Dayton (bio)Manufacturing Awareness and Discovery Experience

Biomanufacturing Trailblazers – Summer 2024

Contents

Intro to Biomanufacturing

Biomanufacturing vs Chemical Manufacturing

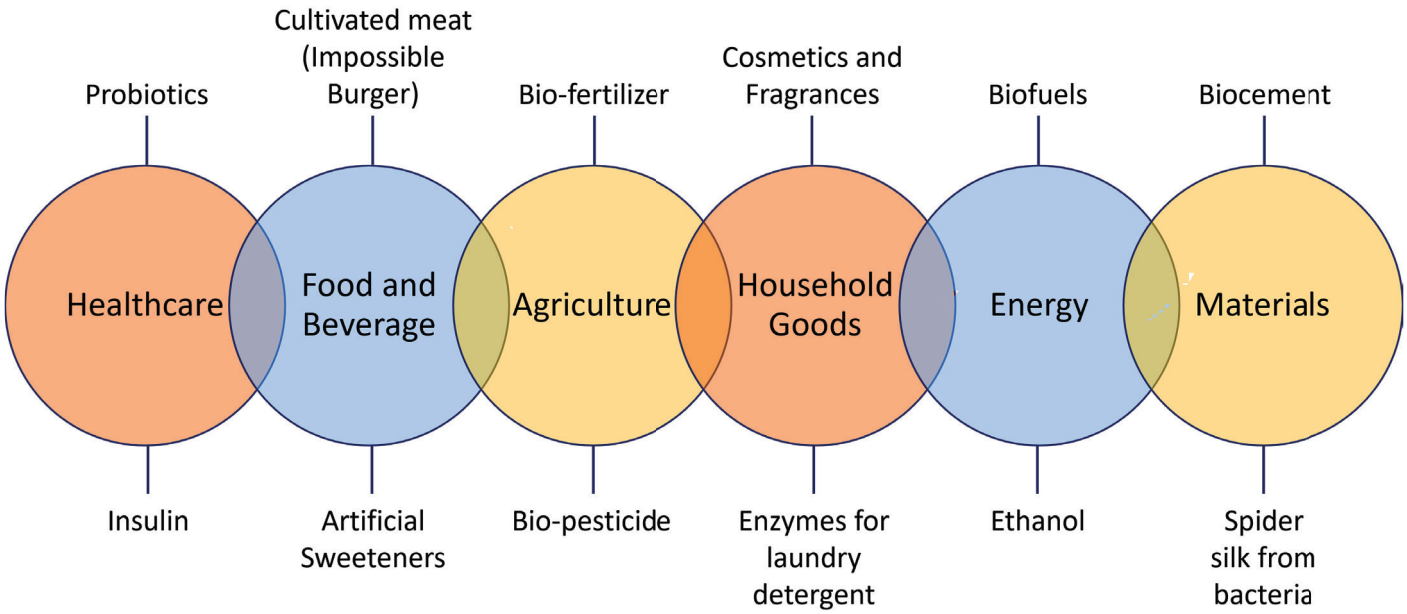
Chemical Synthesis

- Fossil fuels and other less environmentally friendly sources as feedstock
- Unable to produce complex molecules
- Expensive and high carbon footprint

Biomanufacturing

- Renewable feedstock (e.g. sugars, fats)
- Only way to produce complex medicines and proteins (e.g. insulin). Whole cells can be the product
- Typically lower costs and lower carbon footprint

Examples of Biomanufactured Products



Microbiology

Activity: Pouring Plates and Streaking Unknown Samples

Lab Objectives

- □ Use aseptic techniques
- □ Pour agar plates
- □ Streak unknown samples

Safety

PPE - safety glasses, nitrile gloves, lab coats, hot gloves
Hot plates - Use caution. Always assume the surface is hot. Don't leave it unattended when on.

