Biomanufacturing Kit Module 1 Syllabus

New Employee Onboarding: Training, Regulations, Career Exploration

Please Note: These assignments are designed to be flexible. They may be taught in the classroom or assigned as homework – or half and half. All documents are provided as '.doc' or '.ppt' so that teachers can download them and modify them to suit their curriculum, classroom, student population etc

1. Introduction to Biomanufacturing: Module 1, Lesson 1
    a. Insulin or Vaccine Story line and Question Board
    b. Overview of Biomanufacturing
    c. Introduction to Biomanufacturing slide deck
2. New Hire Onboarding: Module 1, Lesson 2
    a. All students read: PPP New Hire Welcome Document
    b. Production Teams are Created
    Team size can be somewhat flexible to suit teacher/class organization needs. Possible choices include:
        - Teams of 5
        - Teams of 4 – The teacher takes on the Quality Assurance Technician role for all teams.

    (Please note: Teams must be at least 4 students in size. Smaller team size will result in a greater number of teams/class, which then results in a need for more reagents and consumables. The SBE budget is limited, which limits the amount of reagents and consumables we can supply per teacher.)

    c. Each team creates a team name and a team logo
3. New Hire Training: Module 1, Lesson 3
    a. Lab Safety
        i. Lab Safety slide deck (if desired)
        ii. lab mapping activity
    b. Introduction to the FDA - video and questions
    c. Introduction to GMP - card sort
    d. Each team fills out the applicable parts of the Team Training Record.
4. Job Assignments: Module 1, Lesson 4
    a. Teams receive job assignments
    Team job/role assignment methods can be flexible. Students are sometimes absent for various reasons, so teachers have suggested

flexibility with selection/assignment of team roles. Possible choices include:

- Each student is assigned one role and keeps that role throughout the kit curriculum.
- Student roles are assigned daily and change from day to day.
- Each student is assigned a primary role and different back-up role.

b. Each team member reads their job description
c. Team members share information about their job assignment with other team members
d. Career Exploration assignment (Option #1 or #2)
e. Each team fills out a Team Role Record.

5. Standard Operating Procedure (SOP) Training: Module 1, Lesson 5
    a. New Hire Training - Importance of SOPs (can be done as a class)
    b. Micropipetting SOP assignment
    Alternatively teachers can use this time to introduce students to SOPs and have them create SOPs in a later module after they have had some experience using SOPs.
    c. Each team finishes filling out the Team Training Record
6. Finish design of Team Logo

Biomanufacturing Module One: New Hire Onboarding: Training/Regulations/Career Exploration

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- Team job/role assignment methods can be flexible. Students are sometimes absent for various reasons, so teachers have suggested flexibility with selection/assignment of team roles. Possible choices include:
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  - Student roles are assigned daily and change from day to day.
  - Each student is assigned a primary role and different back-up role.

	Topic / Activity	**Summary**
Day 1	Introduction to biomanufacturing - ● Insulin or Vaccine Storyline ● Question board ● Overview of biomanufacturing	On day one, students explore a storyline related to either insulin or vaccine production. They generate questions about the process, brainstorm the needs and requirements of such a process, and are introduced to the process of biomanufacturing.
Day 2	New Hire Welcome ● Creation of Teams ● Team Names ● Team Logos	On day two, student teams are created and welcomed as new hires to a biomanufacturing company. Teams create team names and begin designing team logos.

Day 3	New Hire Training Stations ● Lab Safety ● Intro to GMP ● Intro to FDA	Teams participate in new hire training on lab safety, good manufacturing practice, and the FDA by visiting a series of stations with their team.
Day 4	Job Assignments! Career Exploration	Students receive their job assignments! After reading job descriptions, they share a synopsis of their role with the team, and work together to determine how it fits in the biomanufacturing process. Students then learn about the importance of standard operating procedure through a final new hire training.
Day 5	New Hire Training - SOP SOP for Micropipetting	Student teams learn about SOPs. Teams develop SOPs for micropipetting. These are exchanged with another team, which tests the SOP by following it exactly as instructed. Student teams provide one another feedback and make improvements to their own SOP based on feedback.
Day 6	SOP for Micropipetting cont Finish Team Logo design	Student teams provide one another feedback and make improvements to their own SOP based on feedback. Student teams finalize their team logo design.

Biomanufacturing Module 1

Lesson 1 - Introduction to Biomanufacturing

Lesson objectives:

Students will understand:

- Biological pharmaceutical products are made in large scale manufacturing facilities using living organisms (bacteria and other cells) to produce products
- This process must be tightly regulated to ensure consistent and safe supply

Essential Question

- How can biologically produced medical products be mass produced safely and effectively?

Materials:

- How vaccines are made video &/or “How Pfizer Makes its Covid Vaccine” NYTimes interactive
- Butcher Paper and Sticky Notes
- Biomanufacturing Graphic Organizer
- Introduction to Biomanufacturing Slidedeck

What Students Will Do

- Watch a short video and/or interactive showing how mRNA vaccines are produced or how recombinant insulin is produced
- Create prior knowledge and question boards based on video/interactive
- Brainstorm important factors to consider when producing biologics like insulin or mRNA vaccines
- Complete a graphic organizer to summarize the biomanufacturing process, including upstream and downstream processing, quality assurance, and quality control

Teacher Preparation

- Prior to class prepare two pieces of poster paper/ butcher paper. At the top of one, write “What I think I know”. On the top of the other, write “What I want to know”.
- Ensure the video is ready to play
- Make copies of the graphic organizer student handout

Organizer

Time	**Activity**	**Materials**
5 minutes	Watch video/ interactive	Video/interactive
10 minutes	Question Board	Butcher paper and sticky notes
10 minutes	Class brainstorm	Whiteboard or smartboard
25 minutes	Biomanufacturing GO & Slideshow	Slide deck and graphic organizer (1 per student)

Procedure

Introduction (Teachers can choose either insulin or vaccine storyline)

A. Insulin Storyline

1. Ask students what they know about the insulin protein. Likely most students will know that it is used to treat diabetes, or maybe even that it is used to treat Type 1 Diabetes specifically. If you have not yet covered content related to insulin, you could spend a few minutes here reviewing the protein and its function. The first 51/2 minutes of this 12 min video from Novo Nordisk is a good place to start: https://www.youtube.com/watch?v=HJGjNTJgf48
2. Ask students how they think insulin protein is produced on a large scale for the world's population. Take responses from 2 or 3 students. Do not correct misconceptions or add to their explanations.
3. Tell students you will watch a short video about the production of insulin. Play the video for them:

    a. https://www.youtube.com/watch?v=ZVa0BZnSd_A

B. mRNA Vaccine Storyline

4. Ask students what kind of vaccines the Moderna and Pfizer Covid vaccines are. Likely most students will know they are RNA vaccines. If you have not yet covered content related to RNA, you could spend a few minutes here reviewing the molecule and its function.
5. Ask students how they think RNA vaccines are produced on a large scale, for the world's population. Take responses from 2 or 3 students. Do not correct misconceptions or add to their explanations.
6. Tell students you will watch a short video about the production of vaccines. Play the video for them:
    a. https://www.youtube.com/watch?v=H09Lk_MW5Dk (1:47 - 2:29)

    As an alternate, if your school has access to the New York Times, have students visit the interactive, "How Pfizer Makes Its Covid Vaccine" -
    b. https://www.nytimes.com/interactive/2021/health/pfizer-coronavirus-vaccine.html

Question Board

7. Handout two colors of sticky notes to each table group. Designate one color "things I already know" and the other for questions. Tell students to write one thing per sticky note. If students already know things about vaccine or insulin production, they should record this on the first color sticky note and bring to the appropriate poster at the front of the room.
8. Tell students to write at least 2 questions they have about insulin production or vaccine production on the second color sticky note, and put on the class "Things I Want to Know" poster.
9. As students finish, read student ideas and questions from both boards, categorizing questions. Refrain from answering questions at this point.

