**Growth curves for Δ6 (WT) and Δ6ΔspoIIE (ermS)**

We received a *B. subtilis* 168ΔspoIIE::erm strain from the BGSC (BKE00640). Using SPP1 transduction the ΔspoIIE:erm locus was tansfereed to the *B. subtilis* 168Δ6 strain to give Δ6ΔspoIIE (ermR). By expressing a cre-recombinase from plasmid pDR244 the erm resistance gene was removed from this strain and using the plasmid temperature-sensitivity the strain was then cured from the plasmid. This yielded a Δ6ΔspoIIE (ermS), a Δ6 derivative lacking spoIIE which does not carry an additional antibiotic resistance gene compared to its ancestor. The purpose of removing the antibiotic resistance gene is to eliminate the fitness the strain remove the fitness cost associated with experession of that gene, so as to make fitness of ΔspoIIE and WT equal. We note that both Δ6 and Δ6ΔspoIIE (ermS) have a resistance to chloramphenicol.

The goal of this protocol is to test if the Δ6 (WT) and Δ6ΔspoIIE (ermS) indeed have similar fitness. As a first test we will compare the growth curves of these strains. In this run growth will be measured in both LB and DSM media. Cultures will be set in wells of 96-well plate for incubation and OD measurement in the Synergy plate reader.

**Protocol**

1. Inocula culture preparation
   1. Streak out colonies of the 2 strains on LB+Cm 5µg/ml. Grow overnight at 37°C.
   2. Distribute 1.2 ml DSM+Cm 5µg/ml into each of 14 green microtubes.
   3. Distribute 1.2 ml LB+Cm 5µg/ml into each of 14 green microtubes.
   4. Pick 6 colonies of each strain and place it in a separate DSM tube. 2 tubes are left as no culture controls. (5 colonies will be used for the experiment. 6 are picked in case there are problems with any of the colonies.)
   5. Close tubes tightly and incubate overnight at 37°C without shaking.
2. Inocula culture dilutions
   1. Transfer 200µl of each tube to a well of 96-well plate
   2. Measure OD600 in Synergy plate reader.
   3. Choose 5 colonies of each strain to proceed with. Avoid outliers if possible.
   4. Distribute 1 ml DSM+Cm 5µg/ml into each of 11 green microtubes.
   5. Distribute 1 ml LB+Cm 5µg/ml into each of 11 green microtubes.
   6. Dilute innocula cultures to OD600=0.05: remove (50/OD) µl from 1ml tube and replenish to 1ml by adding same volume of overnight culture.
3. Plate setup
   1. In a 96-well plate distribute 200µl/well of diluted cultures according to layout below. Each colony is distributed into 3 replicate wells:
   2. Read OD600 in synergy 2 (37°C, 1200RPM, every 2min, 16hr)

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | LB | LB | LB | LB | LB | LB | DSM | DSM | DSM | DSM | DSM | DSM |
| **B** | LB | WT | A |  |  |  | WT | A |  |  |  | DSM |
| **C** | LB | WT | B |  |  |  | WT | B |  |  |  | DSM |
| **D** | LB | WT | C |  |  |  | WT | C |  |  |  | DSM |
| **E** | LB | ΔspoIIE | A |  |  |  | ΔspoIIE | A |  |  |  | DSM |
| **F** | LB | ΔspoIIE | B |  |  |  | ΔspoIIE | B |  |  |  | DSM |
| **G** | LB | ΔspoIIE | C |  |  |  | ΔspoIIE | C |  |  |  | DSM |
| **H** | LB | LB | LB | LB | LB | LB | DSM | DSM | DSM | DSM | DSM | DSM |
|  | LB | | | | | | DSM | | | | | |