EVOLVINGSTEM

Pseudomonas fluorescens Experimental Evolution Protocol

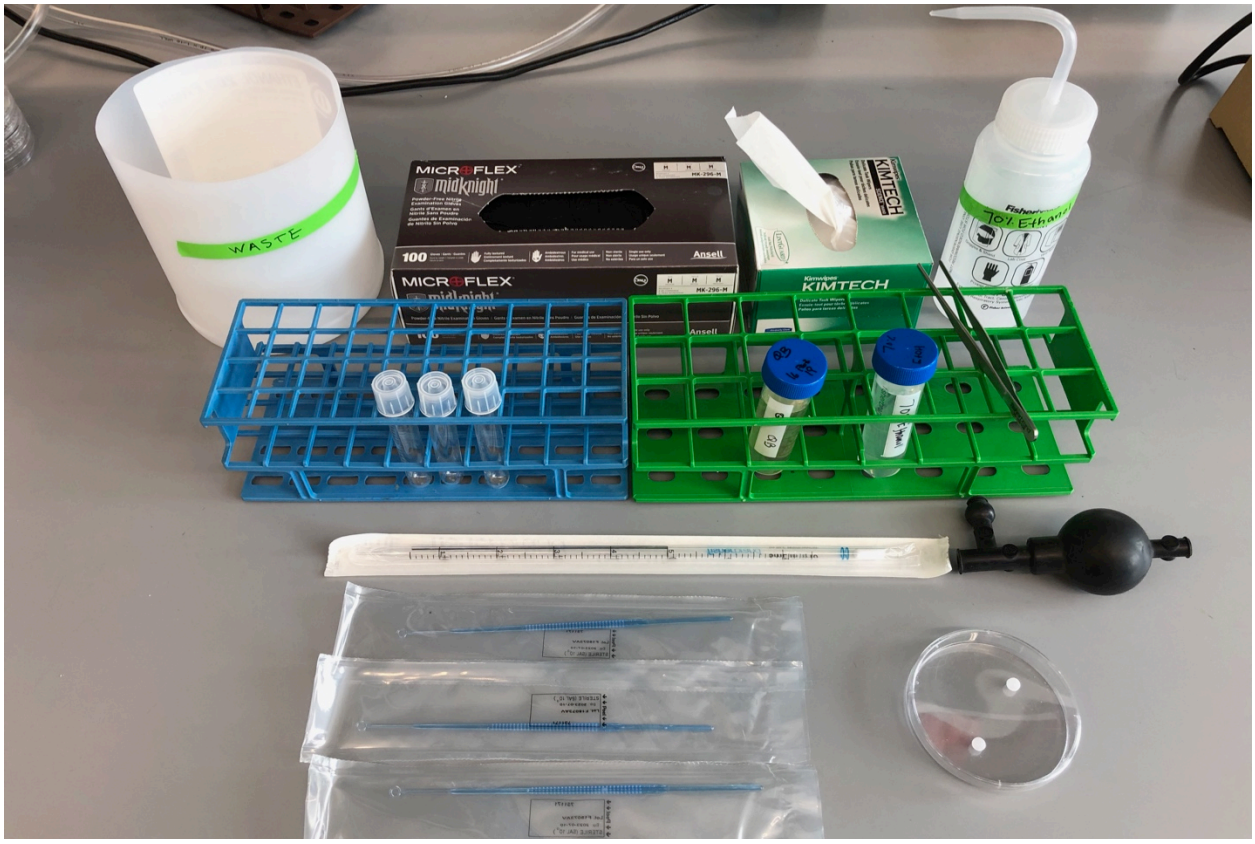
DAY 1: INOCULATING YOUR BACTERIAL CULTURE

