

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36683
MMRRC Strain Name	B6;129S-Zglp ^{1tmEem} /Mmnc
Gene Name(s)	zinc finger, GATA-like protein 1; targeted mutation 1, Edward E Morrisey (Zglp1 ^{tm1Eem})
Breeding Protocol(s)	Sib-mating
Protocol Date	8/12/13

MMRRC #36683 PCR Reaction

	<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

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 61°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: Step 2 to 4; Cycles: 35
 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.

WT-F (36683): GGTGGTTTCTCATACCCTACTCC

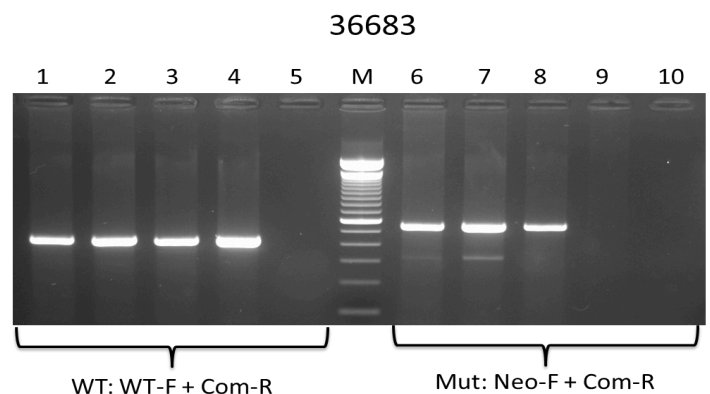
Com-R (36683): CCGCACACAGAAACACACACAAATG

Neo-F (36683): CATTCTCCCACTCATGATCTATAG

Bands expected: WT (primers WT-F + Com-R) 430 bp

MUTANT (primers Neo-F + Com-R) 512 bp

Run on 2% agarose gel in TAE.



Lane 1-3, 6-8: Het; Lane 4, 9: WT; Lane 5, 10: H₂O;

M: 100 bp DNA ladder Invitrogen