

Project Title: Understanding Restriction Enzyme Digests

Authors and Affiliations:

Author	Affiliation
Swati Agrawal	University of Mary Washington
Matthew Mastropaolo	Neumann University
Sara Anderson	Minnesota State University Moorhead
Bryan Gibb	New York Institute of Technology
Dustin Edwards	Tarleton State University

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Description: Background, nomenclature, and activities demonstrating the function of restriction enzymes in DNA characterization.

Prior to submitting genomic DNA for sequencing, SEA students perform a restriction endonuclease digest with several enzymes, which provides a fast and cost-effective way of quickly screening isolated phages. The pattern of bands on the gel should be unique except for very closely related or identical phages, which is useful when trying to identify novel bacteriophages to send for sequencing and ultimately for annotation. Students often struggle with understanding what restriction enzymes are, how they work, and why this is an important step in phage characterization.

The 'Restriction Enzyme Digests' set of teaching resources provides 4 active learning exercises that introduce students to restriction enzymes, demonstrate how they work, and why they are a useful tool in the lab and for bacteriophage identification.

Activities:

Activity 1: Primary Literature reading and Naming of Restriction Enzymes (nomenclature)

Activity 2: Restriction Enzyme Naming (paper)

Activity 3: Virtual DNA cutter (e.g. NEB paper activity and Lambda digest (digital activity)

Activity 4: Virtual Digest of Phage Genomes

Learning objectives:

Objective	Corresponding Activities
Understand why bacteria have restriction enzymes	Activity 1
Examine the classification and uses of different DNA restriction enzymes.	Activity 1, 2 and 4
Understand how restriction enzymes recognize cut sites	Activity 2 and 3
Understanding why restriction enzymes are such useful tools in molecular Biology	Activity 2, 3, and 4

How is the module structured to promote student development as a scientist?

The module contains four activities to help students learn more about restriction enzymes, how they work, and why they are useful in characterizing bacteriophages. Together, this module supports the pedagogical goals of SEA-PHAGES by enhancing student development as a scientist, project ownership, and understanding procedural knowledge with activities that can be conducted before, during and after the restriction digest protocol. This will make the protocol more approachable for students, increase the likelihood of success, enable them to interpret their results, think critically and understand why doing this protocol is important in the context of their research project.

These activities support the first stage of student development as a scientist by aiding their understanding of the procedure, and the second stage with activities that demonstrate expected results so that students will know how to interpret their own data. This in turn supports the third stage of scientific development, as the data from these digests enable better students and their instructors to identify novel candidate phages to submit for genomic characterization.

Summary of activities and suggestions for instructor use

Overview - This resource includes four activities that focus on restriction endonuclease to accompany the protocol in the SEA-PHAGE manual. The activities cover different, but overlapping aspects of restriction endonuclease digestion, and each includes a different and unique activity. While the four activities work well as a unit, it is not necessary to complete all four. Instructors should select the activities they feel will work best for their class.

Activity 1 - Primary literature activity where students read, answer questions and complete an activity from two research articles that discuss the naming and classification of restriction enzymes. This serves as a good introduction to restriction enzymes, both from the natural functions as a bacterial defense system and toward their use in molecular biology. Instructors should consider doing this activity prior to students conducting restriction enzyme digestion on their isolated phage genomes.

Activity 2 - Introduction to RE digests and agarose gels using paper models; good for in-person activity in small groups or individually before doing RE in the lab; portions of this activity could be used during lab waiting times (e.g., DNA extraction or during RE digest incubations).

Activity 3 - Students will learn how to use free tools available at NEB to analyze fragments of DNA for restriction sites. The activity is performed using a computer, and follows Activity