Materials

- □ Powdered agar medium
- □ Weigh boat and spatula
- □ Sterile water
- □ 70% Ethanol
- □ Foil
- □ 4 petri dishes
- □ Sterile swabs
- □ Parafilm or ziploc bag

Equipment

- □ Balance
- □ Hot Plate
- □ Sterile graduated cylinder
- □ Sterile 250mL Erlenmeyer flask

Procedure

1. Clean and sterilize your benchtop with 70% ethanol. ☐
2. Calculate the volume of agar needed to make 4 small agar plates. ☐
   - Number of plates x 12.5 mL each = _____
3. Measure the required volume of sterile water into a graduated cylinder. ☐
4. Pour the sterile water into an Erlenmeyer flask. ☐
5. Use the recipe to calculate how much agar you need to make. ☐
   - _____ mL (from step 2) x 0.04 g = _____ grams of agar needed
6. Measure the required amount of agar and pour into the flask. Swirl the flask to mix. ☐
7. Add a stir bar and cover the flask with foil. ☐
8. Heat with stirring on the hot plate until the liquid is just boiling. ☐
   - **CAUTION:** Agar will boil over quickly. Use heat & spin settings at 40-50%.

Microbiology

Procedure continued

9. Reduce heat to simmer until powder fully dissolves. ☐

10. Turn off the heat and allow the agar to cool to about 60°C, or comfortable to hold. ☐

11. Label your petri dishes with the date and your initials. ☐

12. Pour the agar into the bottom of the petri dish (smaller diameter) until 1/3 full. You should be able to pour 4 plates. ☐

13. Place the lid on the petri dish and allow it to solidify. ☐

14. Once the plates are solid, use a sterile swab to first swab the surface of something (for example: your hand, a lightswitch, your phone) and then streak, or swipe, the swab across the agar back and forth a few times. ☐

15. Cover the plate. Repeat with a second plate. Each partner should be able to swab two plates.Cover the second plate. ☐

16. Flip the agar plates upside down so the part with the agar is on top. ☐

17. Have a staff member parafilm the edges of your plates or stick your plates in a ziploc bag. Place the plates in the incubator at 37°C with the agar side up. ☐

18. Put away all equipment and clean and sterilize your benchtop with 70% ethanol. ☐

Enzymatic Juice Extraction

Activity: Extracting Apple Juice Using Pectinase

Lab Objectives

- □ Monitor enzyme activity by measuring the amount of apple juice released by pectinase or water
- □ Compare enzymatic activity under different temperature conditions

Safety

PPE - safety glasses, nitrile gloves, lab coats
Do not eat or drink the apples or juice in this experiment. The concentration of pectinase used will be much higher than is used in commercial juice production, and the fruit and enzyme have not been handled aseptically.

Materials

- □ Water
- □ Pectinase solution
- □ Diced apple
- □ Parafilm
- □ Plastic spoons or stir rods
- □ Lab tape and marker

Equipment

- □ Water bath set to 40°C or 60°C
- □ Refridgerator (~4°C)
- □ Balance
- □ Weigh boat
- □ Two beakers
- □ Two graduated cylinders
- □ Two funnels
- □ Filter paper

Procedure

1. Label both beakers using lab tape with your group initials. Label one beaker "control" and one beaker "pectinase" ☐
2. Use the balance to weigh 50 g of chopped apple into each beaker ☐
3. Add 2 mL (2000 µL) of water to the control beaker and stir the beaker contents to mix ☐
4. Add 2 mL (2000 µL) of pectinase solution to the pectinase beaker and stir the beaker contents to mix. **Be careful not to put the pectinase sample stirrer into the water beaker!** ☐
5. Cover the beakers with parafilm and incubate them at your group's temperature (4°C, room temperature [~21°C], 40°C or 60°C) for 15 minutes. Group temp: _____ ☐
6. Use your spoons to gently stir/squeeze the apple pieces in each beaker ☐
7. Place a coffee filter in a funnel and then set the funnel in a graduated cylinder. Make two of these set ups. Label the cylinders so you can keep track of the samples. ☐

Continue on next page...