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

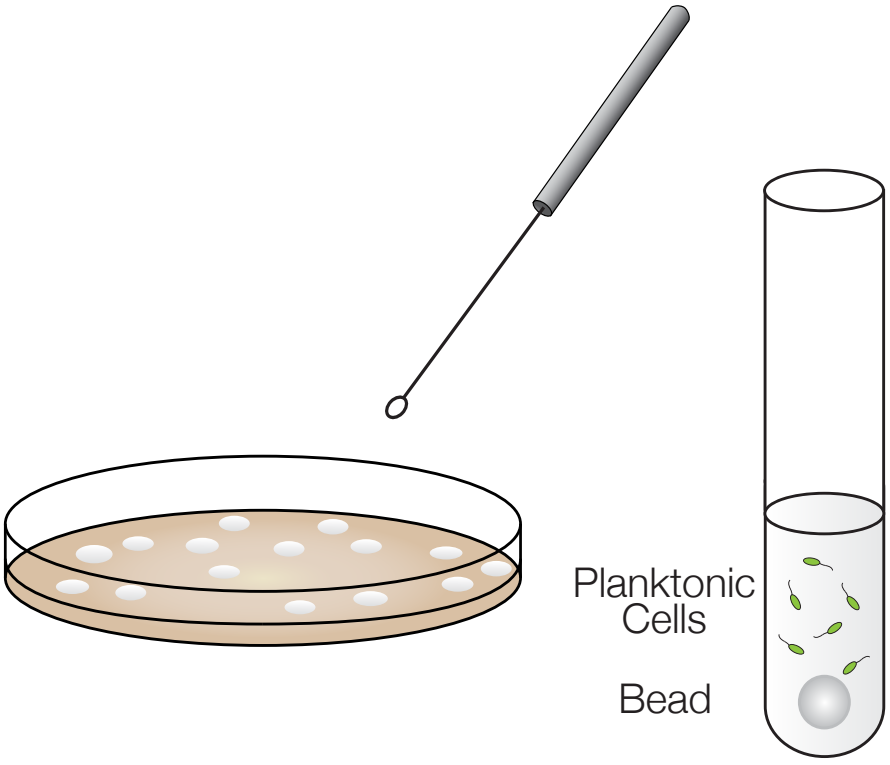
- Gloves
- 70% Ethanol (spray bottle and 50mL conical tube)
- Kimwipes
- Forceps
- 3 Inoculation Loops
- Serological Pipette and Pipette Aid
- 3 Culture Tubes
- 15 and 50mL Tube Racks
- 2 White Polystyrene Beads
- 15mL Queen's B Medium (QB)
- *Pseudomonas fluorescens* SBW25 Colonies on Agar Plates
- Orbital Shaker
- Waste Container with 10% Bleach

LAB BENCH SETUP



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.
- ☐ Add 5mL QB to each culture tube with a serological pipette.
- ☐ 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.
- ☐ 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.



DAY 2: BEAD TRANSFER AND PLATING

Today you will transfer only those bacteria that colonized the bead and formed a biofilm to a new tube with a fresh bead. You will also dilute your bacterial culture and transfer the cells to an agar plate. This will allow you to observe the appearance of the colonies grown from a sample of your bacterial population.

NECESSARY MATERIALS:

- Gloves
- 70% Ethanol (spray bottle and 50mL conical tube)
- Kimwipes
- Vortex
- Forceps
- Serological Pipette and Pipette Aid
- 6 L-shaped Plate Spreaders
- p200 and p1000 Micropipettes and Tips
- 12 Microcentrifuge Tubes
- 3 Culture Tubes
- 2, 15, and 50mL Tube Racks
- 2 **White** Polystyrene Beads
- 6 Tsoy-Agar Plates
- 20mL QB
- 10mL Phosphate Buffered Saline (PBS)
- Orbital Shaker
- Waste Container with 10% Bleach

LAB BENCH SETUP

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 4.5mL QB to each culture tube with a serological pipette.

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”, “E2”, “C” AND 10^{0}. Add 950µL QB to each tube with a p1000 micropipette.

