Welcome to the [Phage Comparative Genomics Lab Manual](https://docs.google.com/document/d/1wINS53ORokW-9gDTN9FLfc1GfHftchGN/edit?usp=sharing&ouid=112209090980686611264&rtpof=true&sd=true)!

First, you and your students can access the lab manual by following the above link to the live Google Doc version of the manual.

Second, please note that the lab manual is a live document and is regularly updated. The latest update will be added to the filename of the Google Doc, and changes will be noted at the very end of the manual under “Change Log.” I welcome any and all suggestions, corrections, and feedback to the manual. I would also be glad to collaborate and add additional tools and instructions to the manual. Please reach out to me at [afreise@ucla.edu](mailto:afreise@ucla.edu) for any of the above.

Third, there is a forum on the SEA-PHAGES website all about [bioinformatic analyses](https://seaphages.org/forums/forum/186/). It’s a great place to post questions and discuss pedagogy related to these tools.

Below, please find some teaching and implementations. Good luck and have fun with these tools!

Best,

Amanda Freise, PhD

Associate Director of Undergraduate Research

Microbiology, Immunology, and Molecular Genetics, UCLA

**Manual Organization**

The manual is organized into three sections:

**Section A** contains some basic data collection techniques that will enable students to pull the files they need from PhagesDB and GenBank. These techniques are important for dot plots and phylogenetic trees, but could be skipped for Pham maps and GCS. However, they do provide a useful exercise in searching for data.

**Section B** outlines the main tools described in the manual: dot plots, comparative Phamerator maps, gene content similarity comparisons, and phylogenetic trees.

**Section C** briefly reviews additional interesting and informative tools for students to explore. These tools generally require more instructor involvement (e.g. use of the Virtual Machine or Linux to pull data files for PhamNexus and OrthoANIu) and/or have limited instructions available. However, enterprising students might enjoy playing with these tools. In fact, most of the tools in this section were added to the manual because a student decided to try something new!

**Implementation Notes**

Most of the entries in the manual has a short description of the entry/tool’s purpose, followed by instructions for how to complete the tool/experiment. Some entries also provide additional background and/or interpretation notes. It is worth discussing these sections briefly so that students recognize the why the tools are being used rather than just carrying out the procedures.

Some sections have **presentations** associated with them which can be used to teach and for students to review. These presentations are linked in relevant sections, as well as in the “[Presentation Quicklinks](https://docs.google.com/document/d/1wINS53ORokW-9gDTN9FLfc1GfHftchGN/edit#bookmark=id.hz7pnyqq38b1)” section near the top of the manual for easy use. You are welcome to use these presentations and teaching materials. They may include UCLA-specific information. All are saved as Google Docs for easy sharing and/or copying and modifying. Please credit UCLA MIMG Research Immersion Labs in any modified versions.

There are also recorded versions of some of the tutorials, also linked in the manual. You are welcome to use these for your own purposes and/or to show them to students. They may also include UCLA-specific information.

Rather than going straight through the manual, you can begin by selecting the tools you feel most comfortable with or interested in, and teach those. Phamerator maps and GCS are the two easiest tools to use: they don’t require any external software, and it is straightforward to come up with research questions about similarities and differences. Dot plots require slightly more work as you and students must download and learn to use the Gepard tool, but don’t be put off – it’s easy (although the tool is not very user-friendly when it comes to annotating and interpreting figures).

You may find it useful to introduce certain analysis tools in parallel with annotation. For example, if you are going over Phamerator/Pham Maps for annotation purposes, you could also discuss the use of comparative Phamerator maps for investigations into phage genomic similarity among clusters and subclusters.

Primary literature on phage comparative genomics is an excellent way to introduce these tools and show students their use in the field of phage diversity. Discussing questions that authors have asked, and analyzing figures that correspond with the tools in the manual, will help orient students as to how to ask questions and interpret data. Any phage paper that has one of the tools above will do. We also provide a few examples in the manual. Finally, we have published two papers on projects conceived of and conducted entirely by students, both of which contain examples of all the tools in the manual, and which can hopefully serve as inspiration for your students.

Demo S\*, Kapinos A\* et al. (2021) [BlueFeather, the singleton that wasn’t: Shared gene content analysis supports expansion of *Arthrobacter* phage Cluster FE](https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0248418). *PLoS ONE* 16(3): e0248418. <https://doi.org/10.1371/journal.pone.0248418> \*equal contributions

Kapinos A et al. (2020) [Novel Cluster AZ *Arthrobacter* phages Powerpuff, Lego, and YesChef exhibit close functional relationships with *Microbacterium* phages](https://www.biorxiv.org/content/10.1101/2020.11.21.392811v1.full.pdf+html). *bioRxiv* 2020.11.21.392811; doi: https://doi.org/10.1101/2020.11.21.392811

Finally, SEA-PHAGES faculty collaborators have assembled formal modules, including learning activities, for the dot plot tool and for creating phylogenetic trees:

Kapinos, A et al (2020). [**Using Dotplots for Comparative Genomic Analysis**](http://dx.doi.org/10.25334/RR9F-HD75). [HHMI Science Education Alliance (SEA) Faculty Group](https://qubeshub.org/groups/hhmi_sea), QUBES Educational Resources. [doi:10.25334/RR9F-HD75](http://dx.doi.org/10.25334/RR9F-HD75)

Tobiason D, Davis T. (2020). [**Introduction to Phylogenetic Trees for Comparative Genomic Analysis**](https://qubeshub.org/publications/2071/1). [HHMI Science Education Alliance (SEA) Faculty Group](https://qubeshub.org/groups/hhmi_sea), QUBES Educational Resources. [10.25334/HWSZ-Q412](http://dx.doi.org/10.25334/HWSZ-Q412)