Brainstorming

10. Ask students to imagine they are picking up a prescription for medicine from the pharmacy, or receiving a vaccine from their doctor. Ask what sorts of assumptions they should be able to make about the medicine or pharmaceutical product. (That the ingredients are as indicated, that the dose is correct, that it has been tested to be safe, that it has been tested to work, that it is not contaminated, etc.) Have students share a few ideas.
11. Next ask student teams to imagine they are responsible for producing a medicine or vaccine. Tell them to brainstorm in small groups as many factors as possible they would be responsible for ensuring. Have a record keeper record the ideas for each group.
12. Ask groups to share ideas, and record on the board. These could be similar to the ideas above, along with ideas such as cost and advertising/marketing.

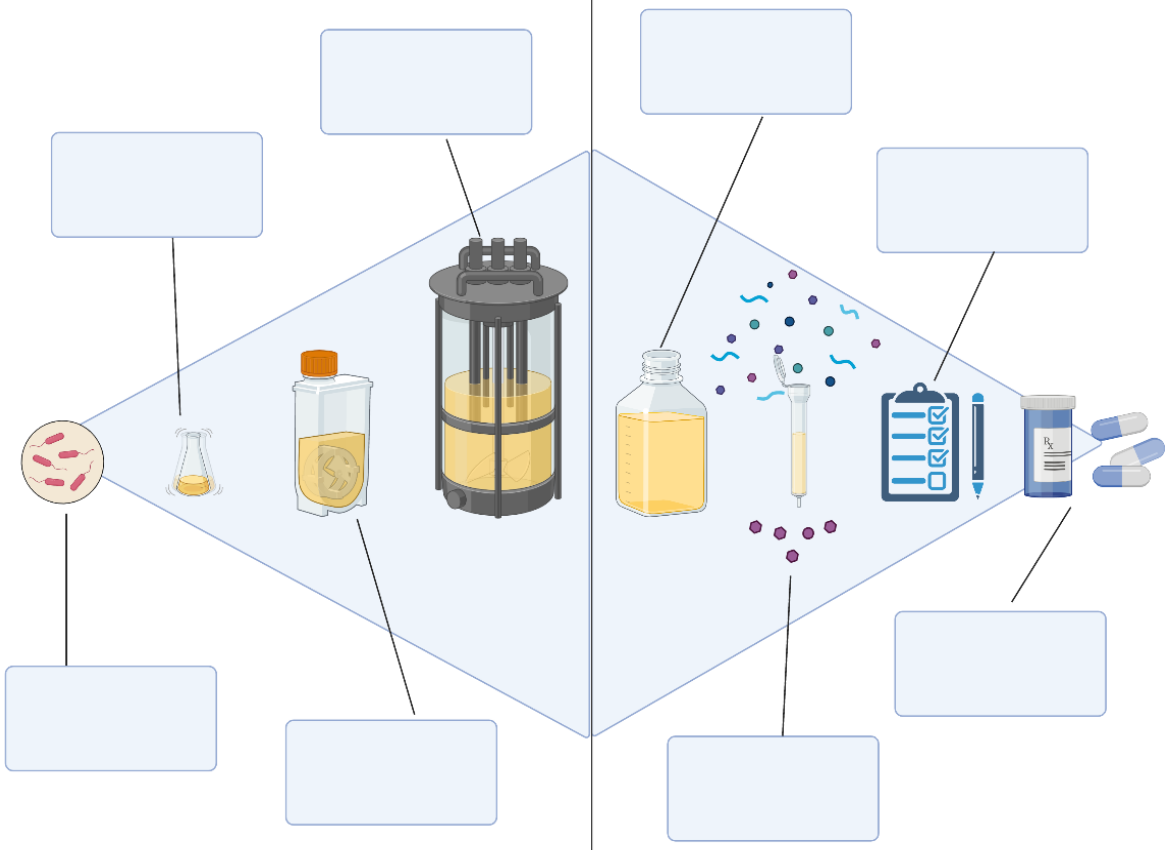
Introduction to biomanufacturing

13. Tell students that because of the ideas shared during the brainstorming session, the production of pharmaceuticals, especially biologics, requires a tightly regulated process.
14. Distribute the Biomanufacturing Process Graphic Organizer to students.
15. Display the Introduction to Biomanufacturing Slide Show, and have students record notes on their graphic organizer as you proceed.

Overview of Biomanufacturing

Upstream Process: _____

Downstream Process: _____



Biomanufacuring_____

Created in BioRender.com bio

Biomanufacturing Module 1

Lesson 2 - New Hire Onboarding

Lesson objectives:

Students will understand:

- Biomanufacturing is done in teams.
- All members of biomanufacturing teams must be properly trained.

Essential Question

- What type of training is necessary for biomanufacturing personnel?

Materials:

- PPP New Hire Welcome Document
- Company Name and Logos slide deck
- Team Name and Logo Design Assignment
- Documentation file folder for each team

What Students Will Do

- Read the PPP New Hire Welcome Document
- Group into Production Teams
- Listen to/watch the Company Name and Logos slide deck
- Create a Team Name
- Brainstorm ideas for a Team Logo
- Sketch initial ideas for Team Logo
- File their Team Logo work in their team file

Teacher Preparation

- Prior to class make a copy of the PPP New Hire Welcome Document for each student. Fill in: Training Start Date, Time, Location
- Make copies of the Team Name and Logo Design Assignment
- Make copies of the graphic organizer student handout
- Identify a storage location for Production Team files

Organizer

Time	Activity	Materials
10 minutes	Read PPP New Hire Welcome Document	PPP New Hire Welcome Document
5 minutes	Form Production Teams	
10 minutes	Company Name and Logo slide deck	Slide deck
10 minutes	Teams meet to choose a name	

15 minutes	Teams meet to brainstorm team logos	Paper and pencils for sketching

Procedure

PPP New Hire Welcome

1. Ask students to read over the 'PPP New Hire Welcome' document. Ask students to discuss the importance of proper training in biomanufacturing.

Creation of Production Teams

2. Break students into Production Teams of 4 or 5 individuals. If the students work in teams of 4, the teacher should take on the role of the Quality Assurance Technician.
3. Display the Company Name and Logos slide deck.

Creation of Team Names and Logos

4. Ask the students in each production team to choose a team name.
5. Ask students to spend some time brainstorming team logos.
6. Hand out scratch paper and pencils so teams can sketch out ideas.
7. Hand out a file folder to each team
8. Ask each team to label the file with their team name.
9. Ask each team to file their preliminary logo work in their team file.

PPP New Hire Welcome Document

Welcome to Protein Production Partners (PPP)! We are happy to have you on the PPP team.

This packet of materials will provide you the information necessary to begin your job at PPP.

Protein Production Partners is a Contract Research Organization (CRO). This means that other companies and research organizations hire us to produce the protein therapeutic products that they have developed. Biomanufacturing is a complex and expensive process which requires space, equipment, and careful monitoring. Many smaller biotech companies or research organizations do not have the capital (funds) to build and staff a biomanufacturing complex. Instead they contract with CROs like PPP to produce their protein therapeutic. Our intelligent, skilled, and meticulous work force (that's you!) grow the cells producing the protein therapeutic and purify it. Welcome on board!

Your first task as a team will be to produce Red Fluorescent Protein (RFP) or Green Fluorescent Protein (GFP) following all company procedures. If you perform this set of tasks well, you will be cleared for production of other protein therapeutics.

All new hires undergo training to learn the policies, regulations, and procedures required for biomanufacturing before beginning any production work. During the training period you will learn about Lab Safety, FDA Regulations, GMP (Good Manufacturing Practice), Quality Systems, Standard Operating Procedures (SOP).

