The Dayton bioManufacturing Awareness and Discovery Experience (DaytonMADE) curriculum and schedule are set up to mimic the biomanufacturing process and covers many general biotechnology techniques that could be applicable in other fields, although presented through a biomanufacturing lens. The Explorers camp covers general techniques and concepts and is designed for students with no experience with biotechnology. The Trailblazers camp dives a bit deeper into biomanufacturing concepts and more advanced techniques and experiences and is designed for students with some prior biotechnology experience, such as a previous Explorers participant or a biotechnology CTE student. Both programs also include industry and military guest speakers, exposure to various higher education programs (including a campus tour and "College 101"), an industry field trip, a soft skills workshop, and a final presentation open house.

This program curriculum was designed specifically for the 2024 DaytonMADE program. The schedule for future programs will continue to evolve and morph based on optimizing activities, new activities, different guest speakers or non-lab sessions, and feedback from students. Many activities utilize kits that come with most reagents and materials required for the activity (refer to their manual), but we modified the protocols and materials lists for use in a lab setting with micropipettes. Please modify the curriculum accordingly to your setup and resources.

Powerpoints and other resources can be found at www.ues.com/dminfo.

Some curriculum notes for further optimization:

- Each camp used different GFP production/purification kits. The version used in the Trailblazers camp was slightly more basic, so perhaps switching these could be more appropriate.
- In all cases of protein purification, there was only one visible component. Ideally, these protocols could be optimized with multiple visible components.

Wright State University provided lab space and classroom space. Ideally these locations would be very near each other to facilitate easy back and forth movement. In our case, they were in neighboring buildings with a ~5 minute walk. Therefore, we tried to minimize transit back and forth.

The lab space was outfitted by Wright State University with equipment such as p20, p200, and p1000 micropipettes, pipette aids, glassware, hot plates, vortexes, centrifuges, tube racks, a plate incubator, a shaking incubator, a thermocycler, a microwave, water baths, and UV spectrometers. There was also access to an autoclave and ice machine. We supplied gel electrophoresis equipment (blueGel kits by miniPCR bio).

DaytonMADE was hosted by BlueHalo, with funding from BioMADE. Partners included BioPharmaceutical Technology Center Institute (BTCI), and additional support was provided by Wright State University. We also thank Primient, Miami University faculty and students, and our numerous guest speakers for their support and for enhancing the program through their participation.

Protocols, activities, and teaching materials were either created or adapted by DaytonMADE staff from the following sources: Edvotek, Bio-Rad, Minipcr, Amino Labs, Carolina Biological Supply Company, BTCI, Shoreline Community College, Miami University faculty, and North Carolina Association for Biomedical Research (NCABR). ChatGPT was also used for inspiration. For commercial kits, we suggest reading the provided manuals.

Any questions about the program can be addressed to DaytonMADE@bluehalo.com, or you can visit www.ues.com/daytonmade.

Supplies not listed in the following pages include:

- Safety items:
  - Safety glasses
  - Lab coats
  - Nitrile gloves
  - Hot gloves
  - Biohazard waste containers and bags
  - Bleach
  - Hand soap
  - Dish soap and cleaning supplies
- Supplies related to lab manuals:
  - Binders
  - Writing utensils
  - Custom puzzles and time fillers
  - Bag for transport
- Custom t-shirts
- Paper towels

Table of Contents

Explorers 2024

Day 1

TIME	Classroom	Lab
9:00 AM	Ice breakers, camp overview, pre-assessment, word cloud Intro to biomanufacturing Lab practices, safety, tools	
9:30 AM		
10:00 AM		
10:30 AM		
11:00 AM		
11:30 AM		Get lab coats and benches
12:00 PM	lunch	
12:30 PM		
1:00 PM		Pour plates, streak unknown samples Pipetting
1:30 PM		
2:00 PM		
2:30 PM		
3:00 PM		
3:30 PM	Reflection	

Day 2

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Microbiology - basic organisms as tools discussion	
10:00 AM	Macromolecules, central dogma, what is DNA	
10:30 AM	DNA models	
11:00 AM		Strawberry DNA
11:30 AM		
12:00 PM	lunch	
12:30 PM		
1:00 PM	Intro to genetic engineering/plasmids Tangles activity	
1:30 PM		
2:00 PM		pGlo transformation During downtine, tour research building Practice loading gels
2:30 PM		
3:00 PM		
3:30 PM	Reflection	

Day 3

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Electrophoresis - intro and safety	
10:00 AM		Electrophoresis of food colors
10:30 AM		
11:00 AM	Soft skills session	
11:30 AM		
12:00 PM	lunch. Guest speaker	
12:30 PM		
1:00 PM	Intro to restriction enzymes/DNA digestion	
1:30 PM		RE Digestion of pUC19 Run digest on gel Check GFP plates, start cultures
2:00 PM		
2:30 PM		
3:00 PM		
3:30 PM	Reflection	

Day 4

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Downstream processing intro and demo	
10:00 AM		GFP purification
10:30 AM		
11:00 AM		
11:30 AM		
12:00 PM	lunch Chalk talk about degrees and education levels	
12:30 PM		
1:00 PM	WSU campus tour	
1:30 PM		
2:00 PM	Biology and art/fashion	
2:30 PM		Tie-dye shirts
3:00 PM		
3:30 PM	Reflection	

Day 5

TIME	Classroom	Lab
9:00 AM	Ice breaker and plan for the day	
9:30 AM	Intro to biomaterials	
10:00 AM		
10:30 AM		Bioplastics
11:00 AM		
11:30 AM		
12:00 PM	lunch Guest speaker	
12:30 PM		
1:00 PM		Finish bioplastics
1:30 PM		
2:00 PM	Guest speaker	
2:30 PM		Finish bioplastics
3:00 PM		
3:30 PM	Reflection	

Day 6

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Miami Univ	
10:00 AM		
10:30 AM		
11:00 AM		
11:30 AM		
12:00 PM	lunch	
12:30 PM		
1:00 PM	Genetic engineering review - introduce chromoprotein	
1:30 PM		Discuss bioplastics and checkerspot results
2:00 PM		Chromoprotein transformation
2:30 PM		
3:00 PM		
3:30 PM	Reflection	

Day 7

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM		Chromoprotein scale-up
10:00 AM		Agar art
10:30 AM	During downtime, did snowman and rock paper scissors tournament	
11:00 AM	Guest speaker	
11:30 AM	lunch	
12:00 PM		
12:30 PM		
1:00 PM	College 101	
1:30 PM		
2:00 PM		Pollinator Kit
2:30 PM		
3:00 PM	Reflection - intro to primient and safety reminders	Poster topic discussion
3:30 PM		

Day 8

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Primient	
10:00 AM		
10:30 AM		
11:00 AM		
11:30 AM		
12:00 PM		
12:30 PM		
1:00 PM		
1:30 PM		
2:00 PM		Check agar art plates Chromoprotein induction Poster prep
2:30 PM		
3:00 PM		
3:30 PM	Reflection	

Day 9

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Chromatography demo	
10:00 AM		Chromoprotein purification
10:30 AM		
11:00 AM		
11:30 AM		
12:00 PM	lunch Guest speaker	
12:30 PM		
1:00 PM	Careers talk part 1	
1:30 PM		
2:00 PM		Poster prep
2:30 PM		
3:00 PM		
3:30 PM	Reflection	

Day 10

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Sinclair CC Admissions	
10:00 AM		
10:30 AM	Careers talk part 2	
11:00 AM		
11:30 AM		
12:00 PM	lunch Post-assessment, word cloud, feedback	
12:30 PM		
1:00 PM		Poster set up and practice
1:30 PM		
2:00 PM		Demo day
2:30 PM		
3:00 PM	Closing ceremony	
3:30 PM		

Trailblazers 2024

Day 1

TIME	Classroom	Lab
9:00 AM	Ice breakers, camp overview, pre-assessment, word cloud	
9:30 AM		
10:00 AM	Intro to biomanufacturing	
10:30 AM	Lab practices, safety, tools	
11:00 AM		
11:30 AM		Get lab coats and benches
12:00 PM	lunch	
12:30 PM		
1:00 PM		Pour plates, streak unknown samples
1:30 PM		
2:00 PM		Pipetting
2:30 PM	Intro to Enzymes	Pectinase - apple juice production
3:00 PM		Graph data
3:30 PM	Reflection/week ahead	

Day 2

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM		
10:00 AM	Microbiology - organisms as tools and factories discussion	
10:30 AM	Yeast balloon demo	
11:00 AM	Yeast/ethanol/biofuels intro	
11:30 AM		Ethanol production - Start yeast cultures
12:00 PM	lunch. Guest speaker	
12:30 PM		
1:00 PM	Macromolecules, central dogma ("how do cells make things?")	
1:30 PM	Intro to genetic eng. Tangles activity	
2:00 PM		GFP Transformation
2:30 PM		Tour research facility during downtime
3:00 PM		Practice pipetting in downtime
3:30 PM	Reflection	

Day 3

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Biomanufacturing process, especially upstream processing	
10:00 AM		Check GFP transformations, scale up
10:30 AM		Ethanol production - take 24hr sample
11:00 AM	Soft skills session	
11:30 AM		
12:00 PM	lunch Chalk talk about degrees and education levels	
12:30 PM		
1:00 PM	Biology and art/fashion	
1:30 PM		Agar art
2:00 PM		
2:30 PM	WSU campus tour	
3:00 PM		
3:30 PM	Reflection	

Day 4

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Downstream processing intro and demo	
10:00 AM		
10:30 AM		GFP purification
11:00 AM		SDS page (intro concept in lab)
11:30 AM		
12:00 PM	lunch Guest speaker	
12:30 PM		
1:00 PM	Discuss residual sugar test and standard curves	
1:30 PM		Ethanol production - take 48hr sample
2:00 PM		Residual sugar test with standard curves
2:30 PM		
3:00 PM	Plot and compare groups/variables	
3:30 PM	Reflection	

Day 5

TIME	Classroom	Lab
9:00 AM	Ice breaker and plan for the day	
9:30 AM	Intro to biomaterials	
10:00 AM		
10:30 AM		Bioplastics
11:00 AM		
11:30 AM		
12:00 PM	lunch Guest speaker	
12:30 PM		
1:00 PM		Finish bioplastics
1:30 PM		
2:00 PM	Guest speaker	
2:30 PM		Finish bioplastics
3:00 PM		
3:30 PM	Reflection	

Day 6

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Review genetic eng. Intro enzyme production experiment, PCR, and gel electrophoresis	
10:00 AM		
10:30 AM		Enzyme production: PCR Pour gels; practice loading Bioplastics analysis
11:00 AM		
11:30 AM	Intro SOPs and PB&J activity	
12:00 PM	lunch Guest speaker	
12:30 PM		
1:00 PM	Intro RE digestion	
1:30 PM		Enzyme production: purify and digest insert
2:00 PM		
2:30 PM		Run gel during digest
3:00 PM		Talk about poster topics
3:30 PM	Reflection	

Day 7

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Intro ligation and review transformation	
10:00 AM		Enzyme production: ligation and transformation
10:30 AM		
11:00 AM		
11:30 AM		
12:00 PM	lunch Guest speaker	
12:30 PM		
1:00 PM	Biomanufacturing process review - quality control, formulation, and packaging	
1:30 PM		
2:00 PM	Mock facility activity	
2:30 PM	Guest speaker	
3:00 PM		Pollinator Kit
3:30 PM	Reflection	

Day 8

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	Enzyme production: scale-up
9:30 AM	Primient	
10:00 AM		
10:30 AM		
11:00 AM		
11:30 AM		
12:00 PM		
12:30 PM		
1:00 PM		
1:30 PM	Review inducers, discuss substrate/reaction	
2:00 PM		Enzyme production: induction
2:30 PM		Poster prep
3:00 PM		
3:30 PM	Reflection	

Day 9

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	College 101	
10:00 AM		
10:30 AM		Enzyme production: observations
11:00 AM		
11:30 AM		
12:00 PM	lunch Guest speaker	
12:30 PM		
1:00 PM	Careers talk part 1	
1:30 PM		
2:00 PM		Poster prep
2:30 PM		
3:00 PM		
3:30 PM	Reflection	

Day 10

TIME	Classroom	Lab
9:00 AM	Ice breaker and review, plan for the day	
9:30 AM	Sinclair CC Admissions	
10:00 AM		
10:30 AM	Careers talk part 2	
11:00 AM		
11:30 AM		
12:00 PM	lunch Post-assessment, word cloud, feedback	
12:30 PM		
1:00 PM		Poster set up and practice
1:30 PM		
2:00 PM		Demo day
2:30 PM		
3:00 PM	Closing ceremony	
3:30 PM		

DaytonMADE Explorers	
Topic:	**Introduction**
Program Schedule:	Day 1
Last Updated:	Sept 2024

Activities

- Introduction to Program powerpoint
- Ice breakers – This or That and Marble Run
- Pre-camp survey
- Word association

Supplies

Introductions, surveys, word association:

- Paper
- Writing utensils
- Name tags
- Markers

Ice breaker "Marble run":

- PVC pipe, cut into ~18" pieces – 1 per student
- Marbles – 1 per group of ~6-8 students

Preparation:

- Prepare powerpoint with program overview and staff introductions
- Print pre-camp survey
- Cut PVC pipe

Agenda/protocol:

1. Introduction powerpoint
    a. Fill out name tags
    b. Introduce staff
    c. Fill out pre-camp survey
    d. Word association
        i. Take 2-3 minutes for students to write down what they think of when they see "Biomanufacturing" and "Biotechnology"
    e. Program overview, including brief biomanufacturing introduction
2. Ice breaker: This or That
    a. Show two options on the screen and have students and staff choose one by moving to the corresponding side of the room. Initiate discussion amongst the group.
3. Ice breaker: Marble run
    a. Break up into groups of 6-8 students. Staff can partake to increase connection with students, but be a follower, not a leader.
    b. Give each student a piece of pipe.
    c. Allow the groups to practice moving the marble from one point to another using only the pipes. No touching the marble. If the marble falls to the ground you must start over. The marble can't stop moving.
    d. After the groups get some practice is, have a race between the groups.
    e. Discuss what strategies worked or didn't work. Connect the soft skills used here to working in a lab.
4. Deeper introduction to biomanufacturing and the biomanufacturing process

Resources:

- DaytonMADE powerpoint "2024Explorers Day 1 Part 1"

Notes:

• Based on student feedback, they wanted more overview and understanding of the schedule for the program.
• Establish expectations early
  ○ Build a good rapport between staff and students
  ○ Establish a safe space that they can learn, explore, and ask questions – even if this ends up not being the field for them
  ○ Respect for staff, other students, equipment, facilities, and guest speakers
  ○ Safety

DaytonMADE Explorers	
Topic:	**Lab Safety, Tools, and Equipment**
Program Schedule:	Day 1
Last Updated:	Sept 2024

Activities

- Powerpoint and interactive discussion about lab safety, tools, and equipment

Preparation:

- Prepare powerpoint with site-specific hazards and procedures

Agenda/protocol:

1. Lab safety powerpoint
    a. Group discussion having students fill in a DOs and DON'Ts table for safety in a lab setting
    b. Go over specific hazards, safety, PPE, cleaning procedures, and a full DOs and DON'Ts
2. Lab tools and equipment powerpoint
    a. Discuss the types of items the students will be using, including photos so they know what to look for in lab
3. Move to the lab
    a. Introduction to the laboratory space, general protocols and safety highlights
    b. Pass out lab coats and have students pick bench space and partners

Resources:

- DaytonMADE powerpoint "2024Explorers Day 1 Part 2"

Notes:

- Establish clear expectations early and often regarding safety, such as the use of PPE, appropriate attire, use of headphones/pods, and cleaning hands.

