**Bioscreen Experiment Protocol**

**Day 0:**

* Design your experiment:
  + Identify strains & growth media.
  + Determine the number of biological and technical replicates and how many wells needed.
  + Design the plate layout.

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| Notes/observations: |

**Day 0-1:**

* Streak the strains needed onto rich media plates.
  + Note: if you have plates less than 1 month old, you can use those.
* Put plates in Ziploc bag and incubate at 42C until colonies are visible (~5-6 days)

Sketch your streak pattern:

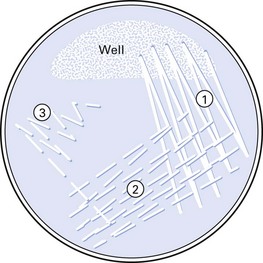
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Figure 1: streaking to single colonies. Source: reddit.com

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| Notes/observations: |

**Day 6:**

* Remove plates from incubator, check for single colonies.
* Label glass test tubes with strain and biological replicate info
* Add 5mL preculture media to labeled glass test tubes.
* Using a pipette tip or toothpick, pick a small amount of single colony and inoculate it in to the appropriate tube.
  + You may leave the tip or tooth pick in the tube, so long as you are consistent with all cultures
* Place tubes in 42C incubator for 24-48hrs

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| Notes/observations: |

**Day 7-8** (Bioscreen day!):

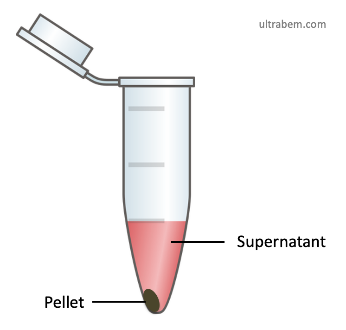
Plate set up, can be done several hours before:

* make media with 2x the amount of glucose (HvCa+ura+50mM glucose, galactose, glycerol, & xylose).
* Print out your plate layout if desired (recommended)
* Add 100ul of 2x media to the appropriate bioscreen well
* 100ul of plain HvCa to blank wells
* Cover plates and carefully set aside

Measure preculture OD:

* Set up spectrophotometer cuvettes (1 blank + 2x # of cultures). Typically, a 1:50 dilution is appropriate (980ul:20ul).
* Remove preculture tubes from incubator.
* Add 20ul culture to each preset up spec cuvette. Vortex briefly before measure optical density. **Record OD in table below.**

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| Tube/strain/biorep | ODspec | Dilution factor (df) | ODtube (ODspec \* df) |
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Wash cultures:

* Add 1mL of culture to a labeled 2mL tube.
* Collect cells in centrifuge at 3000g for 3 minutes
* Remove supernatant. Resuspend cells with 1mL HvCa media, gently pipetting up and down with a p1000.
* Repeat wash step once more. Resuspend pellet in 400ul HvCa.
* Measure OD of washed cells, using remaining set up spectrophotometer cuvettes. **Record OD in table below.**

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| Tube/strain/biorep | ODspec | Dilution factor (df) | ODtube  (ODspec \* df) | ul needed to dilute to 0.1 OD in 1.8mL |
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Dilute washed cells:

* Label new set of 2mL tubes and add 1.8mL HvCa to each.
* Use dilution formula (C1V1 = C2V2) to calculate to amount of each washed culture needed for an OD of 0.1 in the new tubes. **Record in table above.**
* For each tube, remove the calculated amount of HvCa. Then add the same volume of washed cells.
  + Note: remember to vortex the washed cells before pipetting.
* Repeat for all cultures.

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| Notes/observations: |

Finishing plate set up:

* Using the plate layout, add 100ul of diluted cells to the appropriate wells
* Cover plates and prepare to load the bioscreen machine.

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| Notes/observations: |

Starting a bioscreen run:

* Watch the demo video on how to start an experiment.

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| Observations/reflections/questions about the overall protocol: |

**Bioscreen Experiment Worksheet**

Congrats! You’ve set up your first high-throughput growth assay using a Bioscreen C machine. Work though the following questions to check your understanding of the experimental design, set up process, and to begin thinking about how you might analyze data from this experiment. You are welcome to talk through these questions with a partner or group, but please write you answers using your own words.

1. What is the starting OD of the bioscreen experiment? What is the glucose concentration?
2. Define biological replicates. Define technical replicates. What are each designed to capture, and why is it important to have both?
3. State whether each cause of experimental variation is best categorized as biological or technical. If you feel strongly that it could be both, justify your response.

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| Cause of variation | Type of variation |
| Some cells have accumulated unique mutations over time |  |
| Machine temperature varies slightly across each plate |  |
| Optical density of the precultures are different from each other |  |
| Precultures are in different growth stages |  |
| Some wells are inoculated with slightly more cells than others |  |

1. What is the purpose of randomizing the samples in the plate?
2. What is the purpose of putting blank media in the outer/border wells?
3. What hypothesis is this experiment testing?
4. How does this experiment test the hypothesis?
5. For the trmB experiment, why did we use plates and preculture media that had supplemental glucose? *(hint: selection)*
6. Why was it important that we washed the cells and diluted them before adding them to the bioscreen plate?
7. Based on the hypothesis, previously collected data on the function of TrmB in *Hbt. salinarum*, what do you predict the growth curves with and without glucose will look like? Typically, growth curves are plotted with time (the independent variable) on the x-axis and OD (the dependent variable) on the y-axis.
8. Brainstorm and sketch some other graphs you might want to create in order to evaluate the experiment results? Are they any questions you might ask to check that the data is high quality before analyzing it? *Use the back of this page if you run out of space.*

**Bonus section:**

1. In addition to glucose, we also tested the effects of galactose, xylose, and glycerol on Haloferax volcanii growth. The metabolism, or regulation of metabolism, for each of these compounds has been previously published in this system. Use NCBI/PubMed to search and fine at least one relevant article.
2. Johnson et. al. investigated the metabolism of xylose in Hfx. volcanii in 2009[[1]](#footnote-1). Figure 4A specifically shows the difference between WT Hfx. volcanii growth with glucose or with xylose. Can you create a similar figure using WT growth data from this experiment? Is it consistent? If not, what might be some causes?

1. Johnsen U, Dambeck M, Zaiss H, et al. D-xylose degradation pathway in the halophilic archaeon Haloferax volcanii. J Biol Chem. 2009;284(40):27290-27303. doi:10.1074/jbc.M109.003814 [↑](#footnote-ref-1)