

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	117
MMRRC Strain Name	B6;129S4- <i>Gba1</i> ^{tm1Rlp} /Mmnc
Gene Name(s)	glucosidase, beta, acid
Breeding Protocol(s)	Intra-strain mating (hets)
Protocol Date	11/9/21

PCR Reaction

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

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 62C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 35

Step 5: 72C, 7min

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

Bands: WT: 400bp MUTANT: 200bp (after restriction digestion)

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

F: 5' CCC CAG ATG ACT GAT GCT GGA 3'

R: 5' CCA GGT CAG GAT CAC TGA TGG 3'

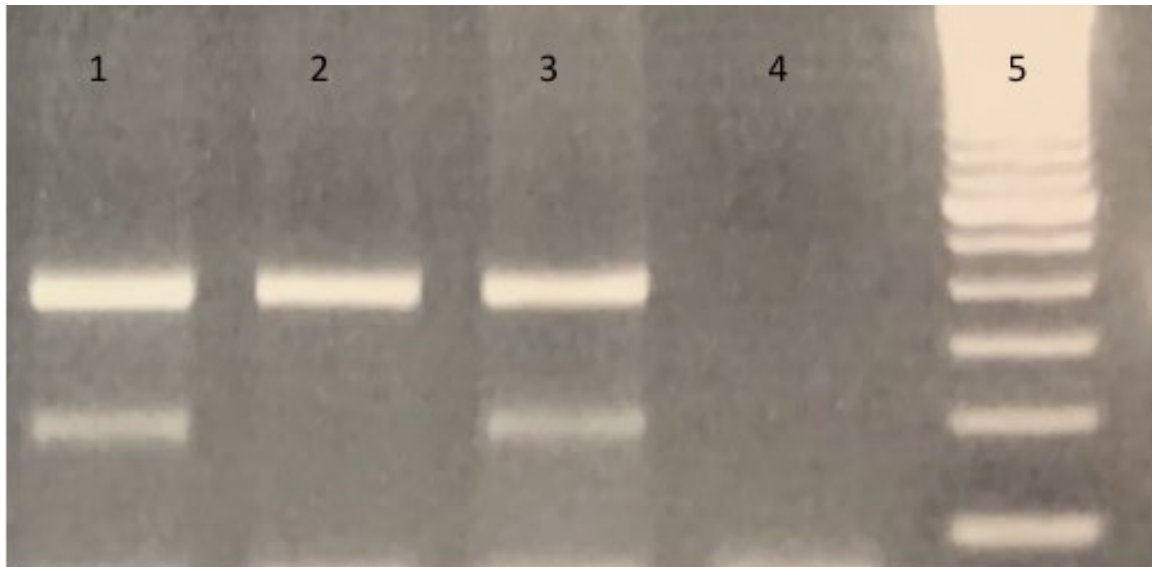
Restriction Digestion is required after the PCR reaction is completed.

To each 25ul PCR reaction add:

10ul HOH
4ul NEB Cutsmart Buffer
1ul NciI

Digest overnight at 37C

Run on 2.0% Agarose gel



1. Mutant Sample
2. Wild-type Control
3. Positive Control
4. Negative Control
5. 100bp Ladder (Invitrogen)