Training Start Date:

Time:

Location:

Dress code: For work in the training lab, we require basic laboratory PPE (personal protective equipment). This includes: lab coats, gloves, safety glasses, masks (may be optional depending on COVID guidelines). You must wear close toed shoes. Long hair must be pulled back.

First week schedule:

- Lab Safety Training
- Job Assignments
- Introduction to Biomanufacturing Regulations: GMP and FDA (Food and Drug Administration)
- Introduction to SOPs (Standard Operating Procedures)

Biomanufacturing Module 1

Lesson 3 - New Hire Training

Lesson objectives:

Students will understand:

- What type of training is needed for biomanufacturing jobs.
- Why the biomanufacturing industry is regulated.
- What types of rules/regulations govern the biomanufacturing industry.

Essential Questions

- Why is the biomanufacturing industry regulated?

Materials:

- Lab Safety Training slide deck - or - your own personal lab safety slide deck
- Paper for laboratory safety mapping
- Lab Safety Assignment
- The FDA and the Origins of GMP slide deck
- Intro to the FDA Assignment
- Intro to GMP Assignment
- GMP Biomanufacturing Card Sort Activity board and cards
- Team Training Record Document
- Documentation file folder for each team

What Students Will Do

- Listen to/watch the Lab Safety Training slide deck
- Work with their team to create a Lab Safety Map (Lab Safety Assignment)
- Listen to/watch the FDA and the Origins of GMP slide deck
- Listen to the ‘Chamber of Horrors’ video about the creation of the FDA
- Work with their team to discuss the questions in the Intro to the FDA assignment
- Work with their team to carry out the GMP Card Sort Activity
- Fill out the appropriate sections of their Team Training Record
- File their Team Training Record in their team file

Teacher Preparation

- Make copies of the Lab Safety Assignment - one per team
- Make copies of the Intro to the FDA Assignment - one per team
- Make copies of the Team Training Record - one per team
- Make sure the video about the creation of the FDA is ready to play.
- Make sure the video(s) about GMP are ready to play.

Organizer

Time	Activity	Materials
10 minutes	Lab Safety slide deck (if needed)	Slide deck
10 minutes	Lab Safety Assignment - Lab mapping	Lab Safety Assignment document, pens
10 minutes	The FDA and the Origins of GMP slide deck	Slide deck
25 minutes	Intro to FDA Assignment Chamber of Horrors video	Intro to FDA Assignment document, video
10 min	Intro to GMP Assignment Video(s)	Intro to GMP Assignment document, video(s)
15 minutes	Assignment discussion GMP Card Sort Activity	GMP Card Sort board and cards
10 minutes	Team Training Record - Documentation	Team Training Record Document

Procedure

Lab Safety Training

1. Display the Lab Safety Training slide deck. If you have your own classroom lab safety presentation, feel free to use it instead. Or if your students are well versed in lab safety, feel free to skip this.
2. Ask the student teams to complete the Lab Safety Assignment

Introduction to the Food and Drug Administration (FDA) and Good Manufacturing Practices (GMP)

3. Display The FDA and Origins of GMP slide deck.
4. Tell the students you will watch a video about the creation of the FDA.
5. Play the 'Chamber of Horrors' video about the creation of the FDA.

https://www.youtube.com/watch?app=desktop&v=jFwhoT1hr0Q

6. Ask the production teams to discuss the questions in the Intro to the FDA Assignment
7. Play the short intro to GMP video and one of the two longer videos that lists the principles of GMP

Short Intro (3 min): https://www.youtube.com/watch?v=wv-77usj0rw

12 min https://www.youtube.com/watch?v=XiOP7ihBOyM

14 min https://www.youtube.com/watch?v=JHkGgFUuZwE

8. Ask the production teams to discuss the questions in the Intro to GMP Assignment
9. Ask the production teams to do the GMP Card Sort Activity
10. Ask each team to fill out the appropriate sections of their Team Training Record. NOTE that the training record is not yet complete.
11. Ask each team to file their partially completed Team Training Record in their team file.

New Hire Training – Laboratory Safety Assignment

Laboratory Safety is of utmost importance. Each employee must do their best to protect themselves and their co-workers by following all lab safety guidelines outlined in this training.

1. Watch the laboratory safety slide deck.

2. Review your school's lab safety rules.

3. Mark the location of the following pieces of safety equipment on a map of your laboratory
    a. Fire extinguisher
    b. Exits
    c. First Aid Kit
    d. Defibrillator
    e. Eye wash
    f. Emergency shower
    g. Spill kit
    h. Location of PPE – gloves, lab coats, safety glasses
    i. Location of waste receptacles – sharps waste, glass waste, liquid biohazard waste, solid biohazard waste

4. Sign off on the Protein Production Partners Lab Safety Training Completion Document.

New Hire Training- Introduction to the FDA Assignment

The Biomanufacturing industry is regulated by the Food and Drug Administration (FDA). At PPP, we want our employees to understand the importance of the regulations that govern our industry.

This video describes the events leading up to the formation of the FDA and the role the FDA plays in ensuring the safety of drugs/therapeutics.

https://www.youtube.com/watch?app=desktop&v=jFwhoT1hr0Q

After watching the video, discuss the following questions in your manufacturing team:

1. What types of issues led to the formation of the FDA?

2. What were some of the limitations of the 1906 Pure Food and Drugs Act?

3. What was the purpose of the 'American Chamber of Horrors' exhibit?

4. How did the 1938 Food, Drug and Cosmetic Act expand the ability of the FDA to protect the American public?

New Hire Training- Introduction to GMP Assignment

Good Manufacturing Practice (GMP) is a system for ensuring that products are consistently produced and controlled according to quality standards of any manufactured product.

In Biomanufacturing, GMP refers to the practices which are **required by law** in order to conform to the guidelines of agencies like the FDA that control the authorization and licensing of the manufacture and sale of food and beverages, cosmetics, pharmaceutical products, dietary supplements, and medical devices.

GMP covers all aspects of production from the starting materials, premises, and equipment to the training and personal hygiene of staff. Detailed written procedures (SOPs) are required for each process that could affect the quality of the finished product. There must be systems to provide documented proof that correct procedures are consistently followed at each step in the manufacturing process - every time a product is made.

Watch the short intro to GMP video and one of the longer videos that list the principles of GMP.

Short Intro (3 min): https://www.youtube.com/watch?v=wv-77usj0rw

(12 min) https://www.youtube.com/watch?v=XiOP7ihBOyM

(14 min) https://www.youtube.com/watch?v=JHkGgFUuZwE

As you watch these videos write down any terms/words that you are not familiar with.

After watching the videos, discuss the following questions in your manufacturing team:

1. What words/terms were unclear to you? Make a list.

   Have each team member look up an unfamiliar term and share the definition with the team. Discuss how you think this term is applied to biomanufacturing given what you saw in the videos or learned from your research.

2. Play the GMP card game. Match each task to the GMP Principle it supports.

Introduction to GMP Training – Card Sort

Teacher Key

Principle 1: Bob looks up the Standard Operating Procedure (SOP) document that describes how to properly operate the spectrophotometer.

Sewite writes a Standard Operating Procedure for using the new shaker/incubator.

Principle 2: Jalisa carefully carries out each step of the Standard Operating Procedure (SOP) for inoculating a liquid culture of bacteria.

Carlos carefully carries out each step of the Standard Operating Procedure (SOP) for using the shaker/incubator.

Principle 3: Jose writes down all of the details regarding the batch of LB (Lysogeny broth) he created in a batch record document that he signs and dates.

Jennifer finishes checking the viability of a scaled-up bacterial culture. She fills out the appropriate parts of the Upstream Process Report Batch Record and signs and dates the sections of the report she has filled out.

Principle 4: Michelle stores the batch reports from each bacterial production run in the appropriate files. This documentation demonstrates that bacterial culture is done the same way every time and that the bacterial cell line produces RFP consistently in every batch.

