# MMRRC UNC – Genotyping Protocol

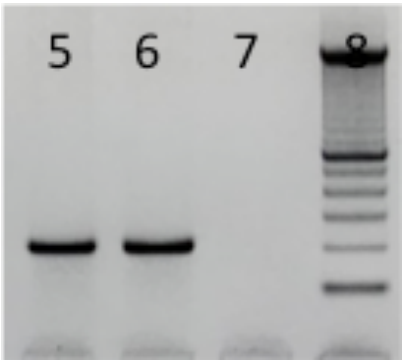
| | |
|---|---|
| **MMRRC Strain ID** | 37477 |
| **MMRRC Strain Name** | B6.Cg-*Vhl*$^{tm1.1Wkr}$ *Ndor1*$^{Tg(UBC-cre/ERT2)1Ejb}$/Mmnc |
| **Gene Name(s)** | von Hippel-Lindau tumor suppressor, cre tamoxifen-dependent recombinase, NADPH dependent diflavin oxidoreductase 1 |
| **Breeding Protocol(s)** | Random Intra-strain Mating |
| **Protocol Date** | 12/2/19 |

## PCR Reaction

### Note: Strain Requires 3 Separate PCR Reactions

### UBCre Reaction

| | 1X |
|---|---|
| dd$H_2O$ | 13 |
| 5X Buffer | 5 |
| 25mM MgCl2 | 2 |
| 10mM dNTPs | 0.5 |
| 10uM Primer 1 | 1 |
| 10uM Primer 2 | 1 |
| Taq | 0.5 |
| DNA | 2 |




**Thermal Cycler:**
Step 1: 94C, 5min
Step 2: 94C, 30sec
Step 3: 55C, 30sec
Step 4: 72C, 30sec
Step 2 to 4 Cycles: 30
Step 5: 72C, 7min

UBCre Reaction
5. Cre+ Sample
6. Cre+ Sample
7. Wild-type Control
8. 100bp Marker (Invitrogen)

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

Bands: WT: No Band MUTANT: 250bp

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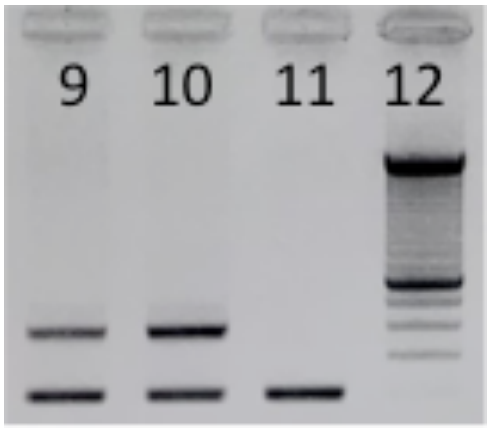
Primer sequences 5' to 3': Primers are 10uM with respect to each primer
UBCreF: GCGGTCTGGCAGTAAAAACTA
UBCreR: CCATGAGTGAACGAACCTGG

## VHL Reaction

| | 1X |
|---|---|
| $ddH_2O$ | 12 |
| 5X Buffer | 5.0 |
| 25mM MgCl2 | 2 |
| 10mM dNTPs | 0.5 |
| 10uM Primer 1 | 1 |
| 10uM Primer 2 | 1 |
| 10uM Primer 3 | 1 |
| Taq | 0.5 |
| DNA | 2 |




VHL Reaction
9. Heterozygous Sample
10. Heterozygous Sample
11. Wild-type Control
12. 100bp Marker (Invitrogen)

**Thermal Cycler:**
Step 1: 94C, 5min
Step 2: 94C, 30sec
Step 3: 55C, 30sec
Step 4: 72C, 30sec
Step 2 to 4 Cycles: 30
Step 5: 72C, 7min

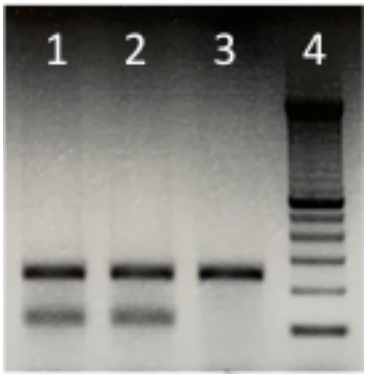
Taq: **Apex Taq and Chromataq 5X Buffer**

Bands: WT: 200bp MUTANT: 300bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer
UBVHLF1: CTGGTACCCACGAAAGTGTC
UBVHLF2: CCGGAGTAGGATAAGTCAGCTGAG
UBVHLR: CTGACTTCCACTGATGCTTGTCACAG

# 2B Reaction

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




2B Reaction
1. Heterozygous Sample
2. Heterozygous Sample
3. Wild-type Control
4. 100bp Marker (Invitrogen)

**Thermal Cycler:**
Step 1: 94C, 5min
Step 2: 94C, 30sec
Step 3: 55C, 30sec
Step 4: 72C, 30sec
Step 2 to 4 Cycles: 30
Step 5: 72C, 7min

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

Bands: WT: ~200bp MUTANT: ~100bp

Primer sequences 5' to 3': Primers are 10uM with respect to each primer
UBscpF: TCTTGGCTCAGTCGCTGTATGTCCTT
UBscpR: AATGTGACTGTTGTTGCTTGGTGGTG

**Note: 2B rxn requires a restriction digest: HpyCh4V@37*C**

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