

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: CR11718 Ostc 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? <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:

Name	Nucleotide Sequence (5' - 3')	Electrophoresis Protocol:		
1. CR_Ostc_comF	CAGAGCCTTGTTACTCTGTTTCTTTGG	Agarose: 1.5%	V: 90	
2. CR_Ostc_wtR*	GTCTCTGATGCCCATGTTTCATCG	Estimated Running Time: 90 min.		
3. CR_Ostc_mutR	AGGGTTCCTGCCTATACATGAGACC	Primer Combination	Band (bp)	Genotype
		1 & 2, 1 & 3	1176,1804	wildtype
		1 & 3	506	mutant

Allele Description: Exon 2 ENSMUSE00000285155 and flanking splicing regions were constitutively deleted from the Ostc Gene ENSMUSG00000041084 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 1298bp deletion from Chr 3: 130499132 - 130500429 GRCm39 was sequence verified. 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)

