

SEA-PHAGES Lysate Archiving Protocol

Objective

A comprehensive archive of lysates for phages found through the SEA-PHAGES program and entered into PhagesDB.org is a valuable asset to the SEA-PHAGES community as well as the larger mycobacteriophage research community. The University of Pittsburgh has agreed to house this archive and distribute samples when necessary. Set forth in these instructions are the procedures needed to ensure your institution's phages are a part of this important resource. Archiving consists of 1) sample preparation and 2) complete and accurate database information.

Major Consideration

1. The phage lysates that you send should match the complete and correct information in PhagesDB. Please be sure to enter all information at PhagesDB before requesting an Archive List of Phages. To obtain an archive list, go to your institution page. (Be sure to log on.) A .pdf is available to download in your archive box on your institution page.
2. As you archive, check your phage page entries for accuracy and completeness.
3. We request 2 samples for each phage lysate archived to be sent to Pitt. We also encourage you save an additional sample at your institution.
4. Bar-coded archive tubes will be sent to your institution (unless otherwise pre-arranged), supplying 2 tubes for each phage lysate (based on student enrollment numbers). These tubes will ship in October. Tube numbers are determined by your section data entered in September each year. Be sure to check that faculty names, shipping addresses, and student numbers are entered on seaphages.org no later than the "Section Data entry" deadline.

Overview

There are 3 sections of this protocol.

1. **Documentation**
 - Part 1: Material Transfer Agreement
 - Part 2: Archive List
2. **Sample Preparation**
3. **Shipping Instructions**

Materials

This year, only bar-coded tubes & a tube box(es) will be sent. No other shipping materials are provided. Exception: Schools in their first year of phage hunting will receive a complete Archive Box (includes tubes, beads, DMSO, Shipping box):

- **Bar-coded screw-capped Microcentrifuge tubes** (MP52345PK: 1.40ml 2D barcoded tubes pack of 960 List price: \$270.00 and MP53101PK: White screw-caps, pack of 960: List price: \$279.00 (www.novabiostorage.com) (2 tubes/lysate) These tubes have unique barcodes for tracking in our inventory. Left-over tubes can be used in subsequent years, so keep them safe and avoid contamination. If they get contaminated, they are autoclavable. **The tubes used for your school inventory are not provided.**
- **Beads** (Fisher Scientific 3mm glass beads solid Catalog # 11-312A List price: \$17.28). You will need to autoclave the beads to sterilize. You will want to autoclave the beads in a vessel that you can pour the beads from easily.
- **DMSO** Dymethyl Sulfoxide (Sigma Aldrich 25 ml bottle Catalog # D8418-50ml List price: \$35.90) Ready to use.

- **Shipping materials (shipping box).** This will only be provided with the initial Archive Kit. (Therefore only the newest cohort will receive shipping materials.) Use a sturdy insulated box and cold packs (not wet or dry ice). We ask that you ship materials overnight and notify us of tracking information. Return shipping cost is at your own expense.
- **Tube Box** – A cardboard box will be shipped with the bar-coded tubes to help ensure that tubes are sent back in the **order of the archive list**.
- **Archive List Print-out.** (See Documentation, part 2 below.)
- **Phage Lysate**, preferably with a titer of at least 10^9 (Provided by you!) We ask that you make 3 samples for archiving purposes: one to be kept at your home institution in your own tubes, and two to be sent to Dr. Graham F. Hatfull at the University of Pittsburgh (Pitt). **Use your own tubes for the sample that is to be kept at your institution.** The following protocol is to be used in preparing your phage samples that you will send to the University of Pittsburgh.

Procedure

Documentation, Part 1: Materials Transfer Agreement

All participating schools have current MTAs in place, no further paperwork is needed at this time. This does not have to be repeated each year.

Documentation, Part 2: “Archive List”

Once you have ALL phages entered at PhagesDB, you can generate an archive list at your [Institution Page at seaphages.org](http://seaphages.org).