DaytonMADE Explorers	
Topic:	**Microbiology and Culturing Microbes**
Program Schedule:	Day 1 (Observations on Day 2)
Last Updated:	Sept 2024

Activities

- Pour agar plates
- Streak unknown samples

Supplies (per group)

Materials:

- Powdered LB agar medium (e.g., Bio-Rad 1660600EDU)
- Weigh boats and spatulas
- Sterile water
- Foil
- Small petri dishes (2 per person; 4 per bench) (e.g., Bio-Rad 1660469EDU)
- Sterile swabs (e.g., Amazon B08111ZV73)
- Lab tape (e.g., Amazon B0889LYH61)
- 70% Ethanol

Equipment:

- Balance
- Hot Plate
- Sterile graduated cylinder
- Sterile 250mL Erlenmeyer flask
- Incubator set at 37°C

Preparation:

- Pre-autoclave flasks and graduated cylinders if possible. Sterilize with 70% ethanol if not.
- Set incubator temperature

Agenda/protocol:

Student Protocol (Day 1):

1. Clean and sterilize your benchtop with 70% ethanol.	☐
2. Calculate the volume of agar needed to make 4 small agar plates. a. 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. a. _____ 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. a. **CAUTION:** Agar will boil over quickly. Use heat & spin settings at 40-50%.	☐
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 tape 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.	☐

Day 2:

1. Observe growth and discuss.
2. Discard plates into biohazard waste.

Notes:

- Don’t have student swab the bottoms of their shoes or inside their mouths.
- Tape the plates closed and don’t open them after incubation.
- Unfortunately, there wasn’t much growth and/or lack of diversity on most of the plates in our experience.

DaytonMADE Explorers	
Topic:	**Micropipetting**
Program Schedule:	Day 1
Last Updated:	Sept 2024

Activities

- Measure and transfer volumes using a micropipette
- Create a piece of pipet art

Supplies (per group)

Materials:
Most materials are from MiniPCR Micropipetting 101 (KT-1510-10) and Micropipetting Art: Full STEAM Ahead (KT-1510-21)
Square and 96-well plate patterns from Bio-Rad: https://www.bio-rad.com/sites/default/files/2022-10/Micropipetting-Practice-Sheets_2022.pptx and https://www.bio-rad.com/webroot/web/pdf/lse/literature/BE14-Pipet-Act.pdf

- Red, yellow, and blue dyed water
- 20 µL tips
- 200 µL tips
- 1000 µL tips
- Practice Pipetting Cards
- Micropipette Art Cards or parafilm
- Pipet art stencils
- 1.5 mL microcentrifuge tubes

Equipment:

- p20 micropipette
- p100 micropipette
- p1000 micropipette

Preparation:

- Aliquot dye as needed
- Print practice art stencils

Agenda/protocol:

Student procedure:
Refer to MiniPCR and Bio-Rad manuals and resources for specific activity protocols and modify as needed for your setting.

An additional activity for students to gain practice in using different size micropipettes is below. We found this to be a useful exercise for catching errors, and it also facilitated discussion about things to look out for when pipetting. For example, if you are at the larger end of the volume range, the tip should be mostly full.

1. Set up 9 1.5 mL microcentrifuge tubes in a rack.
2. In the first 3 tubes, use the p20 micropipette and 20 µL tips to pipet the following volumes (1 per tube)
    a. 2 µL
    b. 10 µL
    c. 20 µL

3. In the next 3 tubes, use the p200 micropipette and 200 µL tips to pipet the following volumes (1 per tube)
    a. 20 µL
    b. 100 µL
    c. 200 µL
4. In the next 3 tubes, use the p1000 micropipette and 1000 µL tips to pipet the following volumes (1 per tube)
    a. 200 µL
    b. 500 µL
    c. 1000 µL

Self-reflection: How do the volumes in the different tubes compare visually? When you pipetted the same volume with different pipettes, did the final amount look the same?

_____
_____
_____
_____

Resources:

MiniPCR and Bio-Rad manuals and resources

Notes:

- We kept design stencils out throughout the camp and some students would use that activity to fill time.
- Some students had success letting the final art dry some and then transferred the design to a paper towel to keep.
- An additional optional activity we did throughout the camps was an accuracy competition with pipetting water into a weigh boat on a balance.

DaytonMADE Explorers	
Topic:	**Molecular Biology and DNA**
Program Schedule:	Day 2
Last Updated:	Sept 2024

Activities

- Introduction to microbes and using microbes and organisms as tools
- Review of macromolecules, central dogma, and DNA
- Make DNA models
- DNA isolation from strawberries

Supplies (per group)

Materials:
DNA models

- Beads (pony beads or large wooden beads)
- Pipe cleaners
- Scissors

Strawberry DNA extraction

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

Equipment:

- Beaker or cup
- 50 mL graduated cylinder

Preparation:

- Prepare lysis buffer solution ahead of time

Agenda/protocol:

1. Powerpoint introduction to microbes and the concept of using microbes and organisms as tools
2. Review of macromolecules of life, Central Dogma, and DNA (function and structure)
3. Classroom activity: Making DNA models
    a. Supply beads and pipe cleaners for students to make their own model of DNA. Provide examples. Students may use specific colors to represent the different parts of DNA (i.e., sugar, phosphate, bases ATGC), or just follow the general double stranded, double helix structure.
4. Lab activity: Extracting DNA from strawberries

Student 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.	☐

Resources:

- DaytonMADE powerpoint “2024Explorers Day 2”

DaytonMADE Explorers	
Topic:	**Genetic Engineering**
Program Schedule:	Day 2 (related to Scale-up Day 3 and Downstream Purification Day 4)
Last Updated:	Sept 2024

Activities

- Introduction to engineering organisms/genetic engineering and plasmids
- "Build a plasmid" with Tangles
- Green Fluorescent Protein (GFP) transformation (Day 2)
    - Next steps: scale up (refer to Day 3) and purification (refer to Day 4)

Supplies (per group)

Materials:
Build a plasmid

- Tangles (6 colors): 7, 1, 1, 2, 2, and 2 links for each color, respectively, per group/demo
    - E.g., Amazon B0CSFZVHBM
- Small ziplock bags

GFP transformation (pGlo Bacterial Transformation Kit; Bio-Rad 17006991EDU)

- 20 µL tips
- 200 µL tips
- 1000 µL tips
- *E. coli* source plate
- Yellow inoculation loops (at least 6)
- Tube of transformation solution (TS)
- Two sterile 1.5 mL microcentrifuge tubes
- Tube of pGlo plasmid
- Four prepoured petri dishes
- Marker
- Tube of LB broth
- 70% Ethanol

Equipment:

- p20 micropipette
- p200 micropipette
- p1000 micropipette
- Beaker of ice
- Water bath set at 37°C
- Water bath set at 42°C
- Incubator set at 37°C
- Waste beaker

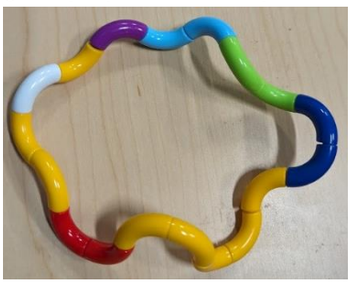
Preparation:

- Prepare bags of Tangles
- Refer to Bio-Rad pGlo kit manual
    - Pouring plates and making aliquots
    - Warming incubator and water baths
    - *E. coli* source plate must be prepared the previous day

Agenda/protocol:

1. Powerpoint introduction to genetic engineering and plasmids
2. Classroom activity: Build a plasmid
    a. Supply groups of 2 with a bag of links. Use one bag of links for the demo.

b. Walk through each step of the plasmid with the students, explaining the purpose of each part.
    i. Build a blank backbone (7 links, yellow)
    ii. Add origin of replication (1 link, white)
    iii. Add antibiotic marker (2 links, red)
    iv. Add promoter (1 link, purple)
    v. Add Multiple Cloning Sites (2 links each, light and dark blue)
    vi. Add gene (2 links, green)



3. Lab activity: GFP transformation

Student procedure:

Refer to Bio-Rad manual and modify as needed for your setting. Include group discussion about transformation predictions (amount and color of bacteria):

- pGlo		+ pGlo	
LB Only	**LB/Amp**	**LB/Amp**	**LB/Amp/Ara**

Resources:

- DaytonMADE powerpoint "2024Explorers Day 2"
- Bio-Rad pGlo Bacterial Transformation Kit manual

Notes:

- The Tangles activity went over really well with the Explorers group and was effective at teaching the parts of a plasmid. The activity was more neutral with the Trailblazers. There were many complaints about the links being hard to work with.
    - Utilize the completed plasmid throughout the program to reinforce the parts of a plasmid and have a visual aid for making and testing hypotheses (e.g., growth after transformation, running a digested plasmid on a gel, etc.)
- Go through predictions as a group to enforce concepts of selection and induction.
- During the incubation after heat shocking (outgrowth/rescue time), we took the students on a tour of a research building. We were able to discuss similarities and differences with their experience and "real-life", undergraduate research, poster presentations, etc
- Also during downtime during the transformation protocol, we used practice gels (e.g., minipcr KT-1510-13; also come in the Micropipetting 101 kit KT-1510-10) to practice loading gels for the following day. We used dye and loading the gel both dry and submerged in water.

DaytonMADE Explorers	
Topic:	**Gel Electrophoresis**
Program Schedule:	Day 3
Last Updated:	Sept 2024

Activities

- Introduction to gel electrophoresis
- Make, load, run, and analyze an agarose gel using dyes

Supplies (per group)

Materials:
Most materials are included in MiniPCR Dye Electrophoresis Lab: Molecular Rainbow (KT-1400-01)

- Six tubes of colored dyes
- 30 mL 1X TBE buffer
- Agarose tab
- 20 µL tips
- Additional 1X TBE buffer to cover gel

Equipment:

- p20 micropipette
- blueGel electrophoresis system (e.g., MiniPCR classroom bundle QP-1500-28/QP-1500-28-INT)
- Microwave
- 250 mL Erlenmeyer flask

Preparation:

- Prepare TBE buffer
- Make aliquots of rainbow dyes

Agenda/protocol:

1. Powerpoint introduction to gel electrophoresis
2. Lab activity: Make, load, run, and analyze an agarose gel using dyes

Student procedure:
Refer to MiniPCR manual for specific protocol and modify as needed for your setting.

Resources:

- DaytonMADE powerpoint “2024Explorers Day 2”
- MiniPCR manuals

DaytonMADE Explorers	
Topic:	**Soft Skills Session**
Program Schedule:	Day 3
Last Updated:	Sept 2024

Activities
• Learn about the 4 Cs of success
Agenda/protocol:
The WSU Student Success Center provided a one-hour session on the 4 Cs of success – Communication, Creativity, Collaboration, and Critical Thinking. The main activity involved splitting the group into teams of 3-4 students. There was a hidden lego structure that the teams had to reproduce, but only one team member could look at the structure at a time for up to 5 seconds. The activity worked well to get the students to interact with each other and work on communication skills.

DaytonMADE Explorers	
Topic:	**Restriction Enzyme Digest**
Program Schedule:	Day 3
Last Updated:	Sept 2024

Activities

- Introduction to restriction enzymes
- Digest a plasmid using restriction enzymes
- Analyze the digest on an agarose gel

Supplies (per group)

Materials:

- Tube of pUC19 plasmid (e.g., NEB N3041S)
- Tube of ScaI enzyme (e.g., NEB R3122S)
- Tube of NdeI enzyme (e.g., NEB R0111S)
- Tube of CutSmart buffer (comes with NEB enzymes)
- Tube of loading dye (comes with NEB enzymes)
- Tube of nuclease-free water
- Four PCR tubes
- Distilled water
- 20 µL tips
- 200 µL tips
- SeeGreen agarose tab (e.g., MiniPCR RG-1500-20)
- 1X TBE buffer (e.g., MiniPCR RG-1502-05)
- Tube of DNA ladder (e.g., MiniPCR RG-1002-01)
- Marker

Equipment:

- p20 micropipette
- p200 micropipette
- Water bath set at 37°C
- 250 mL Erlenmeyer flask
- Microwave
- blueGel electrophoresis system (e.g., MiniPCR classroom bundle QP-1500-28/QP-1500-28-INT)
- Beaker of ice
- Graduated cylinder

Preparation:

- Prepare TBE buffer if needed and prepare aliquots

Agenda/protocol:

1. Powerpoint introduction to restriction enzymes and DNA digestion
2. Lab activity: Digest a plasmid and analyze the results on an agarose gel

Student 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

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

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. ☐
    a. Add 30 mL distilled water to your Erlenmeyer flask.
    a. Add a SeeGreen tab and allow to dissolve
    b. Place the flask into the microwave. Heat until the solution boils and no agarose particles remain. Expect to heat for about 60 seconds.
    c. **Handle the hot flask with a hot glove.**
    d. *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. ☐

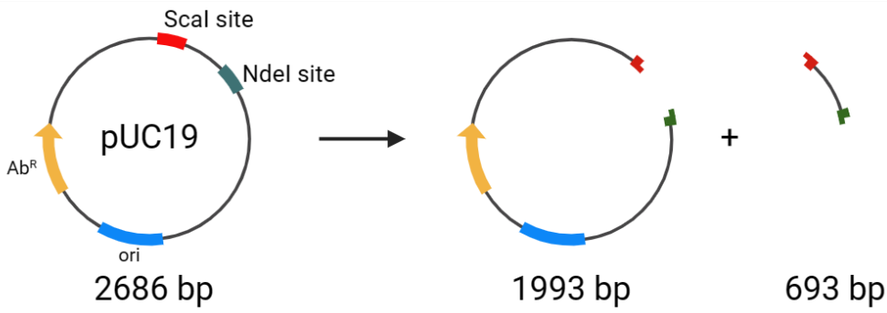
8. Cover your gel in enough 1X TBE buffer to just cover the gel. Get buffer from a camp staff member. ☐

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: ☐
    a. Lane 1: 10 µl Ladder
    b. Lane 2: 10 µl Tube 1
    c. Lane 3: 10 µl Tube 2
    d. Lane 4: 10 µl Tube 3
    e. 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. ☐



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?

_____
_____
_____

Resources:

- DaytonMADE powerpoint "2024Explorers Day 3"
- MiniPCR manual for using SeeGreen tablets and blueGel box

Notes:

- As opposed to other DNA digestion activities like those using lambda phage, we designed this activity to mimic the cloning process for expressing a gene of interest for an application like biomanufacturing.
- We tested the digest with 3 different enzymes individually and pairwise (SacI, NdeI, and BamHI). Using BamHI didn't result in a successful digestion. Additionally, during testing using a different gel electrophoresis set up, the undigested plasmid ran faster/"smaller" than the digested plasmid; however, when the students did the activity, the undigested plasmid ran slower/"larger". The Tangle plasmid served as a visual aid to discuss how plasmids can fold, superfolding, and the idea that experiments work differently sometimes and what can cause that.

DaytonMADE Explorers	
Topic:	**Genetic Engineering - continued**
Program Schedule:	Day 3 (related to Genetic Engineering Day 2 and Downstream Processing Day 4)
Last Updated:	Sept 2024

Activities
• Observe bacterial transformation results
Supplies (per group)
Equipment: • UV light (e.g., Bio-Rad 1660530EDU)
Agenda/protocol:
1. Lab activity: Observe GFP transformations a. Use one group's plates to make observations as a group and compare to hypotheses. b. Allow groups to observe their plates c. Continue onto scale up procedure (next page) Refer to Bio-Rad manual for worksheets and discussion questions.
Resources:
Bio-Rad pGlo Bacterial Transformation Kit manual

DaytonMADE Explorers	
Topic:	**Scale-up**
Program Schedule:	Day 3 (related to Genetic Engineering Day 2 and Downstream Processing Day 4)
Last Updated:	Sept 2024

Activities

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

Supplies (per group)

Materials:
GFP scale up for purification (Green Fluorescent Protein Chromatography Kit; Bio-Rad 1660005EDU)

- Transformed pGlo plates
- Yellow inoculation loops
- Two tubes of LB/Amp/Ara media
- 70% Ethanol
- Marker

Equipment:

- Shaking incubator set at 37°C
- Waste beaker
- UV light

Preparation:

- Refer to Bio-Rad kit manual
    - Making aliquots
    - Warm incubator

Agenda/protocol:

1. Review scaling up and the upstream process
2. Lab activity: Inoculate cultures

Student procedure:
Refer to Bio-Rad manual for specific protocol and modify as needed for your setting.

