

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11675 Nvl EXDEL

Stock # 75969

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_Nvl_comF	GAGTCACCCTACAACTATACAAACACC	Estimated Running Time: 90 min.	
2. CR_Nvl_wtR*	TTGCCACATCTGTTACTACTGAGG	Primer Combination	Band (bp)
3. CR_Nvl_mutR	GTACAACAACAACAGCTCTATCTCC	1 & 2, 1 & 3	370,699
		1 & 3	311
			wildtype
			mutant

Allele Description: Exon 2 ENSMUSE00000160127 and flanking splicing regions were constitutively deleted from the Nvl Gene ENSMUSG00000026516 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 388bp deletion from Chr 1: 180969757-180970144 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)

