Aseptic Technique

Thursday, August 25, 2016

11:30 AM

**Objective**

* + Minimize contamination

**Supplies/Equipment**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + Bunsen burner components
  + Aluminum foil square
  + White tube rack (labeled)
  + Electric pipettor with tips
  + Bottle of chemicals used in process
  + Conical tubes
  + Micropipettors with tips
  + Microcentrifuge tubes
  + Tape
  + Permanent Marker
  + Trash can
  + Biohazard bag

**Procedure**

* + Set Up Work Space
    1. Prepare Work Area
       1. Wash hands in sink
       2. Tidy up area
       3. Use brown detergent bottle to kill contaminants
          1. Wash hands if detergent touches skin! Can be irritant
       4. Wipe down area with a paper towel
          1. Throw away in regular trash
       5. Spray work space with ethanol
       6. Use ethanol to disinfect hands and arms
       7. Wipe down area and hands with paper towel
          1. Throw away in regular trash
       8. Let the ethanol air dry
    2. Ignite Bunsen burner
       1. Feel that the burner still has gas inside
          1. If not, cover the top with blue tape to mark as empty for disposal
       2. Screw top into the gas container
       3. Place aluminum foil square on top
       4. Arrange work space so that nothing is behind the burner, no paper or ethanol is above the flame
       5. Open gas (listen for sound)
       6. Click lighter
          1. If the flame does not light right away, close the gas and ask for help
       7. Once lit, flick the aluminum foil off the flame with the marker, as it is very hot
       8. Adjust the flame so it cannot be heard
       9. Turn off once it is unneeded
  + Transferring Sterile Solutions
    1. Transfer chemicals from large bottle to conical test tube
       1. Move the bottles and test tubes near the flame
       2. Label tube with contents, date, initials
       3. Label bottle (if not already labeled) with contents, date, initials
       4. Loosen (but do not remove) tube and bottle caps
       5. Open electric pipettor tip (when ready to use)
          1. Blue = 5ml
          2. Orange = 10ml
          3. Red = 25ml
          4. Peel down the wrapper from the top and hold the flaps against the outside wrapper at the bottom to prevent contamination
          5. Place tip inside electric pipettor
          6. Be sure not to touch tip to any surface!

Better to be safe than sorry! If unsure, throw away in biohazard and use a new one

* + - 1. Open top of bottle and keep close to bottle and flame during transfer
      2. Place pipette into bottle and draw up desired volume
      3. Close bottle immediately
      4. Open conical test tube and release liquid
      5. Close test tube immediately
      6. Throw the tip into the biohazard bag, using thumb to prevent spillage of extra liquid, the wrapper from the tip into the regular trash can
    1. Transfer chemicals from conical test tube to microcentrifuge tube
       1. Find a container of microcentrifuge tubes with striped tape across (have been sterilized)
       2. Pour out the number of tubes needed
          1. If excess fall out, close immediately and save
       3. Close the tubes from behind
       4. Label tubes with contents, date, initials
       5. Find container of micropipettor tips (of correct size for desired volume to be drawn) with striped tape across top (have been sterilized)
          1. Large = 100-1000 microL
          2. Medium = up to 200 microL
          3. Small = 10-20 microL
       6. Wipe down area on micropipettor
       7. Turn the top of the micropipettor to adjust volume to what is desired
       8. Open box of tips and place one on micropipettor
       9. Before tip is placed in liquid, push top down to first stop
       10. Once inside liquid, release and take out
           1. Make sure there are no air bubbles
       11. In the microcentrifuge tube, push all the way down to the second stop to release all the liquid
           1. If there are any spills, clean with ethanol
       12. Throw tip away into biohazard
  + Clean Up Work Space
    1. Put away/throw away all materials to appropriate locations
    2. Use brown detergent bottle to kill contaminants
    3. Wipe down with paper towel
       1. Throw into regular trash
    4. Spray area with ethanol
    5. Wipe down with paper towel
       1. Throw into regular trash
    6. Let air dry
    7. Wash hands

**Remarks**

* + Use a new pipettor for each sample
  + When transferring a small amount from a big bottle, transfer a small amount (i.e. 1ml) from the big bottle to a smaller intermediate container, then pipette small desired volume into desired container
  + When in doubt, get a new tip!
  + Use all tips ONCE then throw away into biohazard
  + Be aware of surroundings and where everything is in relation to flame!

