

Biomanufacturing Module 2

Lesson 5 – Bacterial Harvest and Determination of Bacterial Yield

Lesson objectives:

Students will understand:

- How to make serial dilutions
- How to use the spread plate technique to calculate bacterial yield

Essential Question

- How can we calculate the number of bacteria in a liquid culture?

Materials:

- Upstream Process Protocol – Days 4 & 5
- Serial Dilution Lab Protocol
- Colored water
- Microfuge tubes
- Induced liquid bacterial culture created in the last class period
- Sterile 50mL tube (1/team)
- Sterile LB/Amp media (1 bottle/team)
- LB/Amp plates (5/team)
- Sterile bacterial spreaders
- Bacterial plate incubator
- Upstream Process Batch Record Document

What Teams Will Do

- Remove their induced bacterial cultures from the shaker/incubator.
- Check the color of these cultures.
- Create 1:10 serial dilution set
- Plate their dilutions on LB/Amp agar
- Place their plates in the bacterial plate incubator
- Place the 50mL tube containing their harvested bacterial culture in the refrigerator.
- Each team fills out the appropriate parts of their Upstream Process Batch Record

Next Day

- Each team removes their plates from the incubator.
- Each team counts colonies and calculates bacterial yield using Colony Forming Unit (cfu) Calculation Record
- Each team fills out their Colony Forming Unit Calculation Record and files it

Teacher Preparation

- Prior to class make copies of
 - Colony Forming Unit (cfu) Calculation Record
- Provide each team
 - Production of RFP+ or GFP+ Bacteria Protocol
 - Serial Dilution Lab Protocol
 - One p200 micropipette

- One box of p200 micropipette tips
- Colored water
- 1.5mL microfuge tubes
- One bottle of LB/Amp media
- 5 LB/Amp agar plates
- Sharpie marker for counting colonies
- Sterile bacterial spreaders
- Spray bottle of 70% ethanol
- Paper towels
- Upstream Process Batch Record
- Colony Forming Unit (cfu) Calculation Record
- Team file folders

Organizer

Time	Activity	Materials
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
5 minutes	Each team takes their induced liquid bacterial culture out of the shaker/incubator and decants it into a sterile 50mL tube.	Production of RFP+ or GFP+ Bacteria Protocol, sterile 50mL tube
20 minutes	Teams practice serial dilutions (if needed)	Serial Dilution Lab Protocol, 1.5mL microfuge tubes, colored water, plain water, p1000 micropipette and tips
30 minutes	Teams create serial dilutions from their bacterial harvest and plate them.	Upstream Process Protocol – Days 4 & 5, harvested bacterial culture, 1.5mL microfuge tubes, p200 micropipette and tips, LB/Amp plates, sterile spreaders
5minutes – NEXT CLASS PERIOD	Teams put on gloves and retrieve their plates from the incubator	
20 minutes	Teams count colonies on the most appropriate plate and calculate their bacterial yield	Colony Forming Unit (cfu) Calculation Record, pencils, scratch paper, sharpie markers

10 minutes	Teams fill out their Colony Forming Unit Calculation Record and file it	Colony Forming Unit Calculation Record Document
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

Procedure

Bacterial Culture Harvest

1. Members of each team put on PPE
2. Each team sanitizes and organizes their bench space
3. Each team retrieves its induced bacterial liquid culture from the incubator
4. Each team examines the color of their induced bacterial culture.
5. Each team moves the bacterial culture to a 50mL tube.

Calculation of Bacterial Yield – Serial Dilution and Plating

6. Each team follows the appropriate section of the Upstream Process Protocol – Day 4 to make a serial dilution of their bacterial culture and plate each dilution on a separate LB/Amp plate.
7. Each team puts their plates in the bacterial plate incubator to incubate at 37 degrees C for 24 hours.

Calculation of Bacterial Yield – Serial Dilution and Plating

8. Each team retrieves their plates from the incubator.
9. Each team uses the instructions in the Colony Forming Unit Calculation Record document to calculate their bacterial yield.
10. Each team fills out their Colony Forming Unit Calculation Record and files it in their team file.
11. Each team fills out the appropriate sections of the Upstream Process Batch Record and files it in their team file.