

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36786
MMRRC Strain Name	CD1.129S(B6)- <i>Arid1a</i> ^{m1Mag} /Mmnc
Gene Name(s)	AT rich interactive domain 1A (SWI-like) (<i>Ariad1a</i> ^{m1Mag})
Breeding Protocol(s)	Backcross to CD-1 (Charles River)
Protocol Date	4/12/13

MMRRC # 36786 PCR Reaction

Note: Requires a HincII restriction digestion after PCR reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 56°C for 30 sec
 Step 4: 72°C for 60 sec
 Step 2 to 4 Cycles: 34
 Step 5: 72°C for 7 min

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM WT Primer	1
10uM Mut Primer	1
Taq	0.5
DNA	2

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

Bands (After Restriction Digest): MUTANT: 334bp, 200bp, 142bp
 WT: 187bp, 147bp

Primer sequences 5' to 3':

45F2: GCCGTTTGAATTTGTCCTGT
 45F2R: TCTTGGGTTGGGACTTCTTG

Restriction Digestion

1. Add 15ul below mix to each 20ul PCR product

- 4.0uL NEB Buffer #3
- 0.4uL 100X BSA
- 9.6uL H₂O
- 1.0uL HincII (NEB)

2. Incubate at 37 °C, overnight. Heat inactivate at 65C for 15 min.
 3. Run on 2% agarose gel in TAE.