### **TOPIC: BUILDING A SOLID FOUNDATION**

This topic introduces students to the fundamentals of lab safety and documentation as they work toward independently weighing solid chemicals and responding to chemical spills.

LESSONS (45 minutes each)	
Safety First!	Students are introduced to key safety equipment in the lab through a scavenger hunt that culminates in creation of a map of their lab space.
Handling Hazards	Students learn how to assess and manage hazards posed by chemicals used in the lab and show their understanding through a role playing activity.
Measuring in Metric	Students learn to collect and record accurate measurements with an electronic balance by weighing solids using different metric units.
Keeping a Lab Notebook	Students begin to take ownership of their experiments by creating their first protocol and lab notebook entry as they weigh solid chemicals.
Lab Practical	Students demonstrate their understanding of lab safety, safe handling of hazardous chemicals, and keeping of a lab notebook.

Students develop professional practices related to	
Lab Safety	<ul> <li>Identifying and rectifying unsafe lab conditions</li> <li>Safely measuring, using, and disposing of hazardous chemicals</li> <li>Safely operating lab equipment such as balances</li> <li>Identifying and correctly using PPE for a specific task</li> <li>Safely cleaning up spills from solid hazardous chemicals</li> </ul>
Documentation & Following Protocol	<ul> <li>Accurately and legibly labeling reagents</li> <li>Safely and accurately carrying out the procedures described in a lab protocol / SOP</li> <li>Recording data accurately and in a timely manner</li> <li>Maintaining an accurate, up-to-date record of their lab activities</li> </ul>
Lab Citizenship	<ul> <li>Conducting lab activities safely and maintain a clean and orderly workspace</li> <li>Maintaining common lab equipment and materials in clean, well-stocked order</li> <li>Collaborate and communicate effectively with lab personnel</li> </ul>
General Lab Skills	<ul> <li>Select the appropriate equipment and materials for weighing solids</li> <li>Safely and accurately measure the mass of solid reagents</li> </ul>



#### SAMPLE SLIDES FOR THIS TOPIC



### Weighing solids

Together with your lab group, you will weigh solids using different metric units to become familiar with these units and develop the ability to estimate how much solid is needed for a given measurement.



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BioTechBuilder LAB SKILLS

### **TOPIC: MAKING SOLUTIONS**

This topic teaches students the math and procedures commonly used to prepare and dilute solutions with units in molarity, %v/v and %w/v. Students prepare single- and multi-solute solutions, measure and adjust pH, and use heat and aseptic technique where needed.

LESSONS (45 minutes each)	
Getting to Know Glassware	Students become familiar with the glassware used to make solutions, as well as how to estimate and read volumes.
Making Stock Solutions	Students practice molarity calculations and prepare a single-solute stock solution.
Diluting Solutions	Students are introduced to serological pipettes and how to prepare serial dilutions.
Micropipetting	Students are introduced to micropipettes and use them to practice transferring liquids.
LAB PRACTICAL I	Students exchange mystery solutions and use spectrophotometry to prepare a standard curve and measure the mystery solution concentration.
Makina Multi-	
solute Solutions	Students review solution calculations and prepare solutions with multiple solutes.
Solute Solutions	Students review solution calculations and prepare solutions with multiple solutes. Students learn how to measure and adjust the pH of a solution.
Solute Solutions Perfecting pH Saturating Solutions	Students review solution calculations and prepare solutions with multiple solutes. Students learn how to measure and adjust the pH of a solution. Students learn how to prepare a saturated solution while practicing %w/v and %v/v calculations and safe operation of a hot plate.
Solute Solutions Perfecting pH Saturating Solutions Preventing Bacterial Contamination	Students review solution calculations and prepare solutions with multiple solutes.         Students learn how to measure and adjust the pH of a solution.         Students learn how to prepare a saturated solution while practicing %w/v and %v/v calculations and safe operation of a hot plate.         Students learn about and practice aseptic technique while pipetting solutions.

