2021 Third Year Phage Hunters

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Chapter 1

Welcome Phage Hunters

Chapter 2

Introduction

Chapter 3

CPL Plan - Week 1

In total, we will have 12 students isolating phages for the CPL. Each student will be assigned a pathogen and will use that to isolate phages from samples crowdsourced by their peers. The first week is spent enriching for phages from their samples. In total there will be 47 samples (plus one negative ctrl).

3.1 Monday 10th May

- Kick off meeting + students given sample packs, with samples to be taken next morning.

BT will give a presentation on the work and the steps involved in phage isolation.

3.1.1 Estimated time: 1 hr

3.1.2 Materials required

- 36 sample jars
- 12 lab pens

3.2 Tuesday 11th May

- Students return to lab with samples
- Samples are transferred to 50 mL falcon tube + centrifuged for 30 mins @ 8000 x g

- Samples are filtered through 0.2 µm syringe filters into fresh 50 mL Falcon tube, then aliquoted into 12 1.5 mL lo-bind microcentrifuge tubes
- Samples are recorded on class shared spreadsheet
- Samples stored O/N at 4 °C.

3.2.1 Materials required

- 6 x 50 mL Falcon tube x 12 students (72 total)
- 3 x 25 mL syringe with luer lock (36 total)
- 3 x 0.2 µm syrige filter (36 total)
- 3 x 12 1.5 mL microcentrifuge tubes (432 total)
- microcentrifuge rack
- Centrifuge capable of 8,000 x g for 50 mL Falcon tubes

3.3 Wednedsay 12th May

- Students assigned a pathogen
- Students provided a set of samples
- Students aliquot 900 µL of each sample into each well of a 96-well deep well plate
- Students aliquot 500 µL of 3x LB + 30 mM $MgCl_2$ + 30 mM $CaCl_2$ into each well
- Students aliquot 100 µL of O/N pathogen culture into each well.
- Cover and incubate overnight at 30 °C on an orbital shaker.
- Students set up fresh O/N culture of pathogen

3.3.1 Materials required

- 10 mL of O/N pathogen culture (200 mL total)
- 50 mL of 3x LB + 30 mM $MgCl_2$ + 30 mM $CaCl_2$ (1L total)
- Deep well plate (12 total)
- PCR film (12 total)
- 2 sterilins with 10 mL LB + 10 mM $MgCl_2$ + 10 mM $CaCl_2$ (24 total)

3.4 Thursday 13th May

- Students transfer 200 µL from each well into a 0.2 µm filter plate atop a regular, sterile 96 well plate.
- Plate is spun at 900 x g for 4 mins to transfer filtrate to bottom plate
- Into a fresh 200 µL 96-well plate, students add 190 µL of LB + 10 mM $MgCl_2$ + 10 mM $CaCl_2$ per well

- 5 µL of phage lysate from bottom plate is added to each well
- 10 µL of O/N host culture added to each well and plate sealed
- Placed in an incubator O/N at 37 °C.
- Students set up fresh O/N culture of pathogen

3.4.1 Materials required

- 0.45 µm filter plate (Merck MSHAS4510) (12 total)
- 2 x sterile 200 µL 96-well plate (24 total)
- PCR film (12 total)
- 2 sterilins with 10 mL LB + 10 mM $MgCl_2$ + 10 mM $CaCl_2$ (24 total)

3.5 Friday 14th May

- Students transfer 200 µL from each well into a 0.2 µm filter plate atop a regular, sterile 96 well plate.
- Plate is spun at 900 x g for 4 mins to transfer filtrate to bottom plate
- Bottom plate is covered with PCR film and stored until Monday at 4 °C.
- Students streak pathogen onto agar plate for growth over weekend.

3.5.1 Materials required

- 0.45 µm filter plate (12 total)
- 1 x sterile 200 µL 96-well plate (12 total)
- PCR film (12 total)
- 2 x LB agar plates for bacterial growth
- Inoculating loops

Chapter 4

CPL Plan - Week 2

In the second week, the students will be performing spot assays and phage purification.

4.1 Monday 17th May

Students will set up four top agar spot plates, with 12 samples per plate (w. neg. ctrl).

- Students will mark plates into quadrants
- Students will add 1 mL of host culture at $OD_{600} of 0.6 to 3 mL of molten top agar (0.6% agar in LB + 10 mM $MgCl_2$ + 10 mM $CaCl_2$), vortex briefly and then pour onto bottom plate
- Once the plates are set, students will spot 5 µL of phage lysate from each sample at 3 per quadrant
- Plates will be incubated O/N at 37 °C for plaque formation

4.1.1 Materials required

- 10 mL pathogen culture
- 5 glass test tubes with lids containing 3 mL molten agar (60 total)
- 5 petri dishes with bottom agar (60 total)
- water bath (or heat blocks if the tubes will fit in).

4.2 Tuesday 18th May

Students will pick plaques and perform first round of O/N purification steps. We are going to assume a maximum of 3 phages per student.

- Students will take a photo of their plates to record plaque morphology
- For each plaque, students will core the plaque using a pipette tip and place it in 200 µL of SM buffer in a microcentrifuge tube, before vortexing briefly.
- Students will then set up a dilution series from 10^0 to 10^{-11} in SM Buffer in a 96 well plate (one row per phage)
- Using the same method as the spot assay, students will spot the 12 dilutions onto a single plate (so one plate per phage).
- Plates will be left O/N for plaques to develop

4.2.1 Materials required

- 10 mL pathogen culture
- 3 glass test tubes with lids containing 3 mL molten agar (36 total)
- 3 petri dishes with bottom agar (36 total)
- water bath (or heat blocks if the tubes will fit in).
- 1 sterile 96 well plate (12 total)
- 3 lo-bind microcentrifuge tubes (36 total)

4.3 Wednesday 19th May

From each plate, students will pick plaque from the biggest dilution (fewest phages) and perform second round of O/N purification. We are going to assume a maximum of 3 phages per student.

