

Production of RFP+ or GFP+ Bacteria – Upstream Process Protocol Day 2

Bacterial Culture Scale Up

Materials for Module 2, Lessons 3:

1. **Lysogeny Broth (LB):** The media used to grow liquid cultures of lab strains of E. coli.
2. **Lysogeny Broth + Ampicillin (LB/Amp):** Ampicillin is the antibiotic used to select bacteria that contain a plasmid that contains the gene encoding GFP or RFP as well as a gene encoding a protein that confers resistance to ampicillin.
3. **125mL sterile glass baffled flask with lid:** The scaled up bacterial culture will be grown in this container.
4. **Sterile 50mL plastic test tubes**
5. **Plastic cuvettes:** The correct container for bacterial cultures that will be read in the spectrophotometer.
6. **Micropipettes and tips:** For measuring small volumes of reagents or bacterial cultures
7. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
8. **Microfuge tube rack:** To hold microfuge tubes.
9. **Sharpie markers:** For correct labeling of samples.
10. **Upstream Process Batch Record Form:** When properly filled out, this form is a record of your team's entire upstream process.

Equipment:

11. **Shaker/Incubator:** The equipment used to shake and warm bacterial cultures for optimal growth in liquid culture.
12. **Spectrophotometer:** The equipment used to measure the optical density of bacterial cultures – for plotting bacterial growth curves.

Protocol: Day 2 (Lesson 3)

Bacterial Culture Scale Up – Bacterial scale up will be carried out by the Upstream Process Technician. The Process Engineers will check and ready the spectrophotometer for use. The QC Technician will take bacterial culture samples at time points and measure OD600.

NOTE: All team members will assist the Process Engineer, the Upstream Process Technician, and the QC Technician as needed.

NOTE: Not all class periods are long enough for bacterial growth OD600 readings to be taken as they are described in this protocol. Your instructor may modify the protocol as needed..

Before starting the protocol, turn on the spectrophotometer to give it time to warm up for the recommended 30 minutes. Your teacher may want to turn it on before the beginning of class.

Use aseptic Technique at all times!!

You are STRONGLY ENCOURAGED to check off each step below as you complete it.

1. Carefully open the sterile baffled flask by pulling the metal cap off and setting it on your sterilized lab bench.
2. The Upstream Process Technician will add 50mL of LB/Amp to a sterile 125mL glass, baffled flask.
Note: This can be done using a Pipet-Aid and sterile 10mL pipettes. If you do not have Pipet-Aids, it can be done by carefully pouring sterile 50mL LB/Amp media into a sterile 50mL tube and then carefully pouring it into the sterile 125mL baffled flask.
3. Place a tape label on the flask with your team name, date, and contents.
4. Using a p1000 carefully pipette up 500uL of your small liquid overnight culture. If this culture was stored in the refrigerator, be sure to swirl the tube to homogeneously mix the contents. Some of the bacterial will have settled out during storage.
5. Add the 500uL of your small liquid overnight culture to the 50mL of LB/Amp in the baffled flask.
6. Cap the flask and swirl gently to mix.
7. The Process Engineers from each team work together to properly blank the spectrophotometer using the 'Spectrophotometer Check SOP'. **Remember:** The spectrophotometer must warm up for 30 minutes before being used.
PLEASE NOTE: Teachers who have other types of spectrophotometers such as: 96 well plate readers, Vernier SpectroVis Plus etc, may choose to use their own equipment. Creation of an SOP for how to use alternate spectrophotometric equipment is up to the teacher.
8. The QC Technician will remove 2mL of bacterial culture and place it into a cuvette.
9. Read the cuvette at OD600. Record the data in the Upstream Process Batch Record. This reading will be your 0 time point reading.
10. Discard the bacterial solution from the cuvette into your 10% bleach beaker. Set the cuvette aside to use again.
11. Place the flask in the shaker/incubator and shake at 250rpm, 37°C.
12. After 15 minutes, remove the flask from the shaker/incubator.
13. Remove 2mL of culture and place it in the cuvette.
14. Take another reading at OD600.
15. Discard the bacterial solution from the cuvette into your 10% bleach beaker. Set the cuvette aside to use again.

16. Record your 15min time point reading in the Upstream Process Batch Record.
17. Place the flask back in the shaker/incubator and shake for another 15 min.
18. Repeat steps 24-30 at 30 min, 45 min, 60 min and 75 min time points.
19. When finished, the Process Engineer cleans the cuvettes according to the 'Spectrophotometer Check SOP'.
20. The flask will shake overnight at 250rpm, 37°C in the shaker/incubator. After the overnight incubation in the shaker/incubator, the flask can be stored in the refrigerator until the next class period.
21. Make sure you have filled out all necessary parts of the Upstream Process Batch Record.
22. Properly dispose of all waste following the guidelines in the Aseptic Technique slidedeck.