EVOLVING**STEM**

A laboratory research-based education program based on an experiment with bacterial biofilms that demonstrates evolution in action by heritable mutations that be seen as colony phenotypes and detected by genomic analysis.

EvolvingSTEM (https://evolvingstem.org/) is a Three-Dimensional, phenomenon-based life sciences curriculum that engages high school students in an authentic research experiment to develop a deeper understanding of genetics, ecosystem dynamics, microbiology, and essential biotechnology skills within the organizing principle of evolution. In essence, students observe "evolution-in-action" as bacterial populations adapt to form complex biofilm communities in their test tubes.

Background. Imagine a mutant bacterium that grows better in a new environment than its ancestor. How exactly does this mutant differ? What gene or genes change in response to new environmental pressures, and how would you find out? How do these genetic changes produce a fitter mutant phenotype?

The ability to capture and decode vast amounts of DNA sequencing data has revolutionized our ability to identify the genetic basis of adaptations. The EvolvingSTEM evolution-in-action experiment provides students with the opportunity to watch bacterial populations adapt to form complex biofilm communities over the course of a week-long experiment. During this experiment, they identify mutants with different phenotypes than their ancestor on petri plates. We can isolate the DNA from these mutants and sequence their complete genomes, which should differ from the ancestor at very few nucleotides. Here, you will identify and interpret the differences.

The laboratory experiment in brief. Students culture a probiotic bacterium, *Pseudomonas fluorescens* SBW25, in test tubes that contain growth media and a plastic bead. Bacteria colonize the bead and form a biofilm, and the biofilm-covered bead is then transferred to a new test tube with fresh media and a sterile bead. Beads are serially transferred to select for biofilm-adapted mutants. In just a few days, colonies mutate from smooth circles to wrinkly rosettes to fill diverse ecological roles. Their results simulate the process of adaptive radiation often illustrated using Darwin's finches in textbooks; moreover, their findings are medically relevant, as pathogenic bacteria involved in chronic infections often evolve to form biofilms through mutations in the same genetic pathways as the harmless bacteria used in the classroom experiment.

A bioinformatics module takes the evolution experiment to the next level by walking students through the process of identifying the genetic basis of their evolved mutants. Students analyze whole genome sequencing data from bacterial populations evolved during evolution experiments performed by high school students. This allows students to understand that changes to the appearance of bacterial colonies was the result of heritable mutations to the bacteria's DNA. Using modern software, students learn how to quickly analyze the massive amount of data produced during genome sequencing and how to identify the new mutations selected in mutant colonies.

Curriculum Overview. Introductory materials (**Lesson 1a, b**) introduce students to the evolutionary concepts of diversity, common ancestry, adaptation, tree thinking, speciation, and extinction through a variety of active learning activities.

Prior to beginning the EvolvingSTEM evolution-in-action experiment, students can practice basic laboratory skills with a pre-lab training lesson (**Lesson 2a**). **Lesson 2b** directs students through the evolution-in-action experiment. Students are first provided with a detailed introduction to the experiment. This introductory information provides a step-by-step overview of the experiment and introduces students to the vast microbial world and importance of studying microbial biofilms. In addition to a written laboratory protocol, daily slideshows direct students on how to perform each experimental step and make connections between the visible changes they identify in their evolving populations and the genetic changes that form the basis of the phenotypic changes they observe. After completing the experiment, students can compare their findings to those of past experiments with images from real experimental populations evolved by former students.

Lesson 3 provides students with evidence that the phenotypic changes to their colonies are a result of heritable genetic changes passed down from parents to their offspring. They will learn how to identify these genomic changes with whole genome sequencing, including the steps involved in extracting DNA from an interesting mutant to using Illumina whole-genome sequencing to read the DNA sequence of the mutant's entire genome.

Lesson 4 introduces modern bioinformatic analysis techniques. Students are provided with representative images and sequencing read data (fastq files) of 10 wrinkly mutants evolved by students from two high schools: Pittsburgh Science and Technology Academy (Pittsburgh, PA, USA), and Pittsburgh Allderdice High School (Pittsburgh, PA, USA). Students select a mutant and follow a step-by-step guide to use online data analysis tools, either provided by the Bacterial and Viral Bioinformatics Resource Center (https://www.bv-brc.org/) or a new online analysis tool (breseq). They process this data with Microsoft Excel or Google Sheets to identify a list of causative mutations for their chosen mutant.

A follow-up activity (**Lesson 5**) connects how changes to the bacteria's DNA leads to changes to its proteins and how these mutations lead to increased biofilm production in their mutants. During this activity, students are directed on how to use the Pseudomonas Genome Database (http://www.pseudomonas.com/) to discover more information about their mutated genes.

The final lesson (**Lesson 6**) reviews the importance of studying bacteria. This lesson demonstrates the relevance of the classroom studies to real world medical applications, and how student research helps support scientific discovery. Students also have the opportunity to reflect on the impact of human directed environmental change on microbial populations.

The Three-Dimensional Learning Approach of EvolvingSTEM
The curriculum materials provide detailed lesson plans to connect the laboratory activity to the cross-cutting concepts, science and engineering practices, and disciplinary core ideas central to effective student learning. In addition, formative and summative assessment materials are provided to gauge student learning throughout the lesson.

Overall Learning Goals.
Goal 1. Students will feel more engaged with science and empowered to practice science.
The EvolvingSTEM curriculum provides students with the opportunity to perform authentic scientific analyses. Students become researchers who need to integrate information from multiple disciplines, use appropriate tools to analyze their data, and communicate their findings. This allows students to build their capacity for argumentation and critical reasoning, as they struggle to understand and explain the phenomena they are studying. We believe investigations such as this one allow students to form a sense of agency to see themselves as scientists and motivate their interest in related careers.

Goal 2. Students will understand why evolution is a unifying principle of biology that is relevant to their everyday lives.
The EvolvingSTEM experiment simulates the process of adaptive radiation often illustrated in textbooks with Darwin's finches and mimics selection for traits associated with adherence that occur in biofilm-associated infections. Adaptations to biofilm growth have been shown to contribute to increased inflammation, prolonged duration of infections, increased health care expense, and worse outcomes.

The bacteria students work with in the classroom, *Pseudomonas fluorescens*, is closely related to the notorious opportunistic pathogen *P. aeruginosa*, which is responsible for >50,000 infections per year, many of which are multidrug-resistant. *P. aeruginosa* is the "P" in ESKAPE, the acronym for the six largest threats to the rising tide of antimicrobial resistance. Importantly, *P. aeruginosa* strains are known to evolve and diversify rapidly, often with worse outcomes for patients. Thus, the evolution of diverse types in biofilms of Pseudomonads may be studied with a safe species (*P. fluorescens*) but can represent the process of pathogenesis in opportunistic infections. Students can therefore connect their classroom experiments to recent findings at the interface of evolutionary biology and medicine. We hypothesize that students will see that basic scientific research impacts their everyday lives with this important example of how laboratory research can produce results that influence the treatment of common medical issues.

Goal 3. Students will demonstrate understanding of microbiology, genetics, and biotechnology through the organizing principle of evolution.
Our Next Generation Science Standards aligned, 3D learning approach connects the activity to the cross-cutting concepts, science and engineering practices, and

disciplinary core ideas central to effective student learning. After completing this module, students will be able to demonstrate a deep understanding of fundamental life science concepts.

EVOLVINGSTEM

Please read through this document for a detailed overview of the EvolvingSTEM Evolution-in-Action Experiment.

Quick Overview of the Experiment

(Please read the detailed protocol before starting.)

Preparing for the experiment

- Several days in advance: **Use stab culture to streak out *P. fluorescens* SBW25 on to the 5 plates without triclosan, and let incubate two days** (28ºC if available, but room temperature works too).
    - Check plates for colony growth
    - Refrigerate plates until Day 1 of experiment
- **One day ahead of time, let the media and plates come to room temperature.**

Day 1: Inoculating Your Bacterial Culture

- Add a **1 white bead** and **1 black bead** to culture tubes E1 and E2.
- Add 2mL Queen's B Media (QB) to each culture tube (E1, E2, and C).
- Transfer a single colony to each culture tube.
- Incubate culture tubes at 28°C on an orbital shaker.

Day 2 (part 1): Bead Transfer

- Add a **white** bead to the E1 and E2, Day 2 culture tubes.
- Add 2mL QB to all Day 2 culture tubes.
- Transfer the Day 1 black beads to the appropriate Day 2 culture tubes.
- Transfer 50µL of the Day 1 C culture to Day 2 C culture tube.
- Incubate Day 2 culture tubes at 28°C on an orbital shaker.

