

## MMRRC UNC – Genotyping Protocol

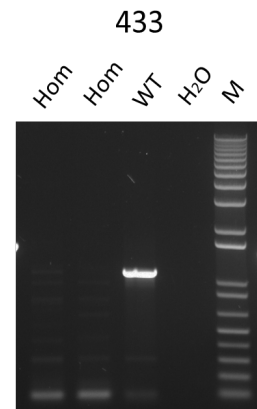
<b>MMRRC Strain ID</b>	433
<b>MMRRC Strain Name</b>	B10.Cg-H2 <sup>k</sup> Cd74 <sup>tm1Liz</sup> /Mmnc
<b>Gene Name(s)</b>	CD74 antigen (Cd74)
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	11/4/13

### MMRRC #433 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: 58°C for 1 min  
 Step 4: 72°C for 3 min  
 Step 5: 32x from step 2 to step 4  
 Step 6: 72°C for 7 min



Primers: WT-F (433) + WT-R (433)  
M: 1Kb plus DNA ladder (Invitrogen)

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

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

WT-F (433): GGCTAGGTCCCAAGTGTAGGC

WT-R (433): TCACTCAAGGCAACCTCC

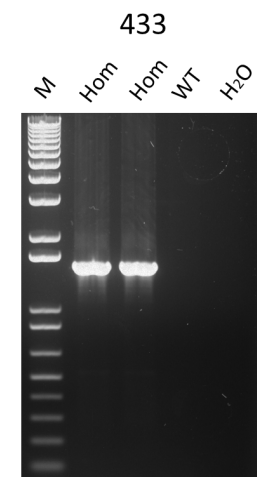
Mut-Neo (433): CGCTGACAGCCGGAACACGG

#### Bands expected:

WT: WT-F (433) + WT-R (433): 1103 bp

MUTANT: WT-F (433) + Mut-Neo (433): 1450 bp

Run on 0.8% agarose gel in TAE.



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