Sample Collection

Monday, August 29, 2016

8:00 PM

**Objective**

* + Obtain an environmental sample containing bacteriophage

**Supplies**

* + 2 collection tubes
  + Marker
  + Camera
  + Sample collection data sheet
  + Optional: SEA-PHAGE app

**Procedure**

* + Collect soil sample
    1. Label tube with location, date, time, initials
    2. Place soil in tube
    3. Mark collection site and characteristics in lab notebook

Sample Preparation

Tuesday, August 30, 2016

11:30 AM

**Objective**

* + Extract phage from an environmental sample

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + Bunsen burner components
  + Aluminum foil square
  + White tube rack (labeled)
  + Electric pipettor with tips
  + 15 ml Conical tubes
  + Micropipettors with tips
  + Microcentrifuge tubes
  + Tape
  + Permanent Marker
  + Trash can
  + Biohazard bag
  + Collected samples
  + Enrichment broth
  + Shaking incubator
  + Syringe
  + Syringe filter
  + Culture tubes
  + Host bacterial culture
  + Negative control phage buffer
  + Top Agar
  + Agar plates
  + Incubator

**Procedure**

* + Clean work area (see Aseptic Technique, # 1)
  + Choose a sample collected using the protocol (See Sample Collection)
  + Extract phage from solid environmental samples, such as soil or compost. If liquid sample collected, proceed to step 4.
    1. Using a 15 ml conical tube, fill 1/3-1/2 full with soil.
    2. Add enrichment broth until the sample is submerged beneath 2-3 ml of liquid.
    3. Cap the tube and invert several times to mix thoroughly
    4. Incubate the tube while shaking vigorously in a shaking incubator at 250 rpm for 1-2 hours
    5. Allow the sample to sit until particulate matter has mostly settled. This may take 2-20 minutes. Record the time taken to settle.
  + Prepare a phage filtrate using aseptic technique (see Aseptic Technique, #2)
    1. Open sample
    2. Label microcentrifuge tubes with sample, date, and initials
    3. Open the package of a syringe filter, leaving the filter in the packaging
    4. Using a syringe, remove approximately 2ml of liquid from the top of the flooded sample
       1. Avoid withdrawing any solid material from the bottom of the tube
    5. Attach the syringe to the top of the filter and then remove the filter from the package
       1. Do not touch the filter
       2. Make sure the filter is screwed firmly into place
    6. Depressing the syringe plunger, dispense at least 0.5 ml of filtrate into a labeled microcentrifuge tube
       1. Be careful depressing the plunger, as the filter is very fine and may break.
       2. Cap the tube immediately
    7. Discard the syringe and filter into biohazard
  + Clean work area (See Aseptic Technique #1)
  + Assemble desired samples
  + Inoculate the host bacteria with phage sample
    1. Obtain 250 microL host bacterial cultures for each sample, plus one for negative control
    2. Label tubes accordingly with tape and markers
    3. Use micropipettor and aseptic technique (see Aseptic Technique #2) to dispense 500 microL of direct isolation sample and 10 microL negative control phage buffer into the sample tube, and 10 microL negative control phage buffer into the control tube
    4. Gently mix each tube
    5. Let the sample sit for 5-10 minutes to allow for attachment
  + Plate samples with top agar.
    1. Obtain an agar plate for each sample, plus one for the negative control. Label accordingly on the bottom of the plate around the edge
       1. LB (media)
       2. CB CHX
       3. Date
       4. Sample
       5. (D) (direct plating)
       6. initials
    2. Obtain a bottle of top agar from the bath IMMEDIATELY before plating and mark with date, initials, section time
    3. Transfer 3ml of top agar to each sample/control tube using the electric pipettor
       1. Return the top agar to the appropriate location right away
    4. Transfer the mixture to the appropriate agar plate
       1. Avoid making air bubbles
    5. Swirl the plate gently to coat the agar plate evenly
    6. Repeat for each sample
    7. Incubate plates to allow bacterial growth and phage infection.
       1. Let plates sit undisturbed for ~20 minutes until the top agar solidifies
       2. Gently invert the plates and place in the proper incubator
       3. Incubate the plates at the specified temperature for 24-48 hours
       4. Record incubation time and temperature in notebook.
  + Clean work area (See Aseptic Technique #3)

Sample Plaque Assay Continuation

Thursday, September 1, 2016

11:30 AM

**Objective**

* + Determine if plaques have grown from collected phage.

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + Trash can
  + Biohazard bag
  + Sample agar plate

**Procedure**

* + Check the plates for plaques
    1. Remove plates from incubator and hold up to light source to look for plaques
       1. If none seen, return plates to incubator for further incubation and check again later
    2. Record results in lab notebook
       1. Be thorough. What do you see on your plates? Count the number of plaques and take note of the size, shape, turbidity, margin type, and other distinctive features of the plaques. Negative results are important, too.

Picking a Plaque

Thursday, September 8, 2016

11:30 AM

**Objective**

* + Isolate and test phage to determine if identified plaques contained phage

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Electric pipettor with tips
  + Micropipettors with tips
  + Microcentrifuge tubes
  + Tape
  + Permanent Marker
  + Trash can
  + Biohazard bag
  + Agar plate with plaque of interest
  + Phage buffer
  + Agar plate
  + Host bacteria
  + Top agar

