

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	32652
MMRRC Strain Name	B6.129-Snap25 ^{tm2M^{cw}} /Mmnc
Gene Name(s)	Synaptosomal-associated protein 25 (Snap25)
Breeding Protocol(s)	Sib-mating
Protocol Date	7/29/13

MMRRC #32652 PCR Reaction

Thermal Cycler:

- Step 1: 94°C for 5 min
- Step 2: 94°C for 45 sec
- Step 3: 55°C for 45 sec
- Step 4: 72°C for 1 min
- Step 5: Step 2 to 4; Cycles: 35
- Step 6: 72°C for 7 min

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	2
10 mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Taq: Apex and Chromataq 5X Buffer

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

Neo F: CTG TGC TCG ACG TTG TCA CT

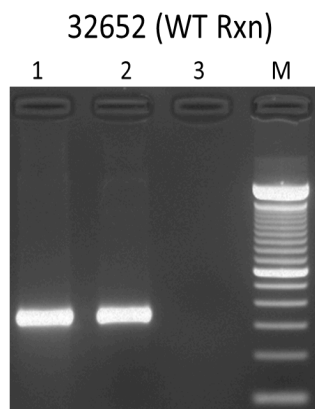
Neo R: GCT CTT CGT CCA GAT CAT CC 3

Snap25 _5b F: TAC TGT GAA AAG ATT TGA TGG TGC

Snap25 _5b R: TAT ATG GAT TTA GGG GGT CAT GTT

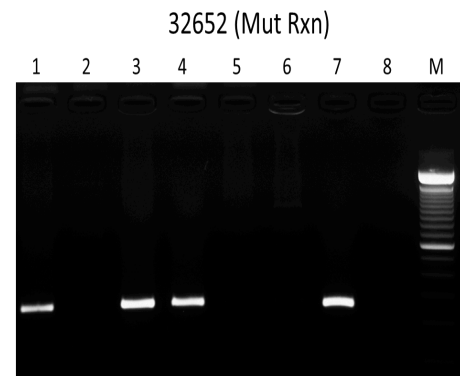
Bands expected: WT 349 bp
 MUTANT 249 bp

Run on 2% agarose gel in TAE.



Primers: Snap25 _5b F + Snap25 _5b R

Lane 1: Het; Lane 2: WT; Lane 3: H₂O; M: 100 bp DNA ladder (Invitrogen)



Primers: Neo F + Neo R

Lane 1, 3, 4 and 7: Het; Lane 2, 5 and 6: WT; Lane 8: H₂O; M: 100 bp DNA ladder (Invitrogen)