Restriction Enzyme Digests: Tips, Modifications, and Suggestions for SEA-PHAGES Classrooms

In the PDG and PDIG

Chapter 10 of the Phage Discovery Guide contains background information about restriction enzymes as well as protocols to perform and visualize restriction digests on an agarose gel. Chapter 10 of the Phage Discovery Instructor's Guide has some practical lab tips along with some pedagogical tips for teaching other concepts alongside restriction digests.

This Document

Has several suggestions from experienced SEA-PHAGES instructors who have tried different approaches to doing/teaching restriction digests in their classrooms.

General Tips

- Digests are a good way to prevent sending duplicate phages for sequencing. The more enzymes you use, the more likely you'll separate truly unique phages and confirm duplicate phages.
- Keep your gel images from previous years, so you can rule out the possibility of year-to-year phage contamination. (It happens!)
- You do not necessarily need a water bath. You can digest overnight at room temperature or in an incubator.
- Some have used the enzyme **Pst1** for estimating genome size, because it cuts most genomes enough times to provide bands within the size range of most ladders, but not so many times that they are too hard to resolve.
- There are less-toxic alternative dyes to Ethidium Bromide, such as GelRed and SybrSafe. If safety is a particular point of concern, you can use one of these.
- With experience, you may begin to learn which enzymes are "diagnostic" for certain clusters. (For example, Cluster CA is only cut by BamHI and HaeIII.)

HaeIII-only Modification

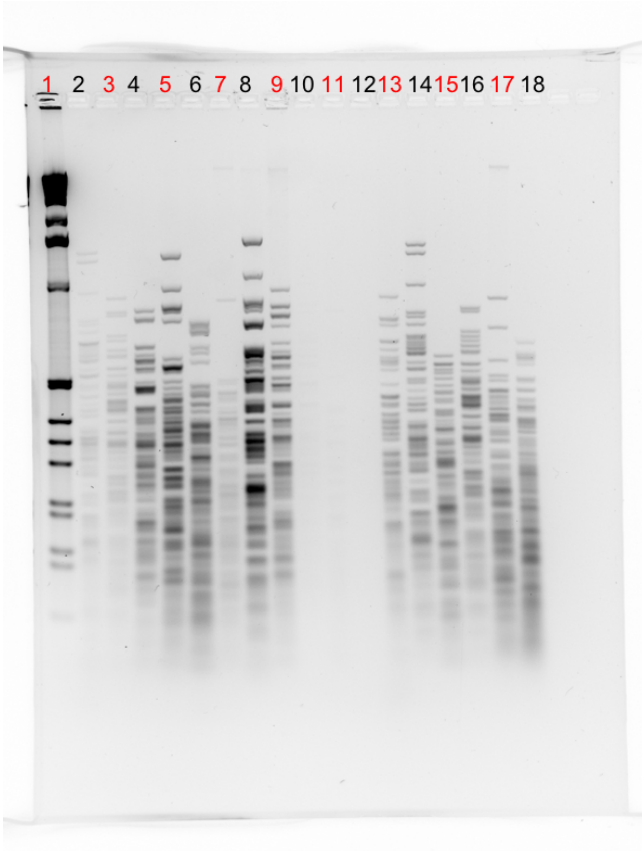
If your primary goal is to compare the phages in your classroom to one another, and/or you're looking for a way to still perform the digest portion of the course while reducing cost, you can use this HaeIII-only setup suggested by Eric Miller of NC State.

This approach is based on the fact that HaeIII's recognition site is GGCC and is thus very common in Actinobacteriophages. (There are dozens to hundreds of sites per phage.) Thus, HaeIII will cut every phage in your classroom multiple times, and the HaeIII pattern will likely differ for each unique phage.

To implement this, have each student digest his/her phage's DNA with HaeIII only. Then, pour a 2% agarose gel (instead of the standard 0.7-1% gel), which will better resolve the small HaeIII fragments. Run all of the students' digests on a single gel, then compare to see whether phages are unique.

An added benefit to this method is that since each student does only a single digest, they use far less of their DNA, which can be beneficial if it's in short supply.

An example of a HaeIII-only gel is shown to the right.



Virtual Gels

You may want to have your students create virtual digest gels of known phages, or use existing virtual digests to compare to their own digest gels.

On PhagesDB, some phages already have virtual gels associated with them. To see all virtual gels from a particular cluster or subcluster, go to http://phagesdb.org/compare/ and select "Virtual Digest" from the "Picture Type" dropdown menu.

Creating your own virtual digests can be done in several ways. DNA Master has a function that allows this, or you can use NEB Cutter (http://nc2.neb.com/NEBcutter2/) to do so. One good option is **Case It**. Case It was developed at the University of Wisconsin, River Falls (a SEA-PHAGES institution) and allows users to run gels for different times or with different agarose concentrations, to better match real student gels. There are enzymes built into the software, but you can also upload any enzyme site.

Case It can be found at http://www.caseitproject.org/

Phage Enzyme Tools

The Phage Enzyme Tools were developed at Louisiana, Monroe, and are a great way to do analysis of digest results. You can use them to quite easily predict your unknown phage's cluster from its digest pattern. Go to phageenzymetools.com to give them a try, and watch a video tutorial on seaphages.org to learn how they can be used.