

Production of RFP+ or GFP+ Bacteria – Upstream Process Protocol Day 3

Induction of RFP or GFP Protein Production

Materials for Module 2, Lesson 4:

1. **Arabinose liquid stock (50X):** This will be added to the bacterial culture to induce bacterial production of GFP or RFP.
2. **125mL sterile glass baffled flask with lid that contains your bacterial culture.**
3. **Micropipettes and tips:** For measuring small volumes of reagents or bacterial cultures
4. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
5. **Microfuge tube rack:** To hold microfuge tubes.
6. **Sharpie markers:** For correct labeling of samples.
7. **Upstream Process Batch Record Form:** When properly filled out, this form is a record of your team's entire upstream process.

Equipment:

8. **Shaker/Incubator:** The equipment used to shake and warm bacterial cultures for optimal growth in liquid culture.

Protocol: Day 3 (Lesson 4) – Induction of RFP or GFP Production

This protocol is carried out by the Upstream Process Technician

NOTE: All team members will assist the Upstream Process Technician as needed.

Use aseptic technique at all times!!

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

1. Determine how much volume remains in the liquid culture in your baffled flask.
You started with 50mL. At various intervals you removed 2mL aliquots to read OD600. The number of readings you took x 2mL = how much volume you have lost. Subtract this amount from 50 to determine how many mL remain in your flask.
2. Calculate how much of the 50X arabinose solution to add to your liquid culture to bring it to a 1X solution.
This can be done using the equation $(C1)(V1) = (C2)(V2)$
Example calculation:
C1 = 1X – the concentration you want the arabinose to be at in your flask
V1 = 32mL remaining in your flask
C2 = 50X - the concentration the arabinose stock is at
V2 = How much of the 50X arabinose you need to add to your 32mL culture to bring it to 1X
 $(1)(32\text{mL}) = (50)(Y\text{ mL})$
 $32 = 50(Y\text{mL})$
 $Y\text{mL} = 32/50 = 0.64\text{mL or } 640\mu\text{L}$

3. Using a micropipette, add the appropriate amount of the 50X arabinose solution to your liquid culture.
4. Put your flask back in the shaker/incubator and let it shake at 250rpm, 37°C until the next day. At this point the flask can be stored in the refrigerator until the next class period.
5. Properly dispose of all waste following the guidelines in the Aseptic Technique slidedeck.
6. Make sure you have filled out all necessary parts of the Upstream Process Batch Record.