

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	67259
MMRRC Strain Name	C57BL/6NTac- <i>Slc7a14</i> ^{em1(IMPC)H} /Mmnc
Gene Name(s)	solute carrier family 7 (cationic amino acid transporter, y+ system), member 14
Breeding Protocol(s)	Intra-strain Mating
Protocol Date	6/9/2022

PCR Reaction

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

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 58C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 30

Step 5: 72C, 7min

Taq: Apex Taq and Chromataq 5X Buffer

Bands: WT: 209bp MUTANT: 209bp

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

Slc7a(67259)F: CGCCTGTTTTCTTTGTCCAT

Slc7a(67259)R: TTTGCCAGGGAGTCAAACAT



This strain requires the PCR products to be sequenced to determine the genotype.

1. Perform PCR Reaction
2. Prep PCR products according to sequencing company guidelines
3. Use forward primer as your sequencing primer
4. Send samples to sequencing company
5. Analyze sequencing results.

Mutant samples will have a deleted cytosine (C) See below

WT: TATGTCACCGTT

MUT: TATGTCACGTT

To determine zygosity you will need to look at the sequencing chromatogram. The above sequences start at approximately base 64.

- Wild-type samples will have a single C peak at the position of interest
- Heterozygous Samples will have two overlapping peaks of C and G at the position of interest
- Homozygous Samples will have a single G peak at the position of interest