# Relative host-phage diversity: experiment 2

Had a meeting with Edze this afternoon (14/10/19) to discuss data from experiment 1.

Three suggestions:

* Include a 48-clone treatment
  + To look for a point of diversity at which host-only and host-x-phage diversity have the same effects on phage titre etc
* Extend the experiment beyond 3 dpi
  + To look for how long phage and CRISPR are stable for under these conditions
  + Possibly extend until phage are extinct or 7 dpi
* Include the host-only treatments as well
  + A reviewer could argue that because the experiments were conducted at different times, there is potentially unknown variation that could explain the differences
  + Would also need to include a host-only 48-clone treatment anyway

The experiment would look like this:

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **Total reps** | **Reps with labelled clone** | **Reps without labelled clone** |
| 3 | 16 | 8 | 8 |
| 6 | 16 | 8 | 8 |
| 12 | 16 | 8 | 8 |
| 24 | 16 | 8 | 8 |
| 48 | 16 | 8 | 8 |
| *Total* | *80* | *40* | *40* |

16 replicates in total because 48/3 = 16

For all 80 replicates, would be able to track phage titre and CRISPR vs. SM selection rate

For 40 replicates, would be able to track labelled BIM vs CRISPR selection rate

This does make the experiment quite large:

|  |  |  |  |
| --- | --- | --- | --- |
| **Per day** | | | |
| **Glass vials** | 80 | **LB hard (ml)** | 1450 |
| **8x3 racks** | 3.33 | **LB soft (ml)** | 150 |
| **M9m (ml)** | 480 | **96 WPs** | 26.66 |
| **Square plates** | 10 | **M9 salts (ml)** | 460.8 |
| **Circle plates** | 80 | **Glycerol** | 4800 |

However this is only about as large as the ecology letters experiment, so on the limit of what is manageable for me each day. Further, I could always simply freeze down the culture samples and assay them at a later time, and focus on the phage while the experiment is running.

One issue is finding the time to do the experiment. Currently, I’m limited by:

* Tattoo appointments (18/10 & 15/11), which means the week is risky for lab work
* Conferences (28-29/10 and 1/11)
* Visit to Oxford (8-10/11)
* Moving house (wb 11/11)

Consequently, as far as I can tell I would need at least a 12 day window in which to complete this experiment, assuming that a) it lasts for 7 days and b) that I have to wait 48hrs for the blue colour to come out and c) includes a couple of days to get things ready.

In the meantime, probably best to get on with other tasks, such as:

* writing an introduction and methods
* writing code
* setting up dataframes
* designing downstream analyses
* updating registration
* making sure stocks and plates are ready
* getting plates etc poured and ready
* ordering materials (x-gal etc)
* could make an M9m freezer stock of the library to save time later?
* Work on thesis
* Probably reviewer comments from Ecol Lett to work on
* Additional writing
* Also personal stuff like moving house and also enjoying my life

So I think the earliest I can start it is the 22/23rd Nov, which is a week after my tattoo sitting. This gives 18 days until I need to go to Notts. The latest I could then start things is the 1/12.

We also discussed downstream analyses:

* Titres of each phage genotype
* Look at the evenness of the host population by picking (24?) CRISPR clones from each replicate at particular timepoints, then challenging them against the phage library. Could find a way of integrating the existing SM data into this?
* Time-shift assays
* Phage evolution assays
  + Could use the same phage isolates as the time-shift