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

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

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

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

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.

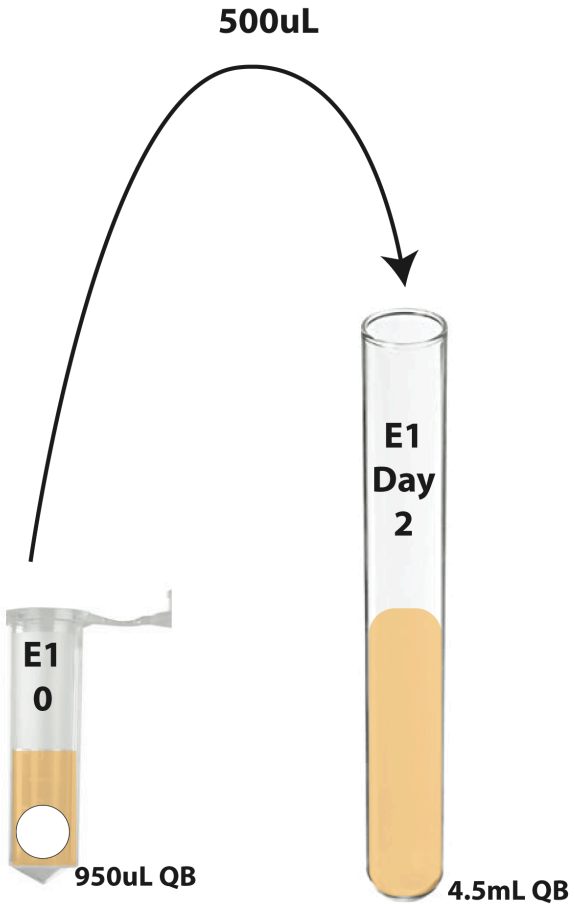
- ☐ For the experimental cultures, use sterile forceps to transfer the DAY 1 bead to the corresponding 10^0 microcentrifuge tube filled with 950µL QB. Be sure to sterilize the forceps between bead transfers to prevent cross-contamination of your bacterial populations.

- ☐ 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 filled with 950µL QB and briefly vortex to mix.

- ☐ For both the experimental and control cultures, use a **p1000** micropipette to transfer 500µL from the 10^0 microcentrifuge tube to the corresponding DAY 2 culture tube filled with 4.5 mL QB.

- ☐ Incubate the culture tubes on an orbital shaker until your next class.



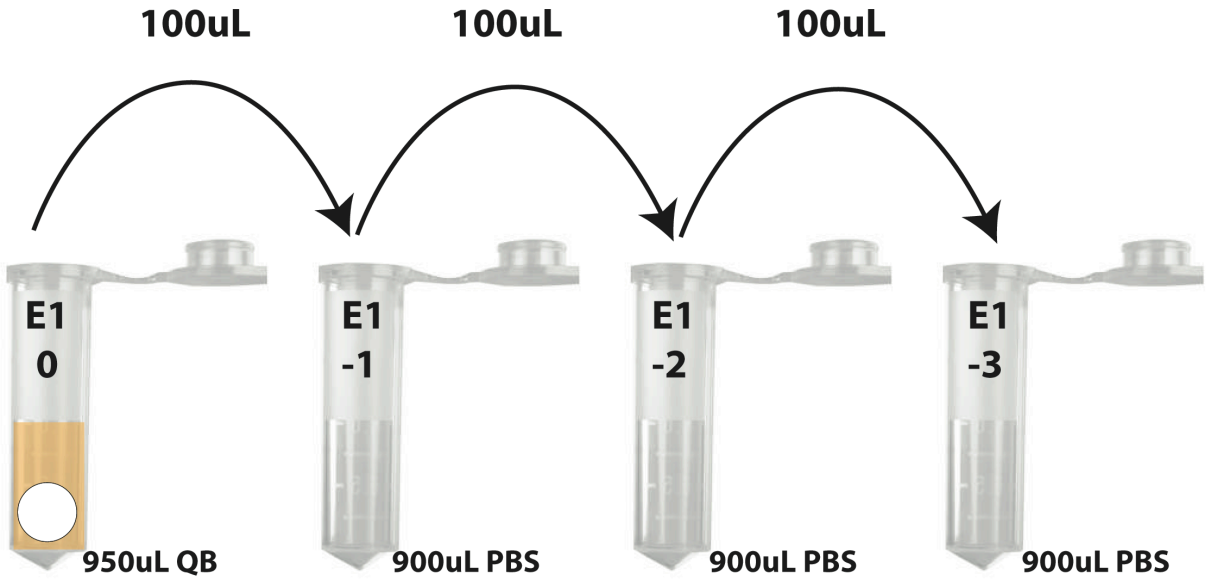
Prepare a dilution series and plate your bacteria:

- ☐ For the following steps, you will reuse the 10^0 microcentrifuge tubes that contain bacteria from your DAY 1 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. 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.

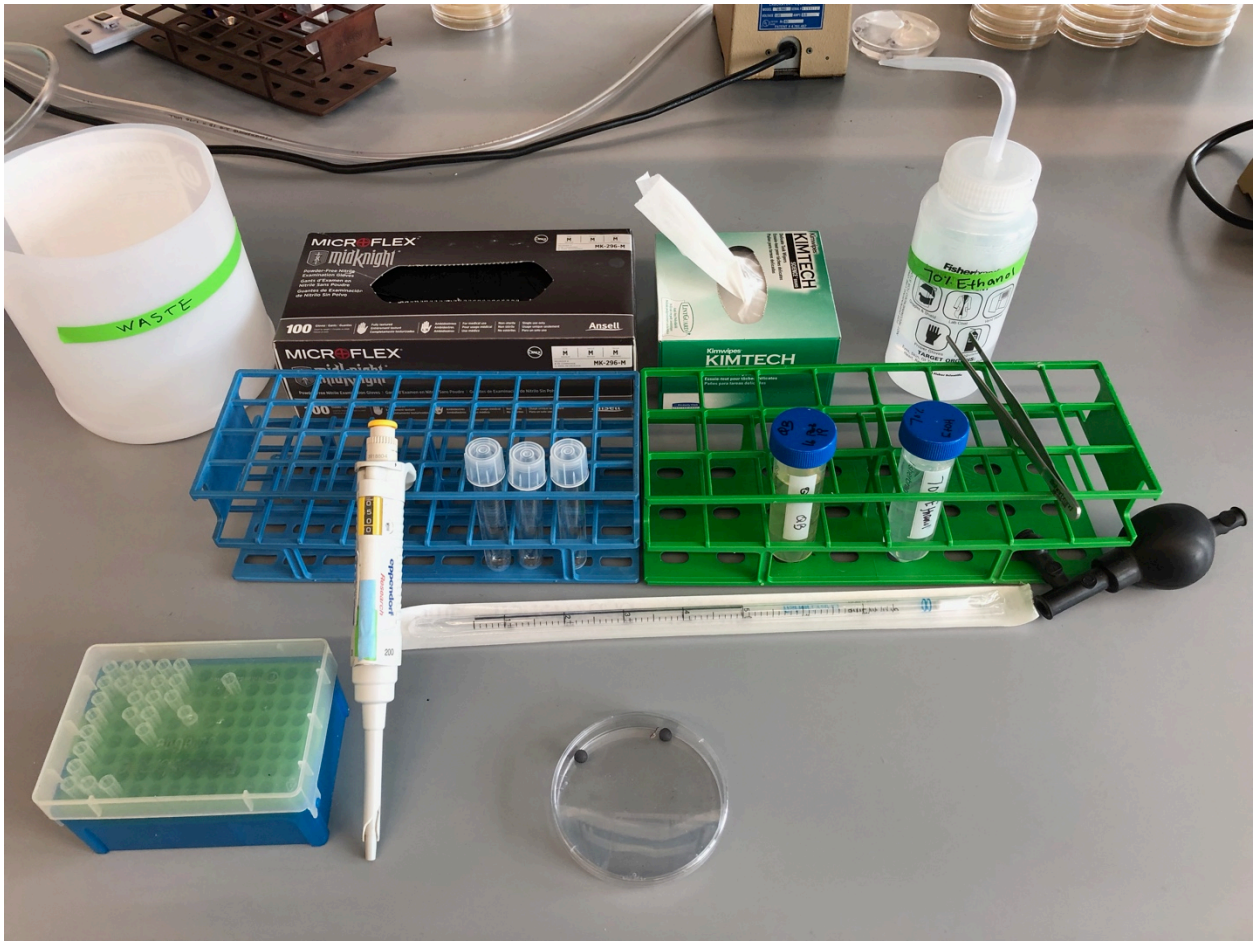
DAY 3: BEAD TRANSFER

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 (spray bottle and 50mL conical tube)
- Kimwipes
- Forceps
- Serological Pipette and Pipette Aid
- p200 Micropipette and Tips
- 3 Culture Tubes
- 15 and 50mL Tube Racks
- 2 **Black** Polystyrene Beads
- 15mL QB
- Orbital Shaker
- Waste Container with 10% Bleach