Junji uses the new shaker/incubator to grow multiple batches of RFP+ bacteria. He records data about each batch to verify that the new equipment works properly every time. Once he has documented that the equipment functions properly and reliably, the equipment can be used by the upstream process team.

Principle 5: The Proteins R Us company designed their new manufacturing plant so that all of the air coming into the manufacturing clean rooms is filtered for sterility.

The MegaPharma Company has installed low pore size filters on all water lines that come into their clean rooms to be sure that all water used in their biomanufacturing processes is sterile.

Principle 6: Asako and Dante carefully conduct maintenance on all equipment used in the clean rooms.

Sheila and Rosa carefully sanitize all clean room surfaces – floors, ceiling, walls, benchtops.

Principle 7: Rubin gets comprehensive training for his job as a production manager before beginning work at Proteins R Us.

Keiko and Amir have finished their aseptic technique training at Proteins R Us. Before being cleared to work in the clean room, they must demonstrate their ability to perform aseptic technique properly.

Principle 8: Jackie follows proper gowning procedures before beginning work in the clean room in order to minimize any contamination coming from herself or her clothing.

George washes his hands before gowning up to begin work in the clean room.

Principle 9: Shari tests all raw materials before releasing them for use in the biomanufacturing process.

Alberto and Yuri test the purity of the protein product they are manufacturing after every phase of the downstream purification process.

Principle 10: Hiro inspects all documentation at Proteins R Us every six months. Ruby verifies that all regulations are being followed I the manufacturing plant.

Muhammed inspects all spectrophotometers every 2 months to verify that they are performing properly.

Biomanufacturing Module 1

Lesson 4 - Job Assignments

Lesson objectives:

Students will understand:

- That biomanufacturing teams are comprised of people with different job descriptions.
- The titles of some of the jobs involved in biomanufacturing.
- That each job title has different responsibilities.

Essential Question

- What type of positions are needed in a biomanufacturing team?

Materials:

- Job Description documents
    - Process Engineer
    - Quality Assurance (QA) Technician
    - Quality Control (QC) Technician
    - Upstream Process Technician
    - Downstream Process Technician
- Career Exploration Assignment
- Team Role Record Document
- Documentation file folder for each team

What Students Will Do

- Read their job description document
- Share information about their job responsibilities with their team
- Listen to their team members describe their job responsibilities
- Discuss questions related to the job descriptions as a class
- Write questions on the white board
- Complete the Career Exploration Assignment #1 or #2 (homework)
- Fill out their Team Role Record
- File their Team Role Record in their team file

Teacher Preparation

- Make copies of the Job Descriptions - one copy of each job description per team
- Make copies of the Team Role Record - one per team

Organizer

Time	Activity	Materials
10 minutes	Assign job roles in each team, or allow teams to choose roles themselves	Job Description Documents
5 minutes	Students read their job descriptions	

20 minutes	Students share information with their team members about their job description	
15 minutes	Class discussion about job descriptions Students identify questions they have about their job descriptions	White board
5 minutes	Students receive the Career Exploration Assignment (homework)	Career Exploration Assignment Document (Option 1 or 2)
5 minutes	Teams fill out their Team Role Record	Team Role Record Document

Procedure

Job Assignments

1. Assign jobs within each team. Or - Hand out job descriptions for each of the 5 team jobs to each team and allow the students to self-select their jobs.
   Team job/role assignment methods can be flexible. Students are sometimes absent for various reasons, so teachers have suggested flexibility with selection/assignment of team roles. Possible choices include:
   a. Each student is assigned one role and keeps that role throughout the kit curriculum.
   b. Student roles are assigned daily and change from day to day.
   c. Each student is assigned a primary role and different back-up role.
2. Ask students to read their job description.
3. Ask students to describe their job to the rest of their team. Each team member describes their job (as they currently understand it from reading the job description) to the rest of their team.
4. The class meets as a whole to discuss questions students have about their jobs.
5. Questions about each job are written on the white board. These can be further discussed after students complete one of the Career Exploration Assignments. These assignments can be worked on in class and/or assigned as homework.
6. Each team fills out their Team Role Record.
7. Each team files their Team Role Record in their team file.

Job Description
Job Title: Downstream Process Technician

Purpose and Scope of Position

The Downstream Process Technician carries out all protocols related to the purification of the protein therapeutic, from lysis of cells through column purification (chromatography). The Downstream Process Technician will provide protein samples at various points throughout the purification process and provide these to the QC Technician for testing. The Downstream Process Technician will draft and review the batch record for the protein therapeutic.

Duties and Responsibilities

- Responsible for chromatography purification of the protein product
- Properly disinfect and stage materials in production room prior to purification
- Assist in drafting and reviewing production batch records.
- Complete all required documentation in an accurate and timely manner.
- Communicate with team members in a timely manner.
- Adhere to safe working practices.
- Identify, escalate, and report deviations.

Vocabulary

QC - Quality Control - You will learn about what quality control is from the Quality Control Technician job description and gain a better understanding as your team works through the biomanufacturing kit curriculum

Protein Therapeutic – The protein you are trying to purify. A protein that will be used as a medicine/drug. Also known as a biologic.

Lysis – The process of breaking cells apart.

Chromatography - A laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a system on which is fixed a material called the stationary phase.

Batch Record - A document that provides the complete manufacturing history of a pharmaceutical product.

Deviations – Results that are incorrect or unexpected that occur during the biomanufacturing process.

Escalate – Report to a higher authority.

Job Description
Job Title: Quality Assurance Technician

Purpose and Scope of Position

Quality Assurance Technicians are responsible for reviewing and approving documentation involving all aspects of the production process. Documentation includes:

- Equipment readiness checklists
- Equipment cleaning and shutdown checklists
- Batch records
- Employee training records

The QA Technician is responsible for reviewing all Biomanufacturing team documentation. If a document is in compliance (filled out properly), the QA Technician will sign off on it and file it. If a document is not in compliance, the QA Technician will meet with the Biomanufacturing team. The team will work under the guidance of the QA Technician to determine the reason for non-compliance and to correct the problem.

Duties and Responsibilities

- Review of all team documentation in an accurate and timely manner.
- Investigation of any documentation that is not in compliance.
- Proper filing of all required documentation.
- Communicate with team members in a timely manner.
- Support of site internal audits, external client audits and regulatory inspections.
- Adhere to safe working practices.

Vocabulary

Documentation – This term refers to all documents that record information about the biomanufacturing process. This includes: employee training, batch records, product testing etc.

Audit - A GMP audit is a process by which an external or internal individual or team verifies that a manufacturer is following its documented Good Manufacturing Practices. It's similar to an audit in accounting or finance, except that the auditor is inspecting production processes for their adherence to quality control guidelines instead of financial records or data.

Job Description
Job Title: Process Engineer

Purpose and Scope of Position

The Process Engineer installs and maintains equipment and processes for manufacturing and testing pharmaceutical products, ensuring that everything runs safely and efficiently and meets regulatory standards.

The Process Engineer is responsible for verifying that equipment is in good working order before use, cleaning equipment after use, and keeping documented records of equipment checks and cleaning.

The equipment the Process Engineer is responsible for includes:

- Spectrophotometer
- Bacterial Incubator
- Shaker/Incubator
- Heat Block
- Micropipettes

Refer to 'Kit Equipment List' document for more information on each piece of equipment.

Duties and Responsibilities

- Verify that equipment is functioning properly prior to use.
- Clean and maintain all laboratory equipment before and after use – according to SOPs.
- Complete all required documentation in an accurate and timely manner.
- Communicate information about equipment to the rest of the team in a timely fashion.
- Adhere to safe working practices.

Vocabulary

SOPs – Standard Operating Procedures – These documents are very detailed laboratory protocols. SOPs must be followed in exactly the same way every time during biomanufacturing.

