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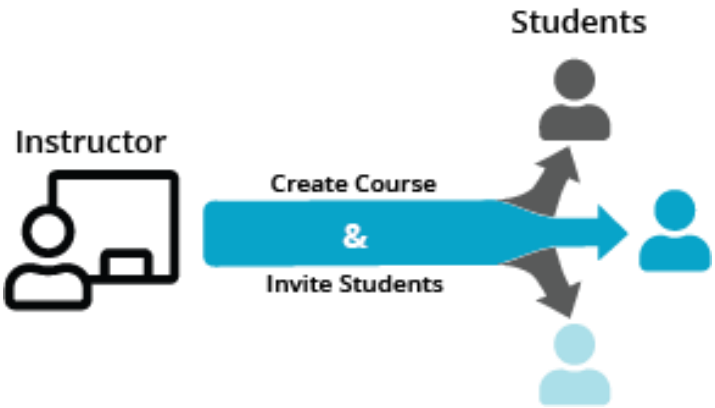

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Lab Builder

Transformation of E. *coli* Lab

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Attribution

Instruction Level

- Undergraduate Lower Division (Freshman, Sophomore)

Topic

- Recombinant production system
- Protein
- E. *coli*
- Transformation
- Sterile technique
- Bacterial Culture
- Characterization of a recombinant protein
- Preparation of Agar Plates
- Transformation
- 4 quadrant streak plate
- Microbiology

Timeline

- **Day 1 -** Starter Culture and Agar Plates: 3 hours
  - Incubation**:** 24-48 hours
- **Day 2 -** Transformation: 2 - 3 hours
  - Incubation: 24 hours
- **Day 3 -** Data Analysis: 30 mins - 1 hour

Learning Objectives

In this lab unit, students will:

- Use aseptic technique to prepare agar plates and media
- Transform *E. coli* with plasmid DNA
- Select for recombinant clones on antibiotic selection plates
- Analyze and troubleshoot the results of a transformation experiment

Instruction Type

- Traditional

Additional Resources

- Course Text: https://drive.google.com/drive/folders/1liqMTi3I5BCulQzcNObk6E-DHe2m3Tcv

Introduction

Background

While bulk enzymes produced for the food and chemical industries are most often isolated directly from microbial or plant sources, biopharmaceuticals are more often isolated from recombinant organisms. Although therapeutic biopharmaceutical protein drugs such as insulin were initially isolated from human and animal tissues, therapeutic proteins are not frequently found in high enough quantities and concentrations needed. Additionally, contaminating substances from natural sources can be unsafe, whether due to allergic responses in patients or due to contaminating viruses or prions.

The disadvantages to extracting therapeutic proteins from natural sources can be overcome by using a recombinant production system. By isolating the gene coding for a target protein and cloning it into a high-expression vector in a recombinant host, the possibility of contaminating viruses and prions is eliminated. The higher level of expression of the protein in a recombinant host can significantly reduce purification costs, and protein engineering can be used to design improvements in stability or effectiveness of a protein product.

In biomanufacturing, the plasmid and cellular host selected is critical to the successful production of the final product. For example, if biomanufacturing a human protein that requires complex post-translational modifications, a company may choose to create a recombinant protein in a mammalian cell. However, for simple proteins that may be used in diagnostic or research assays, a bacterial host may be used.

E. coli was the first host used to produce recombinant proteins because it was well understood genetically and was very amenable to transformation and expression of recombinant genes. However, not all proteins are expressed well in E. coli, in part due to the bacterial host's inability to perform necessary post-translational modifications needed for

eukaryotic proteins. Also, E. coli produces an endotoxin that acts as a pyrogen when injected, and this endotoxin is tough to remove. In the last few decades, the biotechnology industry has turned to other cell systems for recombinant hosts in recombinant protein expression systems: fungal, plant, and animal cell and tissue culture. Below is a selection of interesting and unconventional expression systems currently being used to make an array of drugs.

Drug	Production Process	Indication
ATryn	Purified from the milk of transgenic rabbit goats	Prevention of thromboembolic events
Ruconest	Purified from the milk of transgenic rabbits	Acute hereditary angioedema attacks
Elelyso	Produce in Transgenic carrot-based plant cell expression system	Type 1 Gaucher disease
Kanuma	Purified from the egg white of transgenic hens (*gallus gallus)*	Lysosomal acid lipase deficiency
Pandemic flu vaccine	Virus-like particles transiently expressed in tobacco (*nicotiana benthamiana*) leaves	Prevention of H5N1 influence infection
VEN150	Expressed in the grain endosperm of genetically modified rice	Inflammation in HIV
SBC – 103	Purified from the egg white of transgenic hens (*gallus gallus)*	Mucopolysaccharidoses IIIB
ZMapp	Produced in transgenic tobacco cell culture technology	Ebola virus infection
PRX-106	Produced in carrot and tobacco cell culture technology	Inflammatory bowel disease
Moss-aGal	Produce in glycoengineered *Physcomitrella patens* moss cell line	Fabry disease

Table 1: Selected products using unconventional expression systems on the market or under development.