LAB BENCH SETUP



PROCEDURE:

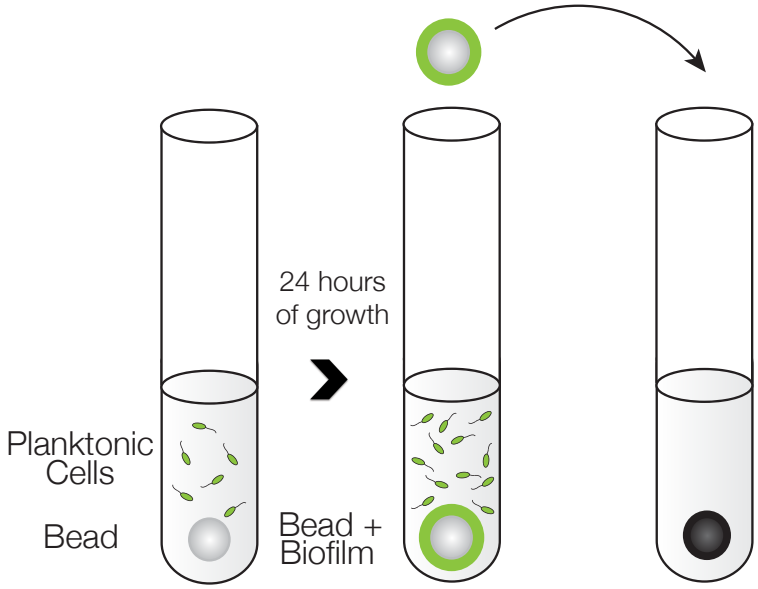
- ☐ Wipe down your lab space with 70% ethanol.

Prepare tubes to continue growing your bacterial cultures:

- ☐ Label your culture tubes: “E1”, “E2”, and “C”, include your group name and “day 3”.
- ☐ Add 5mL QB to each culture tube with a serological pipette.
- ☐ 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.

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 transfer the DAY 2 **white bead** to the corresponding DAY 3 experimental tube with fresh media and a **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.



