

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	30492
<b>MMRRC Strain Name</b>	129S4- <i>Ctla4</i> <sup>tm1Shr</sup> <i>Cd28</i> <sup>tm1Shr</sup> /Mmnc
<b>Gene Name(s)</b>	Cytotoxic T-lymphocyte-associated protein 4(Ctla4) CD28 antigen (CD28)
<b>Breeding Protocol(s)</b>	Intra-Strain Mating (homo x homo)
<b>Protocol Date</b>	10/7/13

### MMRRC# 30492 PCR Reactions

	<b><u>1X</u></b>
DNA	1.0
5X Buffer	5.0
10mM dNTPs	0.5
20 uM Primer F/R	1.0
Taq	1.0
ddH <sub>2</sub> O	14.5
25mM MgCl <sub>2</sub>	2.0

**NOTE:** CTLA4 is KO'd by neo, CD28 is KO'd by hygro. Unfortunately, this line requires 4 PCR's (2 primers apiece): two rxns to test for WT CTLA4 and CD28, and two rxns that test for the presence of neo and hygro. Run each PCR separately. Hygro primers designed by UNC in place of DI primer set.

#### **Thermal Cycler:**

Step 1: 94°C for 45 sec

Step 2: 60°C for 45 sec

Step 3: 72°C for 60 sec

36x

Taq: **Apex and Chromataq 10X Buffer**

**Bands:** CTLA (wt): 217bp; neo ~424bp;  
CD28 (wt): 293bp; hygro ~279bp

**Primer sequences 5' to 3':** Primers are 10uM with respect to each primer (20uM total).

**mCD28 F (30492):** AAC AAG ATT TTG GTA AAG CAG

**mCD28 R (30492):** GAA CTC AAT TTT GCA GAA GTA

**HygroF3(30492):** CGG AAG TGC TTG ACA TTG GG

**HygroB9(30492):** CGT CCA TCA CAG TTT GCC AGT G

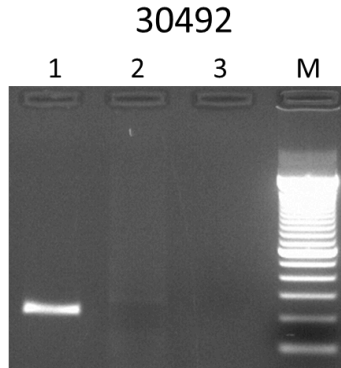
**mCTLA4 F (30492):** TGG TGT TGG CTA GCA GCC ATG

**mCTLA4 R (30492):** TTG GAT GGT GAG GTT CAC TC

**Neo F (30492):** ATT GAA CAA GAT GGA TTG CAC

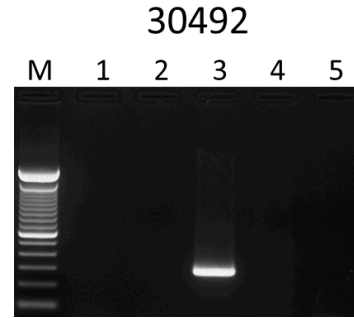
**Neo R (30492):** CGT CCA GAT CAT CCT GAT C

Run on 2.0% agarose gel in TAE.



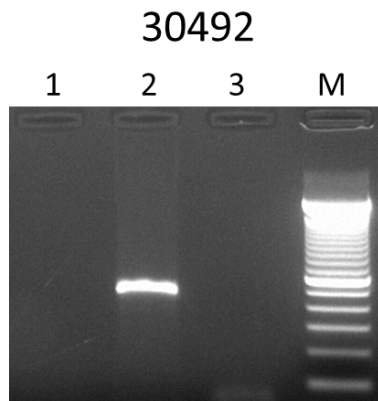
Primers: mCTLA4 F + mCTLA4 R

Lane 1: WT; Lane 2: Hom; Lane 3: H<sub>2</sub>O  
 M: 100 bp DNA ladder (Invitrogen)



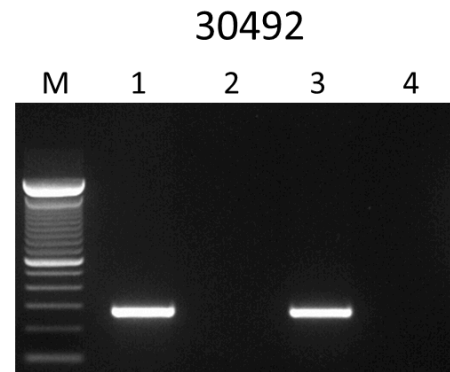
Primers: mCD28F + mCD28R

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



Primers: Neo F + Neo R

Lane 1: WT; Lane 2: Hom; Lane 3: H<sub>2</sub>O  
 M: 100 bp DNA ladder (Invitrogen)



Primers: HygroF3 + HygroB9

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