

Downstream Process - Bacterial Lysis Protocol

Materials:

1. **Harvested RFP+ or GFP+ Bacterial Culture:** This culture contains the bacteria you need to lyse (break open) so you can purify the RFP or GFP.
2. **Micropipette tips:** For measuring small volumes of reagents or bacterial cultures
3. **Microfuge tubes of various sizes:** For containing small volumes of reagents or bacterial cultures
4. **Microfuge tube rack:** To hold microfuge tubes.
5. **Biohazard waste container:** This is the container you will put all solid bacterial waste into.
6. **10% bleach solution:** This is the solution you will put all liquid bacterial waste into.
7. **Sharpie markers:** For correct labeling of samples.
8. **Elution Buffer (TE):** This is a buffered solution you will resuspend your bacteria in prior to lysis.
9. **Lysis Buffer (BPER):** This is the solution you will use to break open bacterial cells.
10. **Downstream Process Batch Record Form:** When properly filled out, this form is a record of your entire upstream process.

Equipment:

1. Microcentrifuge
2. Micropipettes (p1000 and p200)

Protocol:

Done by the Downstream Process Technician

The Downstream Process Technician should work with their team members to carry out this procedure.

Dispose of all waste properly.

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

1. Examine your harvested bacterial culture and record its color.
2. Before you can lyse the bacterial cells, you will need to separate them from the growth medium.
3. Make sure the cap on the 50mL tube containing your team's harvested bacteria is tightly screwed on. The tube has been stored in the refrigerator and the bacteria have settled at the bottom of the tube. Mix the tube well by inverting it repeatedly until the mixture is evenly mixed.
4. Set a p1000 micropipette to 500uL. Place a clean tip on the micropipette and use it to move 1mL of the bacterial mixture into a 1.5mL microfuge tube. Discard the tip into the biohazard waste container when you are through.

5. Repeat step 4 with a second microfuge tube.
6. Label both tubes with your Team name and the date. You will only use one of these tubes initially. The second tube will be saved as a 'back up'.
7. Spin the tubes in a microcentrifuge set at 5,000 rpm for 3 minutes.
NOTE: Be sure to balance the tubes in the microcentrifuge before spinning.
8. Carefully remove the tubes from the microcentrifuge. You should be able to see a bacterial pellet at the bottom of each tube.
9. Set a p1000 micropipette to 300uL. Place a fresh tip on the micropipette and carefully remove the supernatant (liquid) from the tubes without disturbing the cell pellet. The liquid can be dispensed into the 10% bleach solution.
10. Using a p200 micropipette, add 150uL of Elution Buffer to each cell pellet.
11. Close the tube caps and drag the tubes vigorously across the surface of a microfuge tube rack to mix. You can also pipette up and down with the micropipette if you prefer. Mix until you no longer see clumps of bacterial cells.
12. Using the p200 micropipette, add 150uL of Lysis Buffer (BPER) to each tube.
13. Close the tube caps and drag the tubes vigorously across the surface of a microfuge tube rack to mix. You can also pipette up and down with the micropipette if you prefer.
14. Label each tube: Lysate + team name + date.
15. The tube will incubate overnight at room temperature.
16. Place all used tips and tubes into the biohazard container.
17. Place your harvested bacteria back into the refrigerator.
18. Fill out the appropriate parts of the Downstream Process Batch Record.