Day 2 (part 2): Serial Dilution and Plating the Bacteria

- Add Phosphate Buffered Saline (PBS) to the 10^0, 10^{-1}, 10^{-2}, and 10^{-3} microcentrifuge tubes.
- Transfer the Day 1 white beads to the appropriate 10^0 tubes and vortex 60 seconds.
- Transfer 50µL of Day 1 C culture to the 10^0 tube and vortex 1-2 seconds.
- Transfer 100µL from the 10^0 tubes to the 10^{-1} tubes and vortex 1-2 seconds.
- Continue transferring to complete the dilution series.
- Plate 100µL of the 10^{-2} and 10^{-3} dilutions.
- Incubate plates at 28°C.

Day 3: Bead Transfer

- Add a **black** bead to the E1 and E2, Day 3 culture tubes.
- Add 2mL QB to all Day 3 culture tubes.
- Transfer the Day 2 white beads to the appropriate Day 3 culture tubes.
- Transfer 50µL of the Day 2 C culture to the Day 3 C culture tube.
- Incubate the Day 3 culture tubes at 28°C on an orbital shaker.

Day 4: Bead Transfer

- Add a **white** bead to the E1 and E2, Day 4 culture tubes.
- Add 2mL QB to all Day 4 culture tubes.
- Transfer the Day 3 black beads to the appropriate Day 4 culture tubes.
- Transfer 50µL of the Day 3 C culture to the Day 4 C culture tube.
- Incubate the Day 4 culture tubes at 28°C on an orbital shaker.

Day 5 (part 1): Final Plating

- Add PBS to all microcentrifuge tubes.
- Transfer the Day 4 white beads to the appropriate 10^0 tubes and vortex 60 seconds.
- Transfer 50µL of Day 4 C culture to the 10^0 tube and vortex 1-2 seconds.
- Transfer 100µL from the 10^0 tubes to the 10^{-1} tubes and vortex 1-2 seconds.
- Continue transferring to complete the dilution series.
- Plate 100µL of the 10^{-2} and 10^{-3} dilutions.
- Incubate plates at 28°C.

Day 5 (part 2): Colony Examination

- Examine the colony morphology of the Day 2 plates.
- Compare the results of your E1, E2, and C populations.

Day 6: Final Colony Examination

- Examine the colony morphology of the Day 5 plates.
- Compare the results of your E1, E2, and C populations.
- Compare the Day 5 plates to the Day 2 plates.
- Compare your results to the rest of the class.

Recommended Timetable

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Possible prep days. (More time to assess quality of plates.)						
Day 1 Inoculate	Day 2 Transfer & Plate	Day 3 Transfer	Day 4 Transfer	Day 5 Plate & Examine		
Day 6 Examine & Discuss Results						

Helpful Tips

(Please read the detailed protocol before starting.)

General tips

- Vortexing experimental populations for 60 seconds is key to make sure that biofilm associated bacteria are being removed from the bead for plating.
- Whenever using forceps, it is important to make sure that students soak the forceps in Ethanol for 30 seconds, as this is a key step for preventing contamination of the biofilm populations
- Sterilization of lab space and forceps is important for reducing contamination. The media contains a low dosage of an antibiotic to reduce contamination, but it is still important to thoroughly sterilize to prevent contamination (including cross-contamination between populations)

Preparing for the experiment.

By streaking out plates, you can ensure that single, isolated colonies can be picked for inoculating the Day 1 cultures.

Day 1: Inoculating Your Bacterial Culture

This day involves transferring bacteria from the plates to liquid media in both experimental and control populations. Picking from a single colony is important so that each population is started from genetically identical ancestors. Using a white bead on the first day is important for being able to track which bead is from which day.

Day 2: Bead Transfer and Plating

Today students will be transferring the black bead from the previous day (or a small amount of the media, in the case of the control populations) into a new culture tube containing QB media. There are a few important things to focus on: (1) sterilization of the forceps, (2) ensuring that the students know to transfer from the same population from one day to the next, and (3) transferring the correct colored bead.

For the serial dilution and plating, it is important to make sure that students vortex the microcentrifuge tubes containing PBS and the bead for 60 seconds, and we recommend mentioning this during set up for the day to increase the likelihood of success of the experiment. If the bead is not vortexed for 60 seconds, the bacteria associated with the biofilm can fail to be removed from the bead, which in turn could cause a lack of biofilm-associated phenotypes to appear at the end of the experiment. Vortexing control populations does not require a full 60 seconds, as the purpose of vortexing control populations is to make sure the bacteria are thoroughly mixed, not to remove bacteria from a bead.

Days 3 & 4: Bead Transfer

As with Day 2, it is important to focus on: (1) sterilization of the forceps, (2) ensuring that the students know to transfer from the same population from one day to the next, and (3) transferring the correct colored bead (white bead on day 3, black bead on day 4) so that beads being transferred have undergone 24 hours of growth.

Day 5: Final Plating

The primary activity for today is to set-up a dilution series and plate a portion of the bacteria. Be sure to vortex experimental tubes for 60 seconds to ensure that all bacteria are removed from the bead.

Day 6: Final Colony Examination

Students will be able to identify whether their biofilm-adapted populations changed over the course of the experiment. Each experiment is unique, so we can never be completely sure of the outcome, but it is likely that most of the experimental populations will contain colonies with new, unique phenotypes (sometimes more than one!). If possible, use a dissecting microscope to get a fascinating view of the complex shapes these new colonies form.

It is also important to note that students may not observe phenotypic changes in their colonies. **This is not a failure!** Several reasons could account for this observation. One explanation is that, by chance, no mutation arose that allowed the bacteria to be better at living on the bead than the ancestral *P. fluorescens* genotype. A more likely explanation is that a beneficial mutation did arise but did not change the colony morphology of the bacteria, so it is invisible to us with this assay. Comparing the bacteria in a different way may reveal that a phenotypic change occurred (e.g., assaying biofilm production with crystal violet).

Kit Materials

The following list describes the materials provided in the kit, along with additional materials required and their recommended amounts. The kit is designed to perform ten experimental replicates to accommodate a classroom of up to 40 students (we recommend running the experiment with groups of 3-4 students).

All materials have been pre-sterilized. To prevent contamination from environmental microbes, do not open any packaging until the materials are ready for use.

Materials included in the kit

- Serological pipettes (5mL): 40, 4 per group
- Disposable inoculation loops: 30, 3 per group
- Disposable L-shaped plate spreaders: 120, 12 pack per group
- Disposable culture tubes (14mL, round bottom): 125, 25 pack per 2 groups
- Round bottom microcentrifuge tubes (2mL): 250, 50 pack per 2 groups
- Beads: 100, pack of 6 white and 4 black per group
- PBS: 250mL, 25mL bottle per group
- **Queen's B Media: 300mL, 30mL bottle per group
- **Tsoy-agar plates with triclosan: 120, 24 pack per 2 groups
- **Tsoy-agar plates without triclosan: 5
- ***Pseudomonas fluorescens* SBW25 stab culture: 1

**Refrigerate until ready for use (4°C/39.2°F, up to 6 months). All other materials can be stored indefinitely at room temperature.

Additional materials required (yearly replacement)

- Examination gloves (will need at least 6 pairs per student)
- Kimwipes: 5 boxes
- Ethanol, 200 proof: 1L
- Bleach: 500mL
- Micropipette tips: 5-10 sets of p200 and p1000

Additional materials required (one-time purchase)

- Incubator
- Orbital shaker
- Micropipettes: 5-10 p200 and p1000
- Micropipette tip boxes: 5-10
- Vortex: 2
- Pipette aid: 5-10
- Forceps (6" or longer): 10
- Racks to hold 2, 15, and 50mL tubes: 10
- Squirt bottles for bleach and ethanol: 6
- Beakers or conical tubes for forceps sterilization: 5-10
- Waste containers: 5

Media Recipes

500mL Queen's B Media with 30ug/mL Triclosan

- 10g Proteose Peptone No. 3
- 0.75g K_2HPO_4 (Potassium Phosphate Dibasic)
- 12.5mL Glycerol
- 485mL Deionized Water
  1. Autoclave solution for 30-45 minutes.
  2. Let cool to room temperature.
  3. Add 3mL of 1M $MgSO_4$ Stock (recipe below).
  4. Add 500uL of 30mg/mL Triclosan Stock (recipe below).

