

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34254
MMRRC Strain Name	129- <i>At1</i> ^{tm1Jfnk} /Mmnc
Gene Name(s)	atlastin GTPase 1 (<i>At1</i>)
Breeding Protocol(s)	Backcross to 129SvEv/Tac
Protocol Date	11/29/11

MMRRC# 34254 PCR Reaction

NOTE: Run 2 reactions for each sample
(Alt WT and Mutant).

Thermal Cycler:

94°C for 30 sec
59°C for 30 sec
72°C for 120 sec
35x

	<u>1X</u>
DNA	2.0
5X Buffer	5.0
10mM dNTPs	0.5
10uM Primer F/R	1/1
Taq	0.5
ddH ₂ O	13.0
25mM MgCl ₂	2.0

Taq: **Apex and Chromataq 5X Buffer**

Bands: MUTANT: ~1.3 kb
WT: 1.5 kb

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

Mut-F1 (34254): TCCTGAATACCCAGGAGTCACGATTC

Mut-R1 (34254): TGACTAGGGGAGGAGTAGAAGGTGGC

AltWTR(34254): ACA TTG GGT GCC AGA TGT AAA

AltWTF (34254): TGA GGC TTG GTG CTA GAG CTC

Run on 2.0% agarose gel in TAE.