

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	17246
MMRRC Strain Name	C.129(Cg)- <i>Igk-J^{tm2Mwg}</i> <i>Igh-J^{tm2(3H9-VDJ*)Mwg}</i> /Mmnc
Gene Name(s)	immunoglobulin heavy chain, joining region (Igh-J) immunoglobulin kappa chain, joining region (Igk-J)
Breeding Protocol(s)	Sib-mating
Protocol Date	8/12/13

The genotyping for this strain includes 4 reactions: JH1, 3H9/56R, Vk8 and JK1, and runs different cyclers for the reactions.

MMRRC #17246 PCR – JH1 Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM 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: 59 °C for 30 sec
 Step 4: 72°C for 1 min
 Step 5: 34x from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

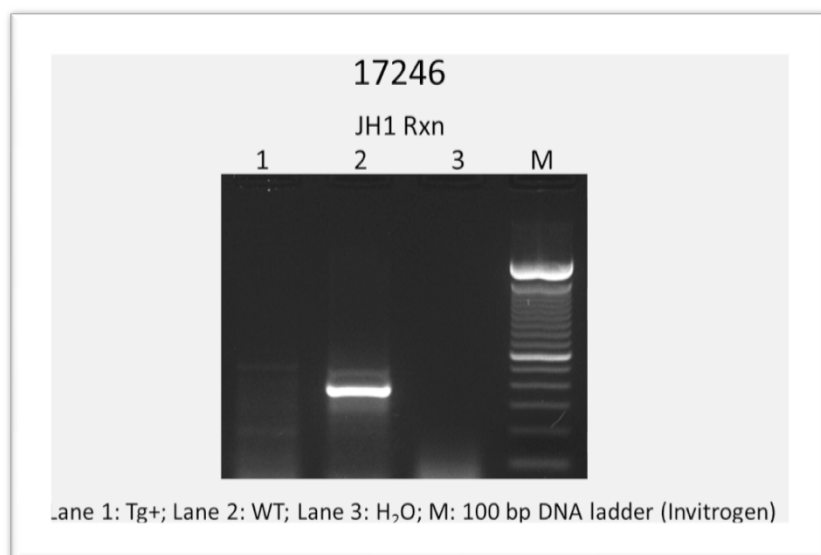
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

MB 631: GCC AAG GAC TTA CCA AGA GG

MB 632: 5GAT GCA GGA CTC ACC TGA CC

Bands expected: WT 376 bp
 Tg+ No product

Run on 1% agarose gel in TAE.



MMRRC #17246 PCR – 3H9 / 56R Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM 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: 50°C for 30 sec

Step 4: 72°C for 45 sec

Step 5: 34x 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.

MB 722: CTG TCA GGA ACT GCA GGT AAG G

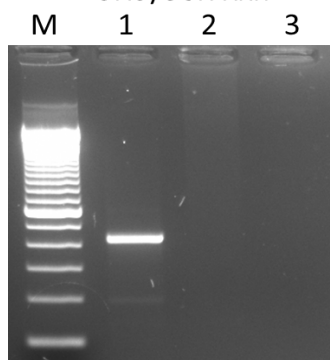
MB 730: AGT CCA TAA CAT AGG AAT ATT TAC TC

Bands expected: Tg+ 400 bp
 WT No band

Run on 1% agarose gel in TAE.

17246

3H9/56R Rxn



Lane 1: Tg+; Lane 2: WT; Lane 3: H₂O; M: 100 bp DNA ladder (Invitrogen)

MMRRC #17246 PCR – JK1 Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM 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 40 sec

Step 5: 34x 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.

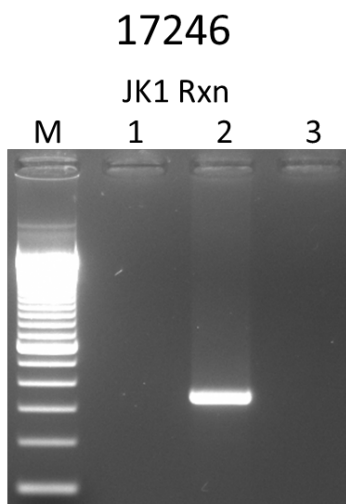
MB 635: CAG CAG TTC TCT GTC AGA GAA G

MB 636: TCT CCA GAG AAC ATG TCT AGC C

Bands expected: WT 350bp

Tg+ No product

Run on 1% agarose gel in TAE.



Lane 1: Tg+; Lane 2: WT; Lane 3: H₂O; M: 100 bp DNA ladder (Invitrogen)

MMRRC #17246 PCR – Vk8 Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM 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 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 34x 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.

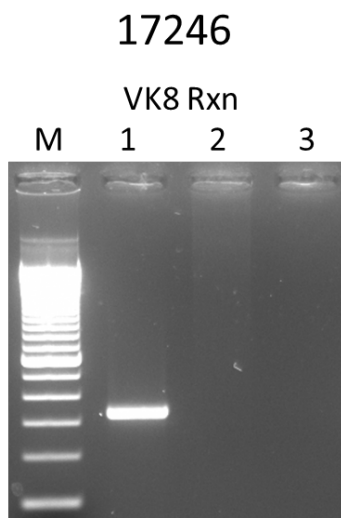
MB 643: AGC ACC GAA CGT GAG AGG

MB 644: GGT ACC TGT GGG GAC ATT GTG

Bands expected: WT no product

Tg+ 330 bp

Run on 1 % agarose gel in TAE.



Lane 1: Tg+; Lane 2: WT; Lane 3: H₂O; M: 100 bp DNA ladder (Invitrogen)