For the remainder of the semester, students will perform a small-scale biomanufacturing batch to purify and characterize a recombinant protein. In this week's lab, students will transform a competent *E. coli* (bacterial) host with a plasmid carrying a GFP-linked enzyme. The GFP in this lab will be used as a selectable marker that will easily identify the bacteria that have successfully taken up and produced recombinant protein from the plasmid template. The transformed *E. coli* will then be used to perform small-scale biomanufacturing, purification, and characterization of this enzyme throughout the semester.

Pre-Lab Assignment

Review Techniques

- To learn more about today's transformation, watch the following video:
  - https://youtu.be/c40UudFIlGw

- Review 4-quadrant streaking for isolated colonies:
  - https://youtu.be/0heifCiMbfY

Questions

1. *Purpose Statement:* In one or two sentences, specifically describe the purpose of the day's experiment/lab work. What are you trying to learn or achieve, and how?
2. Outline the key steps of a transformation. Explain what is happening at each step.
3. What is the purpose of the 4 quadrant streak plate?
4. Bulk enzymes for the food and chemical industries are most often isolated directly from what?
5. When extracting therapeutic proteins from natural sources how can you overcome contaminating substances?
6. In biomanufacturing, what is critical to the successful production of the final product?

Procedure

Safety

- Please refer to the biosafety guidelines for handling microorganisms in teaching laboratories.
- The *Escherichia coli* strain used in this experiment is not considered a pathogen, but *E. coli* bacteria colonize the intestinal tracts of animals. Although it is rarely associated with any illness in healthy individuals, it is good practice to follow simple safety guidelines in handling and disposal:
  1. Gloves and goggles must always be worn.
  2. Wipe down the lab bench with antibacterial cleaner before starting the lab and before leaving the laboratory.
  3. All materials, including plates, pipettes, loops, and tubes that come in contact with bacteria should be autoclaved before disposal in the garbage.
  4. Wash hands thoroughly with soap and water after removing gloves.
  5. Any concerned students should consult with their physician.

Protocol

Part A: Preparation of Agar Plates

Materials:

- Benchtop cleaner
- 55ºC water bath
- Hot gloves
- 70% alcohol
- 40-50 small sterile Petri dishes
- 1L bottle with lid
- Autoclave tape
- Autoclave
- LB Agar Packet
- Lyophilized arabinose
- Lyophilized ampicillin
- 5-mL Serological pipets (x2)

Procedure:

Note: Part of this part may have already been completed. Ask the instructor.

1. Label Petri plates with a sharpie as demonstrated by the instructor. There will be 16 LB, 16 LB/amp, and 8 LB/amp/ara plates.
2. Prepare the plate preparation area and a water bath as previously performed.
3. Prepare nutrient agar: add 500 mL of sterile water to a 1 L or larger sterile Erlenmeyer flask or glass bottle. Add the entire contents of the LB nutrient agar packet. Swirl the flask to mix the agar, and autoclave-sterilize 20min, 121ºC. Allow the LB nutrient agar to cool in a clean 55°C water bath.
4. Prepare arabinose and ampicillin: With a clean, sterile pipet, add 3 ml of transformation solution directly to each vial to rehydrate. This may take 10 minutes to dissolve.
5. Pour 16, LB nutrient agar plates into labeled plates.
6. Now, add the hydrated ampicillin to the remaining LB nutrient agar. Swirl briefly to mix. Pour into the 16 plates that are labeled as LB/amp using the technique utilized above.
7. Last, add the hydrated arabinose to the remaining LB nutrient agar containing ampicillin. Swirl briefly to mix and pour into the eight (or more) plates labeled as LB/amp/ara using the technique utilized above.
8. Allow plates to set at ambient temperature for next class. Keep plastic sleeves for plates! Store long-term at 4ºC.
9. Next class leave out one LB (only) plate per group and put the rest back into the sleeves. Store upside down (agar side up) in the fridge.

Part B: Transformation of E. coli

Preparation of starter culture (Day 1)

Note: Part of this may have already been completed. Ask the instructor.

