

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34
MMRRC Strain Name	129S6/SvEvTac-Slc12a1 ^{tm1Tkh} /Mmnc
Gene Name(s)	Solute carrier family 12, member 1 (Slc12a1)
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	8/19/13

MMRRC #34 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer 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 45 sec
 Step 5: 35x from step 2 to step 4
 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 (34): GAGCTGGGGGTAGGAGAACGGTCAT

WT-R (34): TAGGCTGCTGAGATGAGGCTGGGAC

Mut-R (34): CGCCTTCTATCGCCTTCTTGACGAG

Bands expected: Com-F (34) + WT-R (34): WT 626 bp
 Com-F (34) + Mut-R (34): Mut 500 bp

Run on 2% agarose gel in TAE.