Documentation – This term refers to all documents that record information about the biomanufacturing process. This includes: employee training, batch records, product testing etc.

Batch Record - A document that provides the complete manufacturing history of a pharmaceutical product.

Job Description
Job Title: Quality Control Technician

Purpose and Scope of Position

Quality Control Technicians work in the lab, testing the cells and protein therapeutics during the biomanufacturing process to ensure they meet established criteria (specifications), so the final product is safe to release for sale or distribution.

The QC Technician is responsible for testing raw materials, in-process materials and finished product. During the upstream process, the QC Technician will conduct the required tests on the cells producing the protein therapeutic to determine whether or not they meet the established specifications. During the downstream process, the QC Technician will conduct the required tests on the protein therapeutic.

Duties and Responsibilities

- Test LB broth to confirm whether or not it is sterile.
- Perform required tests on the bacterial cells that are producing the protein therapeutic. This may include graphing cell viability using the spectrophotometer, and monitoring the production of the protein therapeutic by visual inspection for fluorescence.
- Test the concentration of the purified protein therapeutic using the spectrophotometer.
- Complete all required documentation in an accurate and timely manner.
- Communicate with team members in a timely manner.
- Adhere to safe working practices.

Vocabulary

Protein Therapeutic – The protein you are trying to purify. A protein that will be used as a medicine/drug. Also known as a biologic.

Documentation – This term refers to all documents that record information about the biomanufacturing process. This includes: employee training, batch records, product testing etc.

Spectrophotometer – An analytical instrument used to measure the amount of light absorbed (or transmitted) as it passes through a sample.

Sterile – Does not contain live microorganisms (bacteria, viruses, fungi).

Job Description
Job Title: Upstream Process Technician

Purpose and Scope of Position

The Upstream Process Technician carries out all protocols related to the culture of cells producing the protein therapeutic, from inoculation through harvest. The Upstream Process Technician will take samples at various time points throughout the upstream process and provide these to the QC Technician for testing. The Upstream Process Technician is responsible for monitoring cell health and determining when to scale up the cell culture and when to harvest the cells for protein purification.

Duties and Responsibilities

- Use aseptic technique at all times.
- Responsible for cell culture expansions – colony inoculation through culture scale-up
- Responsible for harvest operations – centrifugation, cell lysis.
- Complete all required documentation in an accurate and timely manner.
- Communicate with team members in a timely manner.
- Identify, escalate, and report deviations.
- Adhere to safe working practices.

Vocabulary

QC - Quality Control - You will learn about what quality control is from the QC Technician job description and gain a better understanding as your team works through the biomanufacturing kit curriculum

Aseptic Technique – Specific practices and procedures used in the laboratory to reduce the contamination from unwanted microbes.

Colony Inoculation – The procedure by which a the bacteria contained in a single colony are moved into liquid media.

Centrifugation – The process of using a centrifuge, typically to separate fluids of different densities or liquids from solids.

Lysis – The process of breaking cells apart.

Documentation – This term refers to all documents that record information about the biomanufacturing process. This includes: employee training, batch records, product testing etc.

Deviations – Results that are incorrect or unexpected that occur during the biomanufacturing process.

Escalate – Report to a higher authority.

Career Exploration Assignment Option #1

1. Stand alone research project from a list of careers in biomanufacturing and then students put on a “career fair” to inform the class of the career
    a. https://docs.google.com/document/d/1F5CS_p_gwu-K2xHPs8nyfdbz55n4PLjm9cceCYHp_y0/edit?usp=sharing
2. Students are assigned a role in their biomanufacturing company and they receive a “welcome packet” in the packet is included a job description and qualifications as well as a biography from someone who works in a similar role at an actual manufacturing company
    a. Optional research project
3. Franklin Biologics has an awesome career simulation for a biomanufacturing lab
    a. https://docs.google.com/document/d/1QKBcQIpZhSKRvY8KbE32l-sRS5Ip87A1xnypSR5ETFg/edit This is an assignment I created for it
4. Include virtual interviews with people who work in biomanufacturing

Biomanufacturing Career Exploration

Name:

1. You will work in a group of 4-8 with other students who have the same role as you.
2. You will work to investigate your role in relation to real jobs in biomanufacturing.
3. Assign each person in your group a symbol from below. If you have more than 4 people, 2 students can share a symbol and divide the work.
   ! $ * #

4. Use the following resources to get your information in addition to google.
   - https://www.biotech-careers.org/job/biomanufacturing-technician-downstream#:~:text=Downstream%20Manufacturing%20Technicians%20harvest%20and,formulation%20and%20the%20pure%20product.
   - https://www.indeed.com/jobs?q=Biotech&l=Bothell%2C+WA&aceid=&gclid=CjwKCAjwo_KXBhAaEiwA2RZ8hJm3hikKd1kjr8ksvJTvPlNi-md4rSKxze8XijYJ4xGnIcBMO1wvLRoCInkQAvD_BwE&from=mobRdr&utm_source=%2Fm%2F&utm_medium=redir&utm_campaign=dt&redirected=1&vjk=0f660eb95b1124cb
   - https://www.biospace.com/jobs/washington-state/biotechnology/
   - https://www.simplyhired.com/search?q=biomanufacturing&job=RV7v8mxn7e7JF8TdSh7PXhzL-ZGLTLLjVZQVli3NoIkMiOs7rBSxXA

   !- You will research the starting salary, ending salary, and per hour rate or yearly salary for the national average and in Washington State.

   $- You will research other titles that your role may have as well as what other jobs work closely with your role.

   *- You will research the overall job description for your role, common tasks, and responsibilities

   #- You will research job requirements and current job openings for this role in Washington state

5. Create a presentation to show the class about your role.

Rubric

	0	2	4
Information	Not all information is present in the presentation or some information is inaccurate	Information is complete and accurate	Information is complete and accurate, interesting facts are included about your role
Quality	Presentation is bland and uninteresting	Presentation is engaging, organized, and easy to follow	Presentation is engaging, organized, and easy to follow and Professional
Presentation	No participation from the	Participation from most of	Participation from all

	majority of the group Unclear and difficult to understand and/or hear	the group Clear and easy to understand and hear most of the time	group members Clear and easy to understand and hear

Module 1, Lesson 4: Biomanufacturing Career Video Links

Biomanufacturing Careers

Video links

QA Technician/Associate

https://biotech-careers.org/videos/quality-assurance-associate

QC Technician/Associate

https://biotech-careers.org/videos/quality-control-associate

Upstream Technician

https://biotech-careers.org/videos/john-condosta-manufacturing-technician-upstream

Downstream Technician

https://biotech-careers.org/videos/michael-onufrak-manufacturing-technician-downstream

https://biotech-careers.org/videos/cybelle-mandigo-downstream-manufacturing-technician

Process Engineer

https://www.biotech-careers.org/videos/process-engineer

Biomanufacturing Module 1

Lesson 5 - Standard Operating Procedure (SOP) Training

Lesson objectives:

Students will understand:

- The difference between a lab protocol and a standard operating procedure (SOP)
- Why SOPs are used in biomanufacturing.

Essential Questions

- What is the purpose of an SOP?
- Why are SOPs used in biomanufacturing?

