

MMRRC UNC – Genotyping Protocol

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|-----------------------------|---------------------------------|
| MMRRC Strain ID | 36903 |
| MMRRC Strain Name | B6.Cg-Tg(Il21-mCherry)1Wjl/Mmnc |
| Gene Name(s) | interleukin 21 (Il21) |
| Breeding Protocol(s) | Backcross to C57BL/6J |
| Protocol Date | 9/23/13 |

MMRRC #36903 PCR Reaction

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: Step 2 to 4; Cycles: 35
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

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

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

pmcherry267F: CCTGTCCCCTCAGTTCATGT

pmcherry591R: CTCAGCTTCAGCCTCTGCT

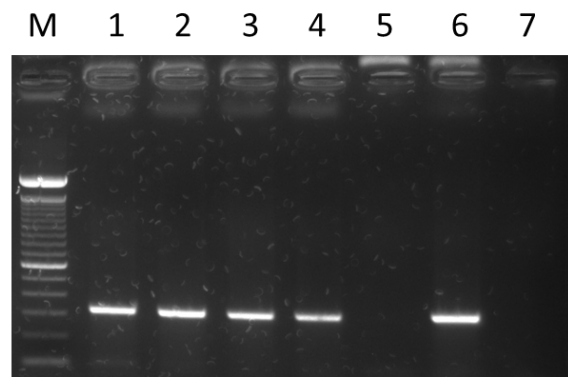
Bands expected:

MUTANT: 320 bp

WT: No Band

Run on 2.0% agarose gel in TAE.

36903



Lane 1 to 4, 6: Mut; Lane 5: WT; Lane 7: H₂O;
 M: 100 bp DNA ladder (Invitrogen)