Students develop professional practices related to	
Lab Safety	Safely operating lab equipment such as heating elements



Documentation & Following Protocol	<ul> <li>Visually communicating how their data addresses a hypothesis</li> </ul>
Lab Citizenship	Storing common materials, glassware and reagents appropriately
General Lab Skills	<ul> <li>Selecting appropriate glassware, equipment &amp; materials for solutions</li> <li>Safely and accurately measuring volumes of liquid reagents (1-1000 ml)</li> <li>Accurately and reliably preparing single-solute solutions</li> <li>Safely operating heating equipment, including a hot plate, Bunsen burner</li> <li>Accurately and reliably preparing solutions (multi-solute, % w/v, %v/v, pH)</li> </ul>
Biotechnology Lab Skills	<ul> <li>Accurately measuring and transferring small volumes (0.1 ul - 20 ml)</li> <li>Use of aseptic technique</li> </ul>

#### SAMPLE SLIDES FOR THIS TOPIC

Agenda

#### Our plan for today

5 min	Introduction	Objectives and pre-lab quiz
5 min	New Ideas	Preparing a solution
15 min	Lab Activity	Prepare blue dye stock solution
10 min	New Ideas	Molarity and dilution calculations
5 min	Wrap-up	Mystery solution concentration

BioTechBuilder LAB SKILLS



#### New Ideas

#### What is a solution?

In today's activity, you will prepare a solution. A solution is created when a solute is dissolved in a solvent.





BioTechBuilder LAB SKILLS

Making Stock Solutions



### **TOPIC: GROWING CELLS**

This topic exposes students to culturing bacterial cells on solid and liquid media. In the lab practical, students use spectrophotometry and plating to measure bacterial viability.

LESSONS (45 minutes each)		
Pouring Plates	Students pour agar plates while learning the fundamentals of bacterial cell structure and growth.	
Streaking for Single Colonies	Students streak for single colonies from a bacterial slant while learning about selective media.	
Culturing a Colony	Students use aseptic technique to prepare liquid selective media and transfer a signal colony into the media for growth overnight.	
Measuring Cell Concentration	Students measure OD600 of their overnight culture and prepare a serial dilution of for viability assay plating.	
LAB PRACTICAL, part 1	Students learn how cell concentration and viable cells differ, then count the colonies on their plates to calculate the viability of their bacterial cells. Each student repeats the viability assay experiment for skills assessment.	
LAB PRACTICAL, part 2	Students repeat the analysis and calculation of the bacterial cell viability using their plates from Lab Practical I, and submit their lab notebook for assessment.	

Students develop professional practices related to	
Biotechnology Lab Skills	<ul> <li>Demonstrating aseptic technique when working with bacterial cultures</li> <li>Culturing bacteria using solid and liquid media</li> <li>Isolating bacterial strains using solid or liquid media and quantifying density</li> </ul>

#### SAMPLE SLIDE FOR THIS TOPIC

**New Ideas** 

BioTechBuilder LAB SKILLS

#### The importance of aseptic technique

In biotechnology labs, we often need to make solutions that will be used to grow bacterial cells. When working with bacteria, it's important to **prevent contamination**, both of you and of your cultures.

- Preventing contamination
- Sterilization, decontamination and disinfection
   Aseptic technique
  - Maintains sterile environment
     Safe use of a flame at the bench



Preventing Contaminatio



### **TOPIC: ANALYZING DNA**

This topic introduces students to DNA techniques used in modern biotechnology. Students begin by growing bacteria in liquid culture, and then they are introduced to DNA analysis techniques including DNA isolation, PCR, restriction digest analysis, gel electrophoresis, and finally sequencing. Other skills covered in this topic include solution preparation and accompanying lab math, micropipette calibration and maintenance, and documentation.

LESSONS (45 minutes each)	
DNA overview	Students streak a transformed <i>E. coli</i> strain for single colonies while learning why it is necessary to verify DNA.
Calibrating Pipettes	Students revisit micropipetting and calibrate their instruments to ensure consistent and accurate measurements.
Making Miniprep Solutions	Students prepare plasmid DNA isolation buffers while learning about the process of DNA isolation by miniprep.
Isolating DNA I	Students learn to safely operate a centrifuge as they begin extracting DNA from their bacterial cultures using miniprep protocol.
Isolating DNA II	Students learn how regulatory elements of the bacterial lac operon apply to the plasmid they are isolating.
Amplifying DNA I	Students learn how the polymerase chain reaction can be used to amplify and verify DNA segments, illustrating their understanding by writing their own PCR protocol using manufacturer's guidelines.
Amplifying DNA II	Students use their own PCR protocol to amplify the DNA they isolated from their bacterial cultures.
QUIZ I	Students design primers, calculate reagent volumes, and predict the length of a PCR product
Digesting DNA	Students learn about restriction enzymes and apply their understanding to verify and analyze their PCR products.
Visualizing DNA I	Students pour gels for electrophoresis of their digested PCR DNA.
Visualizing DNA II	Students practice loading gels.
Visualizing DNA III	Students analyze their PCR DNA by gel electrophoresis.