250mL 1M $MgSO_4$ Stock

- 30g $MgSO_4$ (Magnesium Sulfate, anhydrous)
- 250mL Deionized Water
  1. Autoclave solution for 30-45 minutes.
  2. Let cool to room temperature.

10mL 30mg/mL Triclosan Stock

- 300mg Triclosan
- 10mL DMSO
  1. Measure 300mg Triclosan in a 15mL conical and add DMSO to 10mL.
  2. Vortex to dissolve.

500mL PBS

- 3.825g NaCl (Sodium Chloride)
- 0.36g Na_2HPO_4 (Sodium Phosphate Dibasic, anhydrous)
- 0.105g KH_2PO_4 (Potassium Phosphate Monobasic)
- 500mL Deionized Water
  1. Autoclave solution for 30-45 minutes.
  2. Let cool to room temperature.

1L Half-Strength Tsoy-Agar with 9ug/mL Triclosan

- 15g Tsoy
- 15g Agar
- 1L Deionized Water
  1. Autoclave solution for 30-45 minutes.
  2. Let cool to 55C.
  3. Add 300uL of 30mg/mL Triclosan Stock (recipe above).
  4. Pour 40-50 plates.
  5. Allow agar to solidify and dry for two nights before using.

500mL Half-Strength Tsoy-Agar

- 7.5g Tsoy
- 7.5g Agar
- 500mL Deionized Water
  1. Autoclave solution for 30-45 minutes.
  2. Let cool to 55C.
  3. Pour 20-25 plates.
  4. Allow agar to solidify and dry for two nights before using.

Learning Goals
This lesson allows students to observe evolution occur in real-time, by performing an authentic experiment with a safe bacteria, *Pseudomonas fluorescens*. Students select for bacteria that are best able to colonize a surface (in this case, a plastic bead) and form a biofilm. Over a short period of time, students can observe the population evolve as the frequency of biofilm-adapted bacteria increases over time. By the end of the experiment, students often observe increased biofilm formation in their test tubes and will usually observe changes in the appearance of colonies plated on agar. The primary goal of this experiment is to engage students in real scientific research and help them gain an improved understanding of the ecological, genetic, and evolutionary processes that drive bacterial adaptation and diversification in new environments, including natural and human hosts.

Performance Expectations
HS-LS4-1. Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence.

HS-LS4-2. Construct an explanation based on evidence that the process of evolution primarily results from four factors: (1) the potential for a species to increase in number, (2) the heritable genetic variation of individuals in a species due to mutation and sexual reproduction, (3) competition for limited resources, and (4) the proliferation of those organisms that are better able to survive and reproduce in the environment.

HS-LS4-3. Apply concepts of statistics and probability to support explanations that organisms with an advantageous heritable trait tend to increase in proportion to organisms lacking this trait.

HS-LS4-4. Construct an explanation based on evidence for how natural selection leads to adaptation of populations.

HS-LS4-5. Evaluate the evidence supporting claims that changes in environmental conditions may result in: (1) increases in the number of individuals of some species, (2) the emergence of new species over time, and (3) the extinction of other species.

HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

HS-LS3-1. Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

HS-LS3-2. Make and defend a claim based on evidence that inheritable genetic variations may result from: (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

HS-LS3-3. Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

HS-LS2-1. Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales.

HS-LS2-6. Evaluate the claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem.

HS-LS2-8. Evaluate the evidence for the role of group behavior on individual and species' chances to survive and reproduce.

Disciplinary Core Ideas

Adaptation.

- Evolution is a consequence of the interaction of four factors: (1) the potential for a species to increase in number, (2) the genetic variation of individuals in a species due to mutation and sexual reproduction, (3) competition for an environment's limited supply of the resources that individuals need in order to survive and reproduce, and (4) the ensuing proliferation of those organisms that are better able to survive and reproduce in that environment. (HS-LS4-2)
- Natural selection leads to adaptation, that is, to a population dominated by organisms that are anatomically, behaviorally, and physiologically well suited to survive and reproduce in a specific environment. That is, the differential survival and reproduction of organisms in a population that have an advantageous heritable trait leads to an increase in the proportion of individuals in future generations that have the trait and to a decrease in the proportion of individuals that do not. (HS-LS4-3), (HS-LS4-4)
- Adaptation also means that the distribution of traits in a population can change when conditions change. (HS-LS4-3)
- Changes in the physical environment, whether naturally occurring or human induced, have thus contributed to the expansion of some species, the emergence of new distinct species as populations diverge under different conditions, and the decline–and sometimes the extinction–of some species. (HS-LS4-5)
- Species become extinct because they can no longer survive and reproduce in their altered environment. If members cannot adjust to change that is too fast or drastic, the opportunity for the species' evolution is lost. (HS-LS4-5)

Ecosystem Dynamics, Functioning, and Resilience

- A complex set of interactions within an ecosystem can keep its numbers and types of organisms relatively constant over long periods of time under stable conditions. If a modest biological or physical disturbance to an ecosystem occurs, it may return to its more or less original status (i.e., the ecosystem is resilient), as opposed to becoming a very different ecosystem. Extreme fluctuations in conditions or the size of any population, however, can challenge the functioning of ecosystems in terms of resources and habitat availability. (HS-LS2-6)

Evidence of common ancestry and diversity.

- Genetic information provides evidence of evolution. DNA sequences vary among species, but there are many overlaps; in fact, the ongoing branching that produces multiple lines of descent can be inferred by comparing the DNA sequences of different organisms. Such information is also derivable from the similarities and differences in amino acid sequences and from anatomical and embryological evidence. (HS-LS4-1)

Inheritance of Traits.

- Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function. (HS-LS3-1)

Interdependent Relationships in Ecosystems

- Ecosystems have carrying capacities, which are limits to the numbers of organisms and populations they can support. These limits result from such factors as the availability of living and nonliving resources and from such challenges such as predation, competition, and disease. Organisms would have the capacity to produce populations of great size were it not for the fact that environments and resources are finite. This fundamental tension affects the abundance (number of individuals) of species in any given ecosystem. (HS-LS2-1)

Natural selection.

- Natural selection occurs only if there is both (1) variation in the genetic information between organisms in a population and (2) variation in the expression of that genetic information—that is, trait variation—that leads to differences in performance among individuals. (HS-LS4-2), (HS-LS4-3)
- The traits that positively affect survival are more likely to be reproduced, and thus are more common in the population. (HS-LS4-3)

Social Interactions and Group Behavior

- Group behavior has evolved because membership can increase the chances of survival for individuals and their genetic relatives. (HS-LS2-8)

Structure and Function.

- All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells. (HS-LS1-1), (HS-LS3-1)

Variation of Traits.

- Although DNA replication is tightly regulated and remarkably accurate, errors do occur and result in mutations, which are also a source of genetic variation. Environmental factors can also cause mutations in genes, and viable mutations are inherited. (HS-LS3-2)
- Environmental factors also affect expression of traits, and hence affect the probability of occurrences of traits in a population. Thus, the variation and distribution of traits observed depends on both genetic and environmental factors. (HS-LS3-2), (HS-LS3-3)

Science and Engineering Practices

Analyzing and Interpreting Data.

- Analyzing data in 9–12 builds on K–8 experiences and progresses to introducing more detailed statistical analysis, the comparison of data sets for consistency, and the use of models to generate and analyze data.
- Apply concepts of statistics and probability (including determining function fits to data, slope, intercept, and correlation coefficient for linear fits) to scientific and engineering questions and problems, using digital tools when feasible. (HS-LS4-3), (HS-LS3-3)

Asking Questions and Defining Problems.

- Asking questions and defining problems in 9-12 builds on K-8 experiences and progresses to formulating, refining, and evaluating empirically testable questions and design problems using models and simulations.
- Ask questions that arise from examining models or a theory to clarify relationships. (HS-LS3-1)

Constructing Explanations and Designing Solutions.

- Constructing explanations and designing solutions in 9–12 builds on K–8 experiences and progresses to explanations and designs that are supported by multiple and independent student-generated sources of evidence consistent with scientific ideas, principles, and theories.
- Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future. (HS-LS4-2), (HS-LS4-4), (HS-LS1-1)

Engaging in Argument from Evidence

- Engaging in argument from evidence in 9-12 builds on K-8 experiences and progresses to using appropriate and sufficient evidence and scientific reasoning to defend and critique claims and explanations about the natural and designed world(s). Arguments may also come from current or historical episodes in science.
- Evaluate the claims, evidence, and reasoning behind currently accepted explanations or solutions to determine the merits of arguments. (HS-LS4-5), (HS-LS2-6), (HS-LS2-8)

Obtaining, Evaluating, and Communicating Information.