The screenshot shows the SEA PHAGES website interface. At the top, there is a navigation bar with links for HOME, MEMBERS, NEWS/PUBS, EVENTS, RESOURCES, and CONTACT. Below the navigation bar, there is a section titled "Archiving Info" with a purple background. The text in this section reads: "Archive samples are due December 15 of each calendar year. The archiving protocol is posted on PhagesDB." followed by "As of now, you have no outstanding/overdue phages to archive. Great work!". Below this, there is a "Download Archiving Report" section with instructions: "Select a year using the form below, then click 'Download Report' to generate an archiving report. This report should serve as a check that your shipment matches your PhagesDB entries, and should be included as a packing slip." At the bottom of this section, there is a dropdown menu showing the year "2017" and a blue button labeled "Download Report" which is circled in red.

Review this sheet and make sure all information about each phage is accurate and complete. Revise all discrepancies at PhagesDB and make a new list. DO NOT just make changes by writing on the list, you must enter those changes in PhagesDB and request a new list. The exception is “Deleted Phages”. If you need to delete an entry, please email djs@pitt.edu to do so. Only University of Pittsburgh staff can delete a phage entry. Overall, this will ensure that PhagesDB contains accurate and updated information about each phage. Include this printout in the box that you send back to Pitt.

Note: If your Archive Info box at seaphages.org is red, that means you have entries at phagesDB that are not archived. Please investigate and then contact Debbie (djs@pitt.edu) to devise a plan to remedy the discrepancies.

Note: You can also use the data sheet available on PhagesDB to check for complete and accurate entry of data for each phage entered by your students. Go to

phagesDB.org/data. Download “Full Tab-delimited Text Files” and open in Excel. Sort by year and institution for the phages needed here.

Sample Prep (for each phage you are submitting)

We ask that you prepare 2 samples to send to us for each archive phage in the bar-coded tubes that were sent to you. Add at least one more tube (**not provided**) for your archive set. The following directions refer to the tubes shown in the photo below (A – K). A video for sample prep is available at <http://www.youtube.com/watch?v=5GjAjky81D4>.

1. Labeling **2 bar-coded** microcentrifuge tubes (**A**) with the **Phage Name**. (**C**) Ensure that the phage name is legible.
2. Fill each tube (using aseptic technique) with sterile beads to ~ 1.5 cm from the top of the tube. (**D**) This will keep the final mixture away from the tube threads (**B**).
3. Prepare the Lysate and DMSO Mixture for all of your tubes. The DMSO is provided ready-to-use. You want a final concentration of 6% DMSO.
Final volume of Mixture needed is at least 3 ml.
To prepare a 5ml final volume (because the pipetting is a bit easier), add 300 ul of DMSO to 4700 ul of Your Phage Lysate. Vortex. The mixture is now ready to dispense in the 3 tubes.
4. Pipette the mixture into each of the labeled tubes so that the beads are just covered. (**E&F**) Examples of over and under filling are included to help identify what NOT to do! (**G, H, I, J**)
5. Take care to close the tube properly.
6. Place the bar-coded tubes in the box provided in the same order as that designated on your packing list. That will facilitate the handling of these tubes upon their arrival at Pitt. Refrigerate until you are ready to ship.
7. Place your own archive tubes in an appropriate rack and freeze.
8. Any extra tubes can be saved for next year. There is no expiration date and they can be autoclaved.



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|---------------------------------------|-------------------|
| A: Bar Code | G: Too few beads |
| B: Internal threads | H: Too many beads |
| C: Label | I: Underfilled |
| D: space between top of beads and cap | J: Overfilled |
| E/F: space between top of Mix and cap | K: Not provided |

Note: Samples are “pink” to visualize the liquid in the photo. No samples are actually pink.

Shipping Instructions

Once you have the samples prepared and necessary paperwork completed, you are ready to ship. Place the pair of lysates in the rack box provided in the same order as the Archive List, then placed the box in an insulated box with cold packs (do not use ice). Please ship overnight. Please send tracking information to djs@pitt.edu . Thanks!

Current deadline is December 15. If you miss this deadline, be mindful of holiday closures. If sent when the University of Pittsburgh is recessed, your package will be returned to you.

You may also send your archive lysates along with your DNA samples. Just be sure to notify Becky Garlena (cag4@pitt.edu) that they are being shipped together.

Please send this package overnight to:

Becky Garlena
Hatfull Lab
Mycobacteriophage Archive Samples
University of Pittsburgh
303 Clapp Hall
4249 Fifth Avenue & Ruskin
Pittsburgh, PA 15260-3929
(412) 624-4646

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