

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	222
<b>MMRRC Strain Name</b>	STOCK <i>Cd4<sup>tm1Litt</sup></i> Tg(Cd4-CD4)2362Litt/Mmnc
<b>Gene Name(s)</b>	CD4 antigen (Cd4) CD4 antigen (Human)
<b>Breeding Protocol(s)</b>	Intra-strain Mating (homozygous x homozygous)
<b>Protocol Date</b>	10/7/13

### MMRRC# 222 PCR Reaction

	<b>1X</b>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	1.0
Primer F/R	1.0
Taq	1.0
ddH <sub>2</sub> O	13.5
25mM MgCl <sub>2</sub>	2.0

**NOTE:** Run 2 reactions for each sample (mCD41 + mCD42 + mCD43) & (hCD41 + hCD42).

**Thermal Cycler:**

94°C for 45 sec  
57°C for 45 sec  
72°C for 60 sec  
36x

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

**Bands:** MUTANT: 200bp (Murine); 155bp (Human Tg)  
WT: 375bp (Murine); No Band (Human)

Primers are 10uM with respect to each primer (20uM or 30 uM total).

**Primer sequences 5' to 3':**

hCD41: 5': CTAAGCTCCAGATGGGCAAG

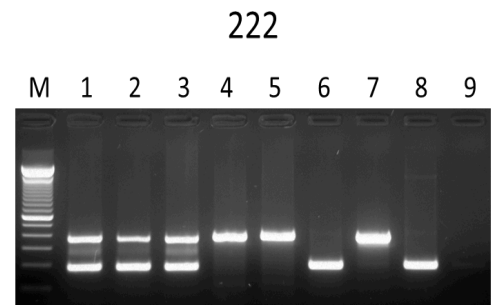
hCD42: 5': CTGAGTGGCTCTCATCACCA

mCD41: 5': GCTTCCTCGTGCTTTACGGTATC

mCD42: 5': AGAGTTGCTATCCAAGGTCAGGG

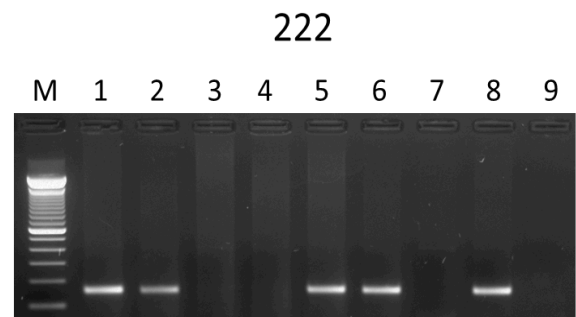
mCD43: 5': GGAGTTGTGGGTGTTCAAAGGTG

Run on 2.0% agarose gel in TAE.



Primers: mCD41 + mCD42 + mCD43

Lane 1, 2, 3: Het; Lane 4, 5, 7: WT; Lane 6, 8: Hom; Lane 9: H<sub>2</sub>O  
M: 100 bp DNA ladder (Invitrogen)



Primers: hCD41 + hCD42

Lane 1, 2, 5, 6, 8: Tg+; Lane 3, 4, 7: WT; Lane 9: H<sub>2</sub>O  
M: 100 bp DNA ladder (Invitrogen)