Materials:

- Importance of SOPs Training Assignment
- SOP Assignment
- p200 Micropipettes
- Tips for p200 micropipettes
- Colored water solution
- 96 well plates
- Documentation file folder for each team

What Students Will Do

- Read the Importance of SOPs training activity document
- Watch the peanut butter sandwich making instructions video
- Discuss the importance of clear instructions (as a team)
- Develop an SOP for micropipetting (as a team)
- Teams test each other's micropipetting SOPs
- Fishing filling out their Team Training Record

Teacher Preparation

- Have the peanut butter sandwich making video ready to show
- Make copies of the Micropipetting SOP assignment

Organizer

Time	Activity	Materials
5 minutes	Read Importance of SOPs training document	Importance of SOPs Training Document
5 minutes	Watch peanut butter sandwich video	Video
10 minutes	Discuss the importance of clear and detailed instructions	
25 minutes	Teams create an SOP for	

	micropipetting	
5 minutes	Complete Team Training Record	Team Training Record Document
25 minutes	Teams test another team's SOP Provide feedback for improvement	Micropipetting SOP documents, Micropipettes, tips, colored water, 96 well plates

Procedure

SOP Training

1. Ask students to read over the 'Importance of SOPs' training document.
2. Play the peanut butter sandwich making video.

https://www.dailymotion.com/video/x5d16ph

3. Ask students to discuss the importance of clear and detailed instructions.
4. Teams finish filling out their Team Training Record and file it in their team file.

Writing an SOP (Micropipetting)

5. Ask students to work through the assignment steps1-5. This can be done as a homework assignment.
6. Ask student teams to write an SOP for micropipetting.
7. Ask each student team to carry out the Micropipetting SOP written by another team
8. Teams give each other feedback on how to improve their SOP

Alternative: Students are introduced to SOPs and read a couple of examples, but do not write an SOP until later in the curriculum.

New Hire Training – The Importance of Standard Operating Procedures (SOPs)

In a tightly regulated industry like Biomanufacturing, it is important that products are made exactly the same way EVERY time. The instructions for how to do this must be written out in extreme detail. These instructions are similar to a recipe or a laboratory protocol, but with every step spelled out in great detail. These detailed instruction documents are called standard operating procedures or SOPs. Biomanufacturing companies are legally required to follow SOPs.

To get you started thinking about how important good instructions can be, watch the following video, in which a father challenges his kids to give him instructions for how to make a peanut butter and jelly sandwich. He follows their instructions to the letter.

https://www.dailymotion.com/video/x5d16ph

After watching the video each biomanufacturing team should discuss:

1. Why did the instructions need to be edited so many times?
2. What types of assumptions were made by the kids when they wrote their instructions?
3. If you wanted the sandwich to not only look the same each time it was made, but to taste exactly the same each time it was made, what other things would need to be specified in the instructions?
   Possible answers (remove this information in student version of this document):
   a. Brand of peanut butter
   b. Brand of jam
   c. Flavor of jam
   d. Brand of white bread

What is a Standard Operating Procedure?

Objective: read an SOP, or Standard Operating Procedure.

Instructions:

I. Read through this example procedure: Found here http://www.webguru.neu.edu/undergraduate-research/structuring-ur-experience/standard-operating-protocols-sops/example-sop

II. Answers the questions below

1. After reading this SOP, how would you define a Standard Operating Procedure?
2. This particular SOP has 8 different components, what are the 8 different components or sections?
3. Why do you think a reference section is often included in an SOP?
4. Describe what kind of information is included in the cautions section.
5. Why might it be important for an SOP to have personnel qualifications?
6. Based on the description of the protocol, do you think that you would be able to recreate a coffee exactly like the person who wrote the SOP did? Why or why not?
7. Do you think it is important for an SOP to be detailed enough for anyone to recreate the protocol exactly? Why or why not.
8. What do you think could make this Standard Operating Procedure more helpful?
9. What do you think was not necessary in the Standard Operating Procedure, if anything. Explain your answer.

How to Write an SOP Information adapted from: lucidchart

Instructions:

1. Read through each section.
2. Summarize each section with 1 or 2 sentences
3. Answer the questions (italicized) at the end of each section

Step 1: Begin with the end in mind

Define what the end result or the goal is for the SOP you are writing. For example, if you are writing a document that describes how to make a diluted solution, your end goal is the finish solution.

This step does not include details such as how to use a micropipette or what a beaker is. You simply want to identify what the procedure will accomplish.

QUESTION: Your boss asked you to put together an SOP for using a micropipette. What would be your goal here? What would you want other people to accomplish?

Step 2: Choose a format

SOP's can take many different formats. You will be using a simple steps format.

Simple steps format: Use this format for routine procedures that are short and easy to follow. In addition to safety guidelines and other mandatory documentation, this type of format is generally a simple numbered or bulleted list with short, simple sentences that are clear and easy for the reader to follow. Please refer to the Simple Steps format template document.

Step 3: Ask for input

Get the team together and ask them how they think the job should be performed. These are the people who you are going to ask to adhere to the SOP, so you want to be sure that it makes sense to them and that all the necessary tasks are included.

There will be multiple drafts and reviews—make sure you invite your team to review the drafts so they can make additional suggestions.

Step 4: Define the scope

It's possible that the SOP you are working on is dependent on other SOPs and teams in other departments in order to be completed successfully. Determine whether it is sufficient to reference those other procedures or if you need to add them to the current standard operating procedure document. Maybe you need a flowchart or a map to clearly define dependencies and responsible parties.

Step 5: Identify your audience

Knowing your audience helps you determine how you should write your SOP document. Consider these questions:

-**What is their prior knowledge?** Are they already familiar with the organization and the procedures? Do they already know the terminology? Have they become complacent and need a refresher? You need to write to your audience's knowledge level—dumb it down too much or make it too complicated, and you'll lose them.

-What are their language skills? Maybe your audience does not natively speak your language. If that is the case, you may want to use more pictures than words.

-Are they new employees? When bringing on new employees, your SOP documents need to be very detailed and training-oriented. You want to ensure consistent outcomes regardless of who is performing a task.

-What is the size of your audience? Will multiple people in different roles across multiple organizations be reading the document? If so, you may want to write the procedures in a way that clearly defines who, or what role, performs each task. This helps your audience understand where they each fit into the process and why their particular part is important.

QUESTION: How would an SOP you are writing for new employees differ than an SOP for experience employees? Explain.

Step 6: Write the SOP

Write a draft of your standard operating procedure. Please refer to the 'Student Example Simple Steps Format SOP'. Consider including some of the following elements:

Title page

This page can include:

The title of the procedure
An SOP identification number
A publication date or revision date
The name of the role, organization, division, or agency that the SOP applies to
Names and signatures of those who prepared and approved the procedures outlined in the SOP
Table of contents: You only need a table of contents if the document is very large with many pages.

The specific procedures

This is the bulk of the document and includes the specific step-by-step procedures that need to be followed in order to successfully comply with company standards and safety regulations. This section could also include:

- A description of the scope and purpose of the SOP, its limits, and how it's used. You can include standards, regulatory requirements, roles and responsibilities, and inputs and outputs.
- Necessary and additional details that are needed to complete each step.
- Clarification of terminology, including acronyms and phrases that may not be familiar to your audience.
- Health and safety warnings. These warnings should be listed in a separate section, and they should accompany applicable steps within the process.
- A complete list of all equipment and supplies that are needed, where to find them, and when each will be needed.
- A troubleshooting section to cover things that can go wrong, what types of things the reader should look for, and what may interfere with the final outcome.

QUESTION: You are writing an SOP for how to use a micropipette. What equipment would you need to list and what safety precautions would you need to describe?

Step 7: Review, test, edit, repeat

After you have written your standard operating procedure document:

1. Send a draft of the SOP to team members for review. Have them note grammatical and technical errors.
2. Test the document yourself to ensure that you achieve the desired outcome.
3. Have other team members test the procedures to ensure that the language is clear, can be easily followed, and can be completed successfully.
4. Incorporate relevant edits and suggestions to improve the document.
5. Repeat these steps until the document is approved and accepted by all stakeholders.
6. Implement the SOP. Make it easily accessible to those who need it to do their jobs.
7. You should review the SOP every six to twelve months or as necessary to identify areas where it can be improved and to reflect any changes that have been made to current procedures.

QUESTION: You have a particularly difficult step in your SOP to understand when read. What can you do to help make this clearer to your audience? List multiple options.

