Name: _____ MOLE BIO/BIOCHEMISTRY

MOLEBIO LAB #13: Bacterial Culture Techniques – Part II

Introduction:

E. coli has simple nutritional requirements and grows slowly on "minimal" medium containing an energy source such as glucose, salts (such as NaCl and MgCl), the vitamin biotin, and the vitamin thiamine (B1). *E. coli* synthesizes all necessary vitamins and amino acids from these precursors and grows rapidly in a complete media, such as LB. Yeast extract and hydrolyzed milk protein (casein) provide a ready supply of vitamins and amino acids.

A liquid bacterial culture goes through a series of growth phases (see **Figure 1**). For approximately 30 – 60 minutes following inoculation, there is a **lag phase** during which there is limited cell growth. The bacteria begin dividing rapidly during **log phase**, when the cell number doubles every 20 – 25 minutes. As nutrients in the media are depleted, the cells nearly stop dividing and the culture enters **stationary phase**. During **death phase**, waste products accumulate and cells begin to die.

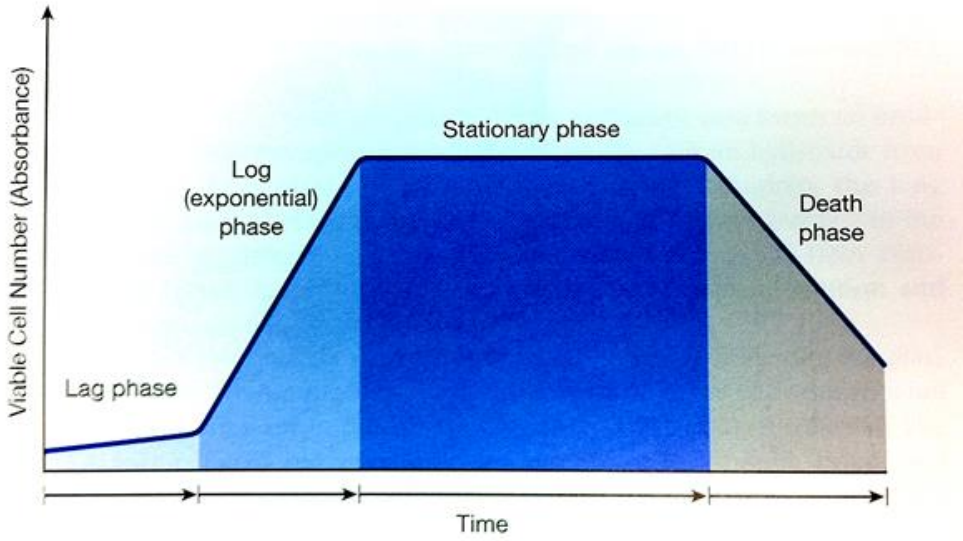

Figure 1: *Escherichia coli* Growth Curve

Optimum growth in liquid culture is achieved with continuous agitation, which aerates the cells, facilitates the exchange of nutrients, and flushes away waste products of metabolism. It can be safely assumed that a culture in complete medium has reached stationary phase following overnight incubation with continuous shaking. However, it is not absolutely essential to grow a culture with shaking. Suspensions can be incubated, without shaking, in a rack within a 37 °C incubator. These cultures will need to incubate for 1 day or more to obtain an adequate number of cells.

A stationary phase culture will look very cloudy and turbid. Discard any overnight culture where vigorous growth is not evident. Expect less growth in cultures incubated for 1 – 2 days *without continuous shaking*. To gauge growth, shake the tube to suspend cells that have settled at the bottom of the tube.

A 50 mL conical tube is preferable for growing overnight cultures. It provides greater surface area for aeration than does a 15 mL culture tube. In overnight cultures for plasmid preparations, it is best, but not essential, to maintain antibiotic selection of the transformed strain by growing in medium containing the appropriate antibiotic. It is prudent to inoculate a "back up" overnight culture, in case the first was not inoculated properly.

Procedure:

Think sterile! A pipette should be considered contaminated whenever the tip end comes into contact with anything in the environment – lab bench, hand, or clothing. When contamination is suspected, discard pipette and start with a fresh one. Plan out steps to perform, organize lab bench, and work quickly.

PREPARING LB BROTH STOCK FOR EACH GROUP

1. The following recipe is for 50 mL of media, we may adjust volume depending on class size…
    a. 0.5 g tryptone
    b. 0.25 g of yeast extract
    c. 0.5 g of NaCl (m.w. = 58.44)

    (We might use a prepared mixture of these instead…)
2. Add all ingredients to a clean 250 mL beaker that has been rinsed with deionized or distilled water.
3. Add 50 mL of deionized or distilled water to the beaker.
4. Add 0.025 mL (which is 25 μL) of 4 N NaOH.
5. Stir to dissolve dry ingredients.
6. Pour LB broth into a 125 mL Wheaton glass bottle. **LOOSELY** screw on the cap.
7. Autoclave for 15 – 20 minutes at 121 °C.

PREPARING OVERNIGHT CULTURE

NOTE: LB broth can be considered sterile as long as the solution remains clear. Cloudiness is a sign of contamination by microbes. Always swirl solution to check for bacterial or fungal cells that may have settled at the bottom of the flask or bottle.

8. Label a sterile 50 mL tube with your name and the date. (This 50 mL conical tube it provides a greater surface area for good aeration of culture than the 15 mL tube – but they are expensive.)
9. Use a 10 mL pipette to sterilely transfer 5 mL of LB broth into the tube.
    a. Attach pipette pump to pipette. Briefly flame pipette cylinder.
    b. Remove cap of LB stock bottle using little finger of hand holding pipette bulb. Flame mouth of LB bottle.
    c. Withdraw 5 mL of LB. Reflame mouth of bottle, and replace cap.
    d. Remove cap of sterile 50 mL conical tube. Briefly flame mouth of tube, and replace cap.
10. Locate a well-defined colony 1 – 4 mm in diameter on a freshly streaked plate.
11. Sterilize inoculating loop in the Bunsen burner flame until it glows red hot. Then, continue to pass lower half of its handle through the flame.
12. Cool loop tip by stabbing it several times into agar near the edge of the plate.
13. Use loop to scrape up a visible cell mass from selected colony.
14. Sterilely transfer colony into culture tube:
    a. Remove cap of the culture tube using little finger of hand holding loop.
    b. Briefly flame mouth of culture tube.
    c. Immerse loop tip in broth, and agitate to dislodge cell mass.
    d. Briefly reflame mouth of culture tube, and replace cap.

15. Reflame loop before placing it on lab bench.
16. Loosely replace cap to allow air to flow into culture. Affix a loop of tape over the cap to prevent it from becoming dislodged during shaking.
17. Incubate for 12 – 24 hours at 37 °C, preferable with continuous agitation.

    NOTE: Shaking is not essential for a culture to be used for plasmid purification. The culture can be incubated at 37 °C, without shaking, for 1 or more days.
18. 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.

Micklos, David A. Freyer, Greg A. *DNA Science*. 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2003. 331-350. Print.

1. Why is 37 °C the optimum temperature for *E. coli* growth?

2. Give two reasons why it is ideal to provide continuous shaking for a suspension culture.

3. What growth phase is reached by a suspension of *E. coli* following overnight shaking at 37°C?