- Obtaining, evaluating, and communicating information in 9–12 builds on K–8 experiences and progresses to evaluating the validity and reliability of the claims, methods, and designs.
- Communicate scientific information (e.g., about phenomena and/or the process of development and the design and performance of a proposed process or system) in multiple formats (including orally, graphically, textually, and mathematically). (HS-LS4-1)

Using Mathematics and Computational Thinking

- Mathematical and computational thinking in 9-12 builds on K-8 experiences and progresses to using algebraic thinking and analysis, a range of linear and nonlinear functions including trigonometric functions, exponentials and logarithms, and computational tools for statistical analysis to analyze, represent, and model data. Simple

computational simulations are created and used based on mathematical models of basic assumptions.
- Use mathematical and/or computational representations of phenomena or design solutions to support explanations. (HS-LS2-1)

Connections to Nature of Science: Scientific Knowledge is Open to Revision in Light of New Evidence

- Scientific argumentation is a mode of logical discourse used to clarify the strength of relationships between ideas and evidence that may result in revision of an explanation. (HS-LS2-6), (HS-LS2-8)

Connections to Nature of Science: Science Models, Laws, Mechanisms, and Theories Explain Natural Phenomena

- A scientific theory is a substantiated explanation of some aspect of the natural world, based on a body of facts that have been repeatedly confirmed through observation and experiment and the science community validates each theory before it is accepted. If new evidence is discovered that the theory does not accommodate, the theory is generally modified in light of this new evidence. (HS-LS4-1)

Cross-Cutting Concepts

Cause and Effect

- Empirical evidence is required to differentiate between cause and correlation and make claims about specific causes and effects. (HS-LS4-2), (HS-LS4-4), (HS-LS4-5), (HS-LS3-1), (HS-LS3-2), (HS-LS2-6), (HS-LS2-8)

Patterns

- Different patterns may be observed at each of the scales at which a system is studied and can provide evidence for causality in explanations of phenomena. (HSLS4-1), (HS-LS4-3)

Scale, Proportion, and Quantity

- Algebraic thinking is used to examine scientific data and predict the effect of a change in one variable on another (e.g., linear growth vs. exponential growth). (HSLS3-3)
- The significance of a phenomenon is dependent on the scale, proportion, and quantity at which it occurs. (HS-LS2-1)

Stability and Change

- Much of science deals with constructing explanations of how things change and how they remain stable. (HS-LS2-6)

Structure and Function

- Investigating or designing new systems or structures requires a detailed examination of the properties of different materials, the structures of different components, and connections of components to reveal its function and/or solve a problem. (HS-LS1-1)

Connections to Nature of Science: Scientific Knowledge Assumes an Order and Consistency in Natural Systems

- Scientific knowledge is based on the assumption that natural laws operate today as they did in the past and they will continue to do so in the future. (HS-LS4-1), (HSLS4-4)

Connections to Nature of Science: Science is a Human Endeavor

- Technological advances have influenced the progress of science and science has influenced advances in technology. (HSLS3-3)
- Science and engineering are influenced by society and society is influenced by science and engineering. (HS-LS3-3)

EVOLVINGSTEM

Pseudomonas fluorescens **Experimental Evolution Protocol**

DAY 1: INOCULATING YOUR BACTERIAL CULTURE

Today, you will begin an experiment with the harmless bacterium, *Pseudomonas fluorescens* strain SBW25. SBW25 was isolated in 1989 from the leaf surface of a sugar beet plant grown at the University Farm, Wytham, Oxford, UK. Before the bacteria got to your classroom, they had been stored in a freezer for a long period of time at -80° Celsius (-112° Fahrenheit). A sample of the frozen bacteria was diluted by streaking it across an agar plate with an inoculation loop. By diluting the bacteria in this way, you can see isolated colonies that grew from an individual bacterial cell. To begin your experiment, you will transfer bacteria from a colony to a culture tube containing a broth that contains the proper nutrients for your bacteria to grow.

NECESSARY MATERIALS

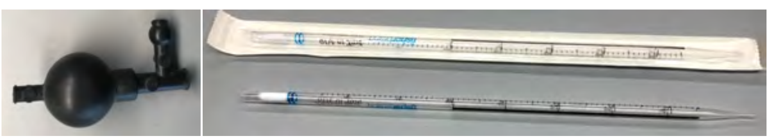
Gloves

70% Ethanol

Waste Container with 10% Bleach

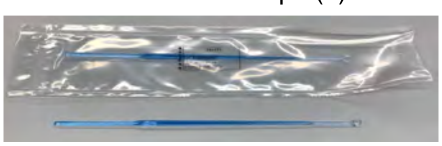
Kimwipes

Bulb & Serological Pipette



Forceps

Inoculation Loops (3)



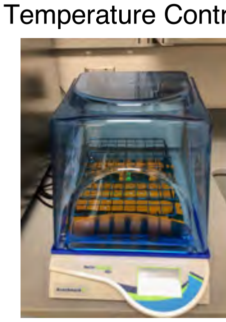
Culture Tubes (3)

QB (6mL)

Beads (2 white & 2 black)

Tube Rack

Orbital Shaker with Temperature Control



P. fluorescens

PROCEDURE

- ☐ Wipe down your lab space with 70% ethanol.

- ☐ Label your culture tubes "E1", "E2", and "C" to identify your experimental and control cultures. Also write your group name and "day 1" on each tube.

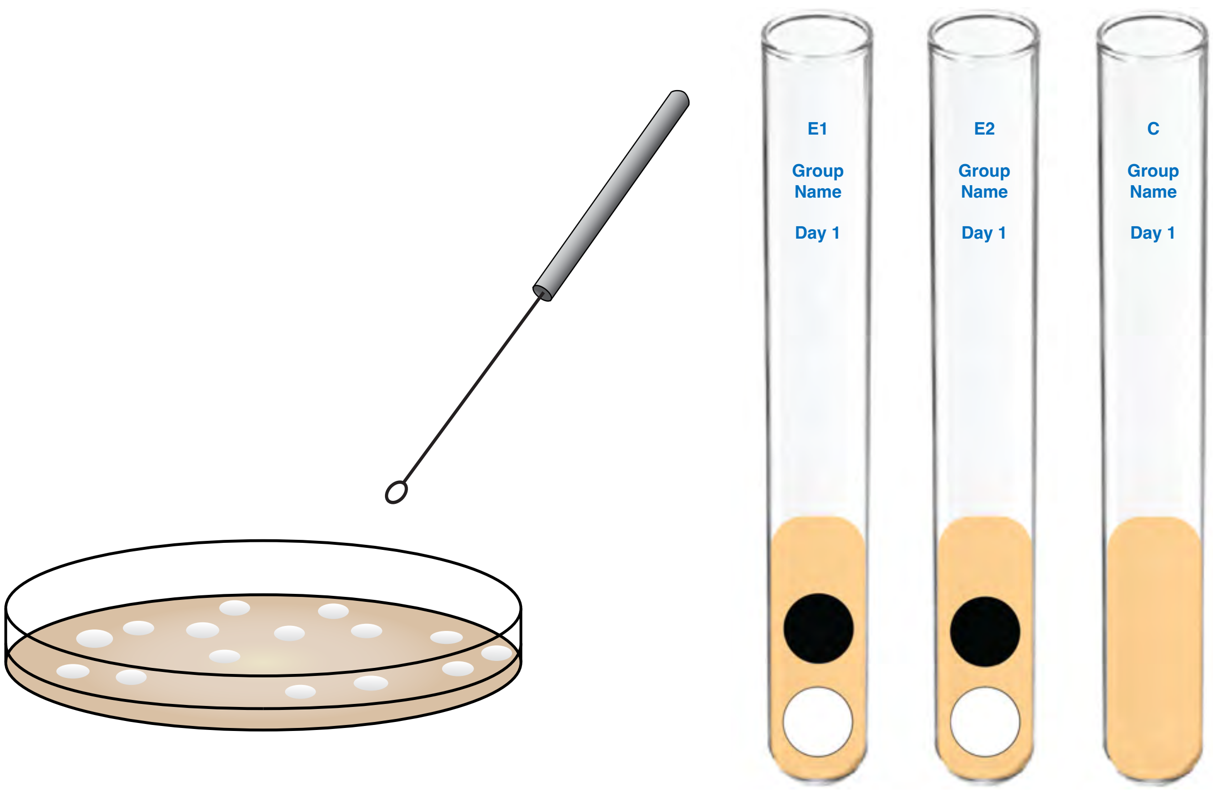
- ☐ Dip your forceps in 70% ethanol for at least 30 seconds. Wipe excess ethanol away with a kimwipe.

