

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) complete and accurate database information and 2) sample preparation. Phages that do not have both – a complete and accurate database information and an archived sample at the University of Pittsburgh will be deleted. Please send the duplicate set of archive samples for each calendar year.

Major Consideration

1. The phage lysates that you send should match the complete and correct information in PhagesDB. Be sure to enter all information at PhagesDB before requesting an Archive List of Phages.
 - a. 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 for each year of lysates.
 - b. As you archive, check your phage page entries for accuracy and completeness.
2. Two samples tubes of lysate are needed for each phage lysate archived to be sent to Pitt. We also encourage you to save an additional sample at your institution.
3. Tube Request
 - a. A yearly tube request will be sent each October. Be sure to fill out the form ([SEA-PHAGES Archiving Materials Form 2023](#)) to request your archiving supplies. If you need tubes early, send an email to debbie (djs@pitt.edu).
 - b. 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.
 - c. Tubes will be sent before the end of October.

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

- **Bar-coded screw-capped Microcentrifuge tubes**
- **DMSO** Dymethyl Sulfoxide (Sigma Aldrich 25 ml bottle Catalog # D8418-50ml List price: \$35.90) Ready to use. *This will only be provided with the initial Archive Kit.*
- **Tube Box** – A cardboard box will be shipped with the duplicate 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**, (titer of at least 5×10^9 (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: “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).



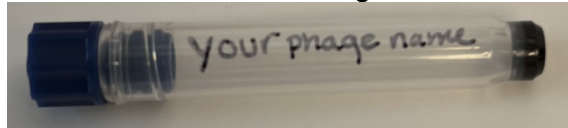
Review this sheet and **make sure all information about each phage is accurate and complete**. Revise all discrepancies at PhagesDB, wait for the changes to be updated, and make a new list. **DO NOT just make changes by writing on the list**, you must enter those changes in PhagesDB and request a new list. The exception is “Deleted Phages”. If you need to delete an entry, please email debbie (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 relay a plan to remedy the discrepancies.

Sample Prep (for each phage you are submitting)

We ask that you prepare 2 provided tubes of sample for each phage to be archived. Add at least one more tube (**not provided**) for your own archive set. A video for sample prep is available at [SEA-PHAGES ARCHIVING PROTOCOL](#).

1. Gather all needed supplies
 - a. Archiving tubes, 15 ml conical tube, DMSO, archiving box, Archiving List, Pipette, Pipette tips, a thin tip Sharpie, Tape to close the box.
2. Label your 2 bar-coded microcentrifuge tubes with the Phage Name exactly how it appears on PhagesDB.org
 - a. Example of how to label tubes for archiving.

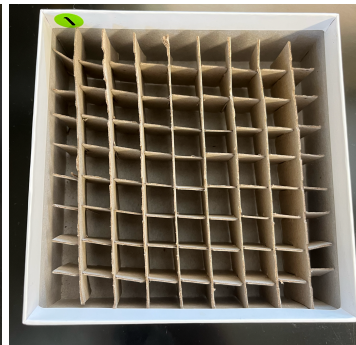
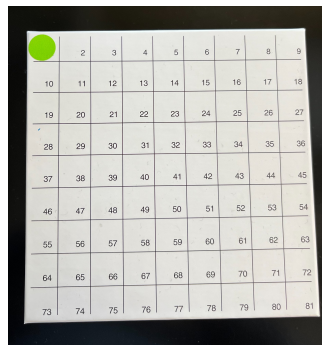


- b. NOTE: Do not:
 - i. Put tape on the bar-coded
 - ii. Use labels
 - iii. Write in all caps
 - iv. Write vertical down the tube
 - v. Write around the tube
 - vi. Parafilm/ Tape the cap of the tube



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 5 ml.
 - a. To prepare a 5ml final volume (because the pipetting is a bit easier), add 300 μ L of DMSO to 4700 μ L of Your Phage Lysate. Vortex. The mixture is now ready to dispense in 2 bar-coded tubes for Pitt and the remainder for your own archives.
4. Pipette 1000 μ L (1 mL) of the mixture into each of the labeled tubes to below the thread line.
 - a. Take care to close the tube properly.
5. 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.
 - a. Start at the green dot (slot 1) in the archiving box

Example of how to package tubes for archiving.



- b. Any phages on your list that you plan to send at a later time should be noted on the archive list with when we can expect to receive them.
 - c. Any phages that do not have a lysate to match, should be marked as “**Delete**” on the archive list. We will delete those phage pages when we receive the list.
 - d. Place your own archive tubes in an appropriate rack and freeze at your institution.
6. Any extra bar-coded tubes can be saved for next year. There is no expiration date so **please do not discard**.

Shipping Instructions

Once you have the samples prepared and necessary paperwork completed, you are ready to ship. Place the pair of lysates into the provided box in the same order as the Archive List, then place the box in a plastic bag and then into an insulated box with cold packs (do not use ice). Please ship overnight. Please send tracking information to djs@pitt.edu . 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. We will happily receive them after the first of the new year.

You may also send your archive lysates along with your DNA samples. Just be sure to notify Dan Russell (dar78@pitt.edu) that they are being shipped together.

Please send this package overnight to:

Attn: Colin Lewis
University of Pittsburgh
Department of Biological Sciences
303 Clapp Hall
4249 Fifth Ave
Pittsburgh, PA 15260
(412) 624-6976

Reconcile Your Institution’s Archive Status

Each year as you prepare to archive your current phage lysates, please go to your institution page at seaphages.org to see if any past samples have not been archived. Even if you were not the instructor of the course when these archived samples were collected, as the current course instructor we ask that you rectify all lysates from your institution. Please send a plan/timeline to send missing lysates to debbie (djs@pitt.edu). If no lysate can be provided, please request that those phage pages be deleted.

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