Resources:

- DaytonMADE powerpoint “2024Explorers Day 3”
- Bio-Rad Green Fluorescent Protein Chromatography Kit manual

DaytonMADE Explorers	
Topic:	**Downstream Purification**
Program Schedule:	Day 4 (related to Genetic Engineering Day 2 and Scale-up Day 3)
Last Updated:	Sept 2024

Activities

- Introduction to downstream processing
- Purify GFP from a culture of *E. coli* cells
    - Previous steps: transformation (refer to Day 2) and scale up (refer to Day 3)

Supplies (per group)

Materials:
GFP scale up for purification (Green Fluorescent Protein Chromatography Kit; Bio-Rad 1660005EDU)

- GFP expressing *E. coli* cultures
- Marker
- 1000 µL tips
- 200 µL tips
- Two 2 mL microcentrifuge tubes
- Three collection tubes
- Tube of TE buffer
- Tube of lysozyme solution
- Tube of equilibration buffer
- Tube of binding buffer
- Tube of wash buffer
- Chromatography column
- 70% Ethanol

Equipment:

- Tube clamp and stand
- Freezer
- Water bath set to 42°C
- Beaker for column waste
- Waste beaker for tips
- p1000 micropipette
- p200 micropipette
- Microcentrifuge
- UV light
- Tube rack

Preparation:

- Refer to Bio-Rad kit manual
    - Making aliquots

Agenda/protocol:

1. Powerpoint introduction to downstream engineering and chromatography
2. Classroom activity: downstream engineering demo
    a. Create a mixture of different sized rocks, sand, and colored sugar/salt (e.g., large, medium, and small white rocks, white sand, and red sugar). Other materials needed are filters/sieves to separate each size rock, containers for filtering into, a coffee filter and funnel, water, and a stir rod.
    b. Relate the mixture to the manufacturing process and you need to purify your product. You can also discuss each “product” being removed can be another product you can sell.

c. Use the materials to progressively purify the mixture and obtain different products:
    i. Use physical separation
        1. Large sieve → purify large white rocks
        2. Medium sieve → purify medium white rocks
        3. Small sieve → purify small white rocks
    ii. Use solubility
        1. Add water, stir, filter, wash → purify white sand
    iii. Discuss evaporation and chromatography to purify sugar and dye
3. Lab activity: GFP purification

Student procedure:
Refer to Bio-Rad manual for specific protocol and modify as needed for your setting.

Resources:

- DaytonMADE powerpoint “2024Explorers Day 4 part 1”
- Bio-Rad Green Fluorescent Protein Chromatography Kit manual

DaytonMADE Explorers	
Topic:	**Campus Tour**
Program Schedule:	Day 4
Last Updated:	Sept 2024

Activities

- Tour a college campus

Agenda/protocol:

1. Tour a college campus

Reflection/Discussion 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?

_____
_____
_____
_____

Notes:

- We found it beneficial to have an informal “chalk talk” about the different degrees and paths after high school during lunch before the tour.

DaytonMADE Explorers	
Topic:	**Biology and Art/Fashion**
Program Schedule:	Day 4
Last Updated:	Sept 2024

Activities
• Powerpoint with videos • Tie-dye t-shirts
Supplies
Materials: *Tie-dying t-shirts* • White t-shirts • Tie-Dye kit (e.g., Amazon B00DV933WY) • Rubber bands • Grocery plastic bags
Preparation:
• Prepare dyes according to kit instructions
Agenda/protocol:
1. Powerpoint introduction to the uses of biology and biotechnology in the fields of art and fashion – mainly photos and videos of examples 2. Activity: tie-dye t-shirts a. Leave shirts in bags overnight, then wash.
Resources:
• DaytonMADE powerpoint “2024Explorers Day 4 part 2”
Notes:
• We had a staff member wash the shirts before giving them back to the students. • Before students take their shirts home, it’s a good opportunity for a fun group photo!

DaytonMADE Explorers	
Topic:	**Biomaterials**
Program Schedule:	Day 5
Last Updated:	Sept 2024

Activities

- Introduction to biomaterials
- Create a variety of bioplastics
- Create bio-based souvenirs using Checkerspot's Pollinator Kit

Supplies

Materials:
Bioplastics (per group) – most materials were part of Amino Labs Bioplastics All-in-One Kit WWG015

- Tube of oil
- Labels
- Silicone tray
- Cotton swab
- Five petri dishes
- Gelatin
- Vegetable starch
- Chitin/chitosan
- Baking soda
- Red, blue, and yellow dyes
- Small plastic pipettes
- Two star molds and a 5-rectangles mold
- Bottle of glycerol
- Bottle of 5% acetic acid
- Tube of liquid soap
- Weigh boats
- Scoopula
- Large plastic pipette
- Toothpicks
- Wooden or glass stir rod
- Distilled water
- 10 mL serological pipets
- Marker

Pollinator Kit

- Pollinator kits (https://www.pollinatorkit.com/)
- Mixing cups and sticks (e.g., Amazon B08FXMYVJ1)
- Various silicon molds
- Optional - Resin pigment (e.g., Amazon B005ZSGQBO)

Equipment:
Bioplastics

- Hot plate
- Serological pipet aid
- Glass beaker
- Three syringes (1 large, 2 small)
- Small balance (included in Amino Labs kit)
- Microwave
- Plastic beaker
- Magnetic stir bar

Preparation:

- For the Pollinator Kit:
    - We found it easiest to pre-pour Sides A and B into separate cups shortly before the students will need them. Work in a well-ventilated space. When giving the cups to the students, we added a tiny bit of resin into Side B (i.e., a pipette tip dipped in resin and then swirled in the liquid).
    - We aliquoted 50 mL Side A and 100 mL Side B for each student which allowed for one large mold and a number of smaller molds.
    - Provide coverage for benchtops. The kit comes with instruction sheets that can be used for this purpose.

Agenda/protocol:

1. Intro to biomaterials: Mix of powerpoint, videos, group discussion, think-pair-share
2. Lab activity: Bioplastics
3. Lab activity: Pollinator Kit

Student procedure - Bioplastics:

Refer to Amino Labs manual for specific protocols and modify as needed for your setting. Specifically, we did individual parts of the Bioplastics All-in-One kit:

- 5 rectangle molds
- Red bioplastic star, Blown texture, Baking Soda Bio-foam, Color Opacity Tests, and Soap Bio-foam
- Strings
- Biocomposites (with chitosan)

Student procedure – Pollinator Kit:

1. Mix resin into Side B very well.
2. Pour Side B into Side A and mix very well.
3. Pour resin into molds to solidify overnight.

Resources:

- DaytonMADE powerpoint “2024Explorers Day 5”
- Amino Labs Bioplastics All-in-One Kit manual and wiki
- Checkerspot Pollinator kit instructions

Notes:

- This protocol incorporates parts of the All-in-one kit from Amino Labs. One major modification was we gave each group a hot plate instead of sharing one microwave. This ended up taking the full day and there was a range of percent completion from the different groups.
- For module purposes, we also include Checkerspot’s Pollinator Kit here, however it didn’t fit in this day as written. The timing of this day needs to be optimized.
    - A Checkerspot employee provided a virtual presentation to the students about the company and the kit. In short, this is a polyurethane resin kit (mix Side A and Side B and get a hard product), but it is produced with oil made from algae and the final product is 56% bio-based. We provided various silicon molds of different sizes and subject matter and gave each student 150 mL of resin to create the items they were interested in. We also added pigments to color the resin (add very little pigment!). The students loved this experiment, especially because they got to keep souvenirs from it. Checkerspot also provided samples of the biomass-to-oil process and other examples of different formulations.
- In the first year of the program, we attempted other activities like biocement and silk enzyme stamping. These needed much more optimization, but could be valid to retry in the future.
- We collected various biomaterial samples to pass around during the introduction (e.g., https://mushroompackaging.com/collections/shop-all)
- Carefully read safety instructions for both kits.

DaytonMADE Explorers	
Topic:	**Enzymes and Scale-up**
Program Schedule:	Day 5
Last Updated:	Sept 2024

Activities

- Professors from Miami University developed a number of “lab quests” exploring enzymes. They, along with some graduate students, guest taught for the full morning of Day 6. Aspects of the visit included:
  - Powerpoint introduction/review of proteins and enzymes
  - Lab activity: lab quests
    - Quests: 1-enzymes are catalysts, 2- how enzymes generate product over time, 3- influence of substrate and enzyme amount on production rate, 4- finding the best enzyme solution
    - Objectives for the lab: show that enzymes catalyze a reaction, learn how to use a spectrophotometer, see the generation of product over time to gain insight into how enzymes function, design and implement single-variable controlled experiments to solve a problem in biotechnology, work together as a team to run reproducible experiments to inform selection of best solution for the class competition, and use economics to evaluate multiple correct solution to select the best solution to a problem
  - Discussion about scaling up in biomanufacturing (bioreactors, etc). They also brought vessels ranging from 384 well plates to 1L bioreactors to discuss scale up and scale down as well as the differences in vessels
  - Discussion about Miami University offerings and the professors’ research

Supplies

- Guests supplied their own materials other than UV spectrophotometers supplied by WSU

Resources

- Lab quests document

DaytonMADE Explorers	
Topic:	**Bioplastics Analysis**
Program Schedule:	Day 6 (related to Biomaterials Day 5)
Last Updated:	Sept 2024

Activities

- Analyze bioplastics and compare results

Agenda/protocol:

1. Analyze bioplastics with lab partner
2. Compare results and group discussion

Student procedure:
Refer to Amino Labs resources for a Bioplastics Analysis manual.

Resources

- We only had students do a brief analysis, mainly looking at how the different formulations affected the basic properties of the material and how results varied between groups along with what might have caused said differences. Amino Labs provides a detailed analysis manual if you want to dive deeper into the analysis.

DaytonMADE Explorers	
Topic:	**Chromoprotein Production – Transformation**
Program Schedule:	Day 6 (related to Scale-up Day 7, Induction Day 8, and Downstream Purification Day 9)
Last Updated:	Sept 2024

Activities

- Transform the pChromo plasmids coding for chromoproteins into *E. coli* cells

Supplies (per group)

Materials:
pChromo transformation (Rainbow Transformation Kit; Edvotek 224)

- Two tubes of E. coli starter culture
- Tube of ice-cold $CaCl_2$
- Tube of ice-cold Competent Cell Solution (“CCS”)
- Tube of Rainbow Transformation Mixture (“RTM”)
- Tube of Recovery Broth
- Four prepoured agar plates
- 200 µL tips
- 20 µL tips
- Two sterile yellow inoculation loops
- 70% Ethanol

Equipment:

- Water bath set to 37°C
- Water bath set to 42°C
- Microcentrifuge
- Beaker or bucket of ice
- p20 micropipette
- p200 micropipette
- Waste beaker for tips
- Incubator set to 37°C

Preparation:

- Refer to Edvotek kit manual
    - Pouring plates and making aliquots
    - Warming incubator and water baths
    - Prepare *E. coli* starter culture

Agenda/protocol:

1. Powerpoint review of genetic engineering and plasmids and introduction to chromoprotein production
2. Lab activity: pChromo transformation

Student procedure:
Refer to Edvotek manual for specific protocol and modify as needed for your setting.

Resources:

- DaytonMADE powerpoint “2024Explorers Day 6”
- Edvotek Rainbow Transformation Kit manual

Notes:

- Again, have students make predictions and discuss as a group. You can use the Tangles plasmid to reinforce the parts of a plasmid and help guide making growth hypotheses.
- The Edvotek kit comes with most reagents and materials required for the activity (refer to their manual), but we modified it for use in a lab setting with micropipettes. For subsequent steps (scale-up, induction, and downstream purification), we used a custom protocol optimized by Biopharmaceutical Technology Center Institute.

DaytonMADE Explorers	
Topic:	**Chromoprotein Production – Scale-up**
Program Schedule:	Day 7 (related to Transformation Day 6, Induction Day 8, and Downstream Purification Day 9)
Last Updated:	Sept 2024

Activities

- Observe transformation results
- Grow a culture of *E. coli* cells from colonies on a plate

Supplies (per group)

Materials:

- Transformed chromoprotein plates
- Yellow inoculation loops
- Marker
- Plastic cuvettes (e.g., Fisher Scientific 14-955-127)
- Tube of LB/Amp media (e.g., Teknova L8100)
- 70% Ethanol
- 1000 µL tips

Equipment:

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

Preparation:

- Making media and aliquots
- Warming incubator

Agenda/protocol:

1. Review scale-up
2. Lab activity: Observe transformation results and start liquid culture

Student procedure:

Refer to Edvotek manual for worksheets and discussion questions.

Scale-up:

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 spectrophotometer. 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.	☐
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.	☐

Notes:

- For this and subsequent steps (scale-up, induction, and downstream purification), we used a custom protocol optimized by Biopharmaceutical Technology Center Institute.

DaytonMADE Explorers	
Topic:	**Agar Art**
Program Schedule:	Day 7
Last Updated:	Sept 2024

Activities

- Create a design using colorful engineered *E. coli*

Supplies (per student)

Materials:

- Pre-transformed bacteria "pallets" (prepared using Amino Labs Engineer-It kit, full rainbow)
- Two LB/Chl plates per person (poured using Amino Labs bulk materials)
- 70% Ethanol
- Toothpicks
- Yellow or blue inoculation loops
- Cotton Swabs

Equipment:

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

Preparation:

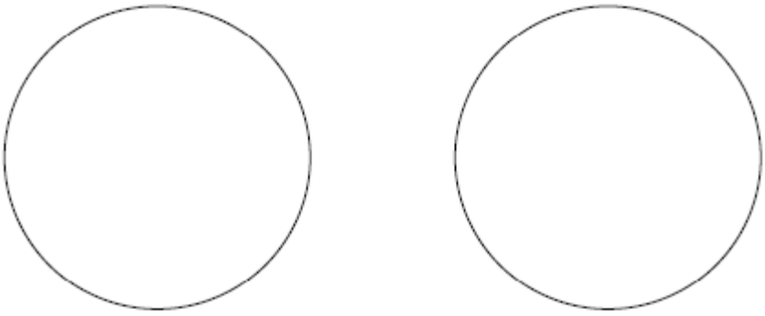
- Transforming and patching "pallets" – refer to Amino Labs Engineer-It kit manual
- Pouring plates
- Warming incubator

Agenda/protocol:

1. Lab activity: Create agar art

Student 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. 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.	☐



Resources:

Amino Labs also sells a Canvas kit that is designed to do agar art using 3 colors. They also have various resources and examples on their website and wiki.

Notes:

- Students can optional use their own transformed colonies as “paint”, however there are only a handful of colonies to use, and they would need LB-Amp plates instead of LB-Chl. We gave students the option and they all chose to use the pallets we made.
- We transformed plasmids expressing 10 different color proteins from Amino Lab’s Engineer-It kit (full rainbow). We took resulting colonies and “patched” them onto new plates to create pallets.
- We attempted to use Amino Lab’s Keep-it kit on the agar art plates so students could take them home as a souvenir. For our first attempt, we also did this for some example plates and a month later they had unfortunately lost their color. For our second attempt, we left the plates to dry over the weekend and the plates completed dried out. Conditions for using the kit would need to be optimized.

DaytonMADE Explorers	
Topic:	**College 101**
Program Schedule:	Day 7
Last Updated:	Sept 2024

Activities

- Learn about how to be college-ready & how to apply for college

Agenda/protocol:

WSU Admissions presented a session on applying to schools, selecting schools, communication best practices, and financial aid.

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?

_____
_____
_____
_____
_____

DaytonMADE Explorers	
Topic:	**Industry Field Trip**
Program Schedule:	Day 8
Last Updated:	Sept 2024

Activities
• Visit an industrial biomanufacturing facility
Agenda/protocol:
Students arrived to camp as normal and then all rode in the van along with staff to the plant. Visit included a presentation overviewing the manufacturing process and safety information, a tour of the site, lunch, and a Q&A session. ● For the tour, the site provided PPE for all students and staff. The group was broken up into two groups. The tour included the microbiology lab, the main control room, the production floor and offices for each area including fermentation and downstream processing, packaging/warehouse, and maintenance shop. ● The Q&A session included employees with various positions and backgrounds. They highlighted the variety of careers and disciplines involved in running a large-scale biomanufacturing facility.

