

Growth Assay Protocol

Day 1-

1. Prepare overnight cultures of all strains to be tested
2. Determine that LB, sodium lactate and synthetic river media are available and sterilized/autoclaved.

Day 2-

1. Create desired sodium lactate solutions
2. Place cells on ice for a short period of time to stop growth
3. Depending on OD₆₀₀ of the cultures, determine the amount of culture to spin down and the amount of Synthetic River Media to resuspend them in to have a cell density of 10,000,000 cells/well, with a final volume of 150 uL/well.
4. Spin down the cell culture at 4000g for 20 minutes at 4 degrees Celsius.
5. Meanwhile, turn on the plate reader and set it to the desired temperature.
6. Resuspend in a small volume of Synthetic River Media and spin again.
7. During this spin, set the program labeled "Shortcut to spf" which is the SoftMax Pro program to take OD595 and set up groups and group blanks.
8. Input correct volume of resuspended cells (about 149 uL) to 96 well plate and add 1 uL of the correct additional food to each one, plus LB controls for each strain used.
9. Insert plate into plate reader and let it take data, every five minutes for 20 or more hours, with a 30 second mix before each read.

Day 3 (or later Day 2)-

1. Perform any desired data analysis using the software.

Alternatively, to use other programs for data analysis,

1. Export data from plate reader as a text file.
2. Copy all from the text file and move it to Microsoft Excel
3. Remove all information besides the data from the wells themselves, making sure that each plate read is in a 96 well chunk.
4. Copy the results only, and paste back into a text document
5. Move the folder to your MATLAB directory, and use the file growthanalysis.m: when it asks you for your input, you must put in the text document name, including file extension, as a string. For example, if you named your file growth, then you'd input 'growth.txt'. This file can be found on DropBox and will convert each well's data into a single vector, A1 through H12, which can be analyzed in MATLAB or copied from the variable editor into Excel.

Desired data may include max OD (or max cells), time of max OD, doubling time, initial growth rate after lag phase, etc.