Enzymatic Juice Extraction

Procedure continued

8. Separately pour the juice from each beaker into its own funnel and allow the juice to drain for exactly 5 minutes ☐

9. Record the amount of juice in your cylinders after 5 minutes in the table below ☐

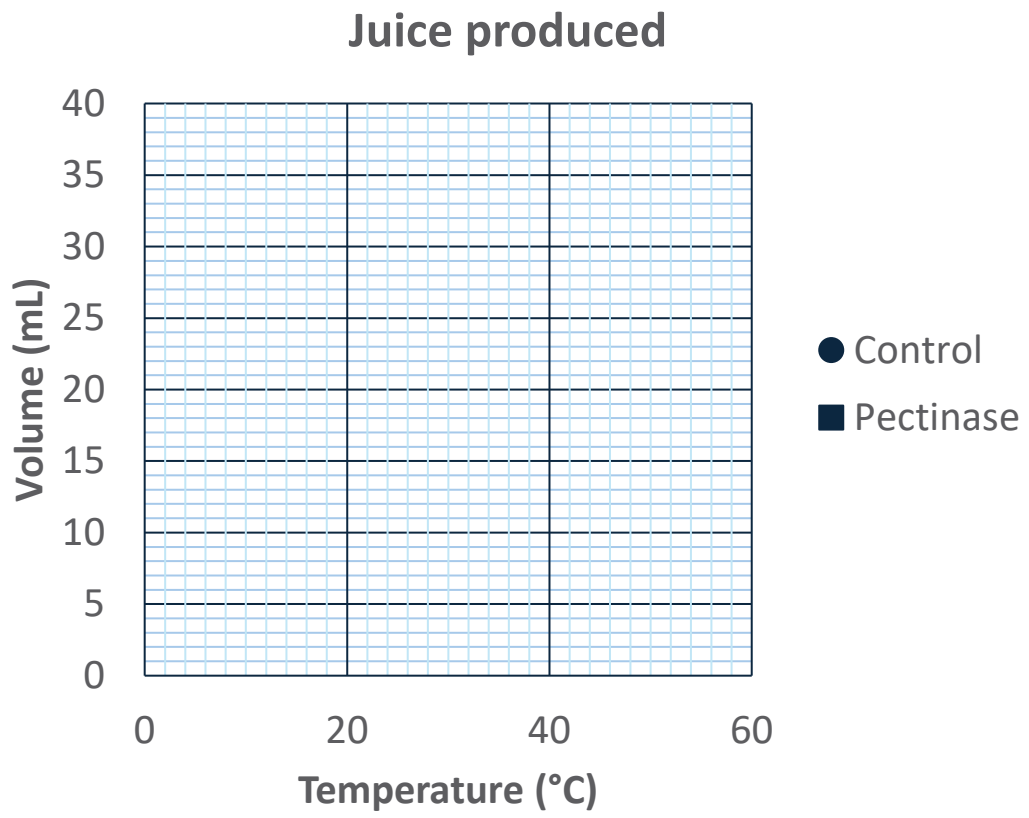
Discussion: What would happen if you added more enzyme or more apple? ☐

_____

_____

_____

	Volume in control cylinder	Volume in pectinase cylinder
Temperature: _____		



Biofuel Production Using Yeast

Plot the calculated sugar remaining for your Day 0, 1, and 2 samples on the chart below. Connect your points with lines. Use the different markers to draw in other group's data.

What happened to the remaining sugar over time?

_____

_____

What condition resulted in the most efficient sugar usage? Did it match the % ethanol results?

_____

_____

_____

Genetic Engineering

Production of GFP in *E. coli*

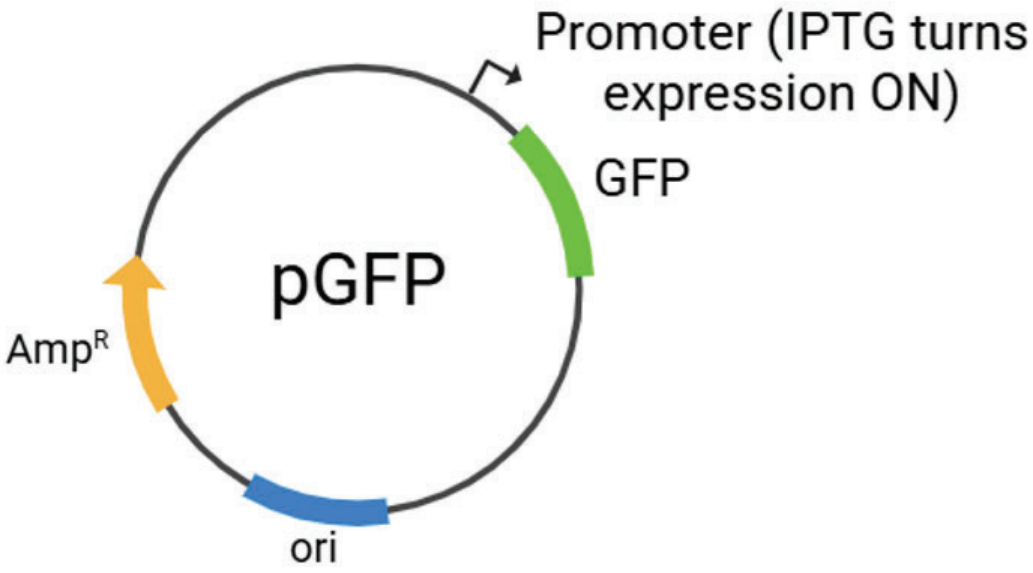
The GFP (Green Fluorescent Protein) gene is normally found in jellyfish. In this experiment, you will add this jellyfish gene to *E. coli* bacteria cells. Adding this new gene to *E. coli* gives them the ability to fluoresce under UV light! When the bacteria are transformed, they receive the jellyfish gene and express the jellyfish trait, now producing our protein of interest (GFP). This technique is called **genetic engineering** and is used to move genes from one organism to another.