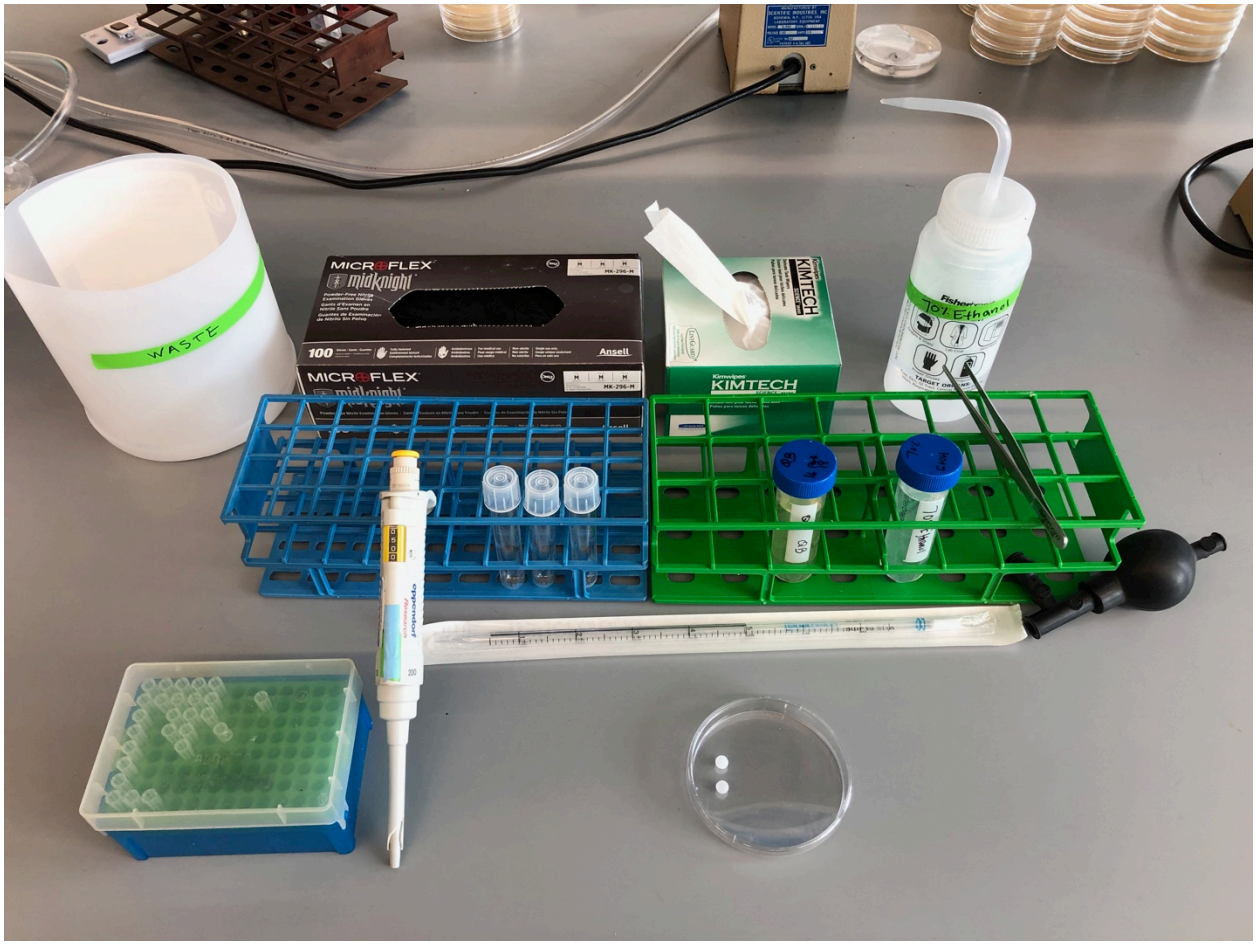
DAY 4: BEAD TRANSFER

Your culture tubes now contain both a white and a black bead. 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 (spray bottle and 50mL conical tube)
- Kimwipes
- Forceps
- Serological Pipette and Pipette Aid
- p200 Micropipette and Tips
- 3 Culture Tubes
- 15 and 50mL Tube Racks
- 2 **White** Polystyrene Beads
- 15mL QB
- Orbital Shaker
- Waste Container with 10% Bleach

LAB BENCH SETUP



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”.
- ☐ Add 5mL QB to each culture tube with a serological pipette.
- ☐ 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.