QUIZ II	Students select enzymes, calculate the length of the DNA digestion products, and predict the results of agarose electrophoresis
LAB PRACTICAL I	Students demonstrate gel electrophoresis competency by pouring, loading, running, and analyzing agarose gel.
Sequencing DNA	Students learn how DNA sequencing works and how to read and interpret DNA sequencing results of their plasmid DNA.
QUIZ III	Students demonstrate their knowledge of techniques used to verify DNA by answering fundamental and situational questions.
LAB PRACTICAL II	Students use different DNA verification techniques to solve a forensic mystery.

Students develop professional practices related to	
Documentation & Following Protocol	<ul> <li>Creation and use of a protocol</li> <li>Recording and analysis of data</li> <li>Keeping a lab notebook</li> </ul>
General Lab Skills	<ul> <li>Selecting the appropriate equipment and materials for weighing solids</li> <li>Safely and accurately measuring the mass of solid reagents</li> <li>Accurately and reliably preparing solutions</li> </ul>
Biotechnology Lab Skills	<ul> <li>Use of aseptic technique and culturing bacteria</li> <li>Performance of DNA-oriented lab techniques and assays (DNA isolation, PCR, restriction digest analysis, gel electrophoresis)</li> <li>Navigating sequence alignment tools</li> </ul>
Biomanufacturing Skills	<ul> <li>Verifying the calibration of single channel micropipettes</li> <li>Identifying, creating, and using SOPs</li> </ul>

#### How do we get from DNA to function in cells?







### **TOPIC: ANALYZING PROTEINS**

This topic teaches students to express and analyze a protein of interest using molecular techniques. Hands-on experiments unify and apply content related to gene expression, protein induction, enzyme activity, protein concentration measurements, and separation techniques including column chromatography and SDS-PAGE.

LESSONS (45 minutes each)	
Protein Overview	Students review central dogma, inducible gene expression (lac operon), and then passage an engineered bacterial strain by restreaking for single colonies.
Bacterial Growth Curves	Students learn about bacterial growth curves, and then aseptically inoculate a bacterial colony from solid to liquid media.
Protein Induction	Students learn how a chemical can induce bio-production of an enzyme, and then initiate the overproduction of an enzyme in the bacterial strain they have grown.
Cell Viability I	Students use spectrophotometry and serial dilution to evaluate the impact of induction on the growth and viability of their induced bacterial strain.
Cell Viability II	Students assess the cell growth data, calculate viability, and analyze the impact of enzyme overproduction on cellular fitness.
Lab Practical I Part 1	Students repeat the viability assay to assess the impact of cold storage on the induced bacterial strain.
Lab Practical I Part 2	Students analyze the viability assay plates and summarize the impact of cold storage on induced bacterial strain, then learn about enzymes as catalysts.
Solution Preparation	Students follow an SOP to prepare reagents necessary for enzyme activity measurement.
Enzyme Activity Measurement	Students learn how colorimetric enzyme assays work, and then measure enzyme activity in their uninduced and induced bacterial cells to calculate enzyme activity.
QUIZ I	Students are evaluated on their understanding of cell growth, induction, viability, activity calculation
Standard Curve	Students learn about the linear relationship between concentration and absorbance, and then serially dilute a known protein to prepare a standard curve.
Calculating Protein Concentration	Students plot absorbance vs concentration and use that standard curve to determine the protein concentration in their induced bacterial cells.



Protein Purification I	Students survey numerous techniques for disrupting cells, then use chemicals to prepare protein lysates from induced cells.
Protein Purification II	Students survey numerous protein separation techniques, then use affinity chromatography to purify the overproduced enzyme.
Visualizing Protein I	Students learn how denaturing gel electrophoresis separates proteins by size, then load select fractions from their protein purification onto SDS-PAGE.
Visualizing Protein II	Students learn how proteins are visualized and how their size is determined, then document predictions for their experiment and use Coomassie to stain the gel.
Visualizing Protein III	Students will compare their documented predictions of the gel to their data, then review the various protein analysis techniques they explored in the lessons.
QUIZ II	Students demonstrate their knowledge of spectrophotometry, cell lysate preparation techniques, and protein gel electrophoresis
LAB PRACTICAL II	Students will be assessed on their ability to generate a standard curve and use that curve to measure the concentration of an unknown solution

