Dayton (bio)Manufacturing Awareness and Discovery Experience

Biomanufacturing Explorers – Summer 2024

Contents

Intro to Biomanufacturing

Biomanufacturing vs Chemical Manufacturing

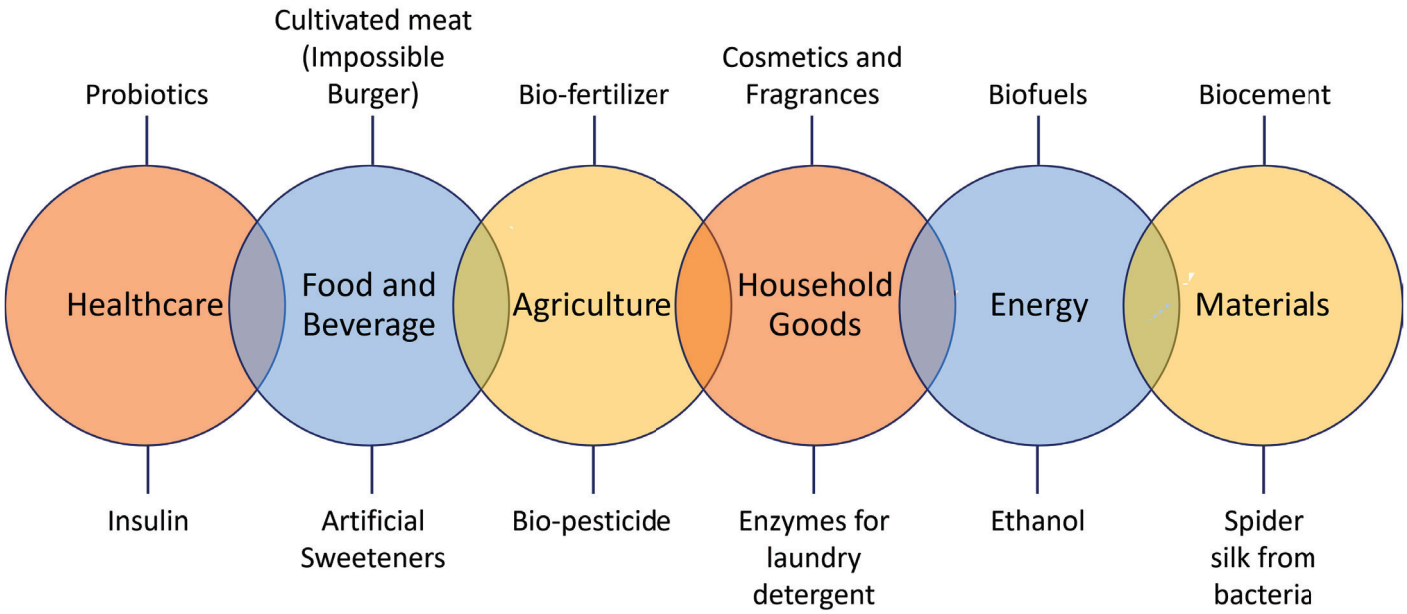
Chemical Synthesis

- 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



DNA Basics

Activity: DNA Isolation from Strawberries

Lab Objectives

- □ Isolate DNA from strawberries

Safety

PPE - safety glasses, nitrile gloves, lab coats
Do **NOT** eat any of the lab materials.

Materials

- □ ~3-5 whole strawberries (fresh or frozen)
- □ Ziploc bag
- □ Beaker
- □ Coffee filter
- □ Lysis buffer (water, soap, salt)
- □ Cold rubbing alcohol
- □ Wooden stirrer
- □ 1.5 mL microcentrifuge tubes

Procedure

1. Place 2-4 strawberries in the bag. Push as much air as possible out of the bag and seal the bag. ☐
2. Without puncturing the bag, smash the strawberries until mostly liquified. ☐
3. Add 10 ml of lysis buffer to the bag and mix by *gently* smushing the bag. ☐
4. Place a coffee filter in a beaker so that it can filter the liquid from the solid. ☐
5. Pour the contents of the bag into the filter (one partner might need to hold it). Wait for the liquid to drain through the filter. You can also *gently* squeeze the filter paper to help the liquid drain into the beaker. ☐
6. Discard the coffee filter and leftover solids and bring the liquid sample to the front. ☐
7. Slowly add about 30 mL of cold rubbing alcohol to the sample and let sit, undisturbed, for 1 minute. Observe the DNA precipitate from solution. ☐
8. Once the DNA has completely precipitated out, use a wooden skewer to *gently* swirl the DNA out of the conical tube and into the microfuge tube. ☐
9. Add about 500 uL of rubbing alcohol to cover the DNA in the microfuge tube. ☐
10. Dispose of the remaining strawberry extract in the sink. Throw away any trash. Return any glassware. ☐
11. Clean up your bench with a damp paper towel. ☐