DaytonMADE Explorers	
Topic:	**Chromoprotein Production – Induction**
Program Schedule:	Day 8 (related to Transformation Day 6, Scale-up Day 7, and Downstream Purification Day 9)
Last Updated:	Sept 2024

Activities

- Induce a culture of *E. coli* cells to express chromoprotein through fermentation

Supplies (per group)

Materials:

- Overnight cultures
- Plastic cuvette with 900 µL media (e.g., Fisher Scientific 14-955-127)
- 1000 µL tips
- 200 µL tips
- Tube of prewarmed LB/Amp media (e.g., Teknova L8100)
- Tube of 100 mM IPTG (e.g., Sigma I1284-5ML)
- Marker
- 70% Ethanol

Equipment:

- Shaking incubator set at 37°C
- UV Spectrometer
- Waste beaker
- p200 micropipette
- p1000 micropipette

Preparation:

- Making aliquots
- Warming incubator

Agenda/protocol:

1. Review induction
2. Lab activity: Induce a culture of *E. coli* cells to express chromoprotein through fermentation

Student procedure:

1. Sterilize your bench with 70% ethanol.	☐
2. Label your tube of media with your group initials.	☐
3. Measure the Abs at 600 nm of your overnight culture using the spectrometer. Camp staff will use plain LB/Amp media to blank the instrument. a. Because your culture is so grown, you will need to dilute it to get a correct reading from the instrument. b. Add 100 µL of your culture to the cuvette prefilled with 900 µL media. Pipette up and down until well mixed. This is now a 10-fold dilution of your culture. c. Write your absorbance value here: _____ d. Multiple the reading by 10 to get the value for your overnight culture: _____	☐
4. Calculate the amount of overnight culture you will need to add to prepare a new 11 mL culture with an absorbance of 0.3: a. _____ ml of overnight culture = 11 mL x 0.3 ÷ Abs of overnight culture	☐

5. Remove and discard the volume of LB Amp from your warmed 50 mL tube of media equivalent to the volume you calculated above.	☐
6. Gently swirl your overnight culture to resuspend all the cells. Measure the volume you calculated above and add it to your 50 mL tube of media. This should now contain overnight culture and fresh LB Amp totaling 11 mL.	☐
7. Place in a shaking incubator at 37°C for 1 hour.	☐
8. Add 25µL of IPTG and place back in the shaking incubator overnight.	☐
9. Empty your waste beaker into the large biohaz box. Spray the beaker and wipe down your bench with 70% ethanol.	☐

Notes:

- For this and subsequent steps (Induction and downstream purification), we used a custom protocol optimized by Biopharmaceutical Technology Center Institute.
- Refer to Edvotek manual for discussion questions.

DaytonMADE Explorers	
Topic:	**Chromoprotein Production – Downstream Purification**
Program Schedule:	Day 8 (related to Transformation Day 6, Scale-up Day 7, and Induction Day 8)
Last Updated:	Sept 2024

Activities

- Purify chromoprotein from a culture of *E. coli* cells

Supplies (per group)

Materials:
Lab demo (one for the class)

- Prepared buffers, sample, and column from Ion Exchange Chromatography kit (Edvotek 243)

Chromoprotein purification

- Chromoprotein expressing *E. coli* cultures
- Two 2 mL microcentrifuge tubes
- Marker
- 1000 µL tips
- 200 µL tips
- 15 mL tube
- 70% Ethanol
- Chromatography column (e.g., empty columns from Edvotek 243)
- Tube of 50% resin slurry (i.e., Nuvia Q resin mixed with pure water, Bio-Rad 1560411)
- Tube of 10 mg/mL lysozyme solution (e.g., Sigma L3790-10X1ML)
- Tube of 50 mM HEPES pH 7.5 buffer (e.g., diluted from Sigma H0887-20ML)
- Tube of DNase solution (i.e., Promega M6101)
- Tube of FastBreak lysis solution (i.e., Promega V8571)
- Six tubes of NaCl solutions of different concentrations (e.g., dilutions made from 5 M NaCl, Fisher Scientific AM9760G)
    - 50 mM, 100 mM, 150 mM, 200 mM, 400 mM, and 800 mM NaCl in 50 mM HEPES pH 7.5
- 1.5 mL centrifuge collection tubes

Equipment:

- Tube clamp and stand
- Freezer
- Water bath set to 30°C
- Beaker for column waste
- Waste beaker for tips
- p1000 micropipette
- p200 micropipette
- Microcentrifuge
- Tube rack

Preparation:

- For the demo, you need to prepare buffers, resin, and sample.
- For the lysis and purification, you need to make many solutions and aliquots
- Warm water bath

Agenda/protocol:

1. Review downstream processing and chromatography – focus on ion exchange chromatography
2. Lab demo: Ion chromatography with dyes
3. Lab activity: Lyse cells and purify chromoprotein

Student procedure:

Self-Reflection: Observe your cultures. Are you surprised by the results? Is it what you expected?	☐
1. Label the 2 mL microcentrifuge tubes with your group initials.	
2. Using a p1000 micropipette, transfer 2 mL of your overnight culture into each tube. Spin the microcentrifuge tube for 2 minutes in the centrifuge at maximum speed.	☐
3. Use a pipet to gently remove and discard the supernatant from each tube, being sure to not disturb the pellet.	☐
4. Add another 2 mL of culture into each of the two tubes and centrifuge for another 2 minutes. Discard the supernatant as before. Repeat until all of the culture has been centrifuged and each tube contains a pellet.	☐
5. Add 800 µL HEPES buffer and 10 µL of lysozyme solution to each tube. Resuspend the cells by vortexing for 3 minutes.	☐
6. Transfer the cells from both tubes into a single 15 mL tube.	☐
7. Freeze the 15ml conical tube horizontally in the freezer until completely frozen.	☐
8. Thaw tube in a 30°C waterbath.	☐
9. Add 10 µL DNAse I. Vortex for 3 minutes.	☐
10. Freeze the 15ml conical tube horizontally in the freezer until completely frozen.	☐
11. Thaw tube in a 30°C waterbath.	☐
12. Add 40 µL FastBreak solution. Vortex for 3 minutes.	☐
13. Freeze the 15ml conical tube horizontally in the freezer until completely frozen.	☐
14. Thaw tube in a 30°C waterbath.	☐
15. Split the contents of the tube between two 1.5 mL microcentrifuge tubes. Centrifuge at max speed for 5 minutes.	☐
16. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.	☐
You've now lysed, or broken, the cells and released the chromoprotein. Through the next steps, you will purify your protein from the other contents of the tube. This process is similar to when you purified GFP but will use changes in salt content to elute the protein instead of relying on a difference in size.	
17. Prepare the chromatography column. Secure your column onto the stand using the clamp. Remove the plug at the bottom of the column. Place a beaker under the column to collect waste.	☐
18. Resuspend your tube of resin by inverting a few times. Pour or pipet the resin into the column. Let the column drain into the waste beaker.	☐
19. Add 2 mL of HEPES pH 7.5 to the resin and let it run through the column.	☐
20. For the rest of the procedure, collect the liquid coming off the column in a new tube for each step.	☐

21. Carefully add 1 mL of your sample to the column and collect the eluate, or liquid coming off, into a 1.5 mL tube. Stop the flow just as the final portion of the solution enters the column using the plug or continue on to the next step. Note the position of the colored protein	☐
22. Add 1 mL 50 mM NaCl to the column. Collect the eluate in a new 1.5 mL tube. Note any change in position of the colored protein: _____	☐
23. Add 1 mL 100 mM NaCl to the column. Collect the eluate in a new 1.5 mL tube. Note any change in position of the colored protein: _____	☐
24. Add 1 mL 150 mM NaCl to the column. Collect the eluate in a new 1.5 mL tube. Note any change in position of the colored protein: _____	☐
25. Add 1 mL 200 mM NaCl to the column. Collect the eluate in a new 1.5 mL tube. Note any change in position of the colored protein: _____	☐
26. Add 1 mL 400 mM NaCl to the column. Collect the eluate in a new 1.5 mL tube. Note any change in position of the colored protein: _____	☐
27. Add 1 mL 800 mM NaCl to the column. Collect the eluate in a new 1.5 mL tube. Note any change in position of the colored protein: _____	☐

Congratulations! You've now taken a plasmid encoding a gene from one organism, transformed it into bacteria, induced the bacteria to make the protein coded by the gene, grew a liquid culture of (fermented) the bacteria, extracted the protein from the bacteria, and purified the protein!
Way to go!

Resources:

- DaytonMADE Powerpoint "2024Explorers Day 9"
- Edvotek Ion Exchange Chromatography kit manual

Notes:

- For lysis and purification, we used a custom protocol optimized by Biopharmaceutical Technology Center Institute.

DaytonMADE Explorers	
Topic:	**Biomanufacturing Careers – part 1**
Program Schedule:	Day 9
Last Updated:	Sept 2024

Activities

- Discuss types of STEM roles in biomanufacturing
- Explore “a day in the life” of a biomanufacturing professional using provided biographies

Agenda/protocol:

1. Powerpoint discussion about types of STEM roles in biomanufacturing
2. Activity looking at biographies and a-day-in-the-life (activity from NCABR)
3. Highlight non-STEM roles also related to the role of biomanufacturing

Resources:

Much of the information, and the activity itself, in this section came from North Carolina Association for Biomedical Research’s (NCABR) “Mapping your Future: Careers in Biomanufacturing” document: https://www.ncabr.org/wp-content/uploads/2015/12/mapping-your-future.pdf.

DaytonMADE Explorers	
Topic:	**Biomanufacturing Careers – part 2**
Program Schedule:	Day 10
Last Updated:	Sept 2024

Activities

- Explore real life job ads related to biomanufacturing

Agenda/protocol:

1. Job ad activity – Think, Pair, Share activity exploring different job ads using a work-sheet as a guideline. Students were provided with 2 or 3 of ~12 job ads for various biomanufacturing positions (e.g. lab tech, operator, fermentation scientist, senior scientist), including openings at companies they toured or learned about. Discussion included what they found interesting or surprising and similarities/differences. The discussion can morph into a general lesson/advice on applying for jobs and reading job postings.
2. Discussion about what the "ideal" candidate is and guidance on finding their career paths

	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.			

Resources:

DaytonMADE powerpoint "2024Explorers Day 9"

DaytonMADE Explorers	
Topic:	**Demo Day and Closing Ceremony**
Program Schedule:	Day 10 (Student prepare 2-3 days prior)
Last Updated:	Sept 2024

Activities

- Work in groups of 1-3 to create a poster presentation
- Present to a diverse audience, including family and industry professionals

Supplies

Materials:
Demo Day

- White poster board
- Cardstock or construction paper
- Bulletin board letters
- Scissors
- Glue sticks
- Masking tape
- Black and colored sharpies
- Thick markers
- Rulers
- Colored pencils
- Other general craft supplies as appropriate
- Science materials as applicable for hands-on demos (e.g., agarose tablets, Minipcr RG-1500-03)

Closing Ceremony

- Refreshments such as coffee, donuts, and water
- Folders containing final materials (e.g., a signed certificate, photos, staff contact info, a skills checklist, and student stipend

Preparation:

- Prepare demos for students as applicable
- Print contents for folders
- Print surveys

Agenda/protocol:

1. During lunch, have students fill out post-camp survey, feedback survey, and a post-camp word association activity.
2. Students finish preparing and practicing their presentations after lunch.
3. Visitors arrived at 2:00 for an open house in the lab.
4. At ~3:00, students and visitors moved to a separate room for the closing ceremony. The ceremony consisted of a short presentation/introduction to UES, BlueHalo, and DaytonMADE, followed by some speeches. Each student was then called up front to receive their folder and handshakes/photos with the staff. The event ended with a final group photo.

Notes:

- A slideshow of photos from the camp was shared during lunch for the students and then projected throughout the open house event.
- Provide students a heads-up on the first day that Demo Day will be happening. Form a list of topics sometime mid-camp and continue adding to it. Have students pick topics early/mid week 2 so that they work on their presentations during downtime. Dedicate time on days 8, 9, and 10 for preparation and practice.

- Students present about something they learned about during the camp that interested them most, such as bacterial transformation, downstream processing, and biomaterials. They create a poster and prepare a prop (e.g., transformed bacteria on plates) or hands-on demo (e.g., teaching visitors how to use a pipette and load a gel). We had the students set up around the laboratory and visitors were invited to join for an open house style event.

DaytonMADE Explorers	
Topic:	**Guest Speakers**
Program Schedule:	Throughout
Last Updated:	Sept 2024

Activities
• Virtual or in-person sessions presented by industry or military professionals.
Notes:
• Set strong expectations ahead of time, discussing professional etiquette with the students with regards to guest speakers. • We had a range of formats for the speakers – some in person and some virtual. The in-person speakers were received slightly better by the students, but in the second year we prepared the virtual speakers to have content more appropriate to the level of the audience and include more pictures and videos/tours which helped. • We attempted to have diversity in speakers. In the first year, all speakers had PhDs, but in the second year we improved to include speakers without PhDs and speakers from a larger variety of organizations (e.g., start ups, CMOs, military) and backgrounds (e.g., using soft skills to be involved in STEM fields without a STEM degree). The topics from the speakers ranged from vignettes through a career path (e.g., “lessons learned”) to more in-depth introductions to companies. • For future speakers, we would like to showcase scientists at various levels and highlight the multidisciplinary nature of biomanufacturing. We would also like to continue showcasing different environments visually, whether through in-person or virtual tours. • Most speakers were found through the network of DaytonMADE staff, but we also created an online form to share broadly.

DaytonMADE Trailblazers	
Topic:	**Introduction**
Program Schedule:	Day 1
Last Updated:	Sept 2024

Activities

- Introduction to Program powerpoint
- Ice breakers – This or That and Traffic Jam
- Pre-camp survey
- Word association

Supplies

Introductions, surveys, word association:

- Paper
- Writing utensils
- Name tags
- Markers

Preparation:

- Prepare powerpoint with program overview and staff introductions
- Print pre-camp survey

Agenda/protocol:

1. Introduction powerpoint
    a. Fill out name tags
    b. Introduce staff
    c. Fill out pre-camp survey
    d. Word association
        i. Take 2-3 minutes for students to write down what they think of when they see "Biomanufacturing" and "Biotechnology"
    e. Program overview, including brief biomanufacturing introduction
2. Ice breaker: This or That
    a. Show two options on the screen and have students and staff choose one by moving to the corresponding side of the room. Initiate discussion amongst the group.
3. Ice breaker: Traffic Jam
    a. Break up into groups of 6 ir 8 students. Staff can partake to increase connection with students, but be a follower, not a leader.
    b. Follow the set up and rules for the activity, for example from https://brilliantpathways.org/wp-content/uploads/2019/04/traffic_jam_teamwork.pdf
    c. Discuss what strategies worked or didn't work. Connect the skills used here to working in a lab.
4. Deeper introduction to biomanufacturing and the biomanufacturing process

Resources:

- DaytonMADE powerpoint "2024Trailblazers Day 1 Part 1"

Notes:

- Based on student feedback, they wanted more overview and understanding of the schedule for the program.
- Establish expectations early
    - Build a good rapport between staff and students
    - Establish a safe space that they can learn, explore, and ask questions – even if this ends up not being the field for them
    - Respect for staff, other students, equipment, facilities, and guest speakers
    - Safety

DaytonMADE Trailblazers	
Topic:	**Lab Safety, Tools, and Equipment**
Program Schedule:	Day 1
Last Updated:	Sept 2024

Activities

- Powerpoint and interactive discussion about lab safety, tools, and equipment

Preparation:

- Prepare powerpoint with site-specific hazards and procedures

Agenda/protocol:

1. Lab safety powerpoint
    a. Group discussion having students fill in a DOs and DON'Ts table for safety in a lab setting
    b. Go over specific hazards, safety, PPE, cleaning procedures, and a full DOs and DON'Ts
2. Lab tools and equipment powerpoint
    a. Discuss the types of items the students will be using, including photos so they know what to look for in lab
3. Move to the lab
    a. Introduction to the laboratory space, general protocols and safety highlights
    b. Pass out lab coats and have students pick bench space and partners

Resources:

- DaytonMADE powerpoint "2024Trailblazers Day 1 Part 2"

Notes:

- Establish clear expectations early and often regarding safety, such as the use of PPE, appropriate attire, use of headphones/pods, and cleaning hands.