Review an SOP

Instructions:

1. You will exchange SOPs with another team.
2. Each team will the SOP they have been given using the materials provided
3. Once complete, provide feedback to the other team on their SOP
    a. You must provide useful feedback
        i. Things like well done are encouraging but do not help your groupmate get better
        ii. Make suggestions to your groupmate about what can be improved or what was tricky about their SOP
        iii. Look for places in the SOP that could be understood differently by different people and suggest to your groupmate a way to make it more straightforward
4. Once reviewing is completed, each team will retrieve their SOP from the team that reviewed it and edit their SOP

Team Logo	**Xxxxxx Department** **Xxxxxx Division/Function**	**SOP #**	
		Revision #	
		Implementation Date	
Page #	1 of xx	**Last Reviewed/Update Date**	
SOP Owner		**Approval**	

Standard Operating Procedure

1. Purpose

Describe the process for <official name of SOP>.

Describe relevant background information.

2. Scope

Identify the intended audience and /or activities where the SOP may be relevant.

3. Prerequisites

Outline information required before proceeding with the listed procedure; for example, worksheets, documents, IFAS reports, etc.

4. Responsibilities

Identify the personnel that have a primary role in the SOP and describe how their responsibilities relate to this SOP. If necessary, include contact information.

5. Procedure

Provide the steps required to perform this procedure (who, what, when, where, why, how). Include a process flowchart.

6. References

List resources that may be useful when performing the procedure; for example, Diagrams, Admin policies, Municipal Code, government standards and other SOPs.

7. Definitions

Identify and define frequently used terms or acronyms. Provide additional and/or relevant information needed to understand this SOP.

OlsonBio

SOP #2006

The Effects of Bleach on Yeast Growth

Authors: Ruthu Mavnur, Julia Kary, Daniel Moran

Effective Date: January 28, 2022

Page	Description/Section
1	Title Page
2-3	Basic Information
4-5	Procedure and Checklist
6	Results

Signatures:



(Julia Kary, Senior Scientist)



(Ruthu Mavnur, Owner of OB)



(Daniel Moran, Leading Author)

Purpose: The purpose of this Standard Operating Procedure is to determine how bleach affects the growth of yeast in a petri dish. We want to accomplish knowing the results of the experiment based on bleach's role in affecting yeast's growth.

SOP Format: This Standard Operating Procedure uses simple steps format, because it has a routine procedure and it is easy for the reader to follow along. It is simple and also precise for the readers.

Scope: Our new employees will not need to cooperate with other departments, because they will have all the material to complete the SOP on their own, and it will get them used to not relying on other people to do their job.

Introduction: There are many ways to grow and change the growth conditions of yeast and bacteria. In this scenario, we want to use bleach, yeast, and a petri dish to explore the effects that bleach has on yeast growth.

References:
How to use a micropipette:
https://solutions.pipette.com/use- /
How to use a bunsen burner:
https://research.wayne.edu/oehs/pdf/factsheet-bunsen-burner.pdf
https://www.youtube.com/watch?v=VLpClJHT9bQ&t=151s
How to swab a petri dish:
https://www.youtube.com/watch?v=DAm21yPGMRo
How to label a petri dish:
https://www.youtube.com/watch?v=aK9nDAzjyv0&t=30s timestamp: (0:52 - end of video)

Terminology Definitions:
Petri Dish: A round, shallow dish used to grow bacteria
Inoculating loop: A tool used to pick up and transfer a small sample from a culture of microorganisms
Streaking: A technique used to isolate bacteria, a zigzag motion
Culture: To grow living organisms in a prepared mixture of nutrients (media).
Media: Substance containing nutrients needed for cell growth.
Sterile/Aseptic Technique: Laboratory procedures used in handling cultures, media and equipment that prevent contamination.
Yeast: A single celled fungus that ferments sugar to produce alcohol
Dilution: the process of reducing the concentration of a solute in solution, usually simply by mixing more solvent
Beaker: A thin glass vessel used for containing liquids
Incubator: A heated, insulated box used to grow and maintain microbiological or cell cultures
Bunsen Burner: A source of open flame that is used to sterilize

Materials and Supplies:
- Container for yeast
- Yeast broth (1 gram of baker's yeast + 50 mg of warm water)



Use beaker as the yeast broth's container

- Warm water
- Incubator
- Household bleach (10% bleach)
- PPE (safety gloves, safety goggles, etc)
- 5 petri dishes (already prepared with agar)
- Inoculating loop
- Bunsen burner (if using metal inoculator)
- Pen
- Ruler

Cautions: Keeping the skin and face safe, being careful around the bleach, staying away from the bunsen burner when not using it, making sure things are disinfected when finished. If bleach gets in your eyes, wash your eyes out immediately and have someone call 911. If bleach touches skin, immediately wash the body and have someone call 911.

Personnel Qualifications: The new employees need to be second year Biotechnology students. They need to know how to use a micropipette, how to incubate agars, how to label agars, and how to use agar stroke techniques.

Procedure:

1. **Put on all PPE and collect materials.**
2. **With a pen, label the petri dishes. Labeling the petri dish should be around the rim on the outside of the base. It includes your initials, the date, type of agar, and type of organism, respectively.**

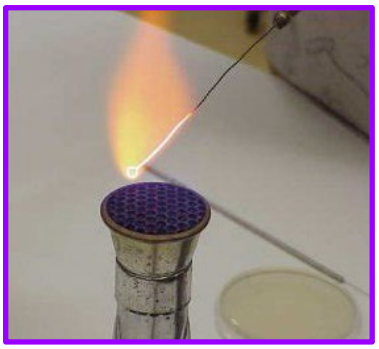
Example:



3. **Draw a grid (1cm x 1cm squares) on each agar plate.**



4. **Prepare all of the materials and spray 4 of the petri dishes with bleach. Let the petri dishes dry. Leave one petri dish without bleach, as that is the control for this experiment.**
5. **Then, using the clamshell method (by lifting the lid of the petri dish slightly upwards/open), dip the inoculating loop into the yeast broth and streak one plate using the T-streak method.**
6. **If the inoculating loop is metal, then, between plates, use the bunsen burner and heat the loop in the middle of the flame, and allow it to cool to room temperature, to clean it. Otherwise, if the loop is plastic, use a new swab each time.**



7. **Repeat steps 5 and 6 for four more petri dishes.**
8. **Incubate the petri dishes for 24 hours at 30 °C.**
9. **Clean up all materials and PPE, if necessary. Then put the PPE in its respected place.**
10. **After the full incubation period, put on respected PPE and remove the petri dishes from the incubator.**
11. **To calculate the amount of growth on the petri dishes: count the number of colonies in each petri dish and divide by the number of squares on the Petri dish to get the percentage of yeast colonies in each dish. Use the equation below for each petri dish.**

$$\frac{\text{\# of colonies}}{\text{total \# of squares on Petri dish}} = \text{\% of yeast colonies in each petri dish}$$



12. **To end the procedure, spray all petri dishes with 10% bleach. Throw all materials, including hand gloves, in the trash. Put the rest of the PPE back to their designated spot and clean according to instructions.**

As you go…

Did you:

- Put on all PPE and gather materials?
- Label and grid the petri dishes?
- Spray 4 petri dishes with bleach and one without?
- Swab ALL petri dishes?
- Incubate?
- Calculate growth?
- Clean up?

