**Virulence Index Assay - repeat**

1. Inocula culture preparation
   1. Streak out colonies of the 2 strains on LB or DSM plate. Grow overnight at 37°C.
   2. Distribute 1.2 ml DSM+Cm into each of 12 green microtubes.
   3. Pick 5 colonies of each strain and place each colony in a separate DSM tube. 2 tubes are left as no culture controls. (4 colonies will be used for the experiment. 5 are picked in case there are problems with any of the colonies.)
   4. Close tubes tightly and incubate overnight at 37°C without shaking.
   5. Set WT culture for lawn in plaque assay.
2. Growth to late exponential

We would like to grow the cultures to OD600 ~ 1. To facilitate OD measurements, we will track OD by measuring 200μl samples in the Synergy plate reader. We will aim for an OD value of 0.5 in that reader.

* 1. Transfer 200µl of each tube to a well of 96-well plate
  2. Measure OD600 of overnight oxygen-limited culture (200μl) in Synergy plate reader.
  3. Choose 4 colonies of each strain to proceed with. Avoid outliers if possible.
  4. Subculture: From each of the selected cultures transfer 200μl into 10 ml DSM in 50ml flask. Adjust inoculation volume if there are great discrepancies in OD.
  5. Incubate in 37°C, 200RPM and monitor growth by plate reader.
  6. Stop incubation at OD ~ 0.5.

1. Make phage dilutions (10-3 – 10-10) in DSM (150μl => 1350μl)
2. Plate setup (see layout below)
   1. Distribute cultures into wells or DSM into blank wells (100µl/well)
   2. Add to wells 100µl of phage dilution or DSM into no-phage columns.
   3. Read OD600 in synergy 2 (37°C, 1200RPM, every 2min, 10hr)
3. Titer hosts and phages
   1. Plaque assay: use leftovers from phage dilutions to do a plaque assay with WT host lawn. Plate phage dilutions 10-7 – 10-10. Make replicates.
   2. Make serial dilutions of host cultures used to setup plate and spread plate 100μl of 10-5 and 10-6 dilutions onto LB plates.

To facilitate serial dilutions of multiple cultures I make such dilutions in a 96-well plate using multi-channel pipettes:

* Distribute 135μl PBS into wells of plate.
* Transfer 15μl into first well to make 10-1 dilution.
* Mix using plate mixer at 1200RPM for ~5 seconds
* For each of the following dilutions use multi-channel to transfer 15μl of previous well to new wells with PBS and repeat mixing as above.

**Plate layout**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | SPO1 dilution |
| **A** | Blank | 10-3 | 10-3 | 10-3 | 10-3 | COL1 | COL1 | 10-3 | 10-3 | 10-3 | 10-3 | Blank | 100 |
| **B** | Blank | 10-4 | 10-4 | 10-4 | 10-4 | COL2 | COL2 | 10-4 | 10-4 | 10-4 | 10-4 | Blank | 10-1 |
| **C** | COL1 | 10-5 | 10-5 | 10-5 | 10-5 | COL3 | COL3 | 10-5 | 10-5 | 10-5 | 10-5 | COL1 | 10-2 |
| **D** | COL2 | 10-6 | 10-6 | 10-6 | 10-6 | COL4 | COL4 | 10-6 | 10-6 | 10-6 | 10-6 | COL2 | 10-3 |
| **E** | COL3 | 10-7 | 10-7 | 10-7 | 10-7 | COL1 | COL1 | 10-7 | 10-7 | 10-7 | 10-7 | COL3 | 10-4 |
| **F** | COL4 | 10-8 | 10-8 | 10-8 | 10-8 | COL2 | COL2 | 10-8 | 10-8 | 10-8 | 10-8 | COL4 | 10-5 |
| **G** | Blank | 10-9 | 10-9 | 10-9 | 10-9 | COL3 | COL3 | 10-9 | 10-9 | 10-9 | 10-9 | Blank | 10-6 |
| **H** | Blank | 10-10 | 10-10 | 10-10 | 10-10 | COL4 | COL4 | 10-10 | 10-10 | 10-10 | 10-10 | Blank | 10-7 |
| **host** | WT | WT | WT | WT | WT | WT | spoIIE | spoIIE | spoIIE | spoIIE | spoIIE | spoIIE |  |
| **colony** | All | COL1 | COL2 | COL3 | COL4 | All | All | COL1 | COL2 | COL3 | COL4 | All |  |
| **phage** | no phage | SPO1 | SPO1 | SPO1 | SPO1 | no phage | no phage | SPO1 | SPO1 | SPO1 | SPO1 | no phage |  |