Genetic Engineering

Bacterial Transformation

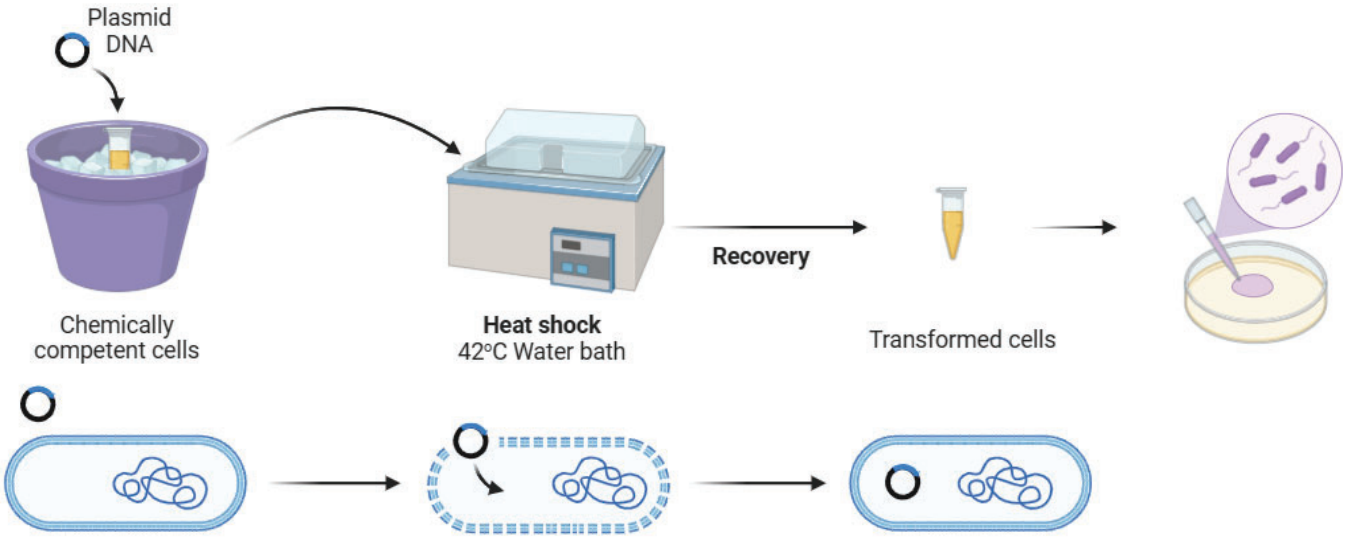
1. **Preparing Competent Cells** - You first need to make the cells ready to accept a plasmid. This typically entails treating the cells with certain salts. It's important to keep the cells cold and treat them gently

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.



Restiction Enzymes

Activity: Restriction Digest of a Plasmid

Lab Objectives

- □ Digest a plasmid using restriction enzymes
- □ Analyze the digest on a gel

Safety

PPE - safety glasses, nitrile gloves, lab coats, hot gloves

Materials

- □ Tube of pUC19 plasmid
- □ Tube of ScaI enzyme
- □ Tube of NdeI enzyme
- □ Tube of water
- □ Tube of CutSmart buffer
- □ Four PCR tubes
- □ Distilled water
- □ 20 µL tips
- □ 200 µL tips
- □ SeeGreen agarose tab
- □ 1X TBE buffer
- □ Tube of loading dye
- □ Tube of DNA ladder
- □ Marker

Equipment

- □ p20 micropipette
- □ p200 micropipette
- □ Water bath set at 37°C
- □ 250 mL Erlenmeyer flask
- □ Microwave
- □ blueGel electrophoresis system
- □ Beaker of ice
- □ Graduated cylinder

Procedure

1. Label your tubes: 1, 2, 3, and 4. Include your group initials. □

2. Add materials to each tube according to the table below. Mix well after each addition. Add enzymes last. Keep material tubes on ice when not using them. All tubes should have the same total volume. *Tip*: It can help to keep track by crossing each amount off as you add it.

