

MMRRC UNC – Genotyping Protocol

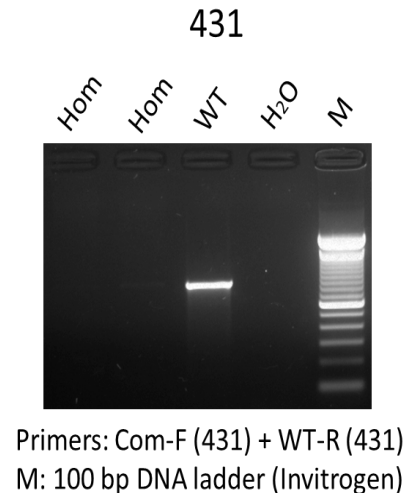
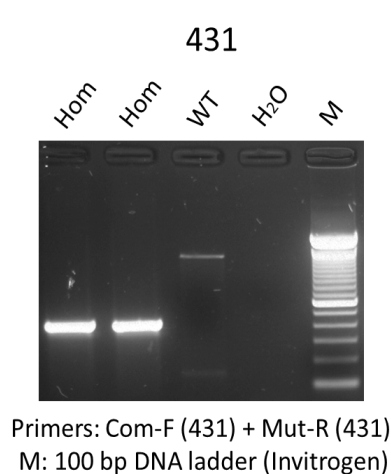
MMRRC Strain ID	431
MMRRC Strain Name	B6.129S- <i>Cd74^{tm3Liz}</i> /Mmnc
Gene Name(s)	CD74 antigen (Cd74)
Breeding Protocol(s)	Sib-mating
Protocol Date	11/4/2103

MMRRC #431 PCR Reaction

	1X
ddH ₂ O	13
5X Buffer	5.0
25 mM MgCl ₂	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

MUTANT: Com-F (431) + Mut-R (431), ~430 bp; WT 1.3 kb (inefficient)

Run on 2% agarose gel in TAE.