**Procedure**

* + Prepare work area (See Aseptic Technique #1)
  + Label the plaques
    1. Mark the plaques intended to pick by drawing a small circle around the plaque on the bottom of the plate. If multiple, label each plaque with a unique letter or number
    2. Label plaques from more than one agar plate, label in a way to keep track and record in lab notebook
  + Record the detailed morphology of each plaque (e.g., size, cloudy/clear, margin type) circled.
  + Label and prepare microcentrifuge tubes
    1. Obtain one tube for each picked plaque
    2. Close tube
    3. Label each tube according to identifier used for each plaque
    4. Using aseptic technique (see Aseptic Technique #2), transfer 100 microL of phage buffer into each microcentrifuge tube
  + Pick a plaque
    1. Place a sterile tip onto a p200 micropipettor (yellow)
    2. Open plate lid slightly near the flame area
    3. Holding the pipettor perpendicular to the agar surface, stab the top agar in the center of the plaque
       1. Avoid touching the bacteria surrounding the plaque
    4. Place the end of the tip into the phage buffer in the corresponding microcentrifuge tube. Tap the tip on the wall of the tube, swirl the tip, and pipett up and down to dislodge phage barticles. Discard the tip.
    5. Mix well by vortexing
    6. Repeat steps a - d for each plaque picking.
  + Clean work bench (see Aseptic Technique #1).

Serial Dilution

Thursday, September 8, 2016

11:30 AM

**Objective**

* + To prepare liquid phage samples of decreasing concentrations

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + Micropipettors with tips
  + Microcentrifuge tubes
  + Trash can
  + Biohazard bag
  + Phage buffer

**Procedure**

* + Prepare work area (See Aseptic Technique #1)
  + Obtain 8 new microcentrifuge tubes and label as 10^-1, 10^-2,…, 10^-8 for 10, 100,…, 10000000 times dilution
  + Add 90 microL of phage buffer to each tube
    1. Do not have to change tip between tubes
  + Transfer 10 microL of picked plaque to each serial dilution tube
    1. Swirl and pipette up and down the liquid to mix well
    2. Transfer 10 microL of the 10^-1 to the 10^-2 sample and vortex.
    3. Continue each successive dilution until last tube
  + Clean work area (See Aseptic Technique #3)

Plating a Sample

Thursday, September 8, 2016

11:30 AM

**Objective**

* + Prepare sample for plating and incubation

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + Bunsen burner components
  + Aluminum foil square
  + White tube rack (labeled)
  + Electric pipettor with tips
  + 15 ml Conical tubes
  + Micropipettors with tips
  + Microcentrifuge tubes
  + Tape
  + Permanent Marker
  + Trash can
  + Biohazard bag
  + Host bacterial culture
  + Negative control phage buffer
  + Top Agar
  + Agar plates
  + Incubator

**Procedure**

* + Clean work area (see Aseptic Technique, # 1)
  + Assemble desired samples
  + Inoculate the host bacteria with phage sample
    1. Obtain 250 microL host bacterial cultures for each sample, plus one for negative control
    2. Label tubes accordingly with tape and markers
    3. Use micropipettor and aseptic technique (see Aseptic Technique #2) to dispense 500 microL of direct isolation sample and 10 microL negative control phage buffer into the sample tube, and 10 microL negative control phage buffer into the control tube
    4. Gently mix each tube
    5. Let the sample sit for 5-10 minutes to allow for attachment
  + Plate samples with top agar.
    1. Obtain an agar plate for each sample, plus one for the negative control. Label accordingly on the bottom of the plate around the edge
       1. LB (media)
       2. CB CHX
       3. Date
       4. Sample
       5. (D) (direct plating)
       6. initials
    2. Obtain a bottle of top agar from the bath IMMEDIATELY before plating and mark with date, initials, section time
    3. Transfer 3ml of top agar to each sample/control tube using the electric pipettor
       1. Return the top agar to the appropriate location right away
    4. Transfer the mixture to the appropriate agar plate
       1. Avoid making air bubbles
    5. Swirl the plate gently to coat the agar plate evenly
    6. Repeat for each sample
    7. Incubate plates to allow bacterial growth and phage infection.
       1. Let plates sit undisturbed for ~20 minutes until the top agar solidifies
       2. Gently invert the plates and place in the proper incubator
       3. Incubate the plates at the specified temperature for 24-48 hours
       4. Record incubation time and temperature in notebook.
  + Clean work area (See Aseptic Technique #3)

Flooding a Plate

Thursday, September 15, 2016

11:30 AM

**Objective**

* + Generate a highly concentrated liquid phage sample

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + Webbed plate with clonal phage population
  + Phage buffer

**Procedure**

* + Prepare work bench for aseptic work (See Aseptic technique, #1)
  + Identify 1 or more plates for lysate collection
    1. Webbed plate may be one prepared from last round of plaque purification.
  + Flood Webbed Plates
    1. Apply 8ml of sterile phage buffer to webbed plate
    2. Store plate overnight (12-14 hours) at 4 degrees C.
    3. Swirl phage buffer gently, taking care not to splash
  + Clear work area (See Aseptic Technique, #3)

Collecting Lysates

Friday, September 16, 2016

3:30 PM

**Objective**

* + Collect the highly concentrated liquid phage sample

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + 0.22 microL filter
  + 5 ml syringe
  + 15 ml sterile conical tube for lysate storage

