

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	31069
<b>MMRRC Strain Name</b>	B6.Cg-Tg(Igh-2-12Vh/Ighm <sup>a</sup> )1Shc Tg(IgkH220-17)1Mwg/Mmnc
<b>Gene Name(s)</b>	transgene insertion 1, Martin Weigert transgene insertion 1, Stephen Clarke
<b>Breeding Protocol(s)</b>	Backcross to C57BL/6J
<b>Protocol Date</b>	7/16/13

### MMRRC #31069 PCR Reaction

This is a double transgenic, and must be typed for the 212H and Vk8 transgene in 2 PCR reactions which are run separately.

#### 212H Reaction

**Thermal Cycler:**

- Step 1: 94°C for 5 min
- Step 2: 94°C for 45 sec
- Step 3: 66°C for 45 sec
- Step 4: 72°C for 1 min
- Step 5: 35x from step 2 to step 4
- Step 6: 72°C for 7 min

	<b>1X</b>
ddH <sub>2</sub> O	32.0
5X Buffer	10.0
25 mM MgCl <sub>2</sub>	3.0
10 mM dNTPs	1.0
10 μM each Primer 1 & 2 mix	2.0
Taq	1.0
DNA	1.0

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

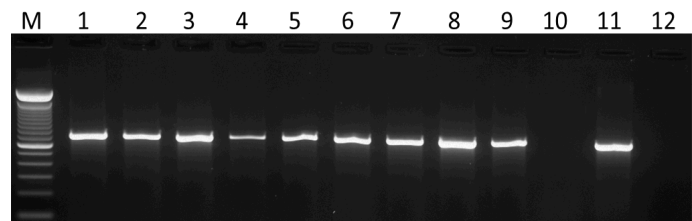
**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

Primer #1 (5' primer): GCA AGG CCA CAC TGA CTT CAG AC

Primer #2 (JH 3' Xba): CAG GCT CCA CCA GAC CTC TCT AGA      31069 (212H Rxn)

**Bands expected:** 212H Tg<sup>+</sup>: ~700bp  
WT: no band

Run on 2% agarose gel in TAE.



Primers: Primer #1 (5' primer) + Primer #2 (JH 3' Xba)

Lane 1-9, 11: Tg<sup>+</sup>; Lane 10: WT; Lane 12: H<sub>2</sub>O;

M: 100 bp DNA ladder Invitrogen

### Vk8 Reaction

**Thermal Cycler:**

- Step 1: 94°C for 5 min
- Step 2: 94°C for 45 sec
- Step 3: 62°C for 45 sec
- Step 4: 72°C for 1 min
- Step 5: 35x from step 2 to step 4
- Step 6: 72°C for 7 min

	<b>1X</b>
ddH <sub>2</sub> O	32.0
5X Buffer	10.0
25 mM MgCl <sub>2</sub>	3.0
10 mM dNTPs	1.0
10 μM each Primer 1 & 2 mix	2.0
Taq	1.0
DNA	1.0

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

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

Primer #1 (leader): GGT ACC TGT GGG ACA TTG TG

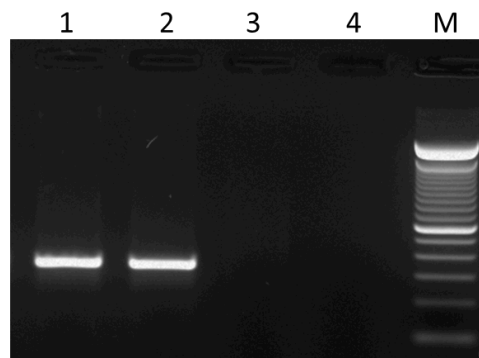
Primer #2 (Jk5 primer): AGC ACC GAA CGT GAG AGG

**Bands expected:** Vk8 Tg: ~350bp

WT: no band

Run on 2% agarose gel in TAE.

### 31069 (Vk8 Rxn)



Primers: Primer #1 (leader) + Primer #2 (Jk5 primer)

Lane 1-2: Tg+; Lane 3: WT; Lane 4: H<sub>2</sub>O; M: 100 bp DNA ladder Invitrogen