Bacterial (Worm Food) Growth Protocol for HTA

PART 1: Bacterial Growth Curves - Determination of Mid-log Phase Time-point for each Strain.

1. Streak out one bacterial strain from a frozen stock to a fresh LB plate.
2. Grow overnight at 37ºC (or temperature appropriate for bacteria). Keep streak plates at 4ºC for no longer than a week.
3. Inoculate a 5 mL Superbroth (recipe below) starter culture with a single colony and grow overnight 12-18 hours, shaking at 200-250 rpm and at 37ºC (or temperature appropriate for bacteria).
4. The next day, determine the OD_{600} of the overnight culture by diluting to the linear range of the spectrophotometer (make dilutions of 1:10, 1:50, 1:100; take the average value). Add 1 ml samples into the disposable cuvettes and use the Morimoto Lab spec. Don't forget to use the same broth (LB or Superbroth) for blank and dilutions!
5. Inoculate 250 mL of Superbroth (U.S. Biological Brand or Andersen Lab Recipe below) in 1 L flask at OD_{600} 0.001 to 0.005.
6. Shake at 200 rpm at 37ºC (or temperature appropriate for bacteria).
7. Take OD_{600} readings at time points every hour until stationary phase (could be up to 12 hours). Dilute samples to the linear range of spec. as needed. (NOTE: If the growth time exceeds one day to reach stationary phase, then repeat the experiment for the same bacteria: start a second culture later in the day and grow overnight. Obtain the OD at timepoints the next morning to plot the late log and stationary phases).
8. Plot logOD vs. time to identify mid-log phase time for each strain (usually between OD 0.6 - 0.9).

PART 2: Preparation of Bacteria Strain Freezer Stocks for Worm Feeding Assays

1. Using the same fresh bacteria streak plate prepared in Part 1, inoculate a 5 mL Superbroth starter culture with a single colony and grow overnight 12-18 hours, shaking at 200-250 rpm.
2. The next day, determine the OD_{600} of the overnight culture by diluting to the linear range of the spectrophotometer (make dilutions of 1:10, 1:50, 1:100; take the average value). Add 1 ml samples into the disposable cuvettes and use the Morimoto Lab spec. Use the same Superbroth stock for blank and dilutions.
3. Inoculate 250 mL of Superbroth (U.S. Biological Brand or Andersen Lab Recipe below) in 1 L flask at OD_{600} = 0.001 to 0.005. If needed, to scale up, use 1 L of Superbroth in a 4 L flask. (NOTE: Use the same batch of Superbroth for Growth Curves and Stock Prep for the same Bacteria Strain to ensure reproducibility of growth time).
4. Shake at 200 rpm at 37ºC (or temperature appropriate for bacteria).
5. Grow for X hours; X is determined by the time that the strain reaches mid-log phase (usually OD_{600} = 0.7 - 0.8). Determine the OD_{600} at hours 1.5, 2.5, 4, 5 etc. to monitor the OD_{600} for each sample. Do not overgrow bacteria!

6. Harvest cells by spinning at 3900 rpm (and 20ºC) in the 5810 clinical centrifuge for 5 minutes using sterile 750 mL bottles (NOTE: We currently have 12 bottles).

7. Decant supernatant and add 5 ml of sterile S Basal (or S Medium). Resuspend the bacteria by vortexing and transfer to a 50 ml sterile, conical tube. (NOTE: use the multi-tube/bottle vortexer to vortex 2 bottles at a time). Collect together in one tube cells of the same bacteria strain from multiple 750 ml bottle samples, as needed.

8. Add 25 ml of sterile S Basal (or S Medium) as a first wash, vortex to resuspend.

9. Spin at 3900 rpm in 5810 clinical centrifuge for 5 minutes.

10. Decant supernatant and add 5 ml of sterile S Basal (or S Medium) as a second wash. Resuspend the bacteria by vortexing.

11. Spin at 3900 rpm in 5810 clinical centrifuge for 5 minutes.

12. Add 5 ml of sterile S Basal (or S Medium), or appropriate amount to get OD_{600} = 100; vortex to resuspend.

13. Determine OD_{600} (Start with a 1:200 dilution in a cuvette) and make appropriate final dilution with S Basal (or S Medium). Final dilution should be at the OD_{600} 100, required for high-throughput assays as a 10X stock.

14. Aliquot 1 mL per pre-labeled microfuge tube. Freeze aliquots and store at -80ºC. (NOTE: Currently, one GWAS HTP assay requires 2 ml of bacteria stock at OD_{600} 100).

Recipes

ECA Superbroth (per Liter)

Reagent	Amount Needed
Bactotryptone	12 g
yeast extract	24 g
50% glycero	8 mL
dH_2O	fill to 1 L

After autoclaving for 30 minutes and cooling to under 60ºC, add phosphate buffer 250 mL of phosphate buffer.

ECA Phosphase buffer (per Liter)

Reagent	Amount Needed
KH_2PO_4	23.1 g
K_2HPO_4	125.4 g
dH_2O	fill to 1 L

Aliquot 250 ml to a 500 ml bottle and autoclave.

S Basal

1. Mix the following:

Reagent	Amount Needed
NaCl, 5 M	20 ml
Potassium Phosphate Buffer, 1 M pH 6	50 ml
Cholesterol, 5 mg/ml in ethanol	1 ml
dH_2O	930 mL

2. Autoclave on liquid cycle for 30 minutes.
    - Note that the solution will be cloudy.

S Medium

1. Make each component below according to the recipe.
2. Using sterile technique, mix the following:

Reagent	Amount Needed
5 M NaCl	40
Potassium Citrate, 1 M pH 6	20 ml
Trace Metals Solution	20 ml
$CaCl_2$, 1 M	6 ml
$MgSO_4$, 1 M	6 ml
Phosphate Buffer, 1 M pH6	20 ml
dH_2O	1886

3. Filter sterilize using a 0.22 µm filter in 499.5 ml aliquots.
4. *Using good sterile technique,* add 0.5 ml of cholesterol (5 mg/ml on EtOH) to each aliquot.