**Procedure**

* + Prepare work bench for aseptic work (See Aseptic technique, #1)
  + Harvest a plate lysate
    1. When the incubation time is complete, flip the plate over, allowing all liquid to collect in the lid.
    2. Open the plate.
    3. Prepare a 0.22 microL filter by opening the packaging, but not removing the filter. Set aside.
    4. Using a 5 ml syringe aspirate the lysate from the plate.
    5. Attach the syringe to the filter. Depress the syringe plunger and collect the filtrate in a 15ml sterile conical tube
    6. Label tubes appropriately.
  + Store lysates at 4 degrees C.
  + Clean work bench (see Aseptic Technique, #3)

Spot Titer and Full Plate Titer

Tuesday, September 20, 2016

11:30 AM

**Objective**

* + Determine the concentration of phage particles in a lysate using a spot test and plaque assay

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + Lysate for titering
  + Agar plates
  + Host bacterial culture
  + Top agar
  + Phage buffer
  + Microcentrifuge tubes
  + 5ml serological pipettes

**Procedure**

* + Prepare work bench (See Aseptic technique #1)
  + Label agar plates, one for spot titer, 9 for full plate titers
    1. Label the bottom with name, date, spot or full plate titer, and concentration for full plate, as well as the medium information
    2. Divide the spot titer plate into 9 sections, one for each dilution and one for negative control, labeling each section appropriately
  + Prepare a bacterial lawn using aseptic technique
    1. Using a sterile 5ml pipette, transfer 3 ml of molten top agar into a 5ml sterile pipette
    2. Transfer the top agar to a culture tube containing 250 microL of host bacteria
    3. Pour the mixture onto the spot titer plate
    4. Gently swirl the plate until the top agar mixture evenly coats the agar plate.
    5. Allow the plate to sit undisturbed until it solidifies.
  + Perform the serial dilutions on the lysate. (see Serial Dilution)
  + Perform plaque assays on the dilutions (see Plating a sample)
  + Spot the dilutions and controls on the bacterial lawn, as labeled
    1. Transfer 3 microL of all samples onto the proper location
    2. Use 3 microL of sterile phage buffer as negative control
    3. Allow liquid to absorb into the agar for 30 minutes or longer
    4. Incubate plates for 24-48 hours
  + Clean the work bench (see Aseptic Technique #3)
  + After incubating the plates, count the number of plaques
    1. Check each spot on the agar plate
    2. Do the numbers of plaques in each spot make sense? Is there a 10-fold decrease in number of plaques as you move through the spots? If so, choose dilutions that contain a countable number of plaques
  + Calculate the titer in pfu/ml using the formula
    1. Titer (pfu/ml) = (# pfu / volume used in microL) \* (10^3 microL/mL) \* dilution factor (reciprocal of dilution used)

Making Webbed Plates from a Lysate of Known Titer

Tuesday, September 27, 2016

11:30 AM

**Objective**

* + Create a plate with a very high density of plaques from a lysate of known titer

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + Lysate for titering
  + Agar plates
  + Host bacterial culture
  + Top agar
  + Phage buffer
  + Microcentrifuge tubes
  + 5ml serological pipettes

**Procedure**

* + Prepare work bench (See Aseptic technique #1)
  + Obtain the titered lysate prepared
  + Calculate the titer
  + Estimate the number of plaques and volume of lysate needed to generate a webbed plate
    1. Estimate how many plaques fill a plate
    2. Count how many plaques are in an area of a plate and multiply by the number of areas on the plate
    3. Estimate how many more plaques are needed to make a webbed plate
  + Calculate the volume of lysate needed to generate a webbed plate
  + Perform the dilutions (see Picking a Plaque #7-10)
    1. Make a dilution of 1/5, 1/2, 1, 2, and 5 times the Original Volume calculated
  + Plate dilutions to create webbed plates
    1. See Plating a Sample #7-9
  + Clean work space (See Aseptic technique #3)
  + Incubate without inverting for at least 24 hours
    1. Choose the best webbed plate and collect a lysate according to the flooding a plate and collecting plate lysates protocols

Plaque Streak Plates

Thursday, September 29, 2016

11:30 AM

**Objective**

* + Generate well-isolated plaques containing only one kind of phage

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + Plate withplaques to purify or liquid sample from picking a plaque
  + Agar plate
  + Top agar
  + Bacterial host culture
  + Sterile sticks or sterile pipette tips