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 **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.

DAY 5: FINAL PLATING

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 (spray bottle and 50mL conical tube)
- Forceps
- Vortex
- p200 and p1000 Micropipettes and Tips
- 6 L-shaped Plate Spreaders
- 12 Microcentrifuge Tubes
- 2, 15, and 50mL Tube Racks
- 15mL PBS
- 6 Tsoy-Agar Plates
- Waste Container with 10% Bleach

LAB BENCH SETUP

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", "E2", "C" AND 10^0. Add 950µL PBS to each tube with a p1000 micropipette.
- ☐ Label 3 microcentrifuge tubes "E1", "E2", "C" AND 10^{-1}. Add 900µL PBS to each tube with a p1000 micropipette.
- ☐ Label 3 microcentrifuge tubes "E1", "E2", "C" AND 10^{-2}. Add 900µL PBS to each tube with a p1000 micropipette.
- ☐ Label 3 microcentrifuge tubes "E1", "E2", "C" AND 10^{-3}. Add 900µL PBS to each tube with a p1000 micropipette.
- ☐ Label 3 Tsoy-Agar plates "E1", "E2", "C" AND 10^{-2}, include your group name and "day 5".
- ☐ Label 3 Tsoy-Agar plates "E1", "E2", "C" AND 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.

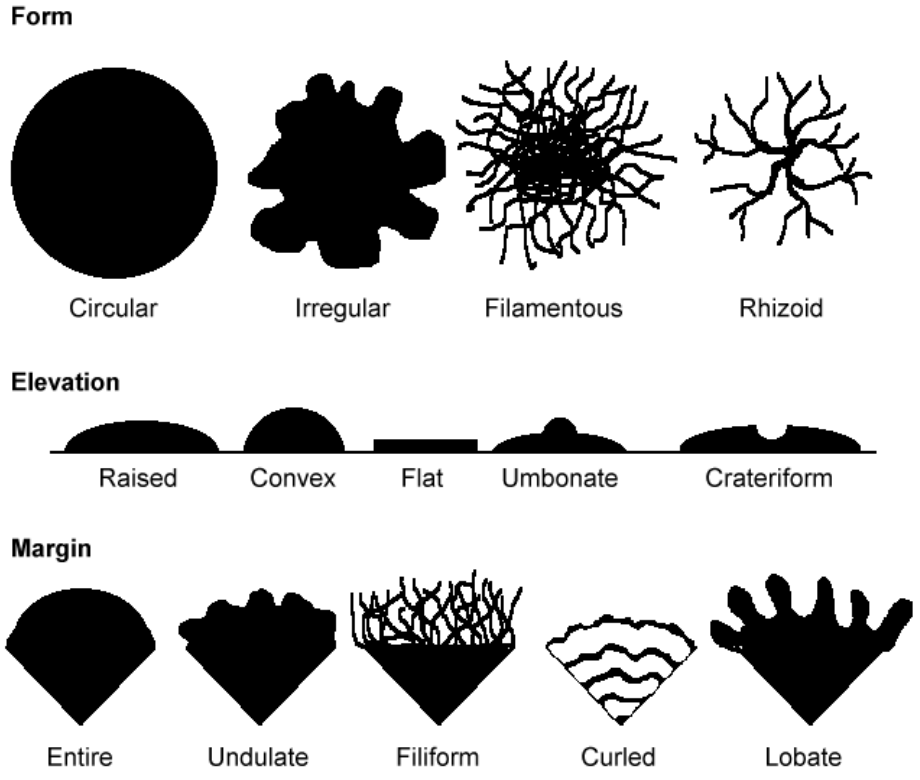
DAY 6: COLONY EXAMINATION

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 about 30-300 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.