

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR10024 Dnmt3b 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
<b>TOTAL VOLUME OF REACTION:</b>	<b>15.00 µL</b>

**Comments on protocol:**

- Protocol may work with other DNA extraction methods.

**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float: right;">HOT START? <input type="checkbox"/></span>	94	2:00	<b>1x</b>
2. Denaturation	94	0:10	
3. Annealing <span style="float: right;">steps 2-3-4 cycle in sequence</span>	65 (↓1°C/cycle)	0:30	<b>10x</b>
4. Elongation	68	2:00	
5. Denaturation	94	0:15	
6. Annealing <span style="float: right;">steps 5-6-7 cycle in sequence</span>	55	0:30	<b>25x</b>
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_Dnmt3b_comF	CCTGTTGATCCCAAGAGCTACTTGC	Estimated Running Time: 90 min.	
2. CR_Dnmt3b_wtR	GGAGACCTCCCTCTTAGACAGCCG	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Dnmt3b_mutR	CAGAGCCATTCCCATCATCTACTTCA	1 & 2, 1 & 3	338,923
		1 & 3	467
			Genotype
			wildtype
			mutant

**Allele Description:** Exon 4 [ENSMUSE00000465132](#) and flanking splicing regions were constitutively deleted from the Dnmt3b gene [ENSMUSG00000027478](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

