# MMRRC UNC – Genotyping Protocol

| | |
|---|---|
| **MMRRC Strain ID** | 36953 |
| **MMRRC Strain Name** | B6.Cg-*Smad3*$^{tm1Par}$ *Smad2*$^{tm2Rob}$ Tg(Cd4-cre)1Cwi/2AchMmnc |
| **Gene Name(s)** | CD4 antigen (CD4)<br>SMAD family member 2 (Smad2)<br>SMAD family member 3 (Smad3 |
| **Breeding Protocol(s)** | Sib-mating |
| **Protocol Date** | 12/23/16 |

There are 3 separate reactions for this strain.

## Cd4Cre Protocol

**Thermal Cycler:**
Step 1: 94°C for 5 min
Step 2: 94°C for 45 sec
Step 3: 59°C for 45 sec
Step 4: 72°C for 60 sec
Step 2 to 4 Cycles: 35
Step 5: 72°C for 7 min

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

| | **1X** |
|---|---|
| $ddH_2O$ | 13 |
| 5X Buffer | 5 |
| 25mM MgCl2 | 2 |
| 10mM dNTPs | 0.5 |
| 10uM WT Primer | 1 |
| 10uM Mut Primer | 1 |
| Taq | 0.5 |
| DNA | 2 |

**Bands:**
MUTANT: 373bp
WT: No Band

**Primer sequences 5' to 3':**
CreF(36953) CCACGACCAAGTGACAGCAATG
CreR(36953) CAGAGACGGAAATCCATCGCTC

Run on 2.0% agarose gel in TAE.



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## Smad2 Protocol

**Thermal Cycler:**
Step 1: 94°C for 5 min
Step 2: 94°C for 45 sec
Step 3: 59°C for 45 sec
Step 4: 72°C for 60 sec
Step 2 to 4 Cycles: 35
Step 5: 72°C for 7 min

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

| | 1X |
|---|---|
| $ddH_2O$ | 12 |
| 5X Buffer | 5.0 |
| 25mM MgCl2 | 2 |
| 10mM dNTPs | 0.5 |
| 10uM Primer F | 1 |
| 10uM Primer R | 1 |
| 10uM Primer Neo | 1 |
| Taq | 0.5 |
| DNA | 2 |

**Bands:**
MUTANT: 415bp
WT: 341bp

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

| | |
|---|---|
| Smad2F(36953) | AGT TAA TTG CCC AGA GCG TTG ACA |
| Smad2R(36953) | GCG GAG TGA ATG GCA AGA TGG |
| Smad2Neo(36953) | AGC ACG TAC TCG GAT GGA AGC |

Run on 2.0% agarose gel in TAE.

## Smad3 Protocol

**Thermal Cycler:**
Step 1: 94°C for 5 min
Step 2: 94°C for 45 sec
Step 3: 59°C for 45 sec
Step 4: 72°C for 60 sec
Step 2 to 4 Cycles: 35
Step 5: 72°C for 7 min

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

| | **1X** |
|---|---|
| $ddH_2O$ | 11 |
| 5X Buffer | 5 |
| 25mM MgCl2 | 2 |
| 10mM dNTPs | 0.5 |
| 10uM P1/P2 | 1/1 |
| 10uM P3/P4 | 1/1 |
| Taq | 0.5 |
| DNA | 2 |

**Bands:**
MUTANT: 200bp
WT: 130bp



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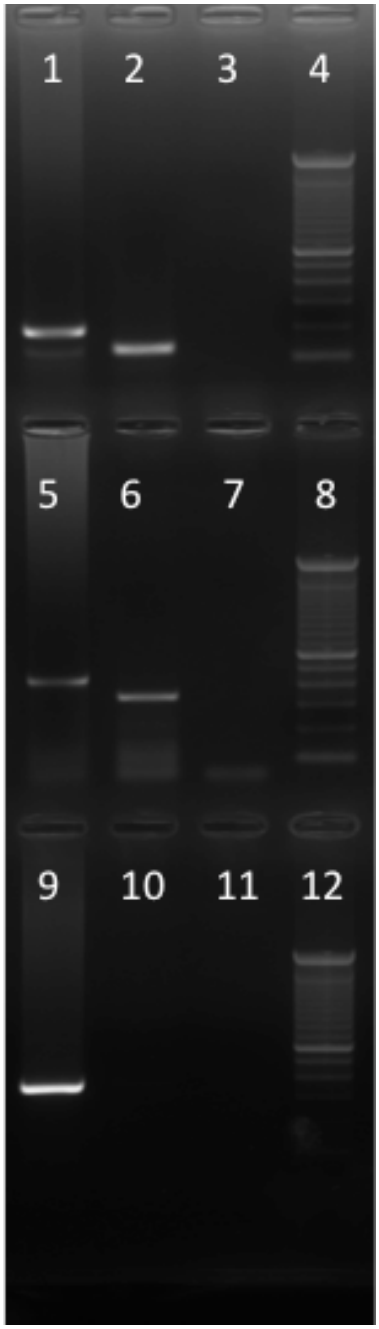
**Primer sequences 5' to 3':** Primers are 10uM each.

| | |
|---|---|
| Smad3P1(36953) | cgg cga gga tct cgt cgt gac cca |
| Smad3P2(36953) | gcg ata ccg taa agc acg agg aag |
| Smad3P3(36953) | gga tgg tcg gctgca ggt gtc |
| Smad3P4(36953) | tgt tga agg caa act cac aga gc |

Run on 2.0% agarose gel in TAE.




1. Smad3 Heterozygous Sample
2. Smad3 Wild-type Control
3. Negative Control
4. 100bp Marker (Invitrogen)
5. Smad2 Homozygous Sample
6. Smad2 Wild-type Control
7. Negative Control
8. 100bp Marker (Invitrogen)
9. Cre+ Sample
10. Wild-type Control
11. Negative Control
12. 100bp Marker (Invitrogen)



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