**Procedure**

* + Prepare bench for aseptic work and assemble supplies (See Aseptic technique #1)
  + Prepare negative control plate
    1. Label bottom of plate with name, date, streak plate, and negative control
    2. Using aseptic technique, remove a sterile stick from its packaging
    3. Gently streak back and forth across the top 1/3 of the plate without lifting the stick from the agar
    4. With a new wooden stick, drag through the area streaked in the previous step, overlapping ONLY on the first two to three strokes. Continue to streak back and forth in an adjacent one-third of the agar, making sure not to overlap the first streak again
    5. Repeat to create a third streak on the remaining one third of the agar
  + Gather phage samples
    1. These may be from a plaque or a liquid sample prepared from picking a plaque protocol. (see Picking a Plaque)
    2. If picking directly from a plate, draw a circle around the plaque on the bottom of the plate and label it. Record details
  + Prepare agar plates
    1. Obtain as many agar plates as you have phage samples to purify
    2. Label each plate according to the phage sample
  + Make a streak plate
    1. See Prepare negative control plate
    2. Instead of using a new wooden stick, gently touch the center of a plaque used to purify once with the edge of the stick, or swirl the stick in the liquid phage sample
    3. Mark an X on the area where the third streak ends (this has the least number of phage
  + Add bacteria to streak plates
    1. In each smaple, use a sterile 5ml pipette to transfer 3ml of molten top agar to a culture tube containing 250 microL of host bacteria
    2. Pour the agar-culture mixture onto the streak plate slowly over the area marked X
    3. Do not swirl the plate
  + Repeat for each sample
  + Incubate the plates at the proper temperature
  + Clean work bench (see Aseptic Technique #3)

Extracting Phage DNA

Tuesday, November 1, 2016

11:30 AM

**Objective**

* + To isolate genomic DNA from phage

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + 1 ml phage lysate
  + Nuclease mix
  + EDTA (0.5 M) (optional)
  + 2 ml DNA clean-up resin (Promega Wizard DNA Clean-Up Kit)
  + 2 DNA clean-up columns (Promega Wizard DNA Clean-Up Kit)
  + 3 ml syringes
  + 6 ml 80% isopropanol, freshly prepared
  + ddH20 pre-warmed 95 degrees C

**Procedure**

* + Prepare bench and assemble supplies (See Aseptic Technique #1)
  + Degrade Bacterial DNA/RNA in high titer phage lysate.
    1. Aseptically transfer 1 ml of phage lysate into a microcentrifuge tube.
    2. Wearing gloves and working in the designated area, add 5 microL nuclease mix to the lysate.
    3. Mix gently but thoroughly by repeated inversions -- do not vortex!
    4. Incubate at 37 degrees C for 10 minutes or room temperature for 30 minutes.
    5. Optional: add 15 microL EDTA to the nuclease-treated lysate and mix gently.
       1. EDTA will inactivate the nucleases by chelating or binding divalent cations required by the nucleases for affinity.
    6. Remove and discard gloves before returning to bench.
  + Denature the protein capsid to release phage DNA.
    1. Put on a fresh pair of gloves.
    2. Add 2 ml of DNA clean-up resin to a 15 ml conical tube.
       1. The DNA resin is a slurry solution containing microscopic polymer beads. Make sure that the bottle of resin is well mixed, the precipitate dissolved by heating to 37 degrees C, and the beads suspended before aliquoting the 2 ml.
    3. Transfer your nuclease-treated phage lysate from the microcentrifuge tube to the 15 ml conical tube containing resin.
    4. Mix the solution by gently inverting the tube repeatedly for two minutes.
  + Isolate the phage genomic DNA
    1. Label two Wizard Kit columns with your initials
    2. Remove the plungers from two 3 ml syringes and attach a column to each syringe barrel.
    3. Follow the steps below for each column at the same time
       1. Set the column and syringe barrel on a new microcentrifuge tube.
       2. Transfer 1.5 ml of phage DNA/resin solution to the column using a pipette.
       3. Do not discard the empty 15 ml conical tube.
       4. Insert a plunger into the syringe and carefully push all the liquid through, collecting the flow-through in the used 15 ml conical tube from above
          1. The DNA is bound to the polymer beads that pack into the column as the liquid is pushed through. It is very important to maintain a firm, gentle, unrelenting, and even pressure on the syringe. Do not let the plunger pop out of the syringe barrel because releasing the vacuum will ruin the column.
       5. Once the liquid is expelled, maintain pressure on the plunger as you dry residual liquid by touching the tip of the column to a paper towel.
       6. Unscrew the column from the syringe barrel before releasing the plunger and set the column into a clean microcentrifuge tube.
       7. Remove the plunger from the syringe barrel, and then reattach the syringe barrel to the column.
    4. Wash the salts from the DNA (now in the column) with the following steps for each column:
       1. Add 2 ml 80% isopropanol to each syringe barrel/column and push the liquid through the column, repeating steps c(iv) - c(vii).
       2. Repeat twice for a total of three isopropanol washes.
    5. Remove residual isopropanol.
       1. With each column in a fresh 1.5 ml microcentrifuge tube, spin at 10,000 x g for 5 minutes.
          1. The column will prevent the microcentrifuge tube lids from closing. Arrange the open tubes in the centrifuge so that the lids point toward the center of the rotor.
       2. Transfer columns to new 1.5 ml microccentrifuge tubes. Spin at 10,000 x g for 1 additional minute to remove any residual isopropanol.
       3. Evaporate the last traces of isopropanol by removing your columns from the microcentrifuge tubes and placing them directly in a 90 degree C heating block for 60 seconds.
          1. Leaving the columns in the heating block for more than one minute can lead to DNA damage.
    6. Elute the phage DNA from the columns.
       1. Place each column in a clean microcentrifuge tube and apply 50 microL of 90 degree C sterile ddH2O directly into each column.
          1. Keep the ddH2O in the heating block so that it remains at 90 degrees C
       2. Incubate columns for 1 minute at room temperature
       3. Spin at 10,000 x g for 1 minute in a microcentrifuge
       4. Combine the products from both microcentrifuge tubes into one tube; this is your eluted phage DNA.
  + Determine the concentration of your phage DNA
    1. Using a spectrophotometer (fluorimeter, or Nanodrop) and a protocol from your instructor, quantify your phage DNA.
    2. Place at 4 degrees C for short-term storage (1-2 weeks) or at -20 degrees C for long-term storage