	Tube 1	Tube 2	Tube 3	Tube 4
Water	38 µL	28 µL	28 µL	18 µL
CutSmart	10 µL	10 µL	10 µL	10 µL
pUC	2 µL	2 µL	2 µL	2 µL
ScaI	0 µL	0 µL	10 µL	10 µL
NdeI	0 µL	10 µL	0 µL	10 µL

□

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Restriction Digest

Procedure continued

3. Cap the tubes. Place in the floating rack in the water bath set at 37°C for at least 15 minuts. ☐

4. While the digest is incubating, pour a gel. This time, you'll be using a SeeGreen tab and water to create your gel. This tablet has TBE already in it, along with a dye that will help us visualize the DNA.
   - Add 30 mL distilled water to your Erlenmeyer flask.
   - Add a SeeGreen tab and allow to dissolve ☐
   - Place the flask into the microwave. Heat until the solution boils and no agarose particles remain. Expect to heat for about 60 seconds.
   - **Handle the hot flask with a hot glove.**
   - *Carefully* swirl the flask to check for particles

5. Set up your gel casting system with the 6-well comb in the center position. ☐

6. Pour the agarose solution into the prepared casting platform with a gel tray and comb. ☐

7. Allow gel to solidify completely. Once solid, remove the comb and place the plastic holder containing your gel into the blueGel box. ☐

8. Cover your gel in enough 1X TBE buffer to just cover the gel. Get buffer from a camp staff member. ☐

9. After your gel is ready to run, and your digest has been in the water bath for at least 15 minutes, remove the tubes from the water bath. Add 10 µL loading dye to each tube. Pipette up and down multiple times to mix well. ☐

10. Load your gel according to the following: ☐
    - Lane 1: 10 µl Ladder
    - Lane 2: 10 µl Tube 1
    - Lane 3: 10 µl Tube 2
    - Lane 4: 10 µl Tube 3
    - Lane 5: 10 µl Tube 4

11. Place cover on and turn on your gel electrophoresis system ☐

12. Conduct electrophoresis for 15-25 minutes.
    - You can place the black cover over the gel and watch the DNA move down the gel and separate using your eyes or a phone. ☐

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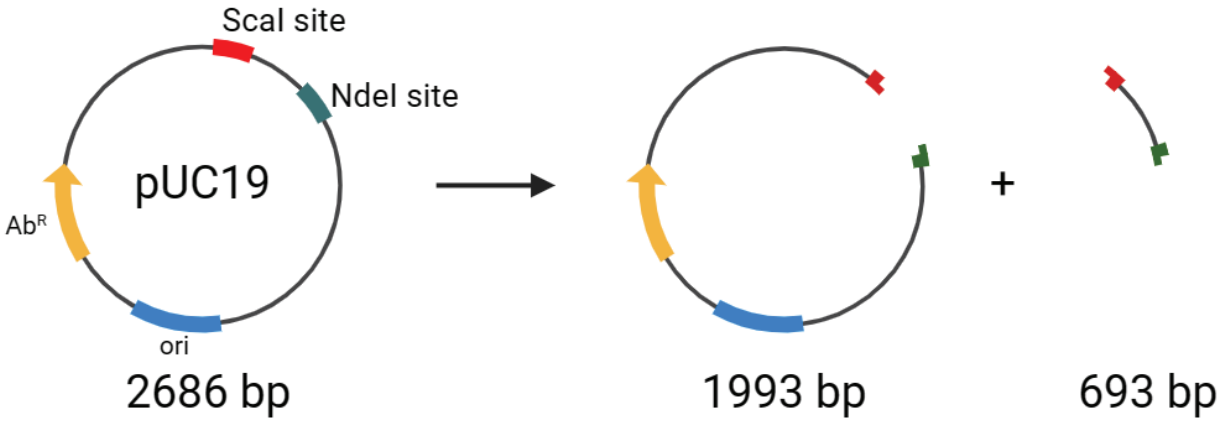
Restriction Digest

Procedure continued



Self-Reflection: While your gel runs, think about what your results will look like. How many bands do you expect in each lane? Why?

