

# 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 request an Archive List, please email [djs@pitt.edu](mailto:djs@pitt.edu).
2. 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.
3. 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 early October. Be sure to check that faculty names, shipping addresses, and student numbers are entered on seaphages.org no later than September 25, 2015.

## 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: Cohort 7 will receive a complete Archive Box (includes tubes, beads, DMSO, Shipping materials):

- **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](http://www.novabiostorage.com)) (2 tubes/lysate) These tubes have unique barcodes for tracking in our inventory. [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, shipping label, and cold packs).** This will only be provided with the initial Archive Kit. (Therefore only Cohort 7 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
- **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.** This list will need to be generated up request.
- **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, 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 Pitt.

## Procedure

### Documentation, Part 1: Materials Transfer Agreement

All participating schools (Cohorts 1-8) have current MTAs in place, no further paperwork is needed at this time.

### Documentation, Part 2: “Archive List”

Once you have ALL phages entered at PhagesDB, including lysate titers, send an email to debbie ([djs@pitt.edu](mailto:djs@pitt.edu)) to request your archive list. She will email you an archive list. Review this sheet and make sure all information about each phage is accurate and complete. Revise all discrepancies at PhagesDB and request a new Archive 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](mailto: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: You can 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](http://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)

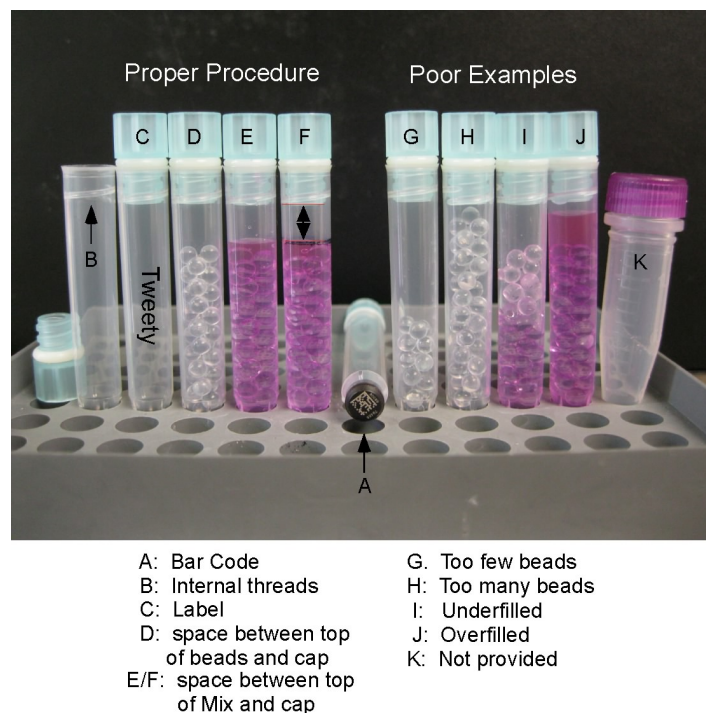
We ask that you prepare 2 samples for each archive phage in the bar-coded tubes that were sent to you. Add at least one more tube (**not provided**) for your archive set. The following directions refer to the tubes shown in the photo below (A – K). A video for sample prep is available at <http://www.youtube.com/watch?v=5GjAjky81D4>.

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.



## Shipping Instructions

Once you have the samples prepared and necessary paperwork completed, you are ready to ship. Place 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. Shipping materials and labels are no longer provided. Please send tracking information to [djs@pitt.edu](mailto:djs@pitt.edu) . Thanks!

Please send this package overnight to:

Hatfull Lab  
Mycobacteriophage Archive Samples  
376 Crawford Hall  
Pittsburgh, PA 15260  
(412) 624-6976

revised 8.21.15 djs