

MMRRC UNC – Genotyping Protocol

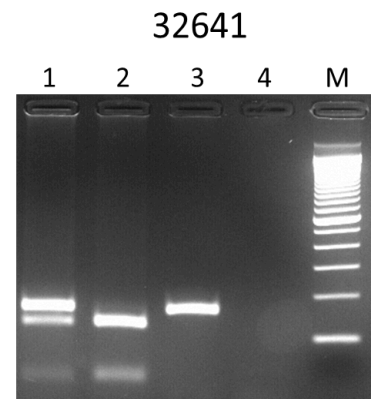
MMRRC Strain ID	32641
MMRRC Strain Name	C57BL/6J- <i>Grhl1</i> ^{m1Btlr}
Gene Name(s)	grainyhead-like 1 (Drosophila); mutation 1, Bruce Beutler (<i>Grhl1m1Btlr</i>)
Breeding Protocol(s)	Random Intra-strain Mating
Protocol Date	7/16/13

MMRRC #32641 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: 55°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



Lane 1: Het; Lane 2: Hom; Lane 3: WT; Lane 4: H₂O; M: 100 bp DNA ladder (Invitrogen)

Taq: Apex and Chromataq 5X Buffer

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

BanditF20 (32641): GAGTCCCTCTCATTGATGTTGACAC

BanditB18 (32641): AAAGCCCTGCCTCTTCACTTG

NOTE: This strain genotyping requires *Ddel* restriction digestion

***Ddel* restriction digestion mix for each PCR sample:**

10 μl H₂O

4 μl NE Buffer 3, 10X

1 μl *Ddel*

Add 15μl reaction mix to each 25μl PCR product; incubate at 37 °C, for 3 hours - overnight. Then 80°C for 20 minutes

Bands expected: Homo: 128 bp + 36 bp
 Het: 164 bp + 128 bp + 36 bp
 WT: 164 bp

Run on a 3% agarose gel