

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	432
<b>MMRRC Strain Name</b>	B10.Cg-H2 <sup>k</sup> Cd74 <sup>tm3Liz</sup> /Mmnc
<b>Gene Name(s)</b>	CD74 antigen (Cd74)
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	11/11/13

### MMRRC #432 PCR Reaction

	<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

**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 45 sec  
 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.

Com-F (431): CCCACCGAGGCTCCACCTAAAG

Mut-R (431): CAGCGTGCGGAGAAGAAGAGA

WT-R (431): CTGGGCCAGGATCTCAGCAGA

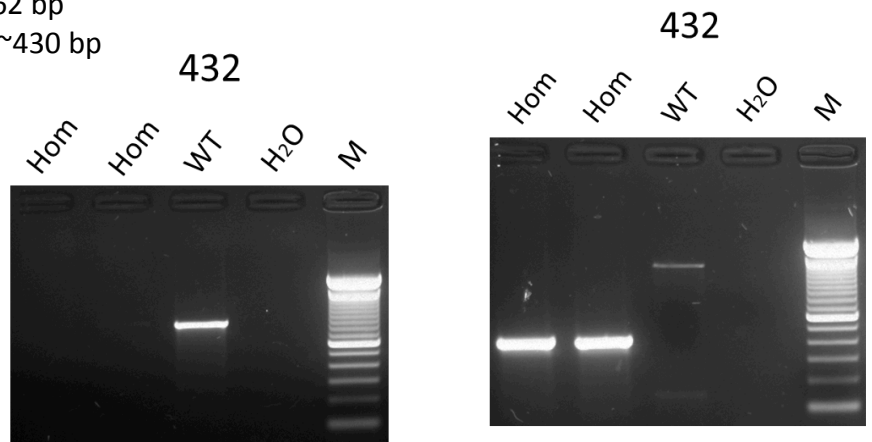
**Bands expected:**

WT, Com-F (431) + WT-R (431): 862 bp

Mut, Com-F (431) + Mut-R (431): ~430 bp

WT 1.3 kb (inefficient)

Run on 2% agarose gel in TAE.



Primers: Com-F (431) + WT-R (431)

M: 100 bp DNA ladder (Invitrogen)

Primers: Com-F (431) + Mut-R (431)

M: 100 bp DNA ladder (Invitrogen)