Here, the pGFP plasmid expressing the GFP gene has already been cloned for you.

The plasmid contains an antibiotic resistance gene that provides resistance to the antibiotic ampicillin. Therefore, once you transform the plasmid into the bacteria, if you grow the bacteria on media containing ampicillin, then only the successfully transformed bacteria should grow.

The GFP gene is controlled by a promoter that turns ON in the presence of the chemical IPTG. If no IPTG is present, then the gene won't be expressed and there will be no protein produced. If IPTG is in the media, then the gene will be turned on and GFP will be expressed.

Simplified map of the pGFP plasmid:



Genetic Engineering

Bacterial Transformation

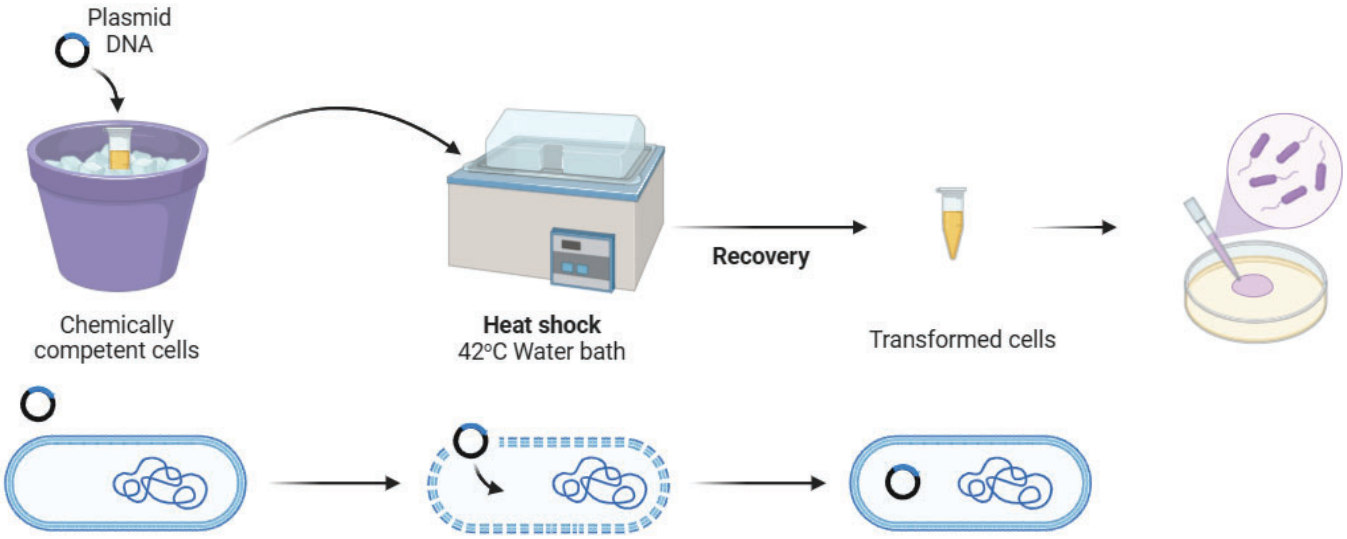
1. **Preparing Competent Cells** - You first need to make the cells ready to accept a plasmid. This typically entails treating the cells with certain salts. It's important to keep the cells cold and treat them gently

2. **Addition of Plasmid** - After you make the cells competent, you add the plasmid and let the cells sit on ice for a bit.

