MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	11668
MMRRC Strain Name	B6;129S5-*Slc17a5*^{tm1Lex}/Mmnc
Gene Name(s)	solute carrier family 17 (anion/sugar transporter), member 5 (Slc17a5)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	7/24/15

MMRRC# 11668 PCR Reaction

	1X
DNA	2.0
5X Buffer	5.0
10mM dNTPs	1.0
20 uM Primer F/R	2.0
Taq	0.5
ddH_2O	12.5
25mM MgCl2	2.0

2, 2-primer reactions: (18+15) and (20+Neo)

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

Thermal Cycler:
Step 1: 94°C for 3 min
Step 2: 94°C for 15 sec
Step 3: 65°C for 30 sec, decrease 1 degree/cycle
Step 4: 72°C for 40 sec
Steps 2-4: 10x
Step 5: 94°C for 15 sec
Step 6: 55°C for 30 sec
Step 7: 72°C for 40 sec
Steps 5-7, 30x
Step 8: 72°C for 5 min

The Mutant Mouse Resource and Research Center – University of North Carolina
CB# 7519, Chapel Hill, NC 27599
Phone: (919)966-4570 Fax: (919)843-1818
www.med.unc.edu/mmrrc www.mmrrc.org

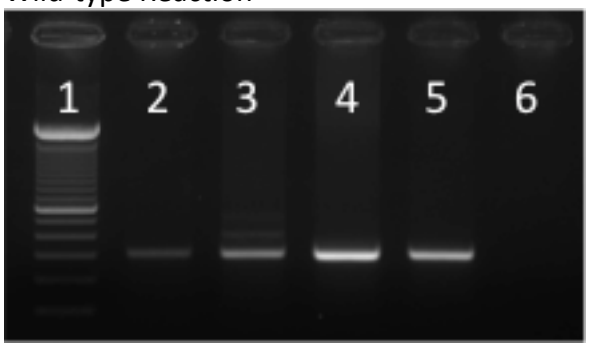
Bands: MUTANT: 450bp
WT: 322bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

NIH1513(11668)Neo	GCAGCGCATCGCCTTCTATC
NIH1513(11668) 20	GCTAAGCGGAACCTGGCG
NIH1513(11668) 18	GCTGGTGACACACATCTTGC
NIH1513(11668) 15	CCGCTTCGGTCTGCCGG

Run on 2.0% agarose gel in TAE.

Wild-type Reaction



1. 100bp Marker
2. Wild-type sample
3. Wild-type sample
4. Wild-type sample
5. Wild-type Control
6. Negative Control

Mutant Reaction

1. Heterozygous sample
2. Wild-type sample
3. Wild-type sample
4. Wild-type sample
5. Wild-type Control
6. Negative Control
7. Empty Lane
8. 100bp Marker

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