



Graham F. Hatfull
Lead Scientist

A warm welcome to the 2015 Fall term of bacteriophage discovery and genomics, both to those SEA-PHAGES schools continuing in the program and the new Cohort 8 faculty and students. This past year witnessed a number of exciting developments in the SEA-PHAGES program and we are looking forward to another year full of interesting and exciting insights into phage biology. As we actively explore the phages of hosts related to *M. smegmatis* the prospects for genomic novelty are enticing, and identifying genetic linkages between phages of different hosts will test the models for the evolution of viral diversity. We are still excited about mycobacteriophages of course, and the recent findings of

a new singleton, a rare Cluster W phage, and a Gaia relative forming the new cluster X illustrate that we still have a lot to learn about the diversity of these particular phages. We have made good progress with various tools that we hope will facilitate course and program implementation including the SEA-PHAGES smartphone app, the seaphages.org database, and the PITS assessment tool. So good luck phage hunting and we look forward to hearing about your successes.

NEW PHAGE DISCOVERY GUIDE FALL 2016



We are delighted to announce that the SEA-PHAGES phage discovery resource guide and lab manual are in the process of a major review and update to facilitate the expansion of our program into new actinobacterial hosts. While the overall workflow remains the

same for the process of phage isolation and amplification, each new host may have its own set of optimal parameters. The new guide will be modular and more flexible, including tips for speeding or slowing incubation times to best fit each instructor's needs. As a special preview, we have revised the DNA extraction protocol and eliminate the requirements for PEG precipitation and a large floor centrifuge. You can access the new protocol [here](http://seaphages.org/media/docs/NewDNAProtocol.pdf): (<http://seaphages.org/media/docs/NewDNAProtocol.pdf>)

SUMMER SUCCESSES WITH NEW ACTINOBACTERIA HOSTS



This summer, a number of new Actinobacteria were tested for growth conditions and phage yield at the University of Pittsburgh; including new species and strains of *Rhodococcus*, *Gordonia*, *Mycobacterium*, and *Dietzia*. The big winner? *Gordonia terrae*, which grows on PYCa medium, and yielded a whopping 20 new isolates through both direct plating and enrichment cultures. In addition, Carlos Guerrero in the Hatfull lab has preliminary data showing successful recombineering in this host. As an added bonus, *G. terrae* is a lovely red-orange color, sure to perk up those

early morning class meetings. Several SEA-PHAGES schools have already signed up to work on their own new Actinobacteria, and preliminary data gathered by SEA-PHAGES faculty, staff, and students, will become available at seaphages.org and PhagesDB.org soon. Watch this space!

IMPORTANT DATES

- **October 30, 2015**
Cohort 9 applications due
- **November 20, 2015**
Phage DNA due at Pitt.
- **December 7-11, 2015**
Bioinformatics workshop at HHMI HQ
- **June 16-20, 2016**
ASM Microbe; General Meeting, Boston, MA

Did you know?

How many phage particles are there in a plaque?

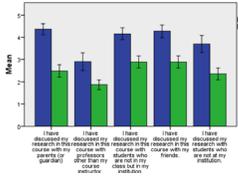
In general, we assert that there are millions of individual phages within a single plaque; however, it turns out that not all plaques are created equal. Different phages have different "burst sizes"; that is, the number of progeny produced per lytic cycle; different "latent periods" or time between absorption and burst; and different phages can diffuse through top agar at different rates due to different physical characteristics. All of these can contribute to the size and particle content of an individual plaque. In *Dedrick et al* (2013), we explored the numbers of phages in individual plaques of phage Giles knockout mutants, noting significant differences in fecundity. Wild-type Giles plaques contain $\sim 8 \times 10^8$ pfu, while some mutant plaques are as low as $\sim 5 \times 10^5$ pfu. So how many phages in a plaque? Millions. But really, it depends.

