

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34765
MMRRC Strain Name	B6;129S- <i>Ghrh</i> ^{tm1Salv} /Mmnc
Gene Name(s)	growth hormone releasing hormone (Ghrh)
Breeding Protocol(s)	Random Intra-strain Mating
Protocol Date	6/27/13

MMRRC #34765 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 60°C for 30 sec
 Step 4: 72° C for 30 sec
 Step 5: Step 2 to 4; Cycles: 35
 Step 6: 72°C for 7 min

Taq: **Apex and Chromataq Buffer**

	<u>1X</u>
ddH ₂ O	12
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer WT-F	1
10 uM Primer R	1
10 uM Primer Mut- F	1
Taq	0.5
DNA	2

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

WT-F: 5'-CACGGGACAACAAAGGGGACCT-3'

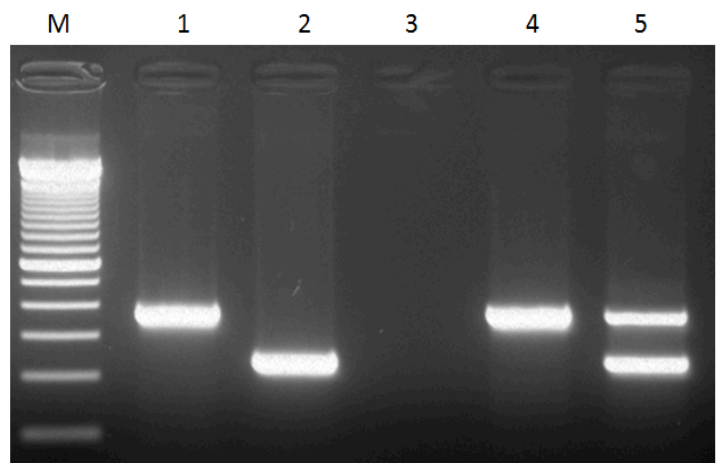
Common -R: 5'-TCAACTGGGCAGAAGCTCCTAGGAT-3'

Mut - F: 5'-AAATGGCCGCTTTTCTGGATTCATC-3'

Bands: WT-F + Common -R: WT 231 bp

Mut-F + Common -R: MUT ~320 bp

Run on 2% agarose gel in TAE.



M: 100 bp DNA ladder; Lane 1 & 4: Hom; Lane 2: WT; Lane 3: ddH₂O; Lane 5: Hom + WT (1:1)