Bacteriophages Bringing Research into the Classroom – Brandl, et al.

Appendix E. Classroom Phage Discovery Protocols

(adapted from The Actinobacteriophage Database, phagesdb.org)

The first classroom outreach day is PI-led INTRODUCTION, personal background, discussion of human health advancements from science, including vaccines, phage and phage discovery project background, phage therapy history and recent successes. Sample collection instructions are provided along with sterile 15 mL conical tubes. This introduction is given to each class of the participating students, up to six iterations in one day.

PHAGEHUNTING Sample Collection Instructions

Collect samples from the environment

Label your tubes!

Take notes/photos/GPS on locations

One location per tube

- Soil (2-3 mL mark)
- Liquid (~10 mL mark)

No animal (including human) waste/fluids

Suggestions:

- Moist samples
- Rich soil (compost, potting soil, decaying mulch, etc.)

Day 2 and Day 3 Protocols for phage discovery and confirmation were modified from SEA-PHAGES (phagesdb,org)

to be completed in two 40-50 minute classroom periods. Exploration of phagesdb.org, and naming protocols.

Discussion of BRIC staff scientists' backgrounds, former research projects

A printed laminated copy of this protocol is placed on each student desk and BRIC staff demonstrated and talked the students through the steps. All students performed the protocol using the soil or water sample they had collected.

DAY 2 PHAGEHUNTING PROTOCOL

1. SOAK

Add Phage buffer to sample
Mix well and let settle

2. FILTER

Remove syringe cap
Suck up liquid in syringe
Open top of filter
Attach syringe to filter
Place over open microcentrifuge tube
Push plunger down

3. INFECT (Add 50 ul sample to bacteria)

Set micropipettor to 50 microliters
Put tip on micropipettor
Push to first stop
Put tip in liquid in microcentrifuge
Slowly let plunger up
Take cap off of tube with bacteria
Put micropipettor vertically over tube with bacteria
Push swiftly to second stop (expel liquid)
Mix phage and bacteria…

**********Wait about 10-15 minutes

4. PLATE

- Add ~5 mls of melted top agar to bacteria
- Pour onto plate
- Swirl plate to spread top agar
- Let plate “set” without bumping

5. INCUBATE

- About 48 hours at 37 degrees

Then test the putative plaques for their ability to kill a new plate of *M. smegmatis*

DAY 3 PHAGEHUNTING PROTOCOL

Students test their putative viral plaques. They pick them into a provided sterile 50 uL buffer solution. They put a 2-5 microliter spot of their solution on a numbered fresh pre-poured plate containing the bacterial host in a standard top agar. A positive control using a previously discovered phage is included.

1. PLAQUES

- Look for death zone in shape of a circle
- Circle plaque on underside of petri dish with marker and write microcentrifuge tube number next to circled plaque

2. EXTRACT

- Touch micropipetter tip to plaque
- Put tip into microcentrifuge tube and mix

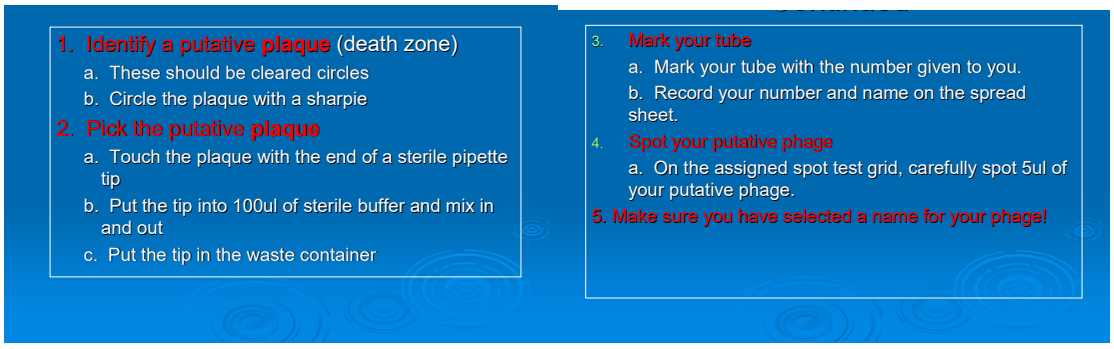
3. PLATE

- Bring sample (microcentrifuge tube) to instructor
- Plate 2 uL of sample onto test plates

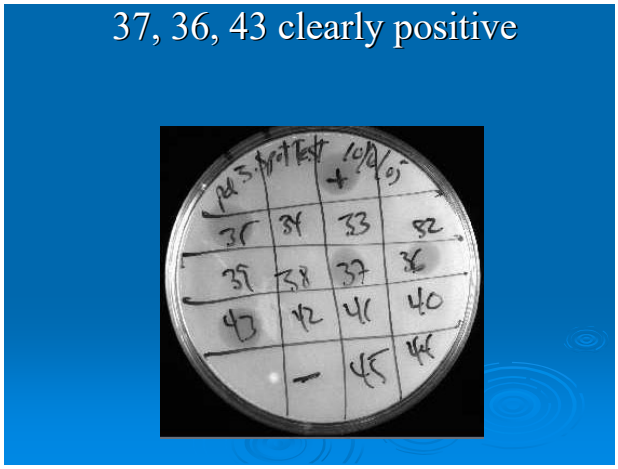
4. DATABASE

- Check database for name of phage
- Enter your name, sample location, and phage name on sheet

5. QUESTIONS??



Plates are incubated 24-48 hours at 37°C.



Positive samples are collected by BRIC scientists for further studies at Montana Tech.