Entering a Phage into the Actinobacteriophage Database

Tuesday, November 8, 2016

11:30 AM

**Objective**

* + To include your phage in the Actinobacteriophage Database to make it available for other scientists

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag

**Procedure**

* + Prepare bench and assemble supplies (See Aseptic Technique #1)
  + Name your phage according to the rules found in the "Phages" dropdown menu and "Naming Rules" on phagesdb.org
  + Log in to phagesdb.org or create an active PhagesDB account by clicking on the "Sign in or Register" button at the bottom of the left column on the page.
  + Enter your phage on the PhagedDB.
    1. If you used the SEA-PHAGES App on your phone or a computer when you collected your soil sample, continue with these instructions. If you did not use the SEA-PHAGES App, continue to step 4b.
       1. Open the SEA-PHAGES App on your smart phone OR access the SEA-PHAGES App on PhagesDB, under the "Social" dropdown menu.
       2. Click or tap on the map icon at the bottom of the screen to view the pins for your samples.
       3. Use the icon with three horizontal lines to view your sample details and select the sample that yielded your phage.
       4. Check the "Phage Found in This Sample" button and "Add to PhagesDB."
          1. If you have not logged in to PhagesDB you will be directed back to the login page. You can log in if you already have an account, or you can create an account by using the green "Sign in or Register" button on the left, at the bottom of the page.
    2. If you did not use the SEA-PHAGES App when you collected your soil sample, you can add phages directly from PhagesDB by selecting the "Data" dropdown menu and clicking on "Add Phage."
       1. If you have not logged in to PhagesDB, you will be redirected back to the login page. You can log in if you already have an account, or you can create an account using the green "Sign in or Register" button on the left, at the bottom of the page.
    3. Fill in as many fields as possible, paying special attention to the following:
       1. Name
       2. Bacterial Host
       3. Institution
       4. Program
       5. GPS coordinates: If your GPS fields were not prepopulated by the SEA-PHAGES App, you can find the GPS coordinates by using a web mapping service application. One such service can be found via this link: <http://www.heywhatsthat.com/profiler.html> OR <http://bit.ly/YqKB>. Simply drag the map to the location where the environmental sample was found, zoom in as much as possible, and click on the map to obtain the GPS coordinates of that location. The output should be to the right of the map and in the N/W Decimal Degree format.
          1. Convert the GPS coordinates to the correct format if necessary. For example, the Coordinates 40 26' 46" N, 79 57' 11" W from the iPhone's "Compass" app will be converted into 40.446111 N, 79.953056 W.
          2. Phages must have GPS coordinates to be submitted to GenBank after sequencing and annotation.
       6. If you found your phage using an enriched culture, click "Yes" to "From Enriched Soil" or "No" if you used a direct isolation.
       7. Fill in the Discovery Notes to explain where the sample was collected and the soil conditions, etc.
       8. Complete the Naming Notes to explain where your phage name came from and why you chose the name.
       9. Enter the details about your phage's plaque morphology, including size, turbidity, margins, etc.
       10. The "Morphotype" of your phage is determined from the TEM data. Details about the different morphotypes can be found in Viewing Phage Particles by Transmission Electron Microscopy.
       11. The Cluster, Subcluster, Sequencing Facility and Method, and GenBank Accession Number can be filled in only after a phage's genome can be sequenced. Therefore, you should not fill in these fields.
       12. If you know the titer of the sample to be archived at the University of Pittsburgh, enter the titer here (or return later and enter the data).
       13. Upload an unaltered image of your phage where indicated. If you do not have a particular image at this time, you will need to return and add it at a later date.
    4. Click "Submit" to submit your request to PhagesDB. Your submission will be reviewed and approved by a scientist at the University of Pittsburgh; therefore, your submission will not be visible immediately. Please submit your phage only ONCE!
       1. Once approved, information is publically available for everyone to see and use.
    5. Once your submission is accepted, you need to create thumbnails of the images that will be displayed with your phage's profile.
       1. From the main PhageDB page select the "Data" dropdown menu and click on "Thumbnails."
       2. Type in your phage's name.
       3. Read the instructions for "Making Thumbnails for Plaque and EM Pictures for PhagesDB."
       4. Upload images that fulfill the requirements in the instructions.
    6. If you need to modify or add data to your phage's PhagesDB profile, you can do so at any time by clicking on the "Data" dropdown menu and selecting "Modify Phage."
  + Once your phage is sequenced, the SEA-PHAGES team will add data, such as the phage cluster, the genome sequence, and the type of ends. Finally, when your phage has been annotated, submitted to GenBank, and published, the SEA-PHAGES team will add the GenBank accession number and publication data. Phages MUST have GPS coordinates for GenBank submission. Only phages that have been, or will be, archived at the University of Pittsburgh should be entered into the database.

