Sampling Protocol

Samples are going to be taken every 3 hours for 24 hrs.

Samples needed:

* qPCR (x2)
* pH
* Dilution series for plate counts

For every sampling event:

For each flask aliquot:

* 1.5 ml in tube for genomic qPCR
* 1.5 ml in tube for gene qPCR
* 1 ml in purple top conical vial for pH
* 0.5 ml for dilution series (not the control flasks but the 4 inoculated flasks) (4 dilutions total)

PROTOCOL:

* Take pH of each beaker first (upstairs with the pH probe)
* Make sure to rinse the probe well and wipe down with bleach
* Wipe down your work area with bleach to keep risk of contamination low
* Spin down the 2 microcentrifuge tubes for 3 min at 8000 rpm (?) to form pellet.
* Take off supernatant and place in freezer box at -80C. One box for genomic and one box for RNA
* Place the boxes back in the -80 and make sure to keep the tubes in order please!
* Start your dilution series. Well go to 10^-8 for most samples (less in the beginning and probably more in the end)
* Take 3 T1N2 plates from the clean room and label them with the dilutions you’ll plate. Again, this will vary – less dilute in the beginning and more dilute in the end.

Sampling Log

|  |  |
| --- | --- |
| **Time:** | **Initials:** |
| T0 |  |
| T1 |  |
| T2 |  |
| T3 |  |
| T4 |  |
| T5 |  |
| T6 |  |
| T7 |  |
| T8 |  |

PH LOG:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Time:** | **Initials:** | **1** | **2** | **3** | **4** | **5** | **6** |
| T0 |  |  |  |  |  |  |  |
| T1 |  |  |  |  |  |  |  |
| T2 |  |  |  |  |  |  |  |
| T3 |  |  |  |  |  |  |  |
| T4 |  |  |  |  |  |  |  |
| T5 |  |  |  |  |  |  |  |
| T6 |  |  |  |  |  |  |  |
| T7 |  |  |  |  |  |  |  |
| T8 |  |  |  |  |  |  |  |