**Measuring growth rates by serial dilution and plating**

Up to now we have Scrambled the synthetic yeast chromosomes and selected for our phenotype of interest. We selected this phenotype based on the size of colonies on the initial selection plates (high temperature, ethanol, or caffeine). Now we need to confirm that these larger colonies really do grow better on the selective conditions. We will do this by:

1. Selecting 5 colonies that you believe show increased growth in the selective conditions
2. Transferring an equal number of cells from each of the 5 colonies to one well of a cell culture dish. You also transfer cells that have not been Scrambled into one well of the cell culture dish.
3. Performing serial dilutions of those cells. This means that in each subsequent well of the cell culture dish, you will have progressively fewer cells
4. Transfer the diluted cells from each well onto a Petri dish containing media with the selective condition
5. Incubate the plates for 2 days and then assess the growth of the diluted cells (both the size and the number of colonies) relative to the non-Scrambled strains.

An example of serial dilutions of yeast cells to compare growth rate on selective conditions (size and number of cells)

**Procedure**

1. Obtain your yeast cells on the selective plate from last week. Also obtain a plate with non-scrambled cells.
2. Obtain a new 96-well plate. When working with the plate, keep the lid on as much as possible; remove the lid to add or pipette the yeast cells, but put the lid right back on when you’re done.
3. Into well A1 of the plate, add 100 ul of nonscrambled cells.
4. Transfer 100 ul of sterile water to wells A2-A6 of a 96-well plate.
5. Using a sterile toothpick, select one of the largest colonies from the scrambled cells on the selective plate. Swirl the toothpick into the water in well A2 in the 96-well plate to disperse all the yeast cells into the liquid.
6. Repeat step 5 for 4 more colonies, resuspending each in a new well (wells A3-A6).
7. Determine the OD of the cells in each well by taking a reading on the NanoDrop at an absorbance of 600 nm. Record in the table below.
8. For each sample, calculate the volume of cells needed for an OD of 0.5 using the equation below

(0.5 OD) = (OD measured from plate reader)(Volume of cells needed).

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Well | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Optical density |  |  |  |  |  |  |  |  |  |  |  |  |
| Volume of cells needed (calculated above) |  |  |  |  |  |  |  |  |  |  |  |  |
| Volume of water needed to achieve a total volume of 50 ul |  |  |  |  |  |  |  |  |  |  |  |  |

1. Obtain a new 96-well plate. Into the first row, transfer the volume of cells calculated above for each well (ie, you are transferring the calculate volume from well A1 on plate 1 to well A1 on plate 2, etc.)
2. To these cells, add the volume of water calculated above to make a total volume of 50 ul.
3. Into each of the other wells in the dish, add 80 ul of water.
4. Using a P20 pipette set to 20 ul, pipette up and down 3-4X in well A1 and then transfer 20 ul of cells to the row beneath it (well B1). Pipette up and down 3-4X in well B1 and transfer 20 ul to well C1. Repeat all the way down to row H1.
5. Repeat step 12 for each column of cells (ie, transferring from A2 to B2 to C2…..)
6. Obtain 2 rectangular petri dishes with media. One should have rich media (YPD) and the other should have your media with your selective condition. Label the back of these plates with your initials and dates.
7. You should now have: your 96-well plate with diluted cells, your YPD plate, and your selective plate. Take 2 ul from the first well (A1) of diluted cells and pipette the liquid onto the corresponding area of the YPD plate (A1). Take another 2 ul from the first well (A1) of diluted cells and pipette the liquid onto the corresponding area of the selective plate (A1).
8. Repeat step 16 for all wells of the 96-well plate.
9. Incubate the cells at 30C (or 40C if that is your selective condition) for 2 days.