

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	71923
MMRRC Strain Name	C57BL/6NJ- <i>Aldh6a1</i> ^{em1(IMPC)Bay} /Mmnc
Gene Name(s)	aldehyde dehydrogenase family 6, subfamily A1
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	4/21/2025

PCR Reaction

	<u>1X</u>
ddH ₂ O	12.5
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	1
DNA	2

2,2 Primer Reactions (CF+WR for wild-type and CF+MR for the mutant reaction)

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 57C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: WT: 512bp MUTANT: 546bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer

Ald(71923)CF: TGTGATAGGGTTTTCCGGGA

Ald(71923)WR: GCATCAGCAAGAGTCTTCCC

Ald(71923)MR: ATCCAAAGGGGGATCATGGC

Run on a 2% agarose gel