DaytonMADE Trailblazers	
Topic:	**Microbiology and Culturing Microbes**
Program Schedule:	Day 1 (Observations on Day 2)
Last Updated:	Sept 2024

Activities

- Pour agar plates
- Streak unknown samples

Supplies (per group)

Materials:

- Powdered LB agar medium (e.g., Bio-Rad 1660600EDU)
- Weigh boats and spatulas
- Sterile water
- Foil
- Small petri dishes (2 per person; 4 per bench) (e.g., Bio-Rad 1660469EDU)
- Sterile swabs (e.g., Amazon B08111ZV73)
- Lab tape (e.g., Amazon B0889LYH61)
- 70% Ethanol

Equipment:

- Balance
- Hot Plate
- Sterile graduated cylinder
- Sterile 250mL Erlenmeyer flask
- Incubator set at 37°C

Preparation:

- Pre-autoclave flasks and graduated cylinders if possible. Sterilize with 70% ethanol if not.
- Set incubator temperature

Agenda/protocol:

Student Protocol (Day 1):

1. Clean and sterilize your benchtop with 70% ethanol.	☐
2. Calculate the volume of agar needed to make 4 small agar plates. a. 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. a. _____ 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. a. **CAUTION:** Agar will boil over quickly. Use heat & spin settings at 40-50%.	☐
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 tape 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.	☐

Day 2:

1. Observe growth and discuss.
2. Discard plates into biohazard waste.

Notes:

- Don’t have student swab the bottoms of their shoes or inside their mouths.
- Tape the plates closed and don’t open them after incubation.
- Unfortunately, there wasn’t much growth and/or lack of diversity on most of the plates in our experience.

DaytonMADE Trailblazers	
Topic:	**Micropipetting**
Program Schedule:	Day 1
Last Updated:	Sept 2024

Activities

- Measure and transfer volumes using a micropipette
- Create a piece of pipet art

Supplies (per group)

Materials:
Most materials are from MiniPCR Micropipetting 101 (KT-1510-10) and Micropipetting Art: Full STEAM Ahead (KT-1510-21)
Square and 96-well plate patterns from Bio-Rad: https://www.bio-rad.com/sites/default/files/2022-10/Micropipetting-Practice-Sheets_2022.pptx and https://www.bio-rad.com/webroot/web/pdf/lse/literature/BE14-Pipet-Act.pdf

- Red, yellow, and blue dyed water
- 20 µL tips
- 200 µL tips
- 1000 µL tips
- Practice Pipetting Cards
- Micropipette Art Cards or parafilm
- Pipet art stencils
- 1.5 mL microcentrifuge tubes

Equipment:

- p20 micropipette
- p100 micropipette
- p1000 micropipette

Preparation:

- Aliquot dye as needed
- Print practice art stencils

Agenda/protocol:

Student procedure:
Refer to MiniPCR and Bio-Rad manuals and resources for specific activity protocols and modify as needed for your setting.

An additional activity for students to gain practice in using different size micropipettes is below. We found this to be a useful exercise for catching errors, and it also facilitated discussion about things to look out for when pipetting. For example, if you are at the larger end of the volume range, the tip should be mostly full.

1. Set up 9 1.5 mL microcentrifuge tubes in a rack.
2. In the first 3 tubes, use the p20 micropipette and 20 µL tips to pipet the following volumes (1 per tube)
    a. 2 µL
    b. 10 µL
    c. 20 µL

3. In the next 3 tubes, use the p200 micropipette and 200 µL tips to pipet the following volumes (1 per tube)
    a. 20 µL
    b. 100 µL
    c. 200 µL

4. In the next 3 tubes, use the p1000 micropipette and 1000 µL tips to pipet the following volumes (1 per tube)
    a. 200 µL
    b. 500 µL
    c. 1000 µL

Self-reflection: How do the volumes in the different tubes compare visually? When you pipetted the same volume with different pipettes, did the final amount look the same?

_____
_____
_____
_____

Resources:

MiniPCR and Bio-Rad manuals and resources

Notes:

- We kept design stencils out throughout the camp and some students would use that activity to fill time.
- Some students had success letting the final art dry some and then transferred the design to a paper towel to keep.
- An additional optional activity we did throughout the camps was an accuracy competition with pipetting water into a weigh boat on a balance.

DaytonMADE Trailblazers	
Topic:	**Enzymes**
Program Schedule:	Day 1
Last Updated:	Sept 2024

Activities

- Introduction to macromolecules, central dogma, proteins, and enzymes
- Monitor enzyme activity by measuring the amount of apple juice released by pectinase or water
- Compare enzymatic activity under different temperature conditions

Supplies (per group)

Materials:

- Water
- Pectinase solution (e.g., Carolina Biological Supply 202380, diluted 4-fold)
- Diced apple
- Parafilm
- Plastic spoons or stir rods
- Lab tape and marker

Equipment:

- Water bath set to 40°C or 60°C
- Refrigerator (~4°C)
- Balance
- Weigh boat
- Two large beakers
- Two 25-50 mL graduated cylinders
- Two funnels
- Filter paper

Preparation:

- Dice up apples
- Dilute pectinase solution
- Warm water baths

Agenda/protocol:

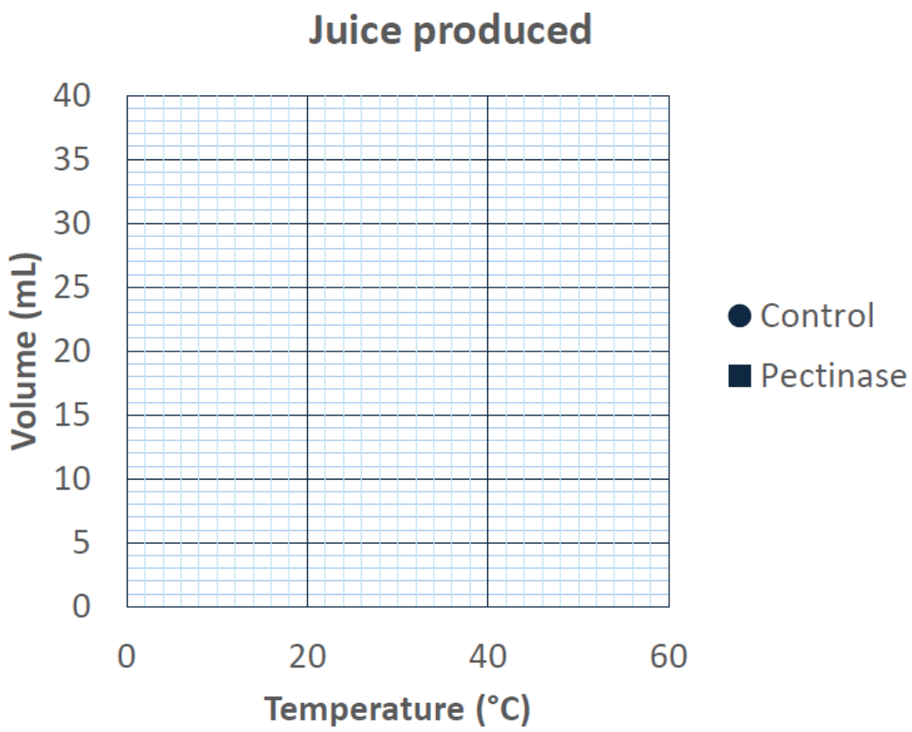
1. Powerpoint intro of macromolecules of life, Central Dogma, and proteins and introduction to enzymes
2. Lab activity: Extracting apple juice using pectinase

Student 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.	☐
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: _____		



Resources:

- DaytonMADE powerpoint "2024Trailblazers Day 1 Part 3"

Notes:

- You can have all students use the same conditions and focus on enzymes, but we adding in the additional environmental variables aspects. This allowed us to introduce the concept before they started their ethanol production experiment, which also compares function at different environmental conditions. This also provided practice at graphing and comparing data.
- The ratio of apple:pectinase and the size of the beaker need to be optimized. We aimed to use 50 g of diced apple and a 50 mL beaker with 2 mL pectinase solution. The beaker was too small so the students used much less apple, and there was too little volume of pectinase to coat the apples. We would suggest larger beakers so that more apple would fit and be exposed to the pectinase solution. Even with less than optimal conditions, the experiment still worked as expected and all groups (except the fridge) were able to produce juice and we saw a temperature dependence.

DaytonMADE Trailblazers	
Topic:	**Microbiology and Cells as Factories**
Program Schedule:	Day 2
Last Updated:	Sept 2024

Activities

- Introduction to microbes, using microbes and organisms as tools, and cells as factories
- Visualize produce of CO_2 by yeast

Supplies (class demo)

Materials:

- 1 packet (7 grams) of active, dry yeast (not rapid-rise)
- 1 cup of warm water
- 2 tablespoons of sugar
- 1 round, rubber balloon (do not use Mylar or water balloons because they do not expand easily)
- 1 small clear, empty water bottle
- Rubber band

Preparation:

- Prepare warm water

Agenda/protocol:

1. Powerpoint introduction to microbes, the concept and examples of using microbes and organisms as tools, and cells as factories
2. Classroom demo: Production of CO_2 by yeast
    a. Stretch out the balloon, then set it aside.
    b. Pour the packet of yeast into the clear water bottle. Add the sugar, then the water.
    c. Swish very slowly to avoid coating the sides of the bottle with yeast.
    d. Attach the balloon to the mouth of the bottle using a rubber band and set it aside.
    e. Within a few minutes you will see bubbling, and then the balloon will begin to inflate.

Resources:

- Demo protocol in this section modified from North Carolina Association for Biomedical Research's (NCABR) "Mapping your Future: Careers in Biomanufacturing" document: https://www.ncabr.org/wp-content/uploads/2015/12/mapping-your-future.pdf.
- DaytonMADE powerpoint "2024Trailblazers Day 2 Part 1"

Notes:

- We did a single classroom demo as part of the introduction to the module, but you could also have groups of students do their own demos. The NCABR document provides additional options.

DaytonMADE Trailblazers	
Topic:	**Biofuel Production in Yeast**
Program Schedule:	Day 2 (related to Day 3 and Day 4)
Last Updated:	Sept 2024

Activities

- Use small-scale flask fermenters to produce ethanol by fermentation using *Saccharomyces cerevisiae* (yeast)
- Observe characteristics of yeast cultures including pH over 48 hours
- Compare the effect of different temperature and aeration conditions on production

Supplies (per group)

Materials:
Most materials were part of The Future of Biofuels - Alcohol Fermentation kit (Edvotek 304)

- 25 mL seed culture of yeast
- 250 mL yeast media
- 1.5 mL screw top tube
- 200 µL tips
- Lab tape
- Marker
- 70% Ethanol

Equipment:

- Sterile 500 mL Erlenmeyer flask
- Sterile 100 mL graduated cylinder
- Hydrometer (one per class)
- pH litmus paper
- p200 micropipette
- Waste beaker
- Stir plate
- Magnetic stir bar
- Water bath set at 30°C
- Shaking incubator set at 30°C
- Incubator set at 37°C
- Refrigerator (~4°C)

Preparation:

- Refer to Edvotek kit manual
    - Prepare media
    - Prepare seed culture
    - Sterilize glassware

Agenda/protocol:

1. Powerpoint introduction to ethanol production and yeast fermentation
2. Lab activity: Start Biofuel Production in Yeast experiment

Student procedure:
Refer to Edvotek manual and modify as needed for your setting.

	Day 0	Day 1	Day 2
Odor			
Color			
Clarity			
pH			
Potential Alcohol %			
Estimated Ethanol %	0%	Day 0 potential minus Day 1 potential	IDay 0 potential minus Day 2 potential

Resources:

- DaytonMADE powerpoint “2024Trailblazers Day 2 Part 1”
- Edvotek The Future of Biofuels - Alcohol Fermentation kit manual

Notes:

- Do whatever aeration/temperature conditions your resources and set up allow for. We had each group of students choose a different condition with regards to temperature and aeration (4°C still, room temp still, room temp stirring, 30°C still, or 30°C shaking).
- We had one hydrometer that a staff member would take bench to bench.
- As written, the kit protocol goes an additional day, however two days worked best for the schedule and was enough to see production and a difference between conditions.

DaytonMADE Trailblazers	
Topic:	**Genetic Engineering**
Program Schedule:	Day 2 (related to Scale-up Day 3 and Downstream Purification Day 4)
Last Updated:	Sept 2024

Activities

- Introduction to engineering organisms/genetic engineering and plasmids
- "Build a plasmid" with Tangles
- Green Fluorescent Protein (GFP) transformation (Day 2)
    - Next steps: scale up (refer to Day 3) and purification (refer to Day 4)

Supplies (per group)

Materials:
Build a plasmid

- Tangles (6 colors): 7, 1, 1, 2, 2, and 2 links for each color, respectively, per group/demo
    - E.g., Amazon B0CSFZVHBM
- Small ziplock bags

GFP transformation (Exploring Biotechnology with GFP kit; Edvotek 303)

- Two tubes of *E. coli* starter culture
- Tube of ice-cold $CaCl_2$
- Tube of ice-cold Competent Cell Solution ("CCS")
- Tube of pGFP plasmid DNA
- Tube of Recovery Broth
- Four pre-poured agar plates
- 200 µL tips
- 20 µL tips
- Two sterile yellow inoculation loops
- 70% Ethanol

Equipment:

- Water bath set to 37°C
- Water bath set to 42°C
- Microcentrifuge
- Beaker or bucket of ice
- p20 micropipette
- p200 micropipette
- Waste beaker for tips

Preparation:

- Prepare bags of Tangles
- Refer to Edvotek kit manual
    - Pouring plates and making aliquots
    - Warming incubator and water baths
    - Prepare *E. coli* starter culture

Agenda/protocol:

1. Powerpoint review macromolecules, Central Dogma, and DNA and introduction to genetic engineering and plasmids
2. Classroom activity: Build a plasmid
    a. Supply groups of 2 with a bag of links. Use one bag of links for the demo.
    b. Walk through each step of the plasmid with the students, explaining the purpose of each part.

i. Build a blank backbone (7 links, yellow)
ii. Add origin of replication (1 link, white)
iii. Add antibiotic marker (2 links, red)
iv. Add promoter (1 link, purple)
v. Add Multiple Cloning Sites (2 links each, light and dark blue)
vi. Add gene (2 links, green)

3. Lab activity: GFP transformation

Student procedure:

Refer to Edvotek manual and modify as needed for your setting. Include group discussion about transformation predictions (amount and color of bacteria.

Resources:

- DaytonMADE powerpoint “2024Trailblazers Day 2 Part 2”
- Edvotek Exploring Biotechnology with GFP kit manual

Notes:

- The Tangles activity went over really well with the Explorers group and was effective at teaching the parts of a plasmid. The activity was more neutral with the Trailblazers. There were many complaints about the links being hard to work with.
    - Utilize the completed plasmid throughout the program to reinforce the parts of a plasmid and have a visual aid for making and testing hypotheses (e.g., growth after transformation, running a digested plasmid on a gel, etc.)
- Go through predictions as a group to enforce concepts of selection and induction.
- During the incubation after heat shocking (outgrowth/rescue time), we took the students on a tour of a research building. We were able to discuss similarities and differences with their experience and “real-life”, undergraduate research, poster presentations, etc
- Also during downtime during the transformation protocol, students practiced pipetting through doing pipet art or testing accuracy by weighing.

DaytonMADE Trailblazers	
Topic:	**Genetic Engineering - continued**
Program Schedule:	Day 3 (related to Genetic Engineering Day 2 and Downstream Processing Day 4)
Last Updated:	Sept 2024

Activities
• Observe bacterial transformation results
Supplies (per group)
Equipment: • UV light (e.g., Bio-Rad 1660530EDU)
Agenda/protocol:
1. Lab activity: Observe GFP transformations a. Use one group's plates to make observations as a group and compare to hypotheses. b. Allow groups to observe their plates c. Continue onto scale up procedure (next page) Refer to Edvotek manual for worksheets and discussion questions.
Resources:
Edvotek Exploring Biotechnology with GFP kit manual

DaytonMADE Trailblazers	
Topic:	**Scale-up**
Program Schedule:	Day 3 (related to Genetic Engineering Day 2 and Downstream Processing Day 4)
Last Updated:	Sept 2024

Activities

- Grow a larger amount of *E. coli* cells on agar plates

Supplies (per group)

Materials:
GFP scale up (Exploring Biotechnology with GFP kit; Edvotek 303)

- Transformed pGFP plates
- Two yellow inoculation loops
- Two LB/Amp/IPTG plates
- 70% Ethanol
- Marker

Equipment:

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

Preparation:

- Refer to Edvotek kit manual
    - Pre-pour plates
    - Warm incubator

Agenda/protocol:

1. Review scaling up and the upstream process
2. Lab activity: Scale up growth on plates

Student procedure:
Refer to Edvotek manual for specific protocol and modify as needed for your setting.

Resources:

- DaytonMADE powerpoint “2024Trailblazers Day 3”
- Edvotek Exploring Biotechnology with GFP kit manual

Notes:

- The Edvotek kit used here is a bit more basic than the Bio-Rad kit used in the Explorers curriculum. If using the Edvotek kit, it would fit into the biomanufacturing process more if the scale-up process was done in liquid culture. This could be optimized in the future. Here, we scaled up on plates as written and the protocol worked great.