3. **Heat Shock** - Applying a brief, rapid burst of heat opens up pores, or holes, in the bacterial cell membrane. After shocking, you need to immediately move the cells to ice to make them happy again and trap the DNA inside.

4. **Recovery** - In order for the transformed bacteria to grow on a plate with antibiotic, you need to allow the cells to start producing the antibiotic resistance gene. Therefore, you recover, or grow, the cells in media containing no antibiotic for a little bit before plating.

5. **Plating** - After recovery, you spread the cells on a petri dish with agar containing antibiotic and your inducer. Controls can be plated as well without these either chemical.



Fermentation and Scale Up

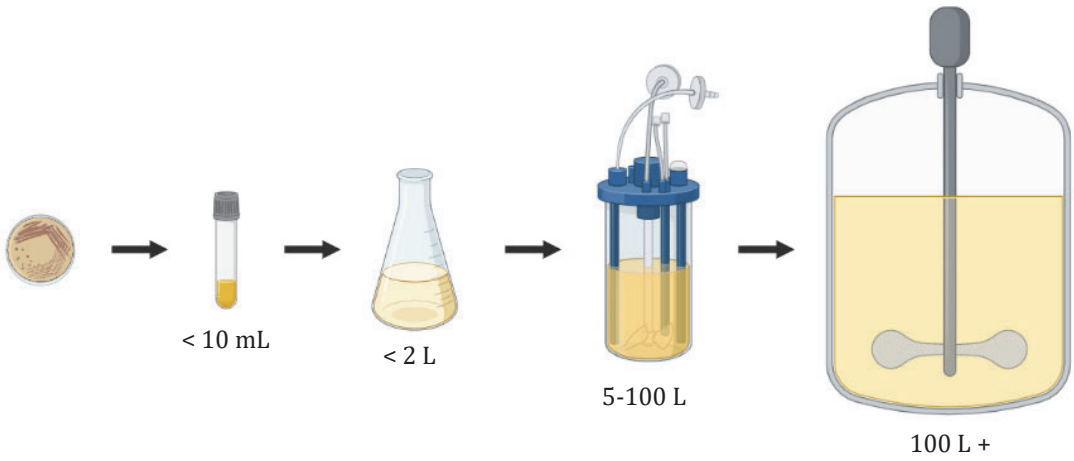
What is Fermentation?

Fermentation is the process used to grow microbes (such as bacteria or yeast) to make a product of interest.

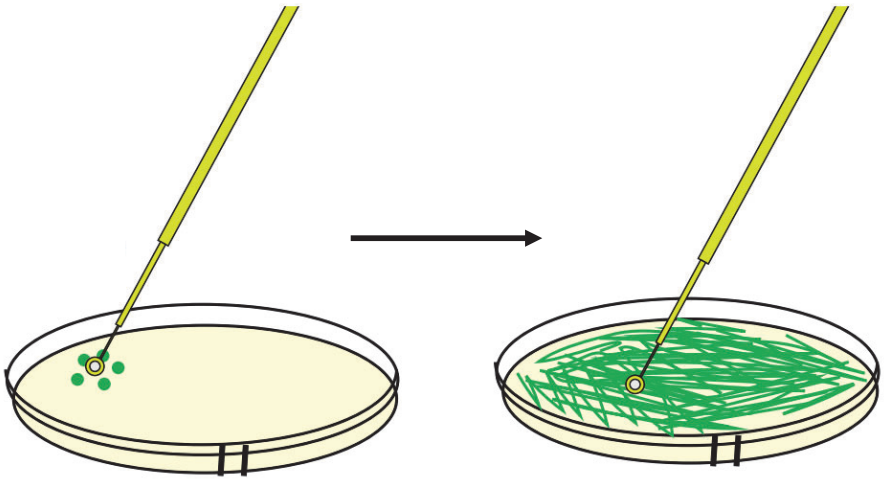
Cells are cultured in increasingly larger volume to build up enough cells to produce the product.

Production takes place in the largest vessels (bioreactors) under tightly controlled conditions.

Example:



In your experiment, you will take colonies from your transformed plate and grow more cells on a new plate. You could also inoculate, or start growth, in a culture tube. Both of these protocols are small scale ups compared to what you would need to produce protein industrially.