Observation: Draw what your gel looks like below. The ladder has been predrawn into Lane 1. Are the results what you expected?

Chromoprotein Production

Activity: Scale Up Growth of Bacteria Expressing Chromopreotein

Lab Objectives

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

Safety

PPE - safety glasses, nitrile gloves, lab coats

Materials

- □ Transformed chromoprotein plates
- □ Yellow inoculation loops
- □ Marker
- □ Plastic cuvettes
- □ Tube of LB/Amp media
- □ 70% Ethanol
- □ 1000 µL tips

Equipment

- □ Shaking incubator set at 37°C
- □ UV Spectrometer
- □ p1000 micropipette
- □ Waste beaker

Procedure

1. Sterilize your bench with 70% ethanol. ☐

2. Label your tube of media with your group initials. ☐

3. Examine your transformation plates. Identify one **pink or purple** colony from your LB/Amp/IPTG + DNA plate that you would like to use for future steps. Blue colonies will not purify correctly with our method. ☐

4. Using a sterile loop, pick the transformed colony you selected from your agar plate and transfer it into the culture tube. Swirl the loop to ensure the colony dissolves fully. ☐

5. Using a p1000 micropipette, mix the culture gently by pipetting up and down until it is well mixed. Using the same tip, add 1000 µL (1 mL) of culture to a clean plastic cuvette. ☐

6. Measure the Abs at 600 nm using the spectrometer. Camp staff will use plain LB/Amp media to blank the instrument. You will track as the absorbance goes up over time, meaning the culture is growing! Write your absorbance value here: _____ ☐

7. Empty the cuvette back into the culture tube. Cap the tube and place it in the shaking incubator until the afternoon. ☐

8. Empty your waste beaker into the large biohaz box. Spray the beaker and wipe down your bench with 70% ethanol. ☐

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Chromoprotein Production

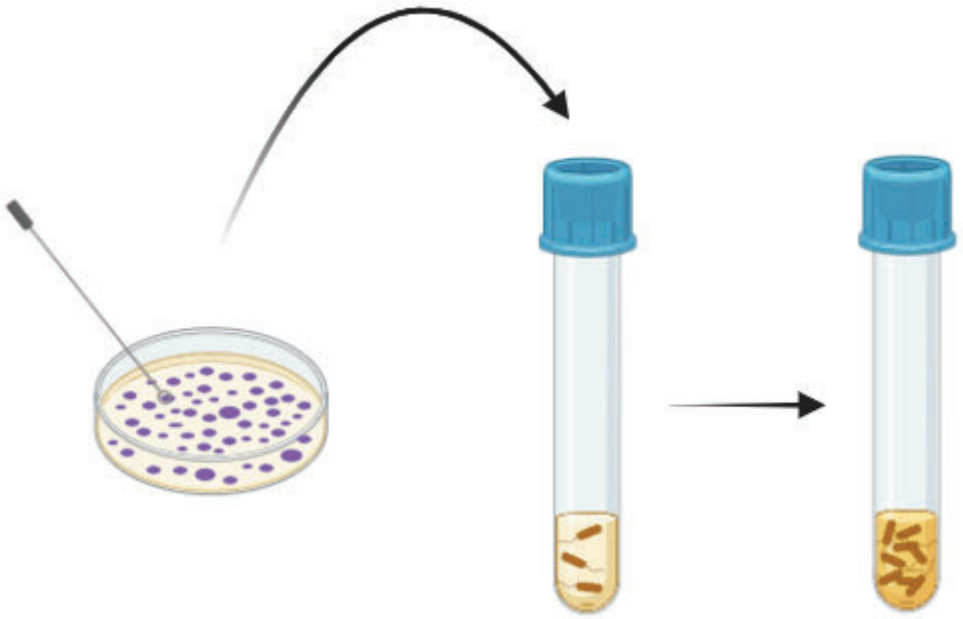
Procedure continued

9. Towards the end of the day, repeat steps 5 and 6. New absorbance value: _____ ☐

Self-Reflection: Is your culture growing? How do you know? ☐

10. Empty the cuvette back into the culture tube. Cap the tube and place it in the shaking incubator overnight. ☐

11. Empty your waste beaker into the large biohaz box. Spray the beaker and wipe down your bench with 70% ethanol. ☐



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
- □ Toothpicks
- □ Yellow or blue inoculation loops
- □ Cotton Swabs
- □ (optional) your chromoprotein plates
- □ (optional) LB/Amp plates

