

GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR11716 Nudt2 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:

Electrophoresis Protocol:

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Nudt2_comF	GGTTGTAAGATCACGATTAGCCTGTCC	Estimated Running Time: 90 min.	
2. CR_Nudt2_wtR*	CTCTACTTTGACAGATGAAGCACTCAGC	Primer Combination	Band (bp)
3. CR_Nudt2_mutR	GTGAGTCCAAGAAAGCATCACAGG	1 & 2, 1 & 3	603,3557
		1 & 3	384
			Genotype
			wildtype
			mutant

Allele Description: Exon 2-3 (ENSMUSE00000179190, ENSMUSE00000632949) and flanking splicing regions were constitutively deleted from the Nudt2 Gene ENSMUSG00000028443 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 3173bp deletion from Chr 4: 41477532 - 41480704 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)