- ☐ Use the sterile forceps to add **1 white bead and 1 black bead** to culture tubes E1 and E2.

- ☐ Add **2mL QB** to each culture tube with a serological pipette.

- ☐ Use a sterile inoculation loop to transfer a **single,** isolated *P. fluorescens* colony to a **single** culture tube. Be sure to use a new inoculation loop to transfer a new colony to each tube.

- ☐ Incubate the culture tubes on an orbital shaker until your next class (shaking speed: 150rpm; temperature: 28 °C).



DAY 2: BEAD TRANSFER AND PLATING
Today you will transfer the bacteria that colonized the bead and formed a biofilm to a new tube with a fresh bead. You will also dilute your bacterial cultures and transfer the cells to agar plates. This will allow you to observe the appearance of the colonies grown from a sample of your bacterial population.

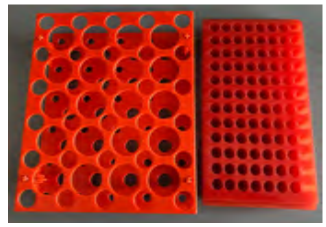
NECESSARY MATERIALS:

Gloves

70% Ethanol

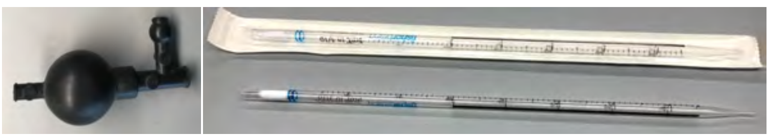
Waste Container with 10% Bleach

Tube Racks



Kimwipes

Bulb & Serological Pipette



Forceps

Culture Tubes (3)

QB (6mL)

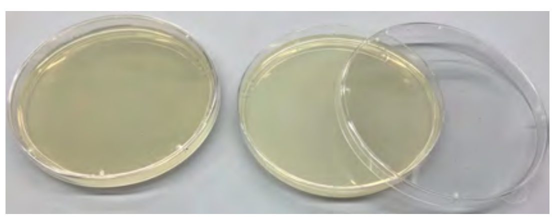
PBS (12mL)

2mL Tubes (12)

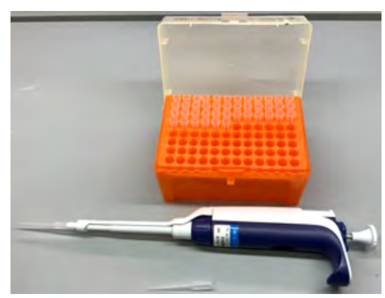
Beads (2 white)

L-shaped Spreaders (6)

Agar Plates (6)

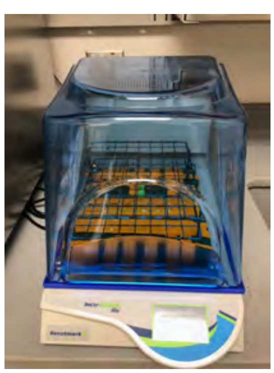


P200 Micropipette & Tips



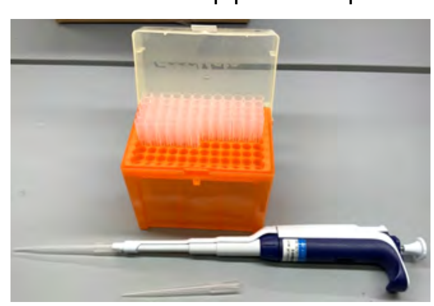
Incubator

Orbital Shaker



Vortex

P1000 Micropipette & Tips



PROCEDURE:

☐ Wipe down your lab space with 70% ethanol.

Prepare tubes to continue growing your bacterial cultures:

☐ Label your culture tubes: "E1", "E2", and "C", include your group name and "day 2".

☐ Dip your forceps in 70% ethanol for at least 30 seconds. Wipe excess ethanol away with a kimwipe.

☐ Use the sterile forceps to add a **white bead** to culture tubes E1 and E2.

☐ Add **2mL QB** to each culture tube with a serological pipette.

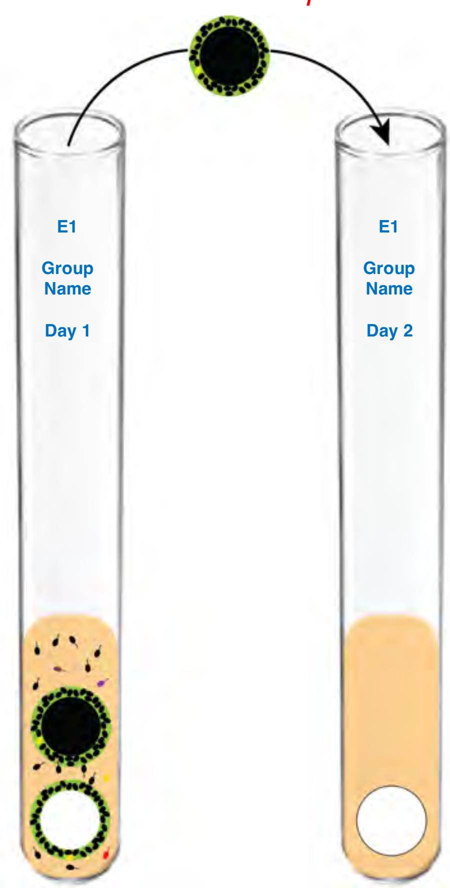
Transfer bacteria to continue growing your experimental and control populations:

☐ Dip your forceps in 70% ethanol for at least 30 seconds. Wipe excess ethanol away with a kimwipe.

☐ For the experimental cultures (E1 and E2), use sterile forceps to **transfer the DAY 1 black bead** to the corresponding DAY 2 experimental tube that you prepared with fresh media and a **new white bead**. *Be sure to sterilize the forceps between bead transfers to prevent cross-contamination of your bacterial populations.*

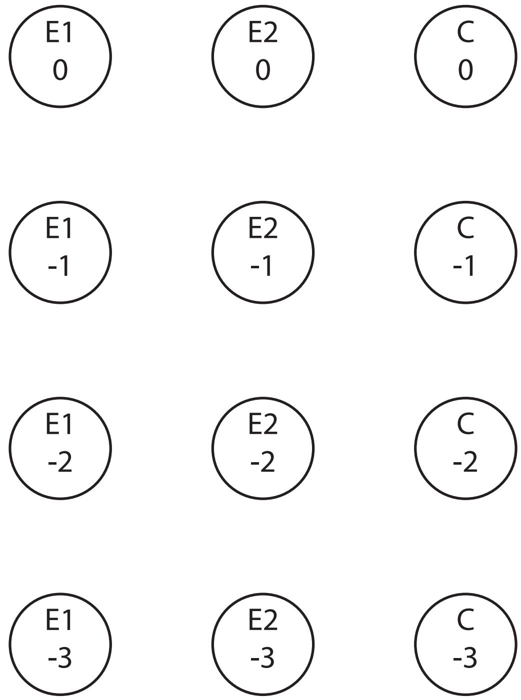
☐ For the control culture, use the p200 micropipette to transfer **50μL** of the DAY 1 culture to the DAY 2 control tube.

☐ Incubate the culture tubes on an orbital shaker until your next class (shaking speed: 150rpm; temperature: 28 °C).



