Bacterial Culture Techniques – Part IV

**Introduction:**
This protocol is for preparing a mid-log culture of *E. coli*. Cells in mid-log growth can generally be rendered more competent to uptake plasmid DNA that can cells at stationary phase. Mid-log cells are used in the classic transformation protocol that we will try later in the year. The protocol begins with an overnight suspension culture of *E. coli*. Incubation with agitation has brought the culture to stationary phase and ensures a large number of healthy cells capable of further reproduction. The object is to subculture a small volume of the overnight culture in a large volume of fresh nutrient broth. This “re-sets” the culture to zero growth, where after a short lag phase, the cells enter the log-growth phase. As a general rule, 1 volume of overnight culture (the inoculums) is added to 100 volumes of fresh LB broth in an Erlenmeyer flask. To provide good aeration for bacterial growth, the flask volume should be at least four times the total culture volume.

A shaking incubator is necessary for growing *E. coli* for competent cells. Proper aeration and nutrient exchange are essential to achieve vigorous growth; only cells collected during the middle part of log (mid-log) phase will produce competent cells with a high transformation frequency.

Timing of the culture to reach mid-log phase is likely to be affected by any change in the protocol. For example, a culture inoculated with an overnight culture that was grown without shaking will take longer to reach mid-log phase. Different strains of *E. coli* display different growth properties. Different nutrient broths also will affect the growth of the culture.

**Procedure:**

**PREPARATION OF A MID-LOG SUSPENSION CULTURE**

1. Sterilely transfer 1 mL of overnight culture into 100 mL of LB broth at *room temperature*.

2. *If using a 1 mL overnight culture:*
   a. Remove cap from overnight culture tube, and flame mouth. *Do not place cap on lab bench.*
   b. Remove foil cap from flask, and flame mouth. *Do not place cap on lab bench.*
   c. Pour entire overnight culture into a flask. Reflame mouth of flask, and replace foil cap.

   *If transferring only a portion of larger overnight culture:*
   a. Flame pipette cylinder.
   b. Remove cap from overnight culture tube, and flame mouth of tube. *Do not place cap on lab bench.*
   c. Withdraw 1 mL of overnight suspension. Reflame mouth of overnight culture tube, and replace cap.
   d. Remove foil cap from flask, and flame mouth. *Do not place cap on lab bench.*
   e. Expel overnight sample into flask. Reflame mouth of flask, and replace foil cap.

3. Incubate at 37°C with continuous shaking.

4. It can safely be safely assumed that an MM294 culture has reached OD$_{550}$ 0.3-0.5 after 2 hours, 15 minutes of incubation with continuous shaking. Note that under ideal conditions, as represented in **Figure 1**, an MM294 culture reaches an OD$_{550}$ 0.3-0.5 in 1 hour, 30 minutes. However, less ideal conditions often result in slower growth.
5. This mid-log culture can be stored on ice until ready for use for up to two hours. During this time, cell growth is temporarily arrested.

6. Take time for a responsible cleanup.
   a. Segregate for proper disposal bacterial cultures *and* tubes, pipettes, and micropipettor tips that have come into contact with the cultures.
   b. Disinfect overnight culture and pipettes and tips with 10% bleach solution, or disinfectant.
   c. Wipe down lab bench with soapy water, 10% bleach solution, or disinfectant.
   d. Wash hands (as always) before leaving lab.

7. Answer questions 1 and 2.

**FOR FURTHER RESEARCH**

8. Start a 500 mL *E. coli* culture as described in the above protocol. Determine the optical density of samples steriley withdrawn at 20-minute intervals, from time zero for as many hours as possible. Using Excel (or any spreadsheet software), construct a data table with all of your measurements and plot a graph of time *versus* OD$_{550}$. Include the answers to the following questions:
   - What is the slope of the curve at a point that corresponds to an OD$_{550}$ of 0.3?
   - Describe the growth of the culture at this point.

**FOR FURTHER, FURTHER RESEARCH**

Perform the following experiment to correlate the optical density of culture with actual number of viable *E. coli* cells. Observe sterile technique!

9. Inoculate 500 mL *(may be modified!)* of LB with 5 mL of *E. coli* overnight culture. Swirl to mix.

10. Immediately remove a 10 mL aliquot of the culture, and place on ice to arrest growth. This will represent time = 0. Then, incubate the remaining culture at 37°C with vigorous shaking.

11. Remove additional aliquots from shaking culture every 20 minutes for a total of 4 hours. Hold each aliquot on ice until ready to perform steps 12 – 15.
12. Determine the OD$_{550}$ of each aliquot.

13. Make a $10^2$ dilution by mixing $10 \mu$L of the aliquot with $990 \mu$L of fresh LB broth. Prepare three serial dilutions of each aliquot for plating in step 14:
   a. $10^4 = 10 \mu$L of $10^2$ culture + $990 \mu$L of LB
   b. $10^5 = 100 \mu$L of $10^4$ culture + $900 \mu$L of LB
   c. $10^6 = 100 \mu$L of $10^5$ culture + $900 \mu$L of LB

14. Spread $100 \mu$L of each dilution onto an LB agar plate, for a total of three plates for each time point (aliquot). *Label each plate bottom with time point and dilution.* There will be a lot of plates, so organization is crucial! Invert plates, and incubate for 15-20 hours at 37°C.

15. For each time point, select a dilution plate that has between 30 and 300 colonies. Multiply the number of colonies by the appropriate dilution factor to give cell number per milliliter in the original aliquot.

16. Using Excel, construct a data table and plot the following:
   - time (x axis) *versus* OD$_{550}$ and cell number (y axis)
   - cell number (x axis) *versus* OD$_{550}$ (y axis)

17. Take time for a responsible cleanup.
   a. Segregate for proper disposal bacterial cultures *and* tubes, pipettes, and micropipettor tips that have come into contact with the cultures.
   b. Disinfect overnight culture and pipettes and tips with 10% bleach solution, or disinfectant.
   c. Wipe down lab bench with soapy water, 10% bleach solution, or disinfectant.
   d. Wash hands (as always) before leaving lab.

18. Answer the remaining questions.
1. What variables influence the length of time for an *E. coli* culture to reach mid-log phase?

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2. What are the disadvantages of beginning a mid-log culture from a colony scraped off a plate, as opposed to inoculums of overnight culture?

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3. Using your graph, an OD$_{550}$ 0.3-0.4 corresponds to what number of cells?

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4. Indicate what is the average cell number at each of the following points:
   a) _____________ lag phase
   b) _____________ first third of log phase (early log)
   c) _____________ second third of log phase (mid log)
   d) _____________ final third of log phase (late log)
   e) _____________ stationary phase

5. Do OD$_{550}$ measurements distinguish between living and dead cells?

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