

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	117
MMRRC Strain Name	B6;129S4- <i>Gba</i> ^{tm1Rlp} /Mmnc
Gene Name(s)	Glucosidase, beta, acid (<i>Gba</i>)
Breeding Protocol(s)	Intra-strain mating (het x het)
Protocol Date	1/27/12

MMRRC# 117 PCR Reaction

	<u>1X</u>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	1.0
ddH ₂ O	14.5
25mM MgCl ₂	2.0

NOTE: Reaction requires a restriction digest!

Add to each 25ul PCR reaction:
10ul HOH
4ul NEB Buffer 4
1ul NciI

Thermal Cycler:

94°C for 45 sec
62°C for 45 sec
72°C for 60 sec
36x

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

Bands: MUTANT: cuts with NciI to ~200bp
WT: 400bp

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

Primer sequences 5' to 3':

K1 F: CCC CAG ATG ACT TGA TGC TGG

R: CCA GGT CAG GAT CTC TGA TGG

Run on 2.0% agarose gel in TAE.