

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36961
MMRRC Strain Name	129P3.B6- <i>Tas1r3</i> ^{Sac-b} /Mmnc
Gene Name(s)	taste receptor, type 1, member 3 (<i>Tas1r3</i>)
Breeding Protocol(s)	Backcross to 129P3/J
Protocol Date	11/4/2013

MMRRC #36961 PCR Reaction

Thermal Cycler:

94°C for 5 min
 94°C for 45 sec
 55°C for 45 sec
 72°C for 60 sec
 Steps 2-4, 35x
 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: MUTANT: 217bp and 114bp (After Restriction Enzyme Digestion)
 WT: 332bp

Primer sequences 5' to 3':

Tas1r3(36961)For CCAGCTGAAGTTCTGCAACA
Tas1r3(36961)Rev CAAAGCATTGCTGCCTACTG

Reaction requires a restriction digest. Add to each 25ul PCR rxn:

9.6ul H₂O
 4.0ul NEB Buffer 4
 0.4ul BSA
 1.0ul HhaI

Incubate at 37°C for 8 hours

Run on 2.0% agarose gel in TAE.