DaytonMADE Trailblazers	
Topic:	**Soft Skills Session**
Program Schedule:	Day 3
Last Updated:	Sept 2024

Activities
• Learn about the 4 Cs of success
Agenda/protocol:
The WSU Student Success Center provided a one-hour session on the 4 Cs of success – Communication, Creativity, Collaboration, and Critical Thinking. To account for second-time participants, they had new activities for each "C". The largest activity involved splitting the group into teams of 2-3 students, giving them a set of materials, and having them build a catapult.

DaytonMADE Trailblazers	
Topic:	**Biology and Art/Fashion**
Program Schedule:	Day 3
Last Updated:	Sept 2024

Activities
• Powerpoint with videos
Agenda/protocol:
1. Powerpoint introduction to the uses of biology and biotechnology in the fields of art and fashion – mainly photos and videos of examples
Resources:
• DaytonMADE powerpoint “2024Trailblazers Day 3”

DaytonMADE Trailblazers	
Topic:	**Agar Art**
Program Schedule:	Day 3
Last Updated:	Sept 2024

Activities

- Create a design using colorful engineered *E. coli*

Supplies (per student)

Materials:

- Pretransformed bacteria “pallets” (prepared using Amino Labs Engineer-It kit, full rainbow)
- Two LB/Chl plates per person (poured using Amino Labs bulk materials)
- 70% Ethanol
- Toothpicks
- Yellow or blue inoculation loops
- Cotton Swabs

Equipment:

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

Preparation:

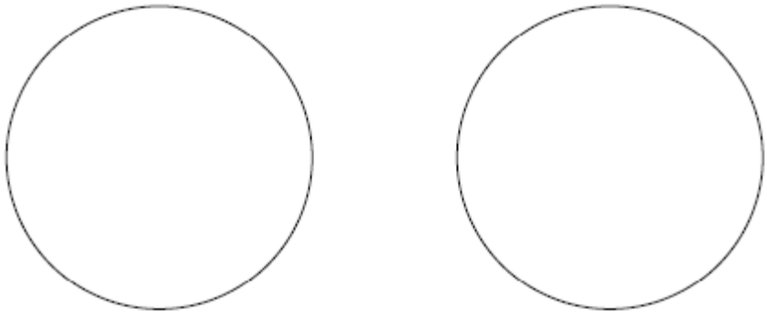
- Transforming and patching “pallets” – refer to Amino Labs Engineer-It kit manual
- Pouring plates
- Warming incubator

Agenda/protocol:

1. Lab activity: Create agar art

Student 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. 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.	☐



Resources:

Amino Labs also sells a Canvas kit that is designed to do agar art using 3 colors. They also have various resources and examples on their website and wiki.

Notes:

- We transformed plasmids expressing 10 different color proteins from Amino Lab's Engineer-It kit (full rainbow). We took resulting colonies and "patched" them onto new plates to create pallets.
- We attempted to use Amino Lab's Keep-it kit on the agar art plates so students could take them home as a souvenir. For our first attempt, we also did this for some example plates and a month later they had unfortunately lost their color. For our second attempt, we left the plates to dry over the weekend and the plates completed dried out. Conditions for using the kit would need to be optimized.

DaytonMADE Trailblazers	
Topic:	**Campus Tour**
Program Schedule:	Day 3
Last Updated:	Sept 2024

Activities

- Tour a college campus

Agenda/protocol:

1. Tour a college campus

Reflection/Discussion 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?

_____
_____
_____
_____

Notes:

- We found it beneficial to have an informal “chalk talk” about the different degrees and paths after high school during lunch before the tour.

DaytonMADE Trailblazers	
Topic:	**Downstream Purification**
Program Schedule:	Day 4 (related to Genetic Engineering Day 2 and Scale-up Day 3)
Last Updated:	Sept 2024

Activities

- Introduction to downstream processing
- Purify GFP from *E. coli* cells
    - Previous steps: transformation (refer to Day 2) and scale up (refer to Day 3)

Supplies (per group)

Materials:
GFP scale up for purification (Exploring Biotechnology with GFP kit; Edvotek 303)

- Plates of GFP expressing E. coli
- Marker
- 1000 µL tips
- 200 µL tips
- 70% Ethanol
- 5 or 10 mL serological pipets
- Tube of lysis buffer
- Two 1.5 mL microcentrifuge tubes
- Chromatography column
- Tube of 1X elution buffer
- Tube of slurry
- Microtiter plate

Equipment:

- Tube clamp and stand
- Freezer
- Water bath set to 55°C
- Beaker for column waste
- Waste beaker for tips
- p1000 micropipette
- p200 micropipette
- Microcentrifuge
- UV light
- Pipet aid

Preparation:

- Refer to Edvotek kit manual
    - Making aliquots
    - Warm water bath

Agenda/protocol:

1. Powerpoint introduction to downstream engineering and chromatography
2. Classroom activity: downstream engineering demo
    a. Create a mixture of different sized rocks, sand, and colored sugar/salt (e.g., large, medium, and small white rocks, white sand, and red sugar). Other materials needed are filters/sieves to separate each size rock, containers for filtering into, a coffee filter and funnel, water, and a stir rod.
    b. Relate the mixture to the manufacturing process and you need to purify your product. You can also discuss each “product” being removed can be another product you can sell.

c. Use the materials to progressively purify the mixture and obtain different products:
    i. Use physical separation
        1. Large sieve → purify large white rocks
        2. Medium sieve → purify medium white rocks
        3. Small sieve → purify small white rocks
    ii. Use solubility
        1. Add water, stir, filter, wash → purify white sand
    iii. Discuss evaporation and chromatography to purify sugar and dye
3. Lab activity: GFP purification

Student procedure:
Refer to Edvotek manual for specific protocol and modify as needed for your setting.

Resources:

- DaytonMADE powerpoint "2024Trailblazers Day 4"
- Edvotek Exploring Biotechnology with GFP kit manual

Notes:

- The Edvotek kit used here is a bit more basic than the Bio-Rad kit used in the Explorers curriculum. Perhaps switching these could be more appropriate.
- We had groups collect the brightest fraction and choose two other fractions to save for SDS-PAGE analysis.

DaytonMADE Trailblazers	
Topic:	**Downstream Purification - Analysis**
Program Schedule:	Day 4 (related to Genetic Engineering Day 2 and Scale-up Day 3)
Last Updated:	Sept 2024

Activities

- Analyze chromatography fractions using gel electrophoresis

Supplies (per group)

Materials:

- Fractions collected from the column chromatography protocol
- 20 µL tips
- Tube of protein denaturing solution *(from Exploring Biotechnology with GFP kit; Edvotek 303)*
- Pre-poured SDS-PAGE gel (one or two per class; e.g., Bio-Rad 4561095)
- Running buffer (one class stock, prepared *from Exploring Biotechnology with GFP kit; Edvotek 303)*
- FlashBlue stain *(from Exploring Biotechnology with GFP kit; Edvotek 303)*
- Standard protein marker *(from Exploring Biotechnology with GFP kit; Edvotek 303)*
- White vinegar – for making destaining solution
- Ethanol – for making destaining solution

Equipment:

- Vertical gel box
- Water bath set to 95°C
- p20 micropipette
- Waste beaker for tips

Preparation:

- Refer to Edvotek kit manual
    - Making aliquots
    - Prepare buffer
    - Prepare destaining solution
    - Warm water bath

Agenda/protocol:

1. Introduce gel electrophoresis at a basic level in lab
2. Lab activity: Analyze GFP production using SDS-PAGE

Student procedure:

Refer to Edvotek manual for specific protocol and modify as needed for your setting.

Resources:

Edvotek Exploring Biotechnology with GFP kit manual

Notes:

- We used one vertical gel box setup and had students share gels. We were able to fit all of the group samples onto two gels that we could then run at once.
- This resulted in a good discussion of purity vs yield, as the brightest fractions were also less pure.
- Interestingly, we also ran some unpurified lysate on our class gel, and all of the wells including this one seemed relatively pure.

DaytonMADE Trailblazers	
Topic:	**Biofuel Production in Yeast – Residual Sugar Test and Analysis**
Program Schedule:	Day 4 (related to Day 2 and Day 3)
Last Updated:	Sept 2024

Activities

- Quantify sugar utilization of yeast over 48 hours
- Compare the effect of different temperature and aeration conditions on ethanol production and sugar usage

Supplies (per group)

Materials:
Most materials were part of The Future of Biofuels - Alcohol Fermentation kit (Edvotek 304)

- Culture samples from Day 0, 1, and 2
- Tube of glucose testing reagent
- 1000 µL tips
- 200 µL tips
- Marker
- Seven screw top microcentrifuge tubes
- Three plastic cuvettes
- 70% Ethanol
- Tube of 10% sugar solution
- Tube of yeast growth media

Equipment:

- p1000 micropipette
- p200 micropipette
- Waste beaker
- Water bath set at 99°C
- Spectrophotometer
- Microcentrifuge

Preparation:

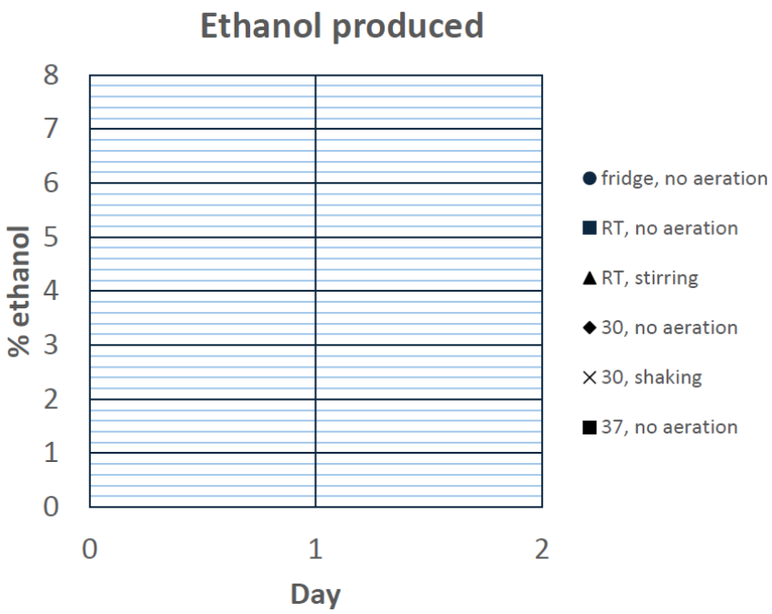
- Refer to Edvotek kit manual
    - Prepare Benedict's Reagent
    - Aliquot solutions
    - Warm water bath

Agenda/protocol:

1. Lab activity: Biofuel Production in Yeast – Residual Sugar Test
2. Group data analysis

Student procedure:
Analysis of ethanol production

Plot the calculated % ethanol produced for your 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 % ethanol produced over time?

_____

_____

What condition resulted in the highest ethanol production? Why do you think that it?

_____

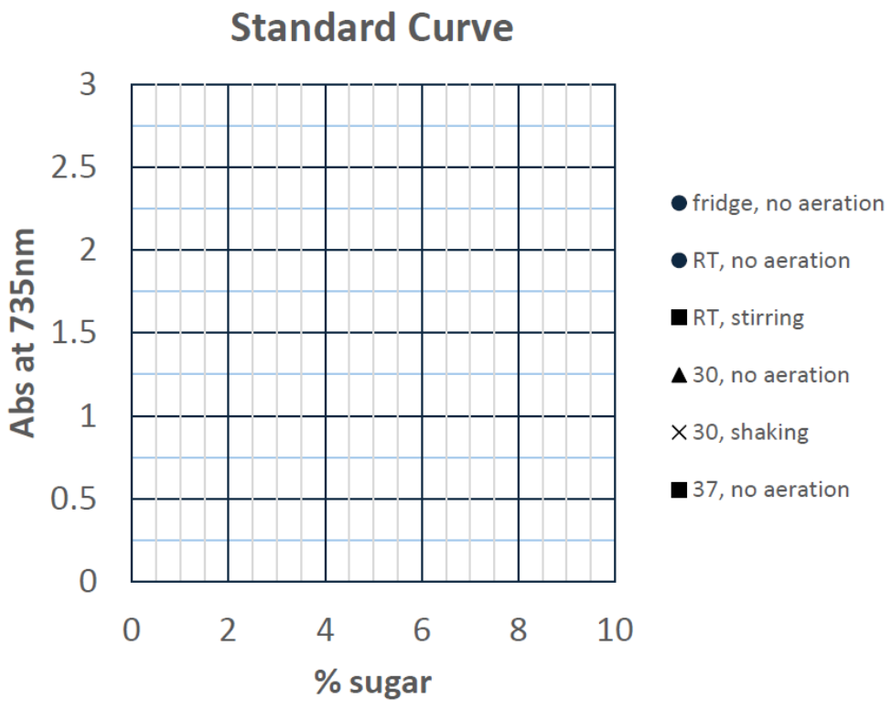
_____

Residual Sugar Test Protocol:

Refer to Edvotek manual for specific protocol and modify as needed for your setting. We had students make observations after heating with the Benedicts Reagent then centrifuge the samples, observe again, and measure absorbance at 735 nm.

	Color after added reagent and heating	Color of pellet after centrifuging	Absorbance at 735 nm
Day 0			
Day 1			
Day 2			
Standard curve # 1: 10% sugar			
Standard curve # 2: 5% sugar			
Standard curve # 3: 2.5% sugar			
Standard curve # 4: 1.25% sugar			
Standard curve # 5: 0.63% sugar			
Standard curve # 6: 0.31% sugar			
Standard curve # 7: 0% sugar			

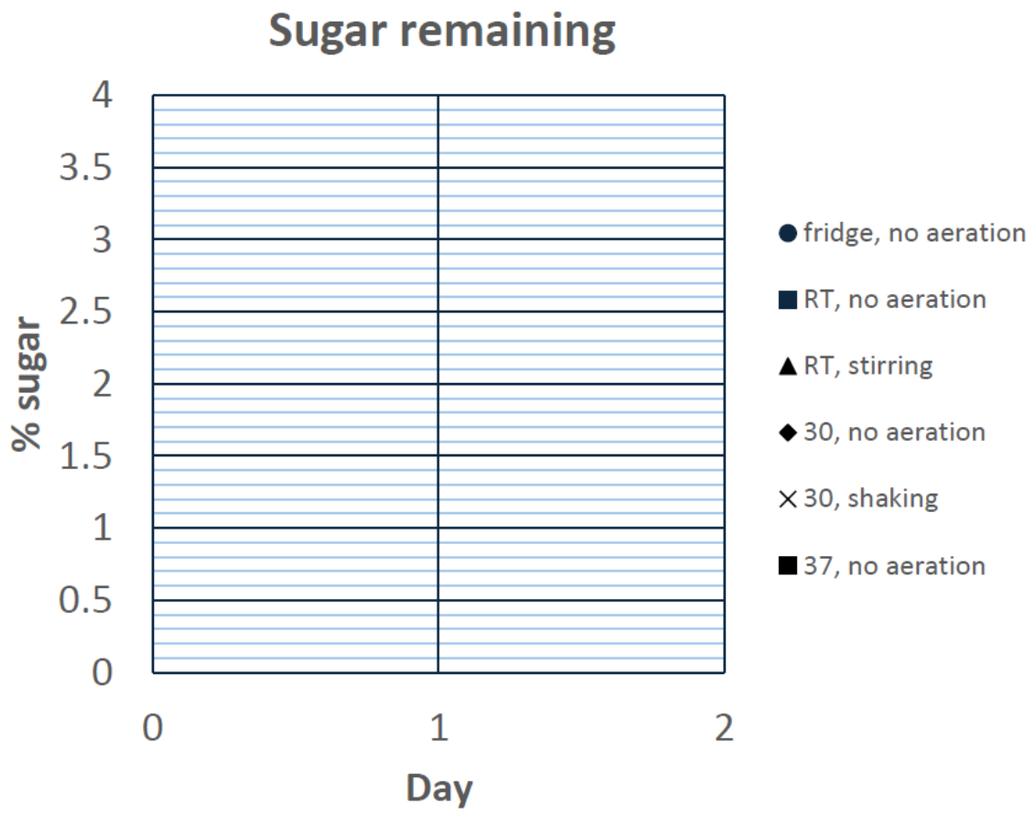
Plot the absorbance values for your seven standard curve samples on the chart below. Connect the points with a line.



