

Biomanufacturing Module 2

Lesson 2 – Bacterial Inoculation

Lesson objectives:

Students will understand:

- How to perform a bacterial inoculation.
- At what stage bacterial inoculation occurs during upstream processing.

Essential Question

- Why do you inoculate with a single bacterial colony?

Materials:

- Intro to Biomanufacturing slide showing upstream and downstream process steps
- Recombinant Protein Production in Bacteria slide deck
- Upstream Process Protocol Day 1 (Production of RFP+ or GFP+ Bacteria)
- Sterile inoculating loop (1/team)
- LB/Amp/Arabinose plate with single colonies of RFP+ or GFP+ E. coli
- LB + ampicillin (LB/Amp) media (1 bottle/team)
- 14mL Falcon snap cap tube (1/team)
- p1000 Micropipette and tips (1/team)
- Shaker/incubator
- Upstream Process Batch Record Document

What Students Will Do

- Review upstream process
- Listen to/watch the Recombinant Protein Production in Bacteria slide deck
- Inoculate a single colony of RFP+ or GFP+ bacteria into LB/Amp media
- Place the tube containing the inoculated bacteria in the shaker/incubator
- Each team fills out the appropriate parts of their Upstream Process Batch Record

Teacher Preparation

- Prior to class make copies of
 - Production of RFP+ or GFP+ Bacteria Protocol (one per team)
 - Upstream Process Batch Record Document (one per team)
- Prior to class turn on the bacterial shaker/incubator to a temperature of 37 degrees Celsius.
- Prior to class secure a tube rack 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.
- Prior to class remove the LB/Amp/arabinose plate that has RFP+ or GFP+ colonies on it from the fridge. Place the plate in a central place so all teams can have access to it.
- Provide each team
 - One sterile inoculation loop
 - One p1000 micropipette and tips OR sterile transfer pipettes
 - One bottle of sterile LB/Amp media

- Sharpie marker for labeling tubes
- One 14mL Falcon snap cap tube
- Spray bottle of 70% ethanol
- Paper towels
- Team file folders

Organizer

Time	Activity	Materials
5 minutes	Review Upstream Process	Slide from Intro to Biomanufacturing slide deck
10 minutes	Present 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, Falcon snap cap tube, micropipette, tips, sterile inoculators, LB/Amp/arabinose plate with RFP+ or GFP+ colonies
15 minutes	Each team creates a bacterial inoculum	Upstream Process Protocol – Day 1, sterile LB/Amp media, Falcon snap cap tube, micropipette, tips, sterile inoculators
5 minutes	Each member of the team labels their plate and places it in the bacterial shaker/incubator	Sharpie markers Bacterial shaker/incubator
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

Introduction to Protein Production in Bacteria

1. Present the Introduction to Recombinant Protein Production in Bacteria slide deck

Preparation of the bacterial inoculum

2. Members of each team put on PPE

3. Each team sanitizes and organizes their bench space
4. Each team follows the appropriate section of the Upstream Process Protocol (Day 1) - Production of RFP+ or GFP+ Bacteria, to create a bacterial inoculum.
5. Each team puts their tube containing the inoculum in the shaker/incubator to shake at 37 degrees C for 24 hours. After 24 hours in the shaker/incubator, tubes can be stored in the refrigerator until the next class period.
6. Each team fills out the appropriate sections of the Upstream Process Batch Record and files it in their team file.