PHAGE DISCOVERY WORKSHOPS



Two SEA-PHAGES Phage Discovery workshops were held at the University of Maryland Baltimore County (UMBC) beautiful new HHMI SEA labs this summer. Each workshop was led by 3 HHMI Staff; 2 Faculty Course Directors, Sharon Isern and Scott Michael; 4 Faculty Facilitators, and a Teaching Assistant-necessary to accommodate the 50 cohort 8 participants from 17 institutions, an all time record number in a single cohort! As usual, everyone worked hard, and played hard (and shared through social media: #seaphages). Cohort 8 faculty spent some “downtime” testing a new PEG-free DNA extraction protocol, which will be available as part of the new SEA-PHAGES Phage Discovery Guide.

PITS ASSESSMENT



We are delighted with our new assessment initiative, designed to measure student Persistence in the Sciences (PITS). This new tool measures various psychosocial components of the student experience in science courses. The statistical validation of the survey looks very promising, suggesting that this will be quite a valuable tool for both for individual schools and for the SEA-PHAGES program as a whole. Please work with your institution’s administration for survey approval, and contact us with any questions.

TALES FROM THE OCEAN BLUE



News from around the SEA:

From Lehigh University, in Bethlehem, PA, Vassie Ware writes:

Lehigh’s SEA-PHAGES program has expanded into new courses and summer internship opportunities offering phage discovery and advanced research in phage biology for undergraduates working with faculty in molecular biology, biochemistry, structural biology, bioengineering, and physics. Based on hypotheses generated from bioinformatics analyses of several phage genomes (e.g., N cluster phage Butters, G cluster phage Sneez), we established long term interdisciplinary research collaborations addressing issues relevant to microbial pathogenesis. Our students are using a variety of strategies to uncover the roles of novel phage proteins in the mycobacteriophage infection cycle and to understand the potential of phage proteins as novel biocontrol agents, including generation of phage mutations by BRED, biochemical, biophysical, and structural analyses of recombinant phage proteins, and advanced imaging technologies.

From Del Mar College, Corpus Christie, TX, Rob Hatherill says:

At Del Mar College we are working on detecting riboswitches (in phage), an ancient and efficient regulatory mechanism for control of gene transcription and translation. We have also investigated the expression of RecA, a DNA repair protein, in the novel bacteriophage ‘Pier’.

And from Western Kentucky University, Bowling Green, KY, Rodney King writes:



SEA Faculty Drs. Rodney King and Claire Rinehart at Western Kentucky University received a grant from the Kentucky Biomedical Research Infrastructure Network (KBRIN) to establish the KBRIN Small Genomes Discovery Program. The goal of the grant is to use the SEA PHAGES model to expand genomics and bioinformatics training at universities and community colleges across the Commonwealth of Kentucky. Ten

schools are now participating in the program and five additional schools will join next fall. The program will support a regional symposium in January. Have news to share? Send it to info@seaphages.org

PUBLICATIONS OF INTEREST

- **Petrova et al (2015)**
Mycobacteriophage-repressor mediated immunity as selectable genetic markers: AdepHagia and BPs repressor-selection. *Microbiology, Aug;161(8):1539-5*
- **Villeneuve et al (2015)**
An unusual repressor encoded by Mycobacteriophage BPs. *PLoS One. 2015 Sep 2;10(9):e0137187*
- **Sun et al (2015)**
CryoEM structure of the bacteriophage T4 portal protein assembly at near atomic resolution. *Nat. Comm. Jul 6 6:7548*
- **Pope and Hatfull (2015)**
Adding pieces to the puzzle: New insights into bacteriophage diversity from integrated research-education programs *Bacteriophage* in press.

THE SEA-PHAGES TEAM:

- Graham Hatfull (Pitt)
- David Asai (HHMI)
- Billy Biederman (HHMI)
- Kevin Bradley (HHMI)
- Steve Cresawn (JMU)
- Debbie Jacobs-Sera (Pitt)
- Crystal Petrone (Pitt)
- Welkin Pope (Pitt)
- Dan Russell (Pitt)
- Vic Sivanathan (HHMI)



SEA-PHAGES: A community of researchers exploring phage diversity

Message in a Bottle
For more information
contact: info@seaphages.org