

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30470
MMRRC Strain Name	B6;129- <i>Bmp2</i> ^{tm1Mis} /Mmnc
Gene Name(s)	Bone morphogenic protein 2 (Bmp2)
Breeding Protocol(s)	Sib-mating
Protocol Date	4/7/14

MMRRC #30470 PCR Reactions

Thermal Cycler:

- Step 1: 94°C for 5 min
- Step 2: 94°C for 30 sec
- Step 3: 57°C for 30 sec
- Step 4: 72°C for 30 sec
- Step 5: 32 x from step 2 to step 4
- Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

	1X
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

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

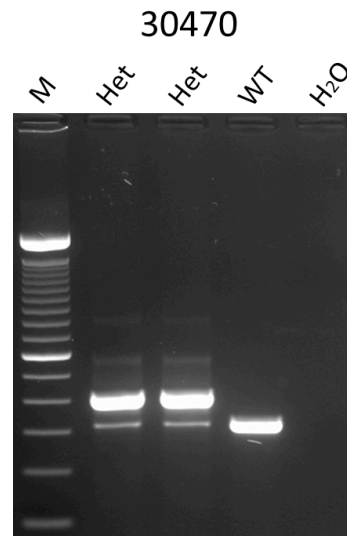
A-F (30470): AGCATGAACCCTCATGTGTTGG

B-R (30470): GTGACATTAGGCTGCTGTAGCA

Bands expected:

- WT 322 bp;
- MUTANT: 357 bp

Run on 2% agarose gel in TAE.



Primers: A-F (30470) + B-R (30470)
 M: 100 bp DNA ladder (Invitrogen)

PCR Reaction for Neo

	<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: 60°C for 45 sec
- Step 4: 72°C for 1 min
- Step 5: 36 x from step 2 to step 4
- 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.

Neo F (30492): ATTGAACAAGATGGATTGCAC

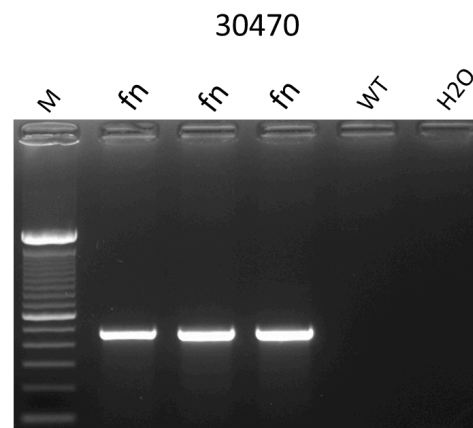
Neo R (30492): CGTCCAGATCATCCTGATC

Bands expected:

Fn: 475 bp

Fx /WT: no band

Run on 1% agarose gel in TAE.



Primers: NeoF (30492) + NeoR (30492)

M: 100 bp DNA ladder (Invitrogen)

fn = Containing Neo