

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	16148
MMRRC Strain Name	B6.Cg-Slc12a3 ^{tm1Ges} /Mmnc
Gene Name(s)	solute carrier family 12, member 3 (Slc12a3)
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	8/12/13

MMRRC #16148 PCR Reaction

	1X
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Primer Com-F	1
10 μM Primer Wt- R	1
10 μM Primer Mut-R	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 60°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 5: Step 2 to 4; Cycles: 28
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

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

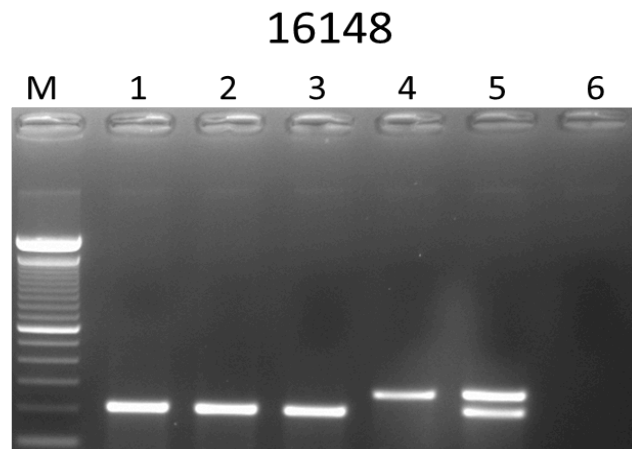
Com-F (16148): AGGGTCAAGGGCACGGTTGGC

WT-R (16148): GGTAAGGGAGCGGGTCCGAGG

Mut-R (16148): GCATGCTCCAGACTGCCTTG

Bands expected: WT 266 bp
Mut 188 bp

Run on 2% agarose gel in TAE.



Lane 1-3: Hom; Lane 4: WT; Lane 5: Het; Lane 6: H₂O;
M: 100 bp DNA ladder (Invitrogen)