Archiving your Phage Sample

Tuesday, November 8, 2016

11:30 AM

**Objective**

* + To prepare a high-titer lysate for long-term storage

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + Barcoded tubes provided by the Hatfull lab
  + Sample tubes provided by your institution
  + DMSO mixture
  + Sterile beads
  + Lysate (at least 5 x 10 ^9 pfu/ml)

**Procedure**

* + Prepare bench and assemble supplies (See Aseptic Technique #1)
  + Certify phage samples on Phagesdb.org
    1. Make certain that the details of your phage samples have been recorded on the phages database at phagesdb.org according to the protocol Entering Phages into the Actinobacteriophage Database.
    2. Double-check the spelling of the phage name and the recorded titer of the lysate. Alert your instructor if you find any discrepancies.
  + Prepare archiving tubes.
    1. Retrieve two barcoded tubes for storage at the University of Pittsburgh and a third tube for storage at your institution. This third tube does not need to be barcoded.
    2. Properly label each tube with your phage's name, making sure it is legible.
    3. Using aseptic technique, carefully fill each tube with sterile beads to 1.5cm from the top of the tube.
  + Prepare DMSO/lysate mixture.
    1. Turn off your flame, as DMSO and its vapors are flammable!
    2. Dispense 2.8 ml of your high-titer lysate into a sterile 15 ml conical tube.
    3. Add 200 microL DMSO to the tube, cap the tube, and then vortex the mixture.
       1. Dimethyl sulfoxide (DMSO) is a cryoprotectant that prevents ice crystals from forming (Ice crystals can cause damage to phage particles during long-term storage at -80 degrees C).
  + Aliquot the mixture into the storage tubes.
    1. Pipette the lysate/DMSO mixture into each of the labeled tubes so that the beads are just covered.
    2. Avoid overfilling and underfilling the tubes.
    3. Take care to close the tubes properly.
  + Prepare the tubes for storage.
    1. Place the barcoded tubes in the storage box provided in the same order as shown on the archive list
    2. Store samples for shipment to University of Pittsburgh at 4 degrees C.
    3. Samples stored at your institution can be frozen immediately.

Setting Up Restriction Enzyme Digests

Tuesday, November 8, 2016

11:30 AM

**Objective**

* + To cut your phage genome into multiple fragments based on its DNA sequence

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + Phage DNA
  + Restriction enzymes with buffers
  + 37 degrees C water bath
  + 65 degrees C heat block
  + Microcentrifuge tubes

**Procedure**

* + Prepare bench and assemble supplies (See Aseptic Technique #1)
  + Prepare genomic DNA
    1. Gently mix your DNA sample by either flicking the closed tube with your finger or vortexing it on low
    2. Incubate the tube at 65 degrees C for 10 minutes, and then quickly place it on ice. Quick spin the tube in a microcentrifuge for less than one minute to move all of the liquid to the bottom of the tube.
    3. Using the concentration of your DNA sample, calculate how many microL of DNA are needed to obtain 0.5 microg of DNA.
  + Set up restriction enzyme digest reactions
    1. Set up a reaction in a microcentrifuge tube for each enzyme and a negative control
       1. Solution diH2O to final volume of 25 microL
       2. 10X reaction buffer = 2.5 microL
       3. Restriction Enzyme = 0.5 microL
       4. Phage genomic DNA = equivalent to 0.5 microg
       5. ADD THE PHAGE DNA LAST TO PREVENT CONTAMINATION OF THE ENZYME STOCKS
    2. Mix the contents of each tube gently and quick spin the tube in a microcentrifuge for less than 1 minute to move all the liquid to the bottom of the tube
    3. Incubate at 37 degrees C for up to 1 hour
  + Quick spin the tube in a microcentrifuge for less than 1 minute to move all of the liquid to the bottom of the tube. Store at -20 degrees C until ready to use.

