

## MMRRC UNC – Genotyping Protocol

|                             |                                                |
|-----------------------------|------------------------------------------------|
| <b>MMRRC Strain ID</b>      | 31132                                          |
| <b>MMRRC Strain Name</b>    | C57BL/6J- <i>Trfr2</i> <sup>m1Btlr</sup> /Mmnc |
| <b>Gene Name(s)</b>         | Transferrin receptor 2 (Tfr2)                  |
| <b>Breeding Protocol(s)</b> | Intra-Strain Mating                            |
| <b>Protocol Date</b>        | 12/2/13                                        |

### MMRRC# 31132 PCR Reaction

**NOTE:** Run 2 reactions for each sample (WT & Mutant).

**Thermal Cycler:**

94°C for 45 sec

55°C for 45 sec

72°C for 60 sec

32x

Taq: **Apex and Chromataq 5X Buffer**

**Bands:** MUTANT: ~300bp

WT: 482bp

|                        | <b><u>1X</u></b> |
|------------------------|------------------|
| DNA                    | 2.0              |
| 5X Buffer              | 5.0              |
| 10mM dNTPs             | 0.5              |
| 20 uM Primer F/R       | 1.0              |
| Taq                    | 1.0              |
| ddH <sub>2</sub> O     | 14.0             |
| 25mM MgCl <sub>2</sub> | 1.5              |

Primers are 10uM with respect to each primer (20uM total).

**Primer sequences 5' to 3':**

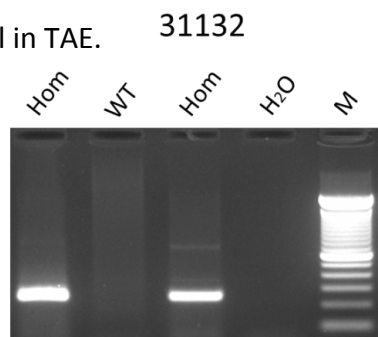
Trfr2new WT F\_(31132): CCCAGCTGGGATGCTGAAGTCT

Trfr2\_WT\_R1 (31132): CATTGAGGTTGCCGATGTGCC

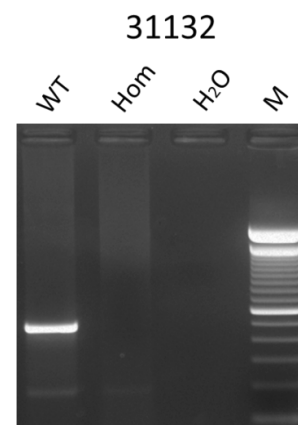
Trfr2 MutF (31132): CCCAGCTGGGATGCTGAAGTCC

Trfr2 MutR1 (31132): TGGGCGCAGGAACGTGGTGCT

Run on 2.0% agarose gel in TAE.



Primers: Trfr2new WT F\_(31132) + Trfr2\_WT\_R1 (31132)  
 M: 100 bp DNA ladder (Invitrogen)



Primers: Trfr2 Mut F (31132) + Trfr2 Mut R1 (31132)  
 M: 100 bp DNA ladder (Invitrogen)

The Mutant Mouse Resource and Research Center – University of North Carolina

CB# 7519, Chapel Hill, NC 27599

Phone: (919)966-4570

[www.med.unc.edu/mmrrc](http://www.med.unc.edu/mmrrc)

[www.mmrrc.org](http://www.mmrrc.org)