Using your standard curve and the absorbance values of your Day 0, 1, and 2 samples, estimate the % sugar remaining in each sample:

	Absorbance at 735 nm	% sugar remaining
Day 0		
Day 1		
Day 2		

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?

_____

_____

_____

Resources:

- DaytonMADE excel sheet
- Edvotek The Future of Biofuels - Alcohol Fermentation kit manual

Notes:

- There was a wide range of results for the standard curve. Make sure that students aren't resuspending their samples before reading the absorbance.
- Each pair of students analyzed their own data, and we graphed and discussed comparisons as a group using an excel sheet projected in the classroom.

DaytonMADE Trailblazers	
Topic:	**Biomaterials**
Program Schedule:	Day 5
Last Updated:	Sept 2024

Activities

- Introduction to biomaterials
- Create a variety of bioplastics
- Create bio-based souvenirs using Checkerspot's Pollinator Kit

Supplies

Materials:
Bioplastics (per group) – most materials were part of Amino Labs Bioplastics All-in-One Kit WWG015

- Tube of oil
- Labels
- Silicone tray
- Cotton swab
- Five petri dishes
- Gelatin
- Vegetable starch
- Chitin/chitosan
- Baking soda
- Red, blue, and yellow dyes
- Small plastic pipettes
- Two star molds and a 5-rectangles mold
- Bottle of glycerol
- Bottle of 5% acetic acid
- Tube of liquid soap
- Weigh boats
- Scoopula
- Large plastic pipette
- Toothpicks
- Wooden or glass stir rod
- Distilled water
- 10 mL serological pipets
- Marker

Pollinator Kit

- Pollinator kits (https://www.pollinatorkit.com/)
- Mixing cups and sticks (e.g., Amazon B08FXMYVJ1)
- Various silicon molds
- Optional - Resin pigment (e.g., Amazon B005ZSGQBO)

Equipment:
Bioplastics

- Hot plate
- Serological pipet aid
- Glass beaker
- Three syringes (1 large, 2 small)
- Small balance (included in Amino Labs kit)
- Microwave
- Plastic beaker
- Magnetic stir bar

Preparation:

- For the Pollinator Kit:
    - We found it easiest to pre-pour Sides A and B into separate cups shortly before the students will need them. Work in a well-ventilated space. When giving the cups to the students, we added a tiny bit of resin into Side B (i.e., a pipette tip dipped in resin and then swirled in the liquid).
    - We aliquoted 50 mL Side A and 100 mL Side B for each student which allowed for one large mold and a number of smaller molds.
    - Provide coverage for benchtops. The kit comes with instruction sheets that can be used for this purpose.

Agenda/protocol:

1. Intro to biomaterials: Mix of powerpoint, videos, group discussion, think-pair-share
2. Lab activity: Bioplastics
3. Lab activity: Pollinator Kit

Student procedure - Bioplastics:

Refer to Amino Labs manual for specific protocols and modify as needed for your setting. Specifically, we did individual parts of the Bioplastics All-in-One kit:

- 5 rectangle molds
- Red bioplastic star, Blown texture, Baking Soda Bio-foam, Color Opacity Tests, and Soap Bio-foam
- Strings
- Biocomposites (with chitosan)

Student procedure – Pollinator Kit:

1. Mix resin into Side B very well.
2. Pour Side B into Side A and mix very well.
3. Pour resin into molds to solidify overnight.

Resources:

- DaytonMADE powerpoint “2024Trailblazers Day 5”
- Amino Labs Bioplastics All-in-One Kit manual and wiki
- Checkerspot Pollinator kit instructions

Notes:

- This protocol incorporates parts of the All-in-one kit from Amino Labs. One major modification was we gave each group a hot plate instead of sharing one microwave. This ended up taking the full day and there was a range of percent completion from the different groups.
- For module purposes, we also include Checkerspot’s Pollinator Kit here, however it didn’t fit in this day as written. The timing of this day needs to be optimized.
    - A Checkerspot employee provided a virtual presentation to the students about the company and the kit. In short, this is a polyurethane resin kit (mix Side A and Side B and get a hard product), but it is produced with oil made from algae and the final product is 56% bio-based. We provided various silicon molds of different sizes and subject matter and gave each student 150 mL of resin to create the items they were interested in. We also added pigments to color the resin (add very little pigment!). The students loved this experiment, especially because they got to keep souvenirs from it. Checkerspot also provided samples of the biomass-to-oil process and other examples of different formulations.
- In the first year of the program, we attempted other activities like biocement and silk enzyme stamping. These needed much more optimization, but could be valid to retry in the future.
- We collected various biomaterial samples to pass around during the introduction (e.g., https://mushroompackaging.com/collections/shop-all)
- Carefully read safety instructions for both kits.

DaytonMADE Trailblazers	
Topic:	**Bioplastics Analysis**
Program Schedule:	Day 6 (related to Biomaterials Day 5)
Last Updated:	Sept 2024

Activities

- Analyze bioplastics and compare results

Agenda/protocol:

1. Analyze bioplastics with lab partner
2. Compare results and group discussion

Student procedure:
Refer to Amino Labs resources for a Bioplastics Analysis manual.

Resources

- We only had students do a brief analysis, mainly looking at how the different formulations affected the basic properties of the material and how results varied between groups along with what might have caused said differences. Amino Labs provides a detailed analysis manual if you want to dive deeper into the analysis.

DaytonMADE Trailblazers	
Topic:	**Enzyme Production – PCR, Gel Electrophoresis, DNA Purification, and Digestion**
Program Schedule:	Day 6 (related to Ligation and Transformation Day 7, Scale-up and Induction Day 8, and Observations Day 9)
Last Updated:	Sept 2024

Activities

- Use PCR to amplify DNA
- Use gel electrophoresis to analyze DNA
- Purify DNA using column chromatography
- Use restriction enzymes to digest DNA

Supplies (per group)

Materials:
Most materials are from Investigating Synthetic Biology kit; Edvotek 331

- PCR tube
- 0.5 and 1.5 mL microcentrifuge tubes
- Tube of primer mix
- Tube of DNA template
- a PCR EdvoBead
- Sterile inoculation loop
- 20 µL tips
- 200 µL tips
- 1000 µL tips
- 70% ethanol
- SeeGreen tablet (MiniPCR RG-1500-20)
- TBE buffer (e.g., MiniPCR RG-1502-05)
- Tube of loading dye
- Four tubes of ultrapure water
- Water
- Tube of DNA ladder
- Spin column
- Tube of purification buffer
- Tube of wash buffer
- Tube of restriction enzyme buffer
- Tube of restriction enzyme mixture
- Marker

Equipment:

- p20 micropipette
- p200 micropipette
- p1000 micropipette
- Benchtop centrifuge
- Thermocycler
- Erlenmeyer flask
- blueGel electrophoresis system (e.g., MiniPCR classroom bundle QP-1500-28/QP-1500-28-INT)
- Microcentrifuge
- Water bath set at 37°C
- Water bath set at 70°C
- Waste beaker for tips
- Microwave

Preparation:

- Refer to Edvotek kit manual
    - Making aliquots and TBE buffer
    - Warming water baths

Agenda/protocol:

1. Powerpoint:
    a. Review of genetic engineering and plasmids
    b. Introduction to enzyme production experiment
    c. Introduction to PCR and gel electrophoresis
2. Lab activity: PCR and pour gel
3. Powerpoint: introduction to restriction enzymes
4. Lab activity: DNA purification, run gel, and digest DNA

Student procedure:
Refer to Edvotek manual for specific protocol and modify as needed for your setting. See notes below.

Self-reflection: What do you expect to see on the gel? How many bands to you expect to see in Lane 2? Lane 3? _____ _____ _____	☐
Observation: Draw what your gel looks like below. The ladder has been pre-drawn into Lane 1. Are the results what you expected?  	☐

Resources:

- DaytonMADE powerpoint “2024Trailblazers Day 6”
- MiniPCR manual for using SeeGreen tablets and blueGel box
- Edvotek Investigating Synthetic Biology kit manual

Notes:

- You can use the Tangles plasmid to reinforce the parts of a plasmid and understand the big picture.
- The Edvotek kit includes agarose, buffer, and stain for pouring gels. We found the miniPCR tablets to be useful and consistent with the rest of the curriculum. While the gels are solidifying, students can practice loading with silicone gels (e.g., from MiniPCR KT-1510-10)
- When running the gel, we had students also load a lane with their unamplified template to show that the PCR reaction amplified the DNA.
- This protocol spans the full day. During the break for lunch, we did the SOP activity before eating lunch. During downtime in the afternoon while the gels were running, we discussed Demo Day topics.
- You can relate running the gel to the Quality Control process in manufacturing.

DaytonMADE Trailblazers	
Topic:	**Standard Operating Procedures**
Program Schedule:	Day 6
Last Updated:	Sept 2024

Activities

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

Supplies

Materials:

- General materials for making multiple sandwiches – enough for 2-3 per group of 2-3 students. For example:
  - Bread (optional: different types)
  - Jelly (optional: different types)
  - Peanut butter (optional: different types)
  - Utensils
  - Plates and napkins
- Paper and writing utensils

Agenda/protocol:

1. Classroom activity: Communication and writing instructions
   a. We provided step-by-step instructions for drawing something (e.g., a train or house), but didn't tell the students what and shared the steps one by one.
2. Powerpoint introduction to Standard Operating Procedures and their importance
3. Classroom activity: Writing an SOP for making a PB&J sandwich

Part 1

a. Students in groups of 2-3 collect possible supplies.
b. Students work within their group to develop a written procedure for making a peanut butter and jelly sandwich. They can make a batch to help determine the steps.
c. Students carefully write out each step of the procedure, making sure the 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

a. Have one group volunteer their SOP and select a volunteer from another group.
b. Have the student use the SOP to make a sandwich while the other groups observe.
   i. Collect supplies from the SOP equipment/materials list.
   ii. Exactly follow the procedure while the other groups observe.
c. While they observe, have students identify problems with the procedures and be prepared to discuss corrections with the class. Have a subsequent group discussion about the successful parts of the SOP and where improvements could be made.
d. Have each group update their protocols based on the full group discussion.
e. Have another group volunteer their SOP and have multiple members of staff make a sandwich using the same updated SOP to see if a consistent, high quality product is made.

Resources:

- DaytonMADE powerpoint "2024Trailblazers Day 6"
- Similar activity in the NCABR "Mapping Your Future" document: https://www.ncabr.org/wp-

content/uploads/2015/12/mapping-your-future.pdf.

Notes:

- Students didn't take the opportunity to create test batches of sandwiches, so we ended up with extra materials.
- We suggest buying smaller containers of materials (e.g., peanut butter) so there's less waste. Alternatively, create aliquots or split bulk purchases into smaller containers.
- Ensure there are no peanut allergies!

DaytonMADE Trailblazers	
Topic:	**Enzyme Production – Ligation and Transformation**
Program Schedule:	Day 7 (related to PCR, Gel Electrophoresis, DNA Purification, and Digestion Day 6, Scale-up and Induction Day 8, and Observations Day 9)
Last Updated:	Sept 2024

Activities

- Use enzymes to ligate two pieces of DNA together to make a plasmid
- Transform a plasmid into bacteria

Supplies (per group)

Materials:
Most materials are from Investigating Synthetic Biology kit; Edvotek 331

- 0.5 mL microcentrifuge tubes
- 1.5 mL tubes of starter culture
- 20 µL tips
- 200 µL tips
- 1000 µL tips
- Tube of T4 ligase
- Tube of cut DNA vector
- Tube of your purified and digested PCR insert
- Tube of ultrapure water
- Two tubes of $CaCl_2$ solution
- Marker
- Tube of competent cell solution (CCS)
- Tube of control DNA
- Tube of recovery broth
- 70% ethanol
- Two agar plates with LB and Kanamycin
- Sterile inoculation loops

Equipment:

- p20 micropipette
- p200 micropipette
- p1000 micropipette
- Ice bucket or beaker with ice
- Waste beaker for tips
- Microcentrifuge
- Water bath set at 42°C
- Water bath set at 37°C
- Incubator set at 37°C

Preparation:

- Refer to Edvotek kit manual
    - Making aliquots and pre-pouring plates
    - Prepare starter culture
    - Warming water baths and incubator

Agenda/protocol:

1. Powerpoint: Introduction to ligation and review transformation
2. Lab activity: Ligation and transformation

Student procedure:

Refer to Edvotek manual for specific protocol and modify as needed for your setting.
Resources:
• DaytonMADE powerpoint “2024Trailblazers Day 7” • Edvotek Investigating Synthetic Biology kit manual
Notes:
• You can use the Tangles plasmid to reinforce the parts of a plasmid and understand the big picture.

DaytonMADE Trailblazers	
Topic:	**Mock Biomanufacturing Facility**
Program Schedule:	Day 7
Last Updated:	Sept 2024

Activities

- Be an employee at a mock biomanufacturing facility.

Supplies

Materials (see resource below for more details):

- Reese's cups
- Saltine crackers
- Vegetable oil
- Paper towels
- Variety of spoons, knives, measuring spoons, mixing bowls
- Small scales if possible (we used the ones that came with the Amino Labs Bioplastics kit)
- Paper and writing utensil

Agenda/protocol:

1. Mock Biomanufacturing Facility activity (activity from NCABR)
    a. The group is split into two "companies" of 5-6 students and they complete an R&D process to determine how best to convert a Reese's peanut butter cup cup into a peanut butter sandwich, including bulk product purification, formulation, and packaging. They then put their process to the test and the different approaches were compared.

Resources:

The activity came from North Carolina Association for Biomedical Research's (NCABR) "Mapping your Future: Careers in Biomanufacturing" document: https://www.ncabr.org/wp-content/uploads/2015/12/mapping-your-future.pdf.

Notes:

- This activity was a hit. It would be useful to have pre-established customer specifications they need to make to aid the "race" at the end.
- Make sure there are no peanut allergies!

DaytonMADE Trailblazers	
Topic:	**Industry Field Trip**
Program Schedule:	Day 8
Last Updated:	Sept 2024

Activities
• Visit an industrial biomanufacturing facility
Agenda/protocol:
Students arrived to camp as normal and then all rode in the van along with staff to the plant. Visit included a presentation overviewing the manufacturing process and safety information, a tour of the site, lunch, and a Q&A session. ● For the tour, the site provided PPE for all students and staff. The group was broken up into two groups. The tour included the microbiology lab, the main control room, the production floor and offices for each area including fermentation and downstream processing, packaging/warehouse, and maintenance shop. ● The Q&A session included employees with various positions and backgrounds. They highlighted the variety of careers and disciplines involved in running a large-scale biomanufacturing facility.

DaytonMADE Trailblazers	
Topic:	**Enzyme Production – Scale-up and Induction**
Program Schedule:	Day 8 (related to PCR, Gel Electrophoresis, DNA Purification, and Digestion Day 6, Ligation and Transformation Day 7, and Observations Day 9)
Last Updated:	Sept 2024

Activities

- Scale up growth of bacteria in liquid culture
- Induce production of an enzyme to produce a scent

Supplies (per group)

Materials:
Continuing Investigating Synthetic Biology kit; Edvotek 331

- Tube of LB broth with kanamycin
- 200 µL tips
- Your 'Ligation' and 'Control' plates
- 70% ethanol
- Sterile inoculation loop
- Tube of IPTG
- Tube of salicylic acid
- Cuvettes

Equipment:

- p200 micropipette
- Waste beaker for tips
- Shaking incubator set at 37°C

Preparation:

- Refer to Edvotek kit manual
    - Making aliquots
    - Warming incubator

Agenda/protocol:

1. Review scale-up
2. Lab activity: Scale-up
   [break for field trip]
3. Lab activity: Induction

Student procedure:
Refer to Edvotek manual for specific protocol and modify as needed for your setting.

Resources:

- Edvotek Investigating Synthetic Biology kit manual

Notes:

- We had students start their cultures before the field trip and continue with induction after lunch.
- Make sure you set up class controls.

DaytonMADE Trailblazers	
Topic:	**College 101**
Program Schedule:	Day 9
Last Updated:	Sept 2024

Activities

- Learn about how to be college-ready & how to apply for college

Agenda/protocol:

WSU Admissions presented a session on applying to schools, selecting schools, communication best practices, and financial aid.

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?