Casting Agarose Gels

Thursday, November 10, 2016

11:30 AM

**Objective**

* + To prepare an agarose gel for electrophoresis

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + Agarose
  + 1X TBE running buffer
  + DNA dye
  + Erlenmeyer flask
  + Electrophoresis apparatus and power supply

**Procedure**

* + Prepare bench and assemble supplies (See Aseptic Technique #1)
  + Pour a 0.8% w/v agarose gel.
    1. Set up gel apparatus according to your instructor's directions.
    2. Prepare enough 0.8% agarose gel to cover the tips of the gel combs by ~ 2-3 mm.
       1. Weigh out the appropriate mass of agarose powder, then transfer the powder to an Erlenmeyer flask.
       2. Add the appropriate volume of 1X TBE buffer to the agarose powser. Swirl gently to mix. Take note of the volume of liquid in the flask.
       3. Heat the mixture in the microwave just until it boils (1-2 minutes). As soon as it boils, stop heating.
       4. Using a heat-resistant glove or mitt, carefully remove the flask from the microwave, remembering to face the open mouth of the flask away from yourself and others.
       5. Very gently, swirl to mix and then examine the solution for small transparent clumps. If clumps remain, return the flask to the microwave and continue heating until the mixture boils again. Repeat this process until no clumps are visible.
          1. Take care not to splash the hot liquid or touch the hot flask.
       6. Check the volume of the solution. If it has decreased, bring it back to the original volume suing ddH2O. Swirl to mix.
       7. Allow the solution to cool between 50 degrees C and 60 degrees C (very warm to the touch but not hot enough to burn) This typically takes 10-15 minutes).
    3. Once the solution is sufficiently cool, put on latex gloves and add the gel dye of your choice.
       1. If using ethidium bromide as a DNA dye, add enough to achieve a final concentration of 0.5 mg/mL in the agarose solution. Swirl to mix.
          1. Use extreme caution when handling EtBr and other DNA dyes. They are mutagens that intercalate between nucleotide bases.
    4. Pour the agarose/EtBr mixture into the prepared gel apparatus, being careful not to introduce any bubbles. Insert the comb to cast the wells.
    5. Allow the gel to cool for 20-30 minutes.
    6. Once the gel has solidified, very carefully remove the comb by pulling it slowly straight up. Once the comb is removed, gently lift the gel platform out of the casting tray.
    7. Place the platform with the solidified gel into the gel box. The wells should be at the (-) cathode end of the box, where the black lead is connected.
    8. Pour 1X TBE buffer into the gel apparatus until your gel is submerged by ~ 1/4 inch of buffer.
  + Load your samples and run the gel according to your instructor's directions and the Gel Electrophoresis protocol.

Gel Electrophoresis of Restriction Enzyme Digests

Thursday, November 10, 2016

11:30 AM

**Objective**

* + To separate DNA fragments via agarose gel electrophoresis

**Supplies**

* + Bottle of brown detergent
  + Paper towels
  + Ethanol bottle
  + White tube rack (labeled)
  + Trash can
  + Biohazard bag
  + Pre-poured agarose gel
  + DNA loading dye
  + Electrophoresis apparatus and power supply
  + DNA cut with restriction enzymes
  + DNA ladder

**Procedure**

* + Prepare bench and assemble supplies (See Aseptic Technique #1)
  + Set up the gel electrophoresis with a gel prepared according to the Casting Agarose Gels protocol
    1. Wearing gloves, orient the gel in such a way that the wells are closest to the cathode (black electrode)
  + Prepare your restriction enzyme digest samples for electrophoresis.
    1. Add 5 microL of concentrated 6x loading dye to each 25 microL restriction enzyme sample.
    2. Place the samples at 65 degrees C, either in a heat block or hot water bath, for 5 minutes. Immediately place the samples on ice to cool, and then spin them in a microcentrifuge for ~ 15 seconds at 10,000 rpm
       1. This step prevents annealing of the cohesive ends of phage DNA
  + Load the gel in the following order from left to right: Ladder, Uncut DNA, BamHI, ClaI, EcoRI, HaeIII, HindIII
    1. Carefully load the gel with the proper volume of DNA ladder. Each manufacturer is different, so follow the manufacturer's instructions.
    2. Using a fresh tip on your micropipettor for each sample, pipette 20 microL of each RE reaction into the wells in the order suggested above
       1. Holding the pipette in both hands, place your elbows on either side of the gel apparatus.
       2. Situate your eyes directly above the wells to make the wells easier to see.
       3. Place the pipette tip directly above the well, just below the surface of the buffer. Do not try to get the pipette tip into the well, or you might puncture the bottom of the gel.
       4. Slowly depress the pipette plunger, allowing the solution to sink into the well.
       5. Remove the pipette from the gel before releasing the plunger
    3. Draw a picture of the gel in your lab notebook, making note of where your samples are relative to your classmates' samples.
    4. Plug the electrodes into the appropriate locations on the power supply. Turn on the power supply and set the voltage to 100 V.
       1. Remember, DNA runs toward the RED electrode.
    5. Run the gel until the blue dye front has migrated at least 3.5 inches from the well. This will take approximately 1 hour.
    6. Turn off the power supply
  + Photograph the gel
    1. Using gloves, carefully remove the gel from the electrophoresis chamber.
    2. Photograph your gel, following your instructor's directions.
    3. Include a copy of the gel photograph in your lab notebook.
  + Clean up your work area.
    1. If another gel isn't going to be run, empty the buffer into the sink, rinse out the gel apparatus, and set it aside to dry. (The buffer can be reused, so check to see if anyone else will need to run a gel in the next few days.
    2. Dispose of your gel as directed by your instructor.