

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11474 Ints5 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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Ints5_comF	AACCACCAGTAACTCCAGCTCCA	Estimated Running Time: 90 min.	
2. CR_Ints5_wtR*	CGATGAAGGAAGATACCAGGGG	Primer Combination	Band (bp)
3. CR_Ints5_mutR	CCATCTTGTACCGGGTTTGCT	1 & 2, 1 & 3	656, 5601
		1 & 3	522
			wildtype
			mutant

Allele Description: Exon 1-2(ENSMUSE00000621617, ENSMUSE00000621616) were constitutively deleted from the 5'UTR through the 3' UTR from the Ints5 Gene ENSMUSG00000071652 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 5079bp deletion from Chr 19: 8870282-8875360 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified)