Measuring Results: Data Table

	Control (without bleach)	Trial 1 (with bleach)	Trial 2 (with bleach)	Trial 3 (with bleach)	Trial 4 (with bleach)
Percentage (%) of yeast in petri dish					

Title: GFP Transformation of *E. coli* Bacteria

Approvals:

Preparer: Lauren Mitchell Date: 12FEB2021
Reviewer: Aubrey Linaman Date: 23FEB2021

1. **Purpose:**
   1.1. To transform the pGLO plasmid into *E. coli* BL21, creating recombinant *E. coli* with ampicillin resistance and GFP.
2. **Scope:**
   2.1. This method is to be used for teaching purposes utilizing small volume bacterial transformations. Transformation volume should not exceed 1mL for this SOP. This SOP also utilizes pre-prepped transformation solution and pGLO plasmid provided by a third party source. This method is only applicable for live cells, and is optimized specifically for *E. coli* cultures.
3. **Responsibilities:**
   3.1. It is the responsibility of the students performing this SOP to follow all the instructions to the best of their ability, and to seek help whenever questions or concerns may rise. Students should document any deviations that they experience while following this SOP.
   3.2. It is the responsibility of the instructor to oversee the proper application of this SOP, answer any questions the students may have, and update the SOP whenever necessary.
4. **References:**
   4.1. BioRad Student Manual: pGLO Transformation
5. **Precautions:**
   5.1. UV light can cause damage when exposed to unshielded eyes. Ensure that any UV light source is pointed away from the eyes, and install/wear extra eye protection as necessary to decrease UV exposure.
   5.2. When handling bacteria wear a lab coat, gloves, and eye protection at all times. Always wash hands before and after handling bacteria. Change PPE as necessary if contamination is believed to have occurred.
6. **Materials:**
   6.1. *E. coli* starter plate
   6.2. Poured agar plates (1 LB, 2 LB/amp, 1 LB/amp/ara)
   6.3. Transformation Solution
   6.4. LB nutrient broth
   6.5. Rehydrated pGLO plasmid (0.08 ug/uL)
   6.6. Inoculation loops
   6.7. Foam microcentrifuge tube holder/float
   6.8. Ice bucket plus crushed ice
   6.9. Microcentrifuge tubes
   6.10. Marking pen
   6.11. UV light
   6.12. 20-200µL micropipette, plus tips
   6.13. 1-10µL micropipette, plus tips

Title: GFP Transformation of *E. coli* Bacteria

6.14. 42°C water bath with thermometer
6.15. 37°C incubator
6.16. Timer

7. Procedure:

7.1. Disinfect workspace and let dry before retrieving reagents.
7.2. Using the lab marker, label one microcentrifuge tube as "+PGLO" and another microcentrifuge tube as "-pGLO".
7.3. Using a sterile transfer pipette, add 250µL of transformation solution to both of the labeled microcentrifuge tubes.
7.4. Place both tubes on ice, ensuring that the bottom half of both tubes is adequately covered.
7.5. Retrieve the *E. coli* starter plate.
7.5.1. Check to ensure that there is no contamination present before moving forward.
7.6. Depending on the size of the colonies present, select 2-4 colonies for each labeled microcentrifuge tube. Ensure that each of the selected colonies are isolated (not touching any other colony) and are uniformly round.
7.7. Using a sterile loop, pick up the colonies selected for the +pGLO tube. Quickly open the +pGLO tube and transfer the colonies to liquid inside.
7.7.1. In order to ensure that the colonies have been properly transferred, submerge the inoculation loop completely within the transformation solution, and quickly swirl the loop back and forth with your fingers. Continue swirling until there are no visible floating chunks.
7.7.2. Discard the inoculation loop in biohazard if using a disposable model. Otherwise, sterilize the loop before moving on.
7.8. Using a fresh, sterile inoculation loop, repeat steps 7.6, 7.6.1, and 7.6.2 with the colonies selected for the –pGLO tube.
7.9. Place tubes back on ice before moving forward.
7.10. Retrieve the rehydrated pGLO plasmid. Visually inspect the tube with the UV light, and note any visual changes.
7.11. Using a p10 micropipette, transfer 10µL of pGLO plasmid to the +pGLO tube.
7.11.1. Note: you can also use a sterile loop to transfer pGLO. Submerge the loop into the pGLO plasmid and withdraw the loop. Ensure that there is a film of liquid across the loop's surface. Submerge the loop in the solution of the +pGLO tube, spinning a few times to ensure that the pGLO has been properly mixed.
7.12. Incubate the tubes on ice for **10 minutes**. Ensure that the bottoms of the tubes are adequately covered in ice.
7.13. During the incubation on ice, retrieve the required plates, and label them as follows. Include the date, initials, and bacterial type on every plate. Keep plates covered and inverted until inoculation.
7.13.1. LB plate, with –pGLO.
7.13.2. LB/amp plate, with –pGLO.
7.13.3. LB/amp plate, with +pGLO.
7.13.4. LB/amp/ara plate, with +pGLO.

Title: GFP Transformation of *E. coli* Bacteria

7.14. After the incubation on ice is complete, bring both the –pGLO and +pGLO tubes over to the 42°C for heat shock.
  7.14.1. Place the tubes in a foam microcentrifuge float while ensuring that the bottoms of the tubes are sticking out from the other side. This ensures the solution in the tubes will be completely submerged.
  7.14.2. After the tubes are secured, double check the temperature of the water bath to ensure that it is 42°C.
  7.14.3. Place the float + tubes into the water bath for exactly **50 seconds**.
7.15. Once the heat shock is complete, immediately move the tubes back into the ice. Let them rest on ice for **2 minutes.**
7.16. After being allowed to rest, remove the tubes from ice and add 250µL of LB nutrient broth to both of the tubes. Incubate the tubes room temperature for **10 minutes**.
7.17. Gently flick the tubes to ensure that the bacteria are resuspended.
7.18. Using a micropipette, add the following:
  7.18.1. 100µL of –pGLO to the LB plate.
  7.18.2. 100µL of –pGLO to one of the LB/amp plates.
  7.18.3. 100µL of +pGLO to the other LB/amp plate.
  7.18.4. 100µL of +pGLO to the LB/amp/ara plate.
7.19. Using a fresh, sterile inoculation loop for each plate, spread the added solution across the surface of each plate.
  7.19.1. Drag the sterile loop across the surface in a back and forth pattern, turn the plate 45° and repeat.
  7.19.2. Be careful not the gouge the surface of the plate with the inoculation loop.
7.20. Tape each plate lid down before storing them, inverted, in the 37°C incubator.
7.21. Disinfect the work station and dispose of any spent reagents and –pGLO and +pGLO tubes.
7.22. Allow plates to incubate for 24 hours before retrieving plates and recording results.

8. History:

Name	Date	Amendment
Rachel Rawle	18AUG2021	Changed Document number from 1 to GFP002. Revised purpose statement to be more succinct.
Freja Krantz	19MAR2022	7.18.4. Revised unit typo

PPP Production Team On-boarding

Complete BEFORE Starting Production

1. Assign a team role to each member of the Production Team.
2. Each team member reads their job description.
3. Team members choose a Team name.
4. Team members fill out the table below.
5. The completed Table is given to the Quality Assurance Technician who signs and dates it and files it.

Team Name:_____

Job Title	**Name (Print)**	**Initials** **(include middle initial)**	**Date** **Month Day Year Ex: 03242022**
Process Engineer			
Quality Assurance Technician			
Quality Control Technician			
Biomanufacturing Associate – Upstream Process			
Biomanufacturing Associate – Downstream Process			

QA Technician Name (Printed)_____

QA Technician Signature_____Date_____

PPP Production Team Training Record Team Name_____

Complete BEFORE Starting Production

1. Complete the activities involved in each training module.
    a. Lab Safety Training
    b. Introduction to Biomanufacturing
    c. Introduction to Biomanufacturing Regulations (FDA, GMP)
    d. Introduction to Standard Operating Procedures (SOPs)
2. Team members fill out the table below.
3. The completed Table is given to the Quality Assurance Technician who signs, dates it, and files it.

Type of Training	Name (Print)	Initials (include middle initial)	Job Title	Date Completed Month Day Year Ex: 03242022
Lab Safety				
Intro to Biomanufacturing				
Intro to GMP and FDA				
Intro to SOPs				

QA Technician Name (Printed)_____

QA Technician Signature_____Date_____