**Primers**

mtCoI-F 61C

mtCoI-R 62C

RpL32-F 63C

RpL32-R 63C

\*RpL32 will be running at suboptimal annealing temps

**Sample Dilutions**

**Calculations for 100uL**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Target Conc.** | **Initial Conc.** | **Sample** | **DI** |
| **WTM1** | 5 | 63.9 | 7.82 | 92.18 |
| **WTF1** | 5 | 96.2 | 5.20 | 94.80 |
| **GRM1** | 5 | 45.9 | 10.89 | 89.11 |
| **GRF1** | 5 | 54.7 | 9.14 | 90.86 |
| **WTM2** | 5 | 48.8 | 10.25 | 89.75 |
| **WTF2** | 5 | 60.3 | 8.29 | 91.71 |
| **GRM2** | 5 | 89 | 5.62 | 94.38 |
| **GRF2** | 5 | 71.5 | 6.99 | 93.01 |
| **WTM3** | 5 | 28.2 | 17.73 | 82.27 |
| **WTF3** | 5 | 68.8 | 7.27 | 92.73 |
| **WTM1\_2.5** | 2.5 | 63.9 | 3.91 | 96.09 |
| **WTM1\_10** | 10 | 63.9 | 15.65 | 84.35 |

**qPCR**

**Design the plate layout**

Determine the layout for your plate before pipetting. (can use the template below)

B 
c 
D 
E 
F 
1 
WTMI 
mtCOl 
WTMI 
mtCOl 
WTMI 
mtCOl 
WTMI 
RpL32 
WTMI 
RpL32 
WTMI 
RpL32 
2 
WTFI 
WTFI 
mtCOl 
WTFI 
mtCOl 
WTFI 
RpL32 
WTFI 
RpL32 
WTFI 
RpL32 
3 
GRMI 
mtCOl 
GRMI 
mtCOl 
GRMI 
mtCOl 
GRMI 
RpL32 
GRMI 
RpL32 
GRMI 
RpL32 
4 
GRFI 
mtCOl 
GRFI 
mtCOl 
GRFI 
mtCOl 
GRFI 
RpL32 
GRFI 
RpL32 
GRFI 
RpL32 
5 
WTM2 
mtCOl 
WTM2 
mtCOl 
WTM2 
mtCOl 
WTM2 
RpL32 
WTM2 
RpL32 
WTM2 
RpL32 
6 
WTF2 
mtCOl 
WTF2 
mtCOl 
WTF2 
mtCOl 
WTF2 
RpL32 
WTF2 
RpL32 
WTF2 
RpL32 
7 
GRM2 
mtCOl 
GRM2 
mtCOl 
GRM2 
mtCOl 
GRM2 
RpL32 
GRM2 
RpL32 
GRM2 
RpL32 
8 
GRF2 
mtCOl 
GRF2 
mtCOl 
GRF2 
mtCOl 
GRF2 
RpL32 
GRF2 
RpL32 
GRF2 
RpL32 
9 
WTM3 
mtCOl 
WTM3 
mtCOl 
WTM3 
mtCOl 
WTM3 
RpL32 
WTM3 
RpL32 
WTM3 
RpL32 
10 
WTF3 
mtCOl 
WTF3 
mtCOl 
WTF3 
mtCOl 
WTF3 
RpL32 
WTF3 
RpL32 
WTF3 
RpL32 
11 
WTM1_2.5 
mtCOl 
WTM1_2.5 
mtCOl 
WTM1_2.5 
mtCOl 
WTM1_2.5 
RpL32 
WTM1_2.5 
RpL32 
WTM1_2.5 
RpL32 
12 
WTMI_IO 
mtCOl 
WTMI_IO 
mtCOl 
WTMI_IO 
mtCOl 
WTMI_IO 
RpL32 
WTMI_IO 
RpL32 
WTMI_IO 
RpL32 

**Set up the software**

* 1. First, download and install "Design
  2. and Analysis Software" from thermo at <https://www.thermofisher.com/us/en/home/global/forms/life-science/quantstudio-3-5-software.html>
  3. Open the software
  4. click "Set Up Plate"
  5. Set Instrument to "QuantStudioTM3"
  6. Set Block to 96-Well 0.2-mL
  7. For a single primer plate, set Analysis to Standard Curve
  8. Select Quantification-SYBR\_with\_Melt

**Run Method**

Set these reaction parameters

Run the reaction:

**Hold**

50oC 2 min

95oC 2 min

**PCR**

40 cycles

95oC 15 sec

59oC 1 min (lowest Tm -5, Thermo Reference suggests 15s)

72oC 1 min

**Melt Curve (default is fine)**

Continuous

95oC 1s

60oC 20s

95oC 1s

**Plate Setup**

Set Passive Reference to none

For each well used in the plate

Enter Sample will be the identifier, ie OreR10ng

Enter Assay will be the primer

Select Reporter is SYBR

Select Quencher is none

Save assay to flash drive

**Set up reagents**

* 1. Dilute the primers to 10μM in water. This should be a 1:10 dilution from the original 100uM stocks.
  2. Reaction composition (using 10ul per well)

|  |  |
| --- | --- |
| PowerUp SYBR Green Master Mix (2x) | 5μl |
| Forward primer (10μM; 300-800ng total) | 0.5μl |
| Reverse primer (10μM; 300-800ng total) | 0.5μl |
| cDNA template dilution | 4μl |
| Water | 0μl |

* 1. Make a master mix for each primer pair.
     + have 36 wells for each primer (rounding to 38 )

|  |  |
| --- | --- |
| PowerUp SYBR Green Master Mix (2x) | 190μl |
| Forward primer (10μM; 300-800ng total) | 19μl |
| Reverse primer (10μM; 300-800ng total) | 19μl |
| Water | 0μl |

C1 has both primers

C12 and F12 are empty

**Load plate**

* 1. Aliquot 6μl master mix per well
  2. Add 4μl cDNA dilution per well
  3. Mix by pipetting
  4. Remove bubbles by pipetting as best as you can
  5. Cover with adhesive plate cover
  6. Centrifuge plate to remove ALL bubbles
  7. Run plate using assay on flash drive
  8. Save the file to a flash drive, put result file in google Wharton/qPCR folder using this label format yymmdd\_name\_experiment title\_results.eds