**Description:**

How to run PCR1 on outside samples to get them ready for NGS analysis.

**Materials:**

1. Quick extract
2. 96/384 well blue plates
3. MyTaq, SuperFii II, QuantaBio, GC enhancer
4. CAGE primers
5. Sterile Water
6. Eppendorf tubes
7. Thermocyclers
8. Gel materials

**Methods:**

1. Run the corresponding program in the thermocycler depending on which Taq you used.
   1. If you used MyTaq use 1st round NGS or MyTaq PCR1.
   2. If you used SuperFii II use SuperFii II PCR1.
   3. If you used QuantaBio 3STEP.
2. Plan out your plates – try to consolidate outside samples as efficiently as possible, while still leaving them in a format similar to what was submitted so that analyzing the data is still easy.
   1. Remember to always have a negative water control as the last sample well.
   2. Label your plates with your initials and #. Ex: EA1, EA2, EA3
   3. Email your plate map to PCR1 analysis team by Wednesday afternoon.
3. Be sure to make/run a gel to check the bands and make sure the PCR worked.
   1. For each sample order run the first 12 wells on a gel.
   2. If less than 12 samples in that order, run all.
   3. Send along with the plate map to the PCR1 analysis team by Wednesday afternoon.
4. Finishing up
   1. Make sure to put a NGS magnet on each thermocycler that contains your sample plates.
   2. If sample is done in thermocycler move it to the top shelf of the NGS freezer (different then where you got the submitted sample plates).
   3. Go into SRM and finish out all your samples in PCR1 and make sure they were moved over to PCR2 (before 9AM on Tuesdays).
   4. Please mark the clear gdna plate with corresponding blue, red, or black dot for the week when you are finished.
5. Reaction Cheat Sheet

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **10 uL rxn** | **None** |  |  | **5%DMSO** | |  | **10%DMSO** | |  | **SF w G/C** | |  |  |  |  |  |  |
|  | 1x | 5x | 10x | 1x | 5x | 10x | 1x | 5x | 10x | 1x | 5x | 10x |  |  |  |  |  |
| dH20 | 3.9 | 19.5 | 39 | 3.4 | 17 | 34 | 2.9 | 14.5 | 29 | 1.9 | 9.5 | 19 |  |  |  |  |  |
| Taq | 5 | 25 | 50 | 5 | 25 | 50 | 5 | 25 | 50 | 5 | 25 | 50 |  |  |  |  |  |
| F | 0.05 | 0.25 | 0.5 | 0.05 | 0.25 | 0.5 | 0.05 | 0.25 | 0.5 | 0.05 | 0.25 | 0.5 |  |  |  |  |  |
| R | 0.05 | 0.25 | 0.5 | 0.05 | 0.25 | 0.5 | 0.05 | 0.25 | 0.5 | 0.05 | 0.25 | 0.5 |  |  |  |  |  |
| DMSO/GCE | 0 | 0 | 0 | 0.5 | 2.5 | 5 | 1 | 5 | 10 | 2 | 10 | 20 |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **20 uL rxn** | **None** |  |  | **5%DMSO** | |  | **10%DMSO** | |  | **SF w G/C** | |  |  |  |  |  |  |
|  | 1x | 5x | 10x | 1x | 5x | 10x | 1x | 5x | 10x | 1x | 5x | 10x |  |  |  |  |  |
| dH20 | 7.8 | 39 | 78 | 6.8 | 34 | 68 | 5.8 | 29 | 58 | 3.8 | 19 | 38 |  |  |  |  |  |
| Taq | 10 | 50 | 100 | 10 | 50 | 100 | 10 | 50 | 100 | 10 | 50 | 100 |  |  |  |  |  |
| F | 0.1 | 0.5 | 1 | 0.1 | 0.5 | 1 | 0.1 | 0.5 | 1 | 0.1 | 0.5 | 1 |  |  |  |  |  |
| R | 0.1 | 0.5 | 1 | 0.1 | 0.5 | 1 | 0.1 | 0.5 | 1 | 0.1 | 0.5 | 1 |  |  |  |  |  |
| DMSO/GCE | 0 | 0 | 0 | 1 | 5 | 10 | 2 | 10 | 20 | 4 | 20 | 40 |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **10 uL rxn** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1x | 5x | 10x | 15x | 20x | 25x | 30x | 50x | 75x | 100x | 150x | 200x | 250x | 375x | 500x | 750x | 1000x |
| dH20 | 3.9 | 19.5 | 39 | 58.5 | 78 | 97.5 | 117 | 195 | 292.5 | 390 | 585 | 780 | 975 | 1462.5 | 1950 | 2925 | 3900 |
| Taq | 5 | 25 | 50 | 75 | 100 | 125 | 150 | 250 | 375 | 500 | 750 | 1000 | 1250 | 1875 | 2500 | 3750 | 5000 |
| F | 0.05 | 0.25 | 0.5 | 0.75 | 1 | 1.25 | 1.5 | 2.5 | 3.75 | 5 | 7.5 | 10 | 12.5 | 18.75 | 25 | 37.5 | 50 |
| R | 0.05 | 0.25 | 0.5 | 0.75 | 1 | 1.25 | 1.5 | 2.5 | 3.75 | 5 | 7.5 | 10 | 12.5 | 18.75 | 25 | 37.5 | 50 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **20 uL rxn** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1x | 5x | 10x | 15x | 20x | 25x | 30x | 50x | 75x | 100x | 150x | 200x | 250x | 375x | 500x | 750x | 1000x |
| dH20 | 7.8 | 39 | 78 | 117 | 156 | 195 | 234 | 390 | 585 | 780 | 1170 | 1560 | 1950 | 2925 | 3900 | 5850 | 7800 |
| Taq | 10 | 50 | 100 | 150 | 200 | 250 | 300 | 500 | 750 | 1000 | 1500 | 2000 | 2500 | 3750 | 5000 | 7500 | 10000 |
| F | 0.1 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 5 | 7.5 | 10 | 15 | 20 | 25 | 37.5 | 50 | 75 | 100 |
| R | 0.1 | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 5 | 7.5 | 10 | 15 | 20 | 25 | 37.5 | 50 | 75 | 100 |

**Mytaq thermocycler conditions**

|  |  |  |
| --- | --- | --- |
| **Temp.** | **Time** |  |
| 95 | 1min |  |
| 95 | 15s |  |
| 56 | 15s | 35 cycles |
| 72 | 40s |  |
| 72 | 2min |  |
| 4 | ∞ |  |

**SuperFi II thermocycler conditions**

|  |  |  |
| --- | --- | --- |
| **Temp.** | **Time** |  |
| 98 | 2min |  |
| 98 | 15s |  |
| 60 | 15s | 35 cycles |
| 72 | 35s |  |
| 72 | 5min |  |
| 4 | ∞ |  |

**QuantaBio thermocycler conditions**

|  |  |  |
| --- | --- | --- |
| **Temp.** | **Time** |  |
| 98 | 10s |  |
| 98 | 10s | 35 cycles |
| 60 | 5s |  |
| 68 | 1s |  |
| 68 | 5min |  |
| 4 | ∞ |  |

**TROUBLESHOOTING**

1. May delete this section if necessary.
2. Otherwise, just leave helpful tips or common problems you run into with the experiment.