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 (blue line) 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. If you want to create agar art with your transformed chromoprotein plates, you need use a different LB/Amp plate. ☐
6. 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. ☐
7. Empty your waste beaker into the large biohaz box. Spray the beaker and wipe down your bench with 70% ethanol. ☐

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

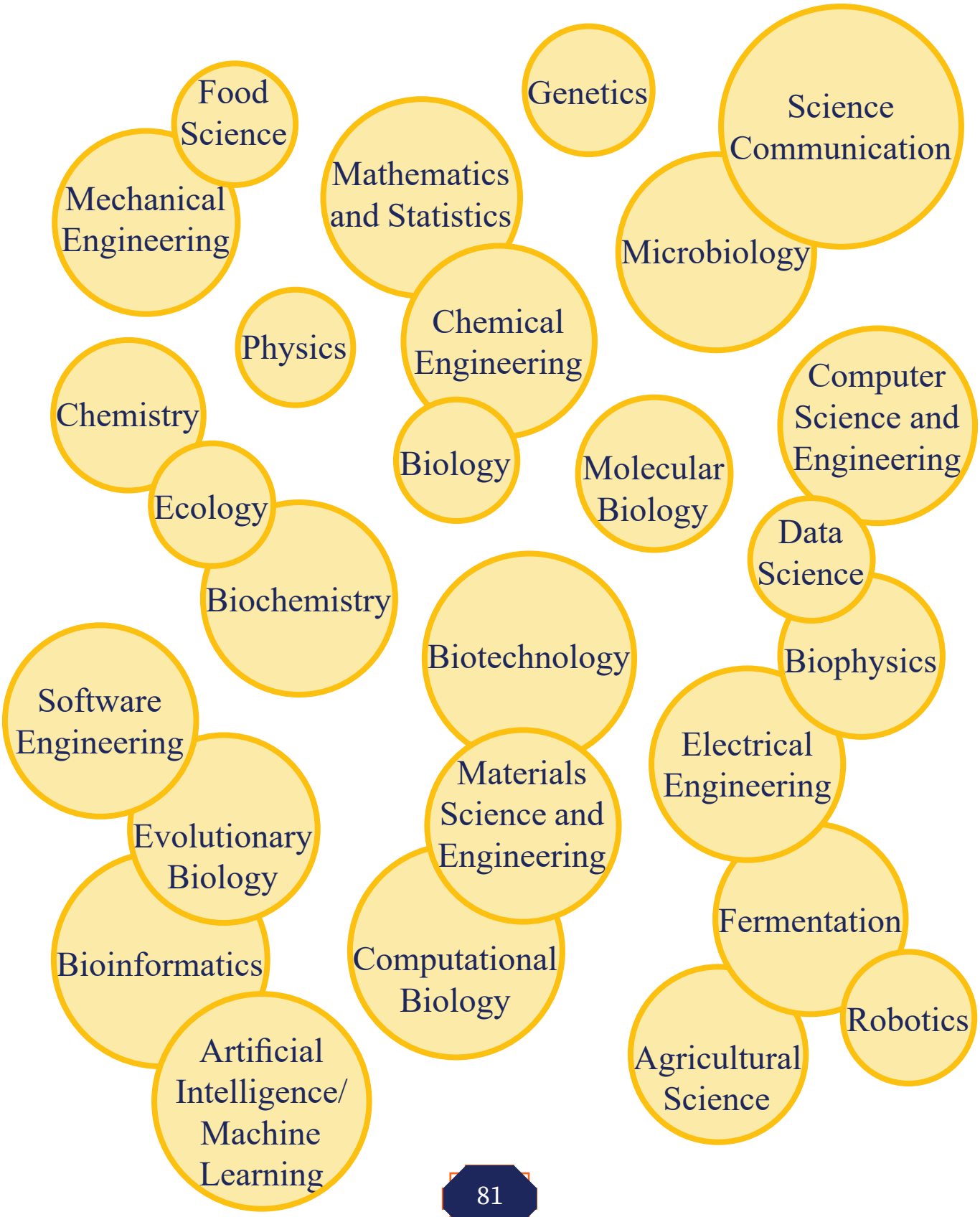
Types of STEM roles in biomanufacturing

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