

# GENOTYPING PROTOCOL

## MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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530-754-MMRRC

Protocol Name: CR1443 Awat2 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:**

Name	Nucleotide Sequence (5' - 3')	Agarose: 1.5%	V: 90
1. CR_Awat2-comF	ATTGGGATGTATTGCCATATATTCACC	Estimated Running Time: 90 min.	
2. CR_Awat2-wtR	AGCTCTGTCCACCCTGCTCTCC	<b>Primer Combination</b>	<b>Band (bp)</b>
3. CR_Awat2-mutR	GAAAGTGGAGACAGGAGAGCTGG	1 & 2, 1 & 3	531,834
		1 & 3	366
			wildtype
			mutant

**Electrophoresis Protocol:**

**Allele Description:** Exon 3 [ENSMUSE00000207389](#) and flanking splicing regions were constitutively deleted from the Awat2 gene [ENSMUST00000033567.14](#) using CRISPR Cas9 gene editing technology in mouse zygotes. Subsequent founders were backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals.