Students develop professional practices related to	
Biotechnology Lab Skills	<ul> <li>Use of aseptic technique and culture bacteria</li> <li>Measurement of concentration using spectrophotometry</li> <li>Performance of protein-oriented lab techniques and assays (chemical induction, protein concentration, and activity measurement)</li> <li>Performance of protein separation techniques (SDS-PAGE and chromatography)</li> </ul>

#### SAMPLE SLIDES FOR THIS TOPIC





### **TOPIC: BIOMANUFACTURING**

This topic explores the life cycle for production and manufacturing of bioproducts from upstream processing to downstream processing. What it means to be GMP compliant and differences between Quality Assurance and Quality Control is emphasized. The labs include seed train process, viability assays, and product activity tests while students document every step using batch production records.

LESSONS (45 minutes each)	
Biomanufacturing Overview	Students are introduced to biomanufacturing, and then investigate commercially available bioproducts and discuss their benefits and importance in the current market.
Fermentation I	Students learn about the stepwise process for growing large cell volumes, and then expand a cell stock into a 10 ml culture.
Fermentation II	Students are introduced to Good Manufacturing Practices and Batch Records, then measure total cell density and perform viability assay of their 10 ml culture.
Fermentation III	Students calculate the viable cell density and % viability of their 10 ml culture, then expand to a 100 ml culture with a known number of viable cells.
Fermentation IV	Students evaluate the 100 ml culture for cell density and viability, and are introduced to hemocytometer-based cell counting.
Fermentation V	Using an online simulation, students perform hemocytometer-based cell counting and data analysis.
QUIZ I	Students calculate cell concentration and viability from microbial and mammalian cell sample data provided by the instructor.
LAB PRACTICAL I	Students use OD600 data to determine TCD and VCD, then determine viable cells needed for the next expansion culture.
Measuring Bioproduct I	Students induce engineered <i>E. coli</i> to produce the bioproduct (β- galactosidase) and learn the importance of SOP for Quality Assurance.
Measuring Bioproduct II	Students follow SOP to make solutions for an enzyme activity assay, record their work in BPR forms, and distinguish Quality Assurance and Quality Control.
Measuring Bioproduct III	Students learn how to make flowcharts, then measure the enzymatic activity of the bioproduct to ensure the scaled-up <i>E. coli</i> cells are behaving as expected.
Industrial-Scale Fermentation I	Students learn bioreactor types and parameters to monitor, then use an online simulation of a 2000 liter bioreactor.



Industrial-Scale Fermentation II	Students develop an SOP as an in-class activity then learn about biosafety cabinet form and function.
QUIZ II	Students demonstrate their knowledge and understanding of GMP regulation, QA, and QC.
LAB PRACTICAL II	Students identify the errors in a Batch Record form, SOP, and a calculation.
Downstream Processing I	Students consider the consequences of extracellular vs intracellular localization of products, and simulate harvesting techniques based on this property.
Downstream Processing II	Students learn about chemical, mechanical, and temperature-dependent cell disruption techniques and carryout procedures to release lysozyme from chicken eggs.
Downstream Processing III	Students are introduced to purification methods that rely on physical properties of the bioproduct including chromatography and filtration. In the lab, students purify egg lysozyme disrupted under different conditions.
Downstream Processing IV	Students learn about product formulation & packaging, the importance of cleanroom to prevent contamination, then analyze the data from their lysozyme purification experiment.
QUIZ III	Students are asked about downstream processing from lysate preparation to purification from contaminants to concentration and packaging a bioproduct.
LAB PRACTICAL III	Students must trace the production of a novel product and troubleshoot errors in workflow.

Students develop professional practices related to	
Biomanufacturing	<ul> <li>Explain the life cycle of a bioproduct starting with seed train, fermentation, purification, and product formulation while monitoring for quality control throughout the process</li> <li>Creation and following of SOPs</li> <li>Accurate documentation using batch production records</li> </ul>



### **TOPIC: SYNTHETIC BIOLOGY**

This topic allows students to explore each stage of the engineering "Design-Build-Test-Learn" (DBTL) cycle, first using existing BioBuilder kits and then through the application of BioBuilder's abstraction hierarchy to design a novel biotechnology of their own. Lab skills focus on microbial culturing, transformation of both bacteria and yeast, and measurement of cellular outputs.

