1661*
		1 & 3	405
			wildtype
			mutant

Allele Description: Exon 6 [ENSMUSE00000357871](#) was constitutively deleted between the 175th coding nucleotide through the 3' UTR from the Hs3st5 gene [ENSMUST0000058738.10](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL/6J to produce sequence confirmed heterozygous animals.

*May not see larger wildtype band

