

Biomanufacturing Module 2

Lesson 3 – Bacterial Culture Scale Up

NOTE: This lesson is best done during a class period that is longer than 50 minutes.

Lesson objectives:

Students will understand:

- How to scale up a liquid bacterial culture.
- Why the bacterial culture is scaled up.

Essential Question

- Why do you scale up the bacterial culture?

Materials:

- Review bacterial growth curve information in Recombinant Protein Production in Bacteria slide deck
- Upstream Process Protocol (Day 2)
- Spectrophotometer Check SOP
- Small liquid bacterial culture created in the last class period
- LB/Amp media (1 bottle/team)
- Sterile 50mL tube (1/team)
- Sterile 125mL baffled flask (1/team)
- Shaker/incubator
- Spectrophotometer
- Cuvettes (1/team and 1 for the Process Engineers to use for blanking the spectrophotometer)
- p1000 micropipette and tips (1/team)
- Upstream Process Batch Record Document
- Bacterial Growth Curve Graphing Protocol
- Bacterial Growth Curve Graphing Spreadsheet

What Students Will Do

- Review bacterial growth curve information in the Recombinant Protein Production in Bacteria slide deck
- Each team will scale up their liquid culture to 50mL
- Each team will label their flask
- Teams place the flask containing their scaled up culture in the shaker/incubator
- Process Engineers will ready the spectrophotometer using the Spectrophotometer Check SOP.

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.

- Teams will take OD600 readings using the spectrophotometer at various time points
- Each team fills out the appropriate parts of their Upstream Process Batch Record

Teacher Preparation

- Prior to class get out the team folders which contain:
 - Upstream Process Protocol (one per team)
 - Upstream Process Batch Record Document (one per team)
 - Spectrophotometer Check SOP (one per team)
- Prior to class, turn on the bacterial shaker/incubator to a temperature of 37 degrees Celsius.
- Prior to class, turn on the spectrophotometer (needs to warm up for 30 min prior to use)
- Prior to class, secure 6 flask holders onto the platform of the shaker/incubator.
- Prior to class, remove the LB/Amp media from the refrigerator and let it warm to room temperature.
- Provide each team
 - Upstream Process - Production of RFP+ or GFP+ Bacteria Protocol
 - Spectrophotometer Check SOP
 - One sterile 50mL tube
 - One bottle of LB/Amp media
 - One p1000 micropipette
 - One box of p1000 micropipette tips
 - One 125mL sterile glass baffled flask
 - Sharpie marker and tape for labeling flasks
 - Spray bottle of 70% ethanol
 - Paper towels
 - Upstream Process Batch Record
 - Team file folders
 - A beaker half full of 10% bleach (in the sink)
 - Bacterial Growth Curve Graphing Protocol

Organizer

Time	Activity	Materials
10 minutes	Review bacterial growth curve information from the Recombinant Protein Production in Bacteria slide deck	Slide deck
5 minutes	Members of all teams put on PPE	Lab coats, gloves, safety goggles
5 minutes	Teams sanitize and prepare their bench space	70% ethanol, paper towels, sterile LB/Amp media, sterile 125mL baffled flask, micropipette, tips, small liquid bacterial culture started in previous class period

15 minutes	Each team takes their small liquid bacterial culture out of the shaker/incubator and uses it to make a scaled up 50mL culture	Upstream Process Protocol – Day 2, sterile LB/Amp media, sterile 125mL baffled flask, micropipette, tips, small liquid bacterial culture started in previous class period
10 minutes	Team Process Engineers prepare the spectrophotometer and blank it	Spectrophotometer, cuvettes, micropipette, tips, 10% bleach solution
60 minutes	Each team takes samples from their flask at specific times and measures the OD600 using the spectrophotometer	Spectrophotometer, cuvettes, micropipette, tips, 10% bleach solution
10 minutes	Teams fill out the appropriate portions of their Upstream Process Batch Record Document and file it	Upstream Process Batch Record Document, Team File Folder
30 minutes – homework or next class	Teams graph their own OD600 data and/or a provided data set to generate bacterial growth curves	Graphing a Bacterial Growth Curve Protocol, Bacterial Growth Curve Graphing Spreadsheet

Procedure

Bacterial Growth Curve Review

1. Review the slides covering bacterial growth curves in the Recombinant Protein Production in Bacteria slide deck

Scale up of the bacterial culture

2. Members of each team put on PPE
3. Each team sanitizes and organizes their bench space
4. Each team retrieves its small bacterial liquid culture from the incubator
5. Each team follows the appropriate section of the Production of RFP+ or GFP+ Bacteria Protocol to scale up their bacterial culture.
6. Each team puts their flask containing the scaled up culture in the shaker/incubator to shake at 37 degrees C for 24 hours.

Plotting a Bacterial Growth Curve

7. All Process Engineers work together using the Spectrophotometer Check SOP to prepare the spectrophotometer for use.
8. Each team takes OD600 readings with samples from their culture flask taken at assigned time points.

9. Each team records their OD600 readings in their Upstream Process Batch Record.
10. Each team plots their own data and/or a provided data set to create a bacterial growth curve graph.
11. Each team fills out the appropriate sections of the Upstream Process Batch Record and files it in their team file.

NOTE: You may want your students to test the difference in production of RFP (or GFP) between cells that are;

- induced at the time of scale-up vs.
- cells that are induced 24 hr after scale-up

If so, one team of students should induce expression of RFP in their 50mL bacterial culture at the time of scale up. Follow the Module 2, Lesson 4 protocol. The difference in expression under these two conditions can be seen in the 'RFP Induction Timeline Results' document (found in the Module 2, Lesson 4 section).