Agar Art

Activity: Create Agar Art Using Engineered Bacteria

Lab Objectives

- □ Grow a culture of *E. coli* cells from colonies on a plate

Safety

PPE - safety glasses, nitrile gloves, lab coats

Materials

- □ Pretransformed bacteria "pallets"
- □ Two LB/Chl plates per person
- □ 70% Ethanol
- □ Yellow or blue inoculation loops
- □ Cotton Swabs
- □ Toothpicks

Equipment

- □ Incubator set at 37°C
- □ Waste beaker
- □ UV light

Procedure

1. Sterilize your bench with 70% ethanol. ☐
2. Using the colors available on the pallet, plan designs in the circles on the next page. You can also freehand your drawing if you prefer. Some colors are fluorescent. ☐
3. Once you have your plan complete, place your LB/Chl plate on top of the design. Using loops, swabs, or toothpicks, touch the tool to the "pallet" to pick up some bacteria (you don't need much!), and trace your design onto a plate. Use a new tool for each colored bacteria to prevent cross-contamination. ☐
4. Complete two plates/designs per person. ☐
5. Allow the plates to sit upright until all the liquid soaks into the agar. Then stack your plates, flip them upside down (agar side up), and place them in the incubator to grow overnight. ☐
6. Empty your waste beaker into the large biohaz box. Spray the beaker and wipe down your bench with 70% ethanol. ☐

WSU Campus Tour

Reflection Questions

What was something new you learned?

What was the most surprising thing you heard during the tour?

What do you think is important when choosing a college campus?

What would you like most about working on a college campus?

Standard Operating Procedures

Activity: Writing a SOP

Objectives

- □ Write a Standard Operating Procedure
- □ Critique a Standard Operating Procedures and offer improvement
- □ Improve a Standard Operating Procedure

Tips: **Say what you do** (writing good procedures) **Do what you say** (following procedures exactly)

Procedure

Part 1

1. Collect possible supplies. ☐

2. Work with your group to develop a procedure for making a peanut butter and jelly sandwich. Make a batch to help you determine the steps. ☐

3. Carefully write out each step of the procedure. Make sure your procedure is precise enough that someone would be able to perform the task successfully by following directions exactly as written. Make sure to also include a list of all materials and equipment needed to perform the procedure, using as much detail as possible. ☐

Part 2

1. One group will use another group's SOP to make a sandwich while the other groups observe. ☐

    - Collect supplies from the SOP equpiment/materials list. ☐
    - Exactly follow the procedure while the other groups observe.

2. While you observe, identify problems with the procedures and be prepared to discuss corrections with the class. ☐

3. Each group will update their protocols based on the full group discussion. ☐

4. Multiple members of staff will make a sandwich using the same updated SOP to see how consistent and high quality product is made. ☐

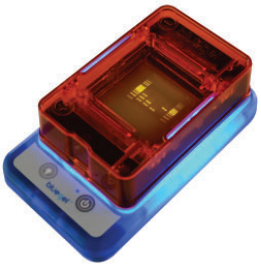
Enzyme Production in Bacteria

PCR

- Polymerase chain reaction (PCR) is used to amplify, or create copies, of DNA. In one reaction, you can make millions of copies of a piece of DNA!
- ***In this experiment***, you will amplify the BSMT gene coding for your enzyme from template DNA:

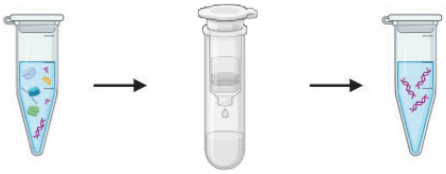
Gel Electrophoresis

- Gel electrophoresis is used to separate and visualize DNA and proteins.
- ***In this experiment***, you will use gel electrophoresis to verify that you have amplified your gene and that it is the right size.



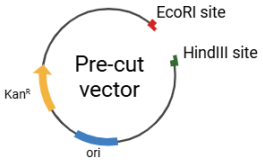
DNA Purification

- Column chromatography can be used to purify your amplified DNA from the PCR reaction.
- ***In this experiment***, you will use a small column and the microcentrifuge to purify your DNA, similar to the larger column you used for purifying the GFP protein.



Digestion

- Restriction enzymes are proteins that cut DNA at a specific site. When two pieces of DNA are cut with the same enzyme, it makes matching "sticky ends" at the end of the DNA that serves like a puzzle piece.
- ***In this experiment***, the empty plasmid (vector) has already been digested. You will digest your purified insert with the same enzymes to make the matching sticky ends.



