The Xeno Project: SEA-PHAGES 2016-2017

The Project

Find a phage against which a Xeno lysogen [*Mycobacterium smegmatis* mc^{2}155 (Xeno)] puts up a defense to infection.

Background

Xeno is a Cluster N phage that encodes several proteins that are expressed from the prophage during lysogeny. Typically, a lysogen is immune to superinfection by similar phages via

1) *Repressor-mediated superinfection immunity*. The repressor prevents lytic gene transcription from the prophage and also blocks lytic expression from superinfecting Xeno particles. The Xeno repressor will confer immunity to any other phages that use a closely related immunity system, including all other Cluster N phages tested thus far.

Cluster N phages, however, are of particular interest because their lysogens have shown immunity (or resistance) to other phages that are NOT members of the same cluster. This resistance/immunity could be due to

2) *Superinfection exclusion*. Xeno, along with the Cluster N phages SkinnyPete and Charlie encode a putative membrane protein (i.e. Charlie gp32) that confers defense against Che9c infection. The phenomenon has only been observed for Che9c but may also affect other phages. It is likely that Charlie gp32 confers exclusion by preventing DNA injection.
3) *Abortive infection*. Xeno encodes a putative type II toxin-antitoxin (TA) system. It has been shown that some Type III and Type IV TA systems confer defense to phage infection through abortive infection, i.e. infection by certain phages reduced antitoxin activity and toxin-mediate death results, preventing phage growth from that cell. Xeno genes *26* and *27* encode a RelEB-like Type II TA system that is expressed during lysogeny. We propose that this also confers defense against phage infection, but we have to identify a phage that is fails to infect a Xeno lysogen because of this activity.

Goal

The goal is to find phages that fail to infect (or infect at a reduced efficiency of plating) a Xeno lysogen, and then to determine if this is repressor-mediated, exclusion, or TA-mediated.

How it works

We will send you a sample of the *M. smegmatis* mc^{2}155 (Xeno) lysogen. You should prepare liquid cultures of the Xeno lysogen and *M. smegmatis* mc^{2}155, prepare plates with top agar layers, and spot serial dilutions of each of your phages on the two plates. Most phages are expected to plate with an efficiency of one on the two strains. The challenge is to identify any phages that show a reduction in efficiency of plating on the Xeno lysogen of 10^{-4} or greater.

Instructions

To participate, you must sign up and complete an MTA to receive *M. smegmatis* mc^{2}155_Xeno by **July 25, 2016**. To sign up, send an email to djs@pitt.edu stating your intentions to participate AND update your seaphages.org contact information to include your institution's legal contact name and email AND make sure the shipping address is correct. We will then process the MTA. You and your

legal contact will need to sign it for its completion, so look for its return to you. We intend to ship the Xeno lysogen **October 1, 2016**.

When you receive the Xeno lysogen, it will be as a cell pellet. Immediately streak it out as you would for mc^2155 or any other strain, and inoculate a single colony into liquid culture. Instructions are provided in the Phage Discovery Guide. Remember to freeze aliquots (we suggest 10!) for long term storage and a seeding source for future cultures.

1. Using PDG protocol 11.4, prepare plates for the two strains (mc^2155 and the Xeno lysogen)
2. Prepare serial dilution of phages to be tested (sequenced or unsequenced), and spot them on both plates in rows as shown in PDG Figure 11.4.1, with the most concentrated spots on the left and most dilute on the right. You can spot six different phages on each set of plates.
3. Take pictures of your plates, and post them on the forum on the Xeno challenge at seaphages.org. Email welkin@pitt.edu about any candidate phages you find.

Findings

There are 3 predictable 'types' of results.

1. No infection is observed. Record and report findings.
2. Infection is observed with a plating efficiency (EOP) of 1. That means that the phage infects the Xeno lysogen at the same frequency as it infects M. smegmatis mc^2155.
3. Infection is observed with a plating efficiency of less than 1. In particular, one that plates at an EOP of $<10^{-3}$.

Interpretations and Further Investigations:

If the phages do not infect the Xeno lysogen, record and report your findings. No further investigation is needed at this time. Photographs are required.

If the phages infect the Xeno lysogen and M. smegmatis mc2155 with an EOP of 1, record and report your findings. Photographs are required. No further investigation is needed.

If the phages infect the Xeno lysogen with an EOP of less than 1 but $>10^{-3}$, you will want to investigate further. This can involve the following:

- A careful repeat of the initial screening
- Purification of the mutant that is infecting the Xeno lysogen
- Sequencing of the the mutant.

But before proceeding, please touch base with the Hatfull lab by emailing welkin@pitt.edu.