_____
_____
_____
_____
_____

DaytonMADE Trailblazers	
Topic:	**Enzyme Production – Observations**
Program Schedule:	Day 8 (related to PCR, Gel Electrophoresis, DNA Purification, and Digestion Day 6, Ligation and Transformation Day 7, and Scale-up and Induction Day 8)
Last Updated:	Sept 2024

Activities

- Analyze production of a scent

Agenda/protocol:

1. Lab activity: Observations

Student procedure:
Refer to Edvotek manual for specific protocol and modify as needed for your setting.

Self-reflection: How might you purify, formulate, and package the wintergreen scent? What products could it be used for? _____ _____ _____ _____	☐

Resources:

- Edvotek Investigating Synthetic Biology kit manual

Notes:

- We wanted to use a spectrophotometer to measure levels of the product in the culture, however we realized we didn't have the details worked out ahead of time.
- Students weren't the biggest fans of the scent, but they did admitted there was a difference in the engineered strains vs non-engineered strains. There was also a range of scents between the groups. This could be optimized or explored more. It would also be interesting to look at the effect of environmental variables or inducer/substrate concentration.

DaytonMADE Trailblazers	
Topic:	**Biomanufacturing Careers – part 1**
Program Schedule:	Day 9
Last Updated:	Sept 2024

Activities
• Discuss types of STEM roles in biomanufacturing • Explore “a day in the life” of a biomanufacturing professional using provided biographies
Agenda/protocol:
1. Powerpoint discussion about types of STEM roles in biomanufacturing 2. Activity looking at biographies and a-day-in-the-life (activity from NCABR) 3. Highlight non-STEM roles also related to the role of biomanufacturing
Resources:
Much of the information, and the activity itself, in this section came from North Carolina Association for Biomedical Research’s (NCABR) “Mapping your Future: Careers in Biomanufacturing” document: https://www.ncabr.org/wp-content/uploads/2015/12/mapping-your-future.pdf.

DaytonMADE Trailblazers	
Topic:	**Biomanufacturing Careers – part 2**
Program Schedule:	Day 10
Last Updated:	Sept 2024

Activities

- Explore real life job ads related to biomanufacturing

Agenda/protocol:

1. Job ad activity – Think, Pair, Share activity exploring different job ads using a work-sheet as a guideline. Students were provided with 2 or 3 of ~12 job ads for various biomanufacturing positions (e.g. lab tech, operator, fermentation scientist, senior scientist), including openings at companies they toured or learned about. Discussion included what they found interesting or surprising and similarities/differences. The discussion can morph into a general lesson/advice on applying for jobs and reading job postings.
2. Discussion about what the "ideal" candidate is and guidance on finding their career paths

	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.			

Resources:

DaytonMADE powerpoint "2024Explorers Day 9"

DaytonMADE Trailblazers	
Topic:	**Demo Day and Closing Ceremony**
Program Schedule:	Day 10 (Student prepare 2-3 days prior)
Last Updated:	Sept 2024

Activities

- Work in groups of 1-3 to create a poster presentation
- Present to a diverse audience, including family and industry professionals

Supplies

Materials:
Demo Day

- White poster board
- Cardstock or construction paper
- Bulletin board letters
- Scissors
- Glue sticks
- Masking tape
- Black and colored sharpies
- Thick markers
- Rulers
- Colored pencils
- Other general craft supplies as appropriate
- Science materials as applicable for hands-on demos (e.g., agarose tablets, Minipcr RG-1500-03)

Closing Ceremony

- Refreshments such as coffee, donuts, and water
- Folders containing final materials (e.g., a signed certificate, photos, staff contact info, a skills checklist, and student stipend

Preparation:

- Prepare demos for students as applicable
- Print contents for folders
- Print surveys

Agenda/protocol:

1. During lunch, have students fill out post-camp survey, feedback survey, and a post-camp word association activity.
2. Students finish preparing and practicing their presentations after lunch.
3. Visitors arrived at 2:00 for an open house in the lab.
4. At ~3:00, students and visitors moved to a separate room for the closing ceremony. The ceremony consisted of a short presentation/introduction to UES, BlueHalo, and DaytonMADE, followed by some speeches. Each student was then called up front to receive their folder and handshakes/photos with the staff. The event ended with a final group photo.

Notes:

- A slideshow of photos from the camp was shared during lunch for the students and then projected throughout the open house event.
- Provide students a heads-up on the first day that Demo Day will be happening. Form a list of topics sometime mid-camp and continue adding to it. Have students pick topics early/mid week 2 so that they work on their presentations during downtime. Dedicate time on days 8, 9, and 10 for preparation and practice.

- Students present about something they learned about during the camp that interested them most, such as bacterial transformation, downstream processing, and biomaterials. They create a poster and prepare a prop (e.g., transformed bacteria on plates) or hands-on demo (e.g., teaching visitors how to use a pipette and load a gel). We had the students set up around the laboratory and visitors were invited to join for an open house style event.

DaytonMADE Trailblazers	
Topic:	**Guest Speakers**
Program Schedule:	Throughout
Last Updated:	Sept 2024

Activities
• Virtual or in-person sessions presented by industry or military professionals.
Notes:
• Set strong expectations ahead of time, discussing professional etiquette with the students with regards to guest speakers. • We had a range of formats for the speakers – some in person and some virtual. The in-person speakers were received slightly better by the students, but in the second year we prepared the virtual speakers to have content more appropriate to the level of the audience and include more pictures and videos/tours which helped. • We attempted to have diversity in speakers. In the first year, all speakers had PhDs, but in the second year we improved to include speakers without PhDs and speakers from a larger variety of organizations (e.g., start ups, CMOs, military) and backgrounds (e.g., using soft skills to be involved in STEM fields without a STEM degree). The topics from the speakers ranged from vignettes through a career path (e.g., “lessons learned”) to more in-depth introductions to companies. • For future speakers, we would like to showcase scientists at various levels and highlight the multidisciplinary nature of biomanufacturing. We would also like to continue showcasing different environments visually, whether through in-person or virtual tours. • Most speakers were found through the network of DaytonMADE staff, but we also created an online form to share broadly.

Standards

The activities in the presented curriculum align with the following standards and competencies:

Ohio Health Career Field Technical Standards (2022)

- Strand 1. Business Operations/21st Century Skills
  - Outcome 1.1. Employability Skills
    Develop career awareness and employability skills (e.g., face-to-face, online) needed for gaining and maintaining employment in diverse business settings.
    - 1.1.1. Identify the knowledge, skills, and abilities necessary to succeed in careers.
    - 1.1.2. Identify the scope of career opportunities and the requirements for education, training, certification, licensure, and experience.
    - 1.1.3. Develop a career plan that reflects career interests, pathways, and secondary and postsecondary options.
    - 1.1.4. Describe the role and function of professional organizations, industry associations and organized labor and use networking techniques to develop and maintain professional relationships.
    - 1.1.5. Develop strategies for self-promotion in the hiring process (e.g., filling out job applications, resumé writing, interviewing skills, portfolio development).
    - 1.1.6. Explain the importance of work ethic, accountability, and responsibility and demonstrate associated behaviors in fulfilling personal, community, and workplace roles.
  - Outcome 1.2. Leadership and Communications
    Process, maintain, evaluate, and disseminate information in a business. Develop leadership and team building to promote collaboration.
    - 1.2.2. Deliver formal and informal presentations.
    - 1.2.3. Identify and use verbal, nonverbal, and active listening skills to communicate effectively.
    - 1.2.5. Communicate information for an intended audience and purpose.
    - 1.2.7. Use problem-solving and consensus-building techniques to draw conclusions and determine next steps.
    - 1.2.10. Use interpersonal skills to provide group leadership, promote collaboration and work in a team.
  - Outcome 1.3. Business Ethics and Law
    Analyze how professional, ethical, and legal behavior contributes to continuous improvement in organizational performance and regulatory compliance.
    - 1.3.1. Analyze how regulatory compliance affects business operations and organizational performance.
    - 1.3.2. Follow protocols and practices necessary to maintain a clean, safe, and healthy work environment.
  - Outcome 1.8. Operations Management
    Plan, organize and monitor an organization or department to maximize contribution to organizational goals and objectives.
    - 1.8.2. Select and organize resources to develop a product or a service.
    - 1.8.4. Identify alternative actions to take when goals are not met (e.g., changing goals, changing strategies, efficiencies).
  - Outcome 1.10. Sales and Marketing
    Manage pricing, place, promotion, packaging, positioning, and public relations to improve quality customer service.
    - 1.10.1. Identify how the roles of sales, advertising and public relations contribute to a company's brand.
- Strand 5. Bioscience Research and Development
  - Outcome 5.1. Handling, Preparation, Storage and Disposal
    Follow standard operating protocols for handling, preparing, storing, and disposing of specimens, supplies and equipment.

        - 5.1.1. Use standard operating procedures for the safe use of instruments, equipment, and gas cylinders.
    - Outcome 5.2. Foundations of Chemistry
      Use standard operating procedure (SOP) when performing systematic and methodical application of general and organic chemistry principles to examine the structures, their functions, their binding to other molecules and the methodologies for their purification and characterization.
        - 5.2.3. Differentiate between organic and inorganic compounds.
        - 5.2.4. Use common and chemical nomenclature for organic and inorganic materials.
        - 5.2.12. Define catalyst and identify materials used as catalysts, including enzymes.
        - 5.2.15. Describe, use, and calibrate precision weighing and measuring techniques (e.g., analytical balance, micropipette) that are based on the metric system.
    - Outcome 5.3. Microbiology Testing and Technology
      Describe the morphology and process of reproduction of microorganisms important in clinical disease and biotechnology applications and perform assays as a diagnostic tool to detect the presence of a pathogen when handling and storing specimens and preservatives for biologicals.
        - 5.3.2. Compare and contrast cellular structure and functions of prokaryotic and eukaryotic cells.
        - 5.3.3. Differentiate between bacterial metabolism, reproduction, cell structures, and their functions.
        - 5.3.8 Describe molecular behavior of large molecules, including carbohydrates, lipids, and proteins and nucleotides.
        - 5.3.9 Explain how chemical energy operates major cell processes (e.g., biosynthesis, movement, transport, growth).
        - 5.3.10 Explain factors that affect and optimize rates of enzyme assay reactions. 5.3.12 Perform biochemical assays of proteins, lipids, carbohydrates, nucleic acids, and enzymes.
        - 5.3.12 Perform biochemical assays of proteins, lipids, carbohydrates, nucleic acids, and enzymes.
    - Outcome 5.4. Bio-Molecular Technology
      Perform molecular and genetic applications using knowledge of nucleic acid structure and function, DNA replication, transcription, translation, chromosome structure and remodeling and regulation of gene expression in prokaryotes and eukaryotes.
        - 5.4.1. Predict and explain offspring genotypes and phenotypes using basic mode of genetics.
        - 5.4.2. Identify complex gene expression and transmission patterns.
        - 5.4.3. Explain and model the structure of DNA from nucleotide to chromosome.
        - 5.4.4. Model the Central Dogma Theory.
        - 5.4.5. Describe the processes involved in gene regulation.
        - 5.4.6. Identify and isolate peptides and proteins.
        - 5.4.7. Summarize the steps in creating a recombinant DNA molecule.
        - 5.4.8. Isolate and purify nucleic acids, including chromosomal and extra-chromosomal DNA molecules.
        - 5.4.10. Perform and interpret the results of restriction enzyme digests.
        - 5.4.12. Perform and interpret the results of a polymerase chain reaction to isolate proteins
        - 5.4.13. Use electrophoresis to separate nucleic acids and determine molecular weight.
        - 5.4.16. Explain the role of RNA and its role in gene expression.
    - Outcome 5.5. Laboratory Standard Operational Procedures
      Perform methods and techniques using protocols in order to conduct an experiment.
        - 5.5.1. Follow standard operating procedure (SOP) to aseptically collect and prepare dry and wet samples for analysis.
        - 5.5.6. Prepare, incubate, and identify colonies microscopically and macroscopically (e.g., colonial morphology, staining procedures, biochemical).
        - 5.5.7. Perform separation techniques, including chemical separations (chromatography), centrifugation, distillation, and filtration and describe their principles and interpret the results.

        - 5.5.10. Use aseptic laboratory techniques while working.
        - 5.5.11 Perform a chromatography separation of a given mixture of substances.
        - 5.5.12 Comply with industry-based and required regulatory quality-assurance practices (e.g., quality control [QC], Good Laboratory Practice [GLP], Good Manufacturing Practice [GMP]) for documentation.
    - Outcome 5.6. Culturing
      Perform experimental techniques used in cell biology to study cell growth, manipulation, and evaluation.
        - 5.6.1. Identify the structure of cells and the functions of their components.
        - 5.6.2. Explain classification, composition and preparation of culture media and prepare media for propagation.
        - 5.6.3. Identify bacteriologic methods necessary for isolation and identification of organisms.
        - 5.6.4. Operate basic microbiology and analytical equipment and examine biological specimens.
        - 5.6.5 Isolate, propagate, maintain, and harvest pure cell lines following standard operating procedure (SOP).
        - 5.6.7 Explain the collection and handling of fungal, mycobacterial, and viral specimens following standard operating procedure (SOP).
        - 5.6.9 Describe how vectors are used to transform host and microorganisms.
        - 5.6.11 Describe physical factors that affect microbial growth and identify a normal bacterium population growth curve.
        - 5.6.12 Calculate values of cell concentration for both batch and continuous cultivation
        - 5.6.14 Test for antibiotic susceptibility.
    - Outcome 5.7. Fermentation Technology
      Describe and perform fermentation procedures.
        - 5.7.1. Maintain, classify, and analyze types and classes of bioreactors and associated materials.
        - 5.7.2. Explain the principles and importance of sterility in industrial fermentations.
        - 5.7.9. Describe the functions and physical properties of simple and complex carbohydrates, lipids and proteins in the fermentation process.
        - 5.7.10. Describe the roles of enzymes as catalysts and the factors that affect enzyme activity in the fermentation process.
        - 5.7.12. Perform applications using benchtop fermenter and bioreactor systems.
        - 5.7.13. Monitor microorganism growth and determine the viability of cells.
    - Outcome 5.8. Biotechnology Research and Experiments
      Conduct a problem-based study, applying scientific methodology and using descriptive statistics to communicate and support predictions and conclusions.
        - 5.8.1. Identify research problems and structure a statistical experiment, simulation, or study related to the problem.
        - 5.8.3. Distinguish between dependent, independent and control variables in an experiment.
        - 5.8.4. Establish and implement procedures for systematic collection, organization and use of data.
        - 5.8.5. Select and apply sampling methods that appropriately represent the population to be studied.
        - 5.8.7. Document results of the experiment in a laboratory notebook, adhering to professional protocol.
        - 5.8.8. Compute measures of central tendency and dispersion to interpret results and draw conclusions.
        - 5.8.9. Describe the relationships between variables using correlations and draw conclusions.
        - 5.8.10. Create, interpret, and use tabular and graphical displays and describe the data.
        - 5.8.11. Draw conclusions and propose next steps based on observations and data analysis, recognizing that experimental results must be open to the scrutiny of others.

Next Generation Science Standards

- HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins, which carry out the essential functions of life through systems of specialized cells.
- HS-LS1-6. Construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids and/or other large carbon-based molecules.
- HS-LS1-7. Use a model to illustrate that cellular respiration is a chemical process whereby the bonds of food molecules and oxygen molecules are broken and the bonds in new compounds are formed, resulting in a net transfer of energy.
- HS-LS2-1. Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales.
- HS-LS2-2. Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales.
- HS-LS2-7. Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.
- HS-LS3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2. Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.
- HS-LS4-3. Apply concepts of statistics and probability to support explanations that organisms with an advantageous heritable trait tend to increase in proportion to organisms lacking this trait.
- HS-PS1-5. Apply scientific principles and evidence to provide an explanation about the effects of changing the temperature or concentration of the reacting particles on the rate at which a reaction occurs.
- HS-PS2-6. Communicate scientific and technical information about why the molecular-level structure is important in the functioning of designed materials.
- HS-ETS1-1. Analyze a major global challenge to specify qualitative and quantitative criteria and constraints for solutions that account for societal needs and wants.
- HS-ETS1-2. Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.
- HS-ETS1-3. Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics as well as possible social, cultural, and environmental impacts.
- HS-ESS3-1. Construct an explanation based on evidence for how the availability of natural resources, occurrence of natural hazards, and changes in climate have influenced human activity.