Prepare tubes to collect your DAY 1 bacteria and prepare a dilution series to plate a sample of your bacteria:

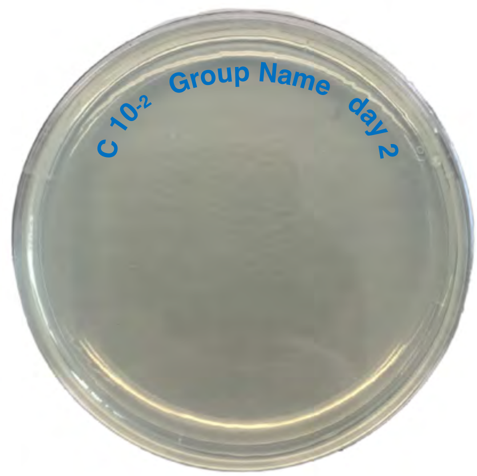
- ☐ Label 3 microcentrifuge tubes:
  - "E1, 10^{0}"
  - "E2, 10^{0}"
  - "C, 10^{0}"
- ☐ Label 3 microcentrifuge tubes:
  - "E1, 10^{-1}"
  - "E2, 10^{-1}"
  - "C, 10^{-1}"
- ☐ Label 3 microcentrifuge tubes:
  - "E1, 10^{-2}"
  - "E2, 10^{-2}"
  - "C, 10^{-2}"
- ☐ Label 3 microcentrifuge tubes:
  - "E1, 10^{-3}"
  - "E2, 10^{-3}"
  - "C, 10^{-3}"

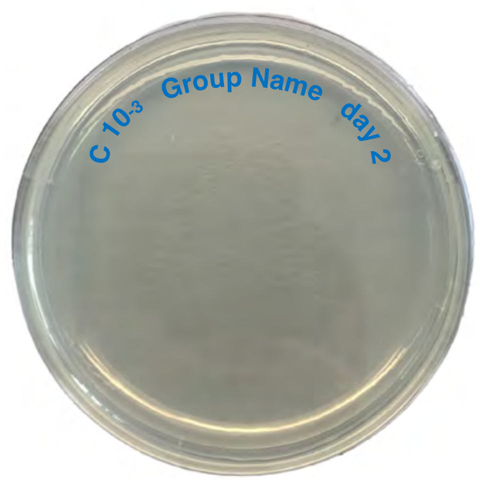


- ☐ Add **950µL PBS** to each 10^{0} tube with a p1000 micropipette.
- ☐ Add **900µL PBS** to each 10^{-1} tube with a p1000 micropipette.
- ☐ Add **900µL PBS** to each 10^{-2} tube with a p1000 micropipette.
- ☐ Add **900µL PBS** to each 10^{-3} tube with a p1000 micropipette.

- ☐ Label 3 Tsoy-Agar plates:
  - “E1, 10^{-2}”, Your Group Name, and “day 2”
  - “E2, 10^{-2}”, Your Group Name, and “day 2”
  - “C, 10^{-2}”, Your Group Name, and “day 2”

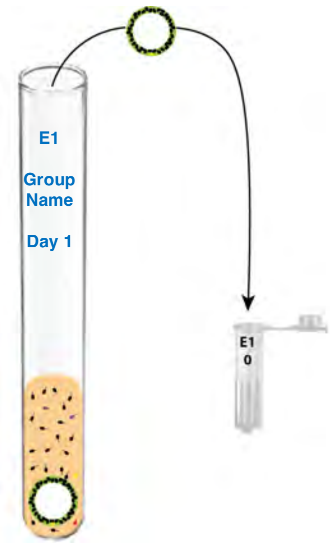
- ☐ Label 3 Tsoy-Agar plates:
  - “E1, 10^{-3}”, Your Group Name, and “day 2”
  - “E2, 10^{-3}”, Your Group Name, and “day 2”
  - “C, 10^{-3}”, Your Group Name, and “day 2”





Prepare a dilution series:

- ☐ For the experimental cultures (E1 and E2), use sterile forceps to transfer the DAY 1 **white bead** to the 10^0 microcentrifuge tube. *Be sure to sterilize the forceps between bead transfers to prevent cross-contamination of your bacterial populations.*
- ☐ Vortex the 10^0 microcentrifuge tube with the bead for at least 60 seconds to remove all attached bacteria from the bead.
- ☐ For the control culture, use a p200 micropipette to transfer **50μL** of the DAY 1 culture to the corresponding 10^0 microcentrifuge tube and vortex for 5 seconds.



For the following steps, you will use the 10^0 microcentrifuge tubes that contain bacteria from your DAY 1 cultures. The same steps are repeated for "E1", "E2", and "C".

- ☐ Use a p200 micropipette to **transfer 100μL** from the 10^0 tube to the 10^{-1} tube and briefly vortex to mix (1-2 seconds).
- ☐ Use a p200 micropipette to **transfer 100μL** from the 10^{-1} tube to the10^{-2} tube and briefly vortex to mix (1-2 seconds).
- ☐ Use a p200 micropipette to **transfer 100μL** from the 10^{-2} tube to the 10^{-3} tube and briefly vortex to mix (1-2 seconds).

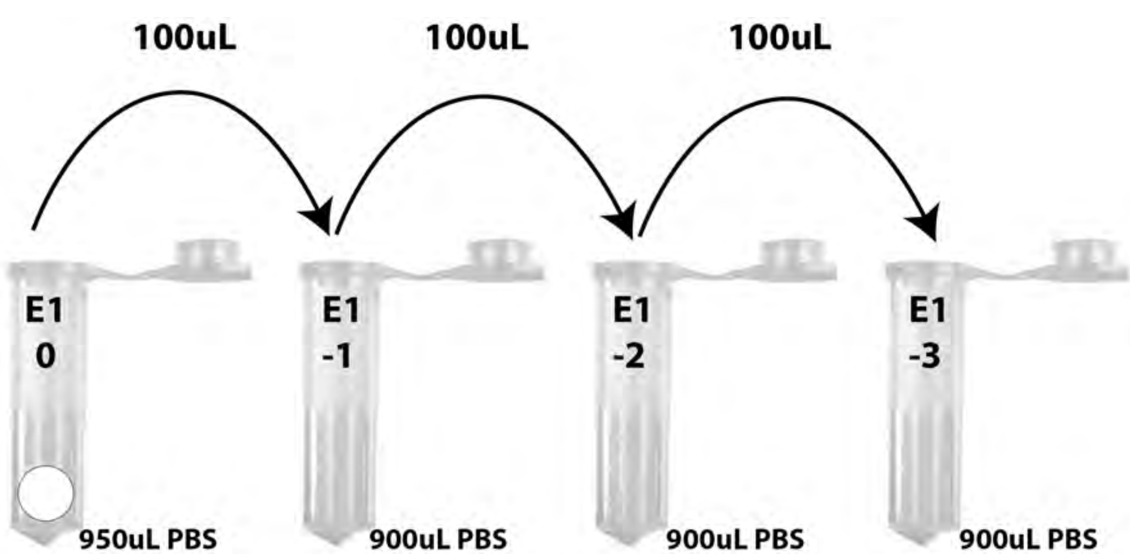
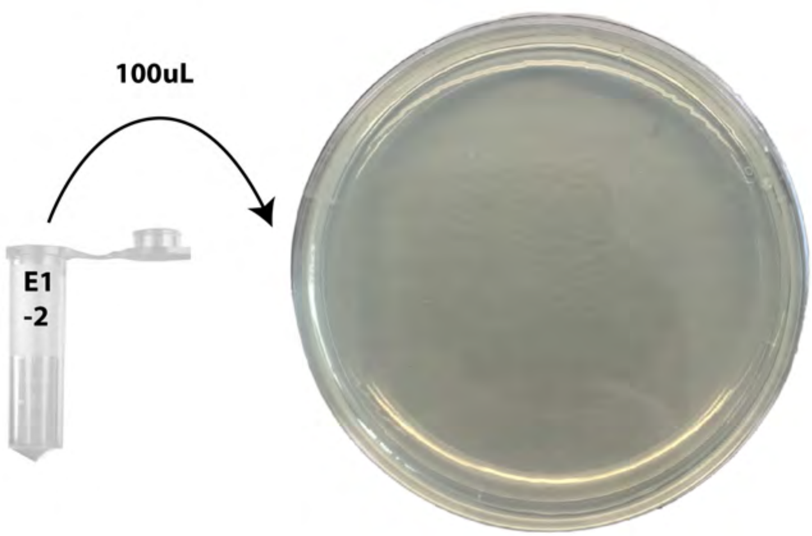


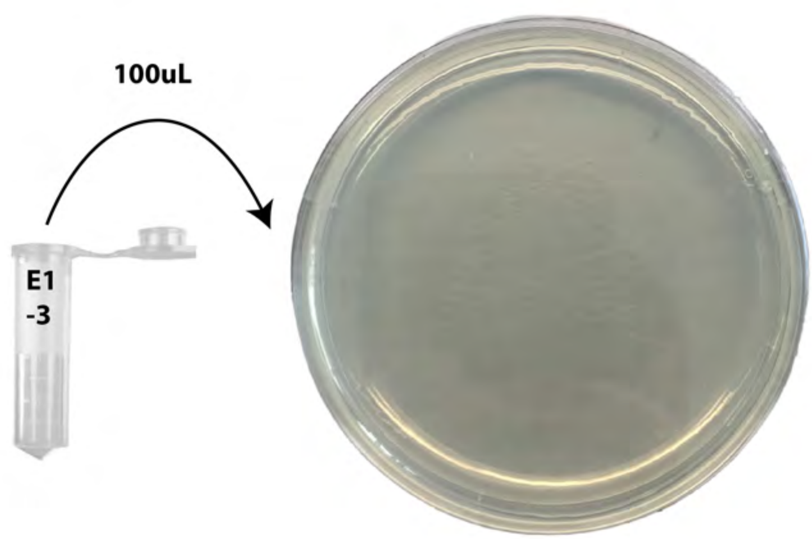
Plate your bacteria:

- ☐ Use a p200 micropipette to **transfer 100µL** of the 10^{-2} and 10^{-3} dilutions to their corresponding agar plates.

