MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	17240
MMRRC Strain Name	CB17.Cg-*Igh-J*$^{tm1(3H9\text{-}VDJ)Mwg}$ *Prkdc*^{scid}/Mmnc
Gene Name(s)	immunoglobulin heavy chain, joining region (Igh-J) protein kinase, DNA activated, catalytic polypeptide (Prkdc)
Breeding Protocol(s)	Sib-mating
Protocol Date	9/7/13

MMRRC #17240 PCR Reactions

Genotyping for this strain includes 3 reactions: one four-primer reaction for scid, and two two-primer reactions for H1 and 3H9/56R. The 101 bp of WT band cannot be shown in the four-primer reaction. A two-primer reaction using the primers MB 575 + MB 576 is needed to confirm the homozygous samples.

	1X
ddH_2O	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: 61 °C for 30 sec
Step 4: 72°C for 45 sec
Step 5: 35x 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 575: GAGAAAAGGAGGATCATG GATTCAAGAAATAAATGTAACG
MB 576: TGGCCCCTGCTAACTTTCTCTTAGCA
MB 577: TGGTATCCACAACATAAAATACGCTAA
M-MB 578: CCTAAGAGTCACTTTCTCCATCTACACAGTGAAGTGCC

Bands expected: MB 575 + MB 576 WT: 101bp
MB 577 + M-MB 578 scid band: 180 bp
MB 575 + M-MB 578 Common band: 229 bp

Run on 3% agarose gel in TAE.

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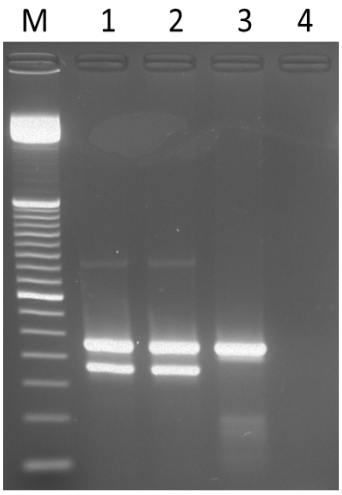
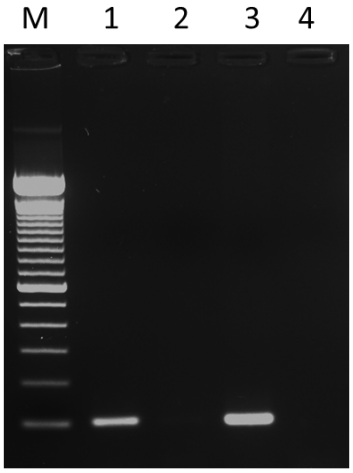
17240



Primers: MB 575 + MB 576 + MB 577 + M-MB 578
Lane 1 and 2: Hom; Lane 3: WT; Lane 4: H_2O
M: 50 bp DNA ladder Invitrogen

17240



Primers: MB 575 + MB 576
Lane 1 and 3: WT; Lane 2: H_2O; Lane 4: Hom;
M: 100 bp DNA ladder Invitrogen

MMRRC #17240 PCR – JH1 Reaction

	1X
ddH_2O	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

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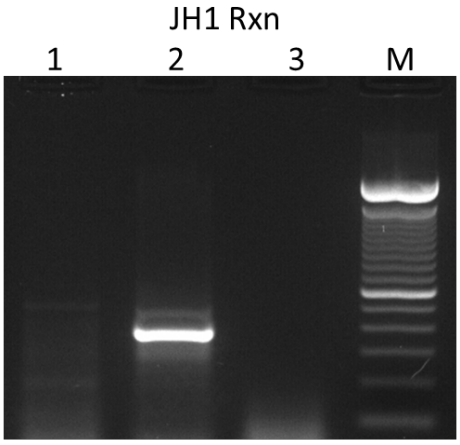
Bands expected: WT: 376 bp
Tg+: No product

Run on 1% agarose gel in TAE.



Lane 1: Tg+; Lane 2: WT; Lane 3: H_2O; M: 100 bp DNA ladder (Invitrogen)

MMRRC #17240 PCR – 3H9 / 56R Reaction

	1X
ddH_2O	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

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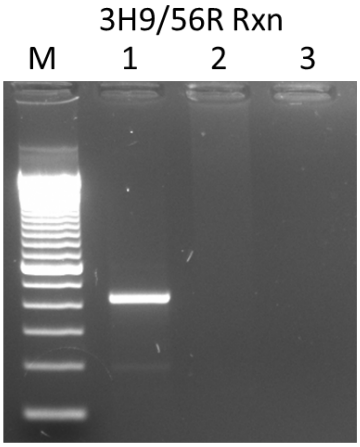
Taq: **Apex and Chromataq 5X Buffer**

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: H_2O; M: 100 bp DNA ladder (Invitrogen)

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