- Students will take a photo of their plates to record plaque morphology
- For each plaque, students will core the plaque using a pipette tip and place it in 200 µL of SM buffer in a microcentrifuge tube, before vortexing briefly.
- Students will then set up a dilution series from 10^0 to 10^{-11} in SM Buffer in a 96 well plate (one row per phage)
- Using the same method as the spot assay, students will spot the 12 dilutions onto a single plate (so one plate per phage).
- Plates will be left O/N for plaques to develop

4.3.1 Materials required

- 10 mL pathogen culture
- 3 glass test tubes with lids containing 3 mL molten agar (36 total)
- 3 petri dishes with bottom agar (36 total)
- water bath (or heat blocks if the tubes will fit in).
- 1 sterile 96 well plate (12 total)
- 3 lo-bind microcentrifuge tubes (36 total)

4.4 Thursday 20th May

From each plate, students will pick plaque from the biggest dilution (fewest phages) and perform third round of O/N purification. We are going to assume a maximum of 3 phages per student.

- Students will take a photo of their plates to record plaque morphology
- For each plaque, students will core the plaque using a pipette tip and place it in 200 µL of SM buffer in a microcentrifuge tube, before vortexing briefly.
- Students will then set up a dilution series from 10^0 to 10^{-11} in SM Buffer in a 96 well plate (one row per phage)
- Using the same method as the spot assay, students will spot the 12 dilutions onto a single plate (so one plate per phage).
- Plates will be left O/N for plaques to develop
- Students will set up O/N cultures of host

4.4.1 Materials required

- 10 mL pathogen culture
- 3 glass test tubes with lids containing 3 mL molten agar (36 total)
- 3 petri dishes with bottom agar (36 total)
- water bath (or heat blocks if the tubes will fit in).
- 1 sterile 96 well plate (12 total)
- 3 lo-bind microcentrifuge tubes (36 total)
- 2 sterilins containing 10 mL LB + 10 mM $MgCl_2$ + 10 mM $CaCl_2$ (24 total)

4.5 Friday 21st May

From each plate, students will pick plaque from the biggest dilution (fewest phages) and bulk it up on hosts overnight.

- Students will take a photo of their plates to record plaque morphology. These will also be used to determine approximate PFU counts.
- Students will pick plaque from the biggest dilution (fewest phages) and add it to 50 mL of LB + 10 mM $MgCl_2$ + 10 mM $CaCl_2$, amended with 1 mL of O/N host culture.
- Cultures will be grown O/N at 30 °C

The next day (Saturday), BT and team will go in and centrifuge the samples down and prepare for each phage 1 x 50 mL tubes of lysate, filtered through a 0.2 µm syringe filter. We will prepare 3 x 2 mL phage stocks in acid washed, autoclaved amber glass vials.

200 µL of each phage filtrate will be provided to the imaging centre.

4.5.1 Materials required

- 3 x 50 mL LB + 10 mM $MgCl_2$ + 10 mM $CaCl_2$ (2L total)
- 3 x 50 mL falcon tubes (36 total)
- 3 x 0.2 µm syringe filter (36 total)
- 3 x 25 mL syringe with luer lock (36 total).
- 3 x 2 mL amber glass vials

Chapter 5

CPL Plan - Week 3

In the third week, the students will be performing DNA extractions on phage DNA

5.1 Monday 24th May

Students will test their lysate for estimating PFU and begin phage DNA precipitation

- Students will prepare a dilution series of their phages as before and spot them in triplicate across 3 plates
- Students will transfer 30 mL of lysate to a fresh Falcon tube
- Students will add 15 µL of nuclease solution and incubate at 37 °C for 30 mins
- Students will then add 15 mL of precipitant solution to each tube and mix gently by inversion
- Samples will be incubated at 4 °C O/N

5.1.1 Materials required

- 10 mL pathogen culture
- 3 glass test tubes with lids containing 3 mL molten agar (36 total)
- 3 petri dishes with bottom agar (36 total)
- water bath (or heat blocks if the tubes will fit in).
- 1 sterile 96 well plate (12 total)
- 3 x Falcon tube (36 total)
- 50 µL of nuclease solution (600 µL total)
- 50 mL of precipitant solution (600 mL total)

5.2 Tuesday 25th May

Students will extract DNA using the Promega Wizard kit using this protocol

Depending on how many phages we isolate, we are anticipating sending two per student for sequencing, with the remaining extractions kept back for future analyses. ### Materials required * 3 x 500 µL resuspension buffer (5 mM $MgSO_4$) (18 mL total) * 2 x Promega Wizard Kits.

5.3 Precipitant solution

This is a ready-mixed 30% w/v PEG-8000, 3 M NaCl solution for adding to phage lysate in a 1:2 ratio precipitant:lysate (10%PEG-8000, 1 M NaCl final conc.)

1. In a sterilized 500 mL bottle add 330 mL of autoclaved MilliQ and 105 g of NaCl and dissolve.
2. Add 180g of PEG8000, cap bottle and shake.
3. Incubate bottle in a 60 °C waterbath for 3 hours, shaking occasionally
4. Remove and let cool to RT, shaking occasionally
5. Add autoclaved MilliQ to 600 mL and store at RT.