Materials:

- Each group
  - LB (only) plate (x1)
  - Sterile loops (x4)
  - Biohazard bag
- Class Shares
  - Lyophilized *E. coli*
  - 250 mL reconstitution solution
  - Benchtop cleaner
  - 37ºC incubator

Procedure:

1. Rehydrate lyophilized *E. coli* by directly adding 250 µL of reconstitution solution directly into the vial. Recap, swirl to mix and allow to stand for 5 min at ambient temperature. *Note: this step may have been already performed.*
2. Each group prepares a starter culture plate by streaking plate for isolated colonies using the 4-quadrant method.
   - Label the top of an LB (only) plate with group initials, date, LB, and the name of bacteria. Always keep the lid on the plate, remember this plate has no antibiotics!
   - Swirl culture to mix just before use.
   - Using a 10 µL loop, insert loop into bacteria vial and remove a loop-full of rehydrated *E. coli.* The loop will have a shine to it (like when you are blowing bubbles).
   - Dispense the 10 µL loop of culture on a labeled LB only plate, spread as on single line on the plate. Dispose of the loop.
   - Obtain a clean loop and repeat streak, dispose of the loop. Repeat two more times. See the demonstration by the instructor.
   - Return lid, turn upside down and incubate 24-48 hrs at 25-37^{o}C. The colonies should be 1mm wide.
   - **NOTE**: Use a fresh starter plate. Prepare 1-2 days before the transformation section

Labs – Biotechnology: Transformation of E. *coli*

Transformation of E. coli (Day 2)

Note: Part of this may have already been completed. Ask the instructor.

Laboratory Safety:

See previous note on lab safety working with bacteria and plasmids! Dispose of all tips, tubes, and gloves in the biohazard trash. Clean benchtop before and after working. Discard gloves in biohazard trash and wash hands before leaving the lab.

Materials:

- Each group:
    - Micropipettes and plugged tips
    - Sterile microcentrifuge tubes & rack
    - Floating microcentrifuge racks
    - Biohazard bag
    - Gloves
    - Bucket of ice
    - LB Petri plates (x2)
    - LB/amp Petri plate (x1)
    - LB/amp/ara plate (x1)
    - Biohazard bags and stands
    - 250 ul warm LB broth
    - 250 ul cold 50 mM $CaCl_2$
    - Sterile loops
    - Starter plate (from last class)
- Class Shares
    - Benchtop cleaner
    - 37ºC incubator
    - 42ºC water bath & floats
    - Plasmid solution

Procedure:

1. Label the top of one closed microcentrifuge tube with group initials.
2. Add 250 µL of transformation solution ($CaCl_2$) into the tube and place on ice.
3. Use a sterile loop to pick up a single colony of bacteria from the starter plate and immerse the loop into the transformation solution at the bottom of the tube. Spin the loop between index finger and thumb to disperse the colony in the transformation solution. Place the tube back on the ice.
4. Add 10 µL of the plasmid into the cell suspension and return it to the ice. Incubate the tubes on ice for 10 minutes.
5. While the tubes are incubating on the ice, label the three agar plates around the bottom of the plate with the agar (not the lid). Each group will need one each of, LB only, LB-amp, and LB-amp-ara.
6. Heat shock the sample by transferring the tube into the 42ºC water bath for exactly 50 seconds (use a timer!). Immediately after 50 seconds, place tube back on the ice. For the best transformation results, the change from the ice (0ºC) to 42ºC and then back to the ice must be immediate!
7. Incubate on ice for 2 minutes.
8. Remove from ice and place on the benchtop. Open the tube and add 250 µL of warm LB broth. Gently pipet up and down to mix and re-close the tube. Incubate the tubes for 10 minutes at room temperature.
9. Mix by inversion and pipette 100µL of transformed bacteria onto each plate. Spread the liquid evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the entire plate surface. Return the lid and allow the liquid to absorb into the plate on the benchtop. This may take 5-10 minutes.
10. Once the liquid had fully absorbed into the surface of the plate, invert, and stack the plates together. They can be taped together and labeled with group initials.
11. Place the inverted stack in the 37ºC incubator overnight. The plates should be removed after approximately 18-24 hours and refrigerated until the next class period. Seal plates closed with Parafilm or place them in a Ziploc baggy, so they do not dry out. Label and store them at 4ºC as directed by the instructor.

Results

- Create a table to organize your results.
- Attach pictures of your plates.

Post-Lab Assignment

1. Briefly summarize experimental results. Include a brief description of the plasmid map and how to determine which colonies have the recombinant protein inserted in them.
2. Briefly discuss the advantage of linking a recombinant protein to a fluorescent protein in biotechnology.
3. The transformed cells (with plasmid DNA) were grown in an LB-ampicillin culture. Why was ampicillin used? How did the cells survive when exposed to this antibiotic?
4. The transformed cells (with plasmid DNA) were grown in an LB-ampicillin-arabinose culture. Why was arabinose used? What happens if you leave out the arabinose?
5. In this lab, you added a transformation solution to your bacteria and plasmid DNA. What is the purpose of this solution and what do you think would happen to the results of your experiment if you left out this step?