Ligation

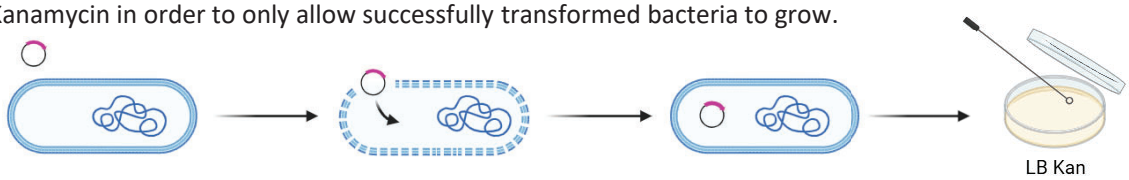
- Ligation uses an enzyme that joins DNA pieces together.
- ***In this experiment***, you will do a ligation to join your gene and your vector to make a new, complete plasmid.

Images: Biorender

Enzyme Production in Bacteria

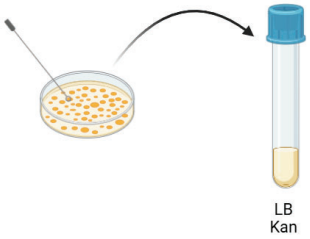
Transformation

- The plasmid is transformed, or inserted, into the bacteria.
- ***In this experiment***, the transformed bacteria is plated on agar plates containing the antibiotic Kanamycin in order to only allow successfully transformed bacteria to grow.



Scale Up

- In order to produce enough protein, you need to scale up, or grow more cells. This is part of the "Upstream Process" in biomanufacturing.
- ***In this experiment***, you will scale up by growing cells in liquid culture.



Induction

- To turn on expression of a gene, you need to add a specific chemical, or inducer. Sometimes you also have to add a substrate, which is the chemical that the enzyme reacts on or changes.
- ***In this experiment***, you will add the inducer IPTG to turn on expression of the BSMT protein. You will also add the substrate Salicylic Acid, which the BSMT protein turns into Methyl Salicylate.

Analysis

- It's important to measure the amount of product made. You can use different methods depending on the product.
- ***In this experiment***, you will use your sense of smell as well as a spectrophotometer to measure the amount of Methyl Salicylate (wintergreen scent) produced.

Downstream Processing

- Downstream processing involves purifying your product.
- ***In this experiment***, we won't be purifying the product, but think about ways that you could!

Images: Biorender

College 101

Participants will learn about how to be college-ready & how to apply for college.

Reflection Questions

What is something new you learned today?

What other questions do you have about applying for colleges?

Did anything surprise you about the college application process?