- ☐ Spread the liquid culture evenly across the plate with a Plate Spreader. Plate the bacteria gently so the agar maintains a smooth surface and **be sure to use a new Plate Spreader for each plate.**

- ☐ Incubate the plates, **upside down**, until your next class (temperature: 28 °C).





DAY 3: BEAD TRANSFER

The millions of cells that you added to your tube have quickly reproduced to become billions. It doesn't take long before the bacteria consume the food and nutrients provided by the media inside of the test tube. In order to make sure that the bacteria continue to reproduce, you have to transfer a subset into a new tube with fresh media. In the case of the experimental cultures, you transfer only the bacteria that are good at forming biofilm and have thus successfully stuck to the bead.

NECESSARY MATERIALS:

Gloves

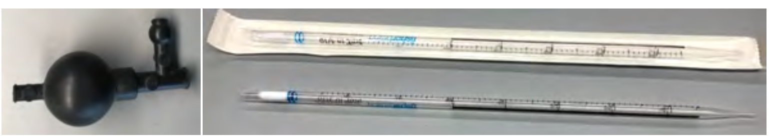
70% Ethanol

Waste Container with 10% Bleach

Beads (2 black)

Kimwipes

Bulb & Serological Pipette



Forceps

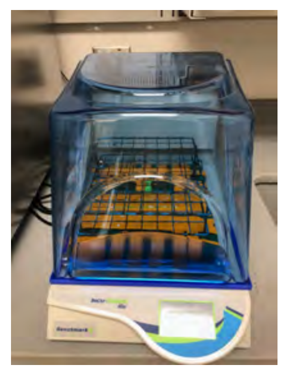
Culture Tubes (3)

QB (6mL)

Tube Rack

P200 Micropipette & Tips

Orbital Shaker



PROCEDURE:

☐ Wipe down your lab space with 70% ethanol.

Prepare tubes to continue growing your bacterial cultures:

☐ Label your culture tubes: “E1”, “E2”, and “C”, include your group name and “day 3”.

☐ Dip your forceps in 70% ethanol for at least 30 seconds. Wipe excess ethanol away with a kimwipe.

☐ Use the sterile forceps to add a **black bead** to each experimental culture tube.

☐ Add **2mL QB** to each culture tube with a serological pipette.

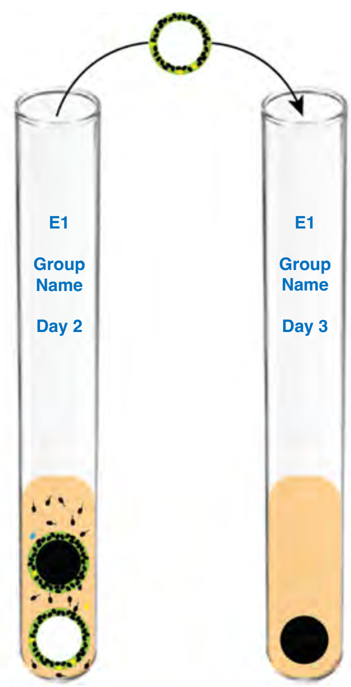
Transfer bacteria to continue growing your experimental and control populations:

☐ Dip your forceps in 70% ethanol for at least 30 seconds. Wipe excess ethanol away with a kimwipe.

☐ For each experimental culture, use sterile forceps to move the DAY 2 black bead out of the way by transferring it to the cap of the DAY 2 culture tube, now **transfer the DAY 2 white bead** to the corresponding DAY 3 experimental tube with fresh media and a **new black bead**. Remember to sterilize the forceps between bead transfers.

☐ For the control culture, use the p200 micropipette to **transfer 50μL** of the DAY 2 culture to the DAY 3 control tube.

☐ Incubate the culture tubes on an orbital shaker until your next class (shaking speed: 150rpm; temperature: 28 °C).



DAY 4: BEAD TRANSFER

Overnight, some of the bacteria detached from the white bead's biofilm and reattached to the surface of the black bead to create a new biofilm. Today, you will transfer your black bead to a new tube containing fresh media and a white bead to continue the biofilm lifecycle of dispersal, recolonization, surface attachment, and biofilm formation.

NECESSARY MATERIALS:

Gloves

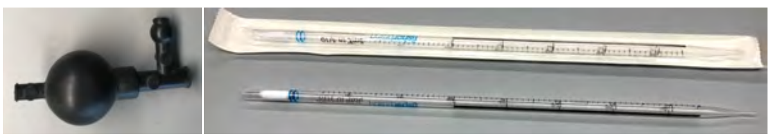
70% Ethanol

Waste Container with 10% Bleach

Beads (2 white)

Kimwipes

Bulb & Serological Pipette



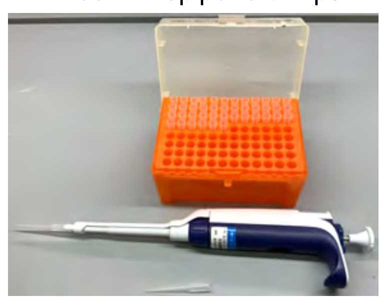
Forceps

Culture Tubes (3)

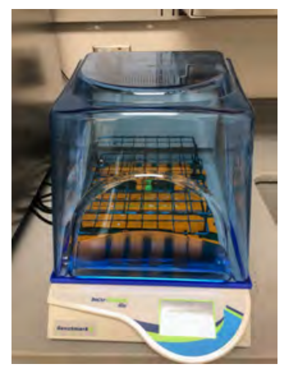
QB (6mL)

Tube Rack

P200 Micropipette & Tips



Orbital Shaker



PROCEDURE:

☐ Sterilize your lab space with 70% ethanol.

Prepare tubes to continue growing your bacterial cultures:

☐ Label your culture tubes: “E1”, “E2”, and “C”, include your group name and “day 4”.

☐ Dip your forceps in 70% ethanol for at least 30 seconds. Wipe excess ethanol away with a kimwipe.

☐ Use sterile forceps to add a **white bead** to each experimental culture tube.

☐ Add **2mL QB** to each culture tube with a serological pipette.

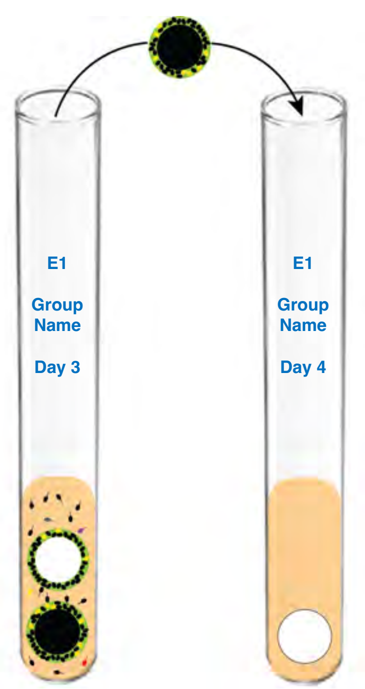
Transfer bacteria to continue growing your experimental and control populations:

☐ Dip your forceps in 70% ethanol for at least 30 seconds. Wipe excess ethanol away with a kimwipe.

☐ For the experimental cultures, use sterile forceps to move the DAY 3 white bead out of the way by transferring it to the cap of the DAY 3 culture tube. Now **transfer the DAY 3 black bead** to the corresponding DAY 4 experimental tube with fresh media and a **new white bead**. Remember to sterilize the forceps between bead transfers.

☐ For the control culture, use the p200 micropipette to **transfer 50µL** of the DAY 3 culture to the DAY 4 control tube.

