

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	17243
MMRRC Strain Name	<i>C.129(Cg)-Rag1^{tm1BaI} Igk-J^{tm2Mwg} Igh-J^{tm1(3H9-VDJ)Mwg}/Mmnc</i>
Gene Name(s)	immunoglobulin kappa chain, joining region (Igk-J) immunoglobulin heavy chain, joining region (Igh-J) recombination activating gene 1 (Rag1)
Breeding Protocol(s)	Sib-mating
Protocol Date	8/12/13

The genotyping for this train includes 5 reactions: JH1, 3H9/56R, Vk8, JK1 and RAG and runs different cyclers for the reactions.

MMRRC #17243 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

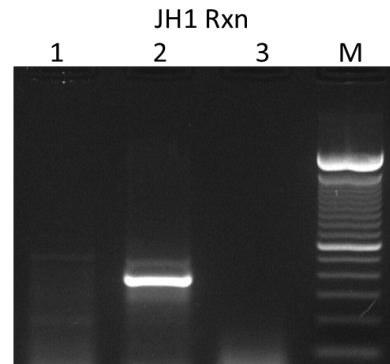
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

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.



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

MMRRC #17243 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

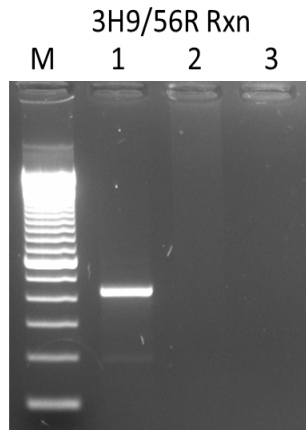
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

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.



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

MMRRC #17243 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

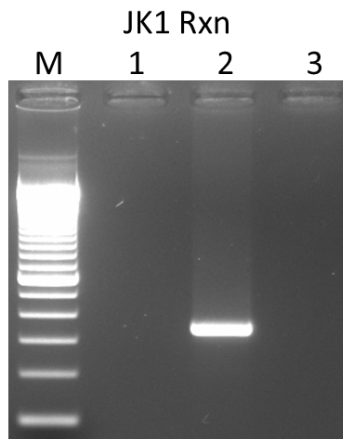
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

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 #17243 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

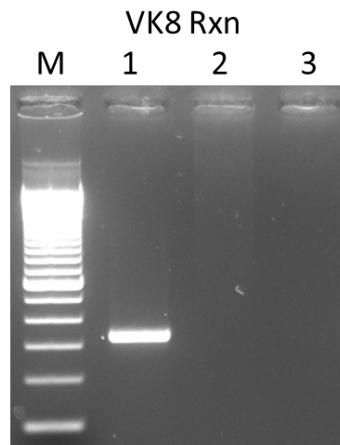
Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

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)

MMRRC #17243 PCR – RAG1 Reaction

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

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 63°C for 30 sec
 Step 4: 72°C for 30 sec
 Step 5: 30 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 10uM with respect to each primer

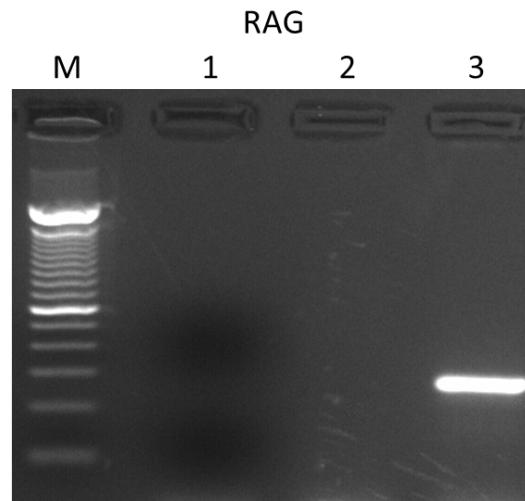
MB 720: CGA ACA CAT TCT GGC TGA TCC C

MB 721: TCT TTG TGG CTT GAC ACA TGG TG

Bands: WT 250bp

Tg+ No product

Run on 1% agarose gel in TAE.



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