Careers in Biomanufacturing

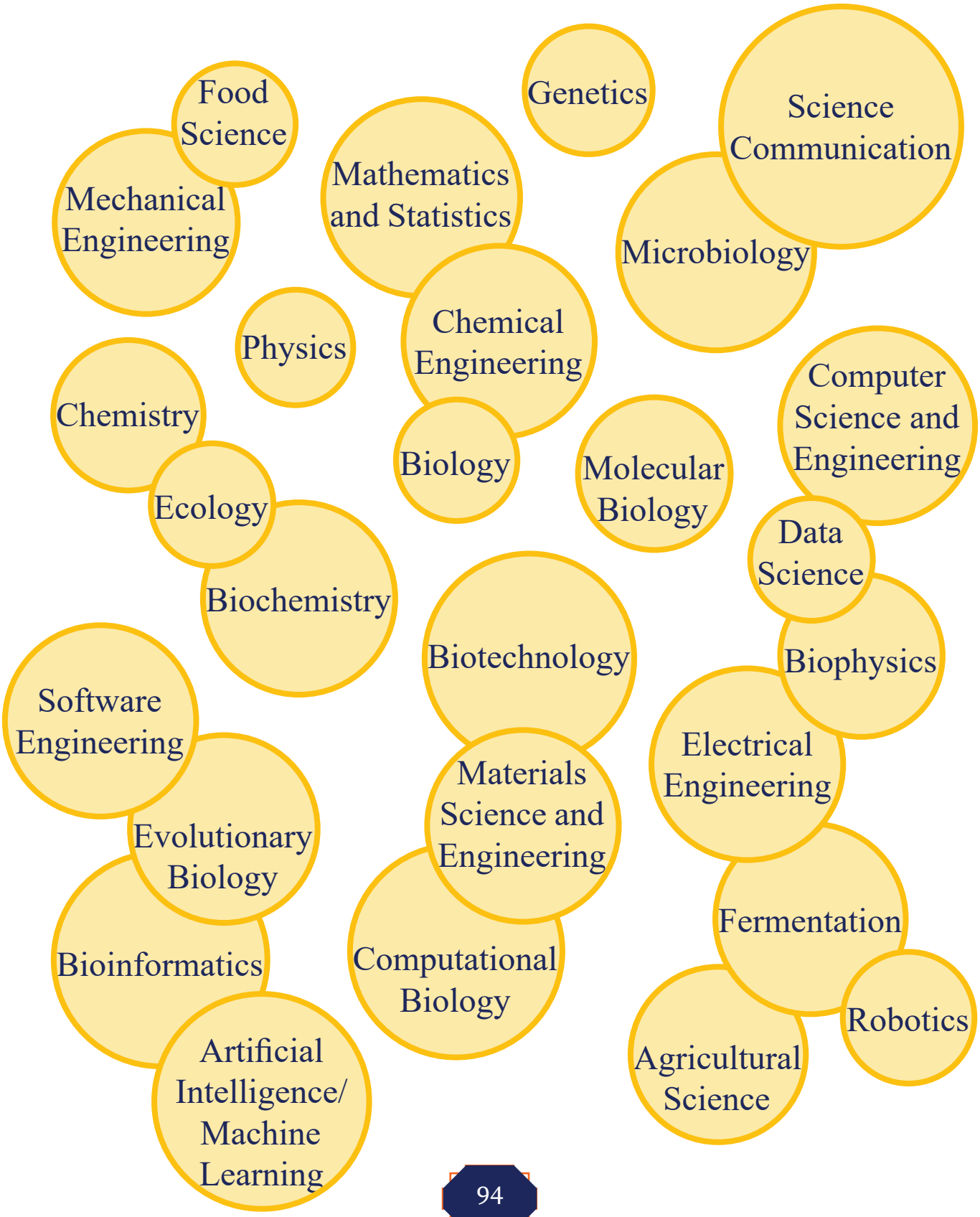
Types of STEM roles in biomanufacturing

JOB TYPE	SAMPLE JOB TITLES, OR KEYWORDS
Scientist	Discovery Research Scientist, Process Development Scientist, Quality Control Manager, Chemist, Microbiologist, Biochemist, Natural Science Manager, R&D Scientist, Research Associate, Environmental Scientist
Laboratory Technician	Research Assistant, Research Associate, Process Development Associate, Laboratory Technician, Quality Control Associate, Validation Technician
Engineer	Process Engineer, Chemical Engineer, Process Development Engineer, Manufacturing Engineer, Quality Engineer, Facility Engineer, Optimization Engineer, Process Control Engineer, Environmental Engineer
Process Technician	Process Technician, Manufacturing Technician, Manufacturing Associate, Packaging Technician, Formulation/Fill Technician, Manufacturing Prep Technician, Operator, Validation Technician
Maintenance and Instrumentation Technician	Maintenance Technician, Instrumentation Technician, Calibration Technician, Manufacturing Support Technician, General Mechanic, Maintenance Mechanic
Corporate Scientific Professional	Quality Assurance (QA) Manager, QA Associate, Quality Inspector, QA Auditor, Validation Specialist, Clinical Research Associate, Regulatory Affairs Specialist, Customer Support Specialist, Project Manager, Sales Representative, Marketing Specialist, Patent Attorney, Corporate Trainer, Lawyer, Technical Writer

Ref: "Mapping Your Future: Careers in Biotechnology"
North Carolina Association for Biomedical Research

Careers in Biomanufacturing

STEM Degrees Related to Biomanufacturing Careers



Careers in Biomanufacturing Activity

Working in groups of 2-3 participants, read 3 job ads and complete the following table.

	Job Ad 1	Job Ad 2	Job Ad 3
Job title and company			
What information is provided about the required education.			
List the three responsibilities that sound most interesting.			
Which terms or series of terms are new to you?			
Which required skills or experience do you think would be the most challenging to acquire?			
List one thing that surprised you when you read the job ad?			
Which school could you attend to complete a major that would prepare you for this job?			
Is this a job that would interest you? Explain.			

Notes and Observations