☐ Incubate the culture tubes on an orbital shaker until your next class (shaking speed: 150rpm; temperature: 28 °C).



DAY 5: FINAL PLATING AND COLONY EXAMINATION

You may notice that the appearance of your test tube has changed from the start of the experiment. Your test tube may have an increased amount of biofilm on its sides and your culture may have a neon-yellow tint and chunks of biofilm in it. Believe it or not, this is normal! It is also normal, however, that you may not observe these changes.

NECESSARY MATERIALS:

Gloves

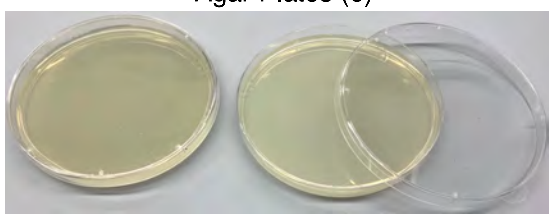
70% Ethanol

Waste Container with 10% Bleach

Kimwipes

L-shaped Spreaders (6)

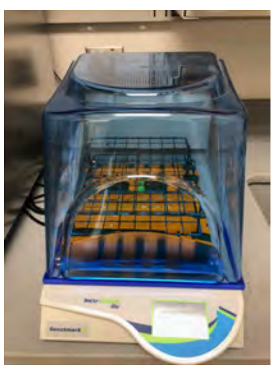
Agar Plates (6)



Forceps

Incubator

Orbital Shaker

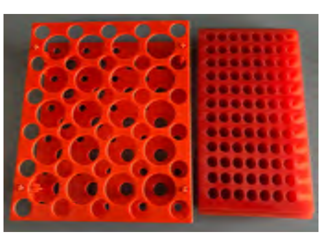


Vortex

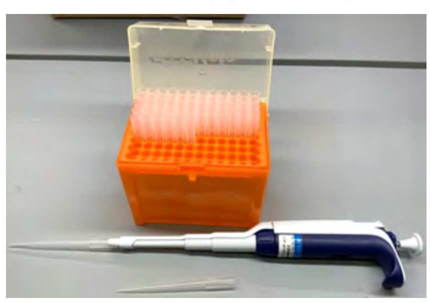
PBS (12mL)

2mL Tubes (12)

Tube Racks



P1000 Micropipette & Tips



P200 Micropipette & Tips

PROCEDURE:

☐ Wipe down your lab space with 70% ethanol.

Prepare tubes to prepare a dilution series to plate a sample of your bacteria:

☐ Label 3 microcentrifuge tubes "E1, 10^0", "E2, 10^0", "C, 10^0". Add **950μL PBS** to each tube with a p1000 micropipette.

☐ Label 3 microcentrifuge tubes "E1, 10^{-1}", "E2, 10^{-1}", and "C, 10^{-1}". Add **900μL PBS** to each tube with a p1000 micropipette.

☐ Label 3 microcentrifuge tubes "E1, 10^{-2}", "E2, 10^{-2}", and "C, 10^{-2}". Add **900μL PBS** to each tube with a p1000 micropipette.

☐ Label 3 microcentrifuge tubes "E1, 10^{-3}", "E2, 10^{-3}", and "C, 10^{-3}". Add **900μL PBS** to each tube with a p1000 micropipette.

☐ Label 3 Tsoy-Agar plates "E1, 10^{-2}", "E2, 10^{-2}", and "C, 10^{-2}", include your group name and "day 5".

☐ Label 3 Tsoy-Agar plates "E1, 10^{-3}", "E2, 10^{-3}", and "C, 10^{-3}", include your group name and "day 5".

Prepare a dilution series and plate your bacteria:

☐ Dip your forceps in 70% ethanol for at least 30 seconds. Wipe excess ethanol away with a kimwipe.

☐ For the experimental cultures, use sterile forceps to transfer the DAY 4 **white bead** to the corresponding 10^0 microcentrifuge tube filled with 950μL PBS. Remember to sterilize the forceps between bead transfers.

☐ Vortex the microcentrifuge tube with the bead for at least 60 seconds to remove all attached bacteria from the bead.

☐ For the control culture, use a p200 micropipette to **transfer 50μL** of the DAY 4 culture to the corresponding 10^0 microcentrifuge tube filled with 950μL PBS and briefly vortex to mix.

☐ Perform the remaining steps on both the experimental and control cultures.

☐ Use a p200 micropipette to **transfer 100μL** from the 10^0 tube to a microcentrifuge tube filled with 900μL PBS (10^{-1} dilution) and briefly vortex to mix.

☐ Use a p200 micropipette to **transfer 100μL** from the 10^{-1} tube to a new microcentrifuge tube filled with 900μL PBS (10^{-2} dilution) and briefly vortex to mix.

☐ Use a p200 micropipette to **transfer 100µL** from the 10^{-2} tube to a new microcentrifuge tube filled with 900µL PBS (10^{-3} dilution) and briefly vortex to mix.

☐ Use a p200 micropipette to **transfer 100µL** of the 10^{-2} and 10^{-3} dilutions to agar plates.

☐ Spread the liquid culture evenly across the plate with a Plate Spreader.

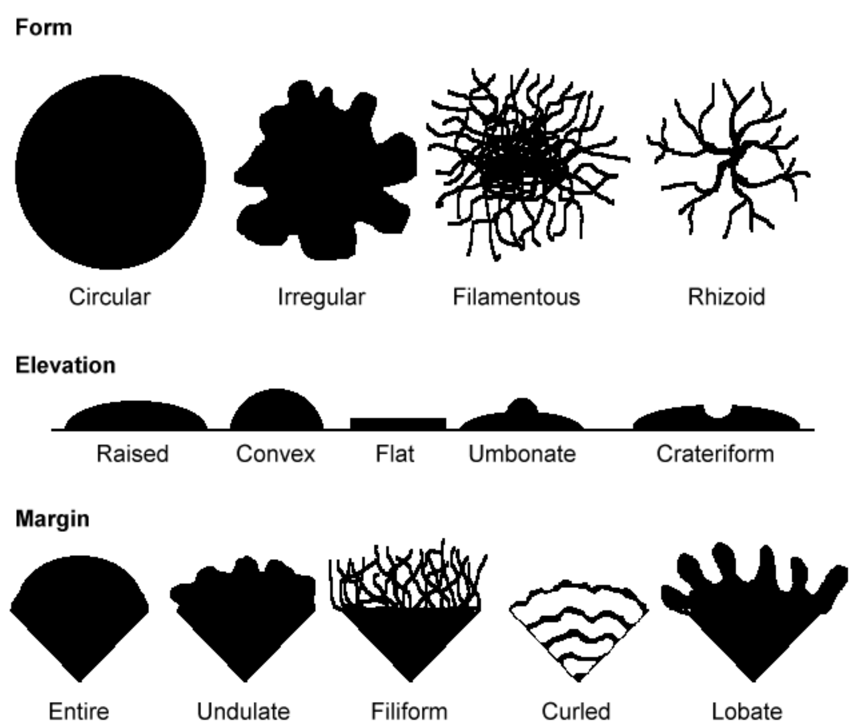
☐ Incubate the plates, **upside down**, until your next class (temperature 28 °C).

Examine your DAY 2 plates:

☐ Closely examine the colony morphology of your DAY 2 plates. Make observations from a plate with colonies that are well-isolated from one another. Be sure to take pictures!

☐ Describe the following for each colony type you see:
- Comparative size: large, medium, or small
- General form: the shape when viewed from above the plate
- Elevation: the shape when viewed in cross-section
- Margin: the magnified shape of the colony's edge
- Surface texture: smooth, glistening, wrinkled, etc.

☐ Store your plates in a refrigerator until your next class.



DAY 6: FINAL COLONY EXAMINATION
Today you can observe if there are changes in the appearance of your bacterial colonies after selection for life on the bead.

PROCEDURE:

- ☐ Closely examine the colony morphology of your DAY 5 plates. Make observations from a plate with colonies that are well-isolated from one another. Be sure to take pictures!

- ☐ Describe the following for each colony type you see:
  - Comparative size: large, medium, or small
  - General form: the shape when viewed from above the plate
  - Elevation: the shape when viewed in cross-section
  - Margin: the magnified shape of the colony's edge
  - Surface texture: smooth, glistening, wrinkled, etc.

- ☐ Compare the results of your experimental and control populations. Document the percentage of colonies that look like the ancestral population (the bacteria you plated on DAY 2) and the percentage that look different.

- ☐ Compare your results to those of another group of classmates.