

## MMRRC UNC – Genotyping Protocol

|                             |   |
|-----------------------------|---|
| <b>MMRRC Strain ID</b>      | 10774   |
| <b>MMRRC Strain Name</b>    | B10.Cg- <i>Il4</i> <sup>tm1Cgn</sup> Tg(HLA-DRA*0101,HLA-DRB1*0101)1Dmz   |
| <b>Gene Name(s)</b>         | Interleukin 4 (Il4), Tg(HLA-DRA*0101,HLA-DRB1*0101)1Dmz, down-regulator of transcription 1, TBP-binding (negative cofactor 2) (DR1 Human) |
| <b>Breeding Protocol(s)</b> | Random Intra-strain Mating  |
| <b>Protocol Date</b>        | 2/24/14   |

### MMRRC #10774 PCR Reaction

**This strain genotyping requires to type DR1 IL4 knockout and DR1 Tg+.**

|                         | <b>1X</b> |
|-------------------------|-----------|
| ddH <sub>2</sub> O      | 13        |
| 5X Buffer               | 5.0       |
| 25 mM MgCl <sub>2</sub> | 2         |
| 10 mM dNTPs             | 0.5       |
| 10 μM Primer F          | 1         |
| 10 μM Primer R          | 1         |
| Taq                     | 0.5       |
| DNA                     | 2         |

#### **Genotyping IL4**

#### **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 1 min

Step 5: 32x 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.

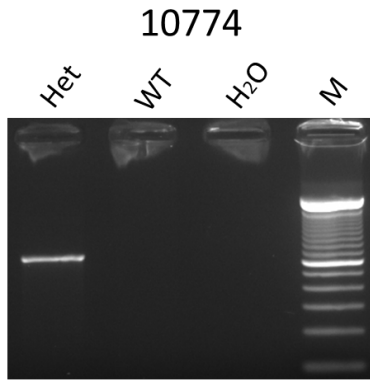
IL-4-Com-F (10774): GGGAGGGGTGTTTCATTTTCCAATT

IL-4-WT-R (10774): GGTACATTCGAGAAAGAAGAGCAGGATG

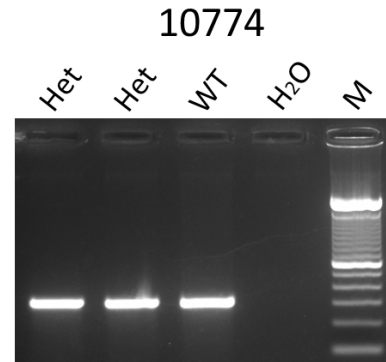
IL-4 Mut-R (10774): AGTCATAGCCGAATAGCCTCTCCACC

**Bands expected:** WT: IL-4-Com-F (10774) + IL-4-WT-R (10774), 313 bp

MUTANT: IL-4-Com-F (10774) + IL-4 Mut-R (10774), ~700 bp



Primers: IL-4-Com-F (10774) + IL-4 Mut-R (10774)  
M: 100 bp DNA ladder (Invitrogen)



Primers: IL-4-Com-F (10774) + IL-4-WT-R (10774)  
M: 100 bp DNA ladder (Invitrogen)

### Genotyping DR1 Tg+

#### Thermal Cycler:

- Step 1: 94°C for 5 min
- Step 2: 94°C for 30 sec
- Step 3: 65°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 10 μM with respect to each primer.

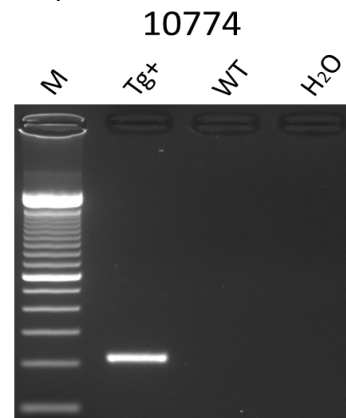
DR1-F (10774): ACATGAGTGTCAATTTCTTCAACGGGAC

DR1-R (10774): CGTAGTTGTGTCTGCAGTAGGTGTCCA

#### Bands expected:

Tg+: DR1-F (10774) + DR1-R (10774): 215 bp  
WT: no band.

Run on 2% agarose gel in TAE.



Primers: DR1-F (10774) + DR1-R (10774)  
M: 100 bp DNA ladder (Invitrogen)