LESSONS (45 minutes each)	
Synthetic Biology Overview	Students are introduced to the Design-Build-Test-Learn cycle and an abstraction hierarchy for designing novel biotechnologies, then grow two bioengineered bacterial strains on solid media.
Design Cycle I	Students consider the design process that invented a banana-smelling bacteria, then start liquid overnight cultures of that strain.
Design Cycle II	Students examine the genetic elements that were engineered to control banana-scent production, and then grow the bioengineered cells to different growth stages.
Design Cycle III	Students discuss qualitative and quantitative data, build a data table, and collect growth and scent data from the cells they have grown.
QUIZ I	Students apply the design process to specify a plastic eating bacteria and design a data table for a preliminary experiment testing its function.
Build Cycle I	Students consider the design process that invented a color-generating bacteria, then patch two untransformed strains on solid media.
Build Cycle II	Students explore the concept of cellular chassis, then prepare solutions for bacterial transformation.
Build Cycle III	Students discuss positive and negative controls, then transform two plasmids into two host strains, documenting their predictions for the experimental results.
Build Cycle IV	Students analyze transformation data, document discrepancies from predictions, develop protocol variation to improve results, and repatch cells for lab practical.
LAB PRACTICAL I	Students repeat transformation using 4-2 under different experimental conditions
Test Cycle I	Students compare bottom-up and top-down design processes, then grow four bioengineered bacterial strains on solid media.
Test Cycle II	Students are introduced to consensus sequence, then follow formulation records to prepare solutions for enzyme activity measurement.

Test Cycle III	Students consider how sequence variations can impact function and measure the enzyme activity in four bioengineered bacterial strains.
QUIZ II	Students analyze the protocol and data for 10 strains that are variations of the four tested.
Test Cycle IV	Students are introduced to standardization of DNA parts, and grow triplicate overnight for one bioproduction strain.
LAB PRACTICAL I (part 1)	Students perform enzyme activity assay on triplicate overnight cultures.
LAB PRACTICAL I (part 2)	Students take measurements of the triplicate enzyme assay and carry out calculation and analysis.
Redesign I	Students consider the engineering approach to reliable performance, then pour YPD petri dishes.
Redesign II	Students look at yeast as a model organism, and then culture commonly available self-rising yeast.
Redesign III	Students learn about yeast that have been re-engineered to produce Vitamin A, then streak VitaYeast strains for single colonies.
LAB PRACTICAL III	Students develop a PCR protocol for a new gene of interest, and predict the size of the PCR product.
Redesign IV	Students explore the concept of codon shuffling, then transform a white colony with a codon shuffled copy of a gene on a plasmid and select for transformants.
QUIZ III	Students illustrate their understanding of genetic complementation and positive and negative controls by predicting the outcome of the yeast transformation they performed.
Biodesign I (no lab)	Students analyze their yeast transformation data, then design a PowerPoint slide to illustrate their understanding of the experimental system.
Biodesign II (no lab)	Students work in groups on BioBistro and TechTokens activities.
Biodesign III (no lab)	Students watch a "Design Process" video and brainstorm biotechnology ideas for various topics (Food/Energy, Environment, Health/Medicine, and other novel ideas).
Biodesign IV (no lab)	Students watch video of Cambridge City Council hearings in 1976, and work through questions adapted from pages 84-89 of the BioBuilder book.
FINAL PROJECT	Students present design idea, ethical analysis of existing project, or data from lab.



#### Students develop professional practices related to

Biotechnology Lab Skills	<ul> <li>Use of aseptic technique and culturing of bacteria and yeast</li> <li>Statistical analysis of replicate samples</li> <li>Bacteria and yeast transformation</li> <li>Use of complementation in yeast transformation</li> <li>Measurement of concentration and enzyme activity using spectrophotometry</li> <li>Documentation of results</li> <li>Written and oral data communication</li> </ul>

#### SAMPLE SLIDES FOR THIS TOPIC

**New Ideas** 

### **Reliability in engineered products**

Manufacturers must consistently produce reliably functioning products.

To achieve this, they employ several engineering tools:

- Mean-Time to Failure (MTF)
- Redundancy



**BioTechBuilder** Applications

### **Upcoming Lab**

#### **Golden Bread**

In the next several lessons, we will be examining a synthetic system called VitaYeast.

The goal is to engineer a yeast strain that **reliably** produces β-carotene, which is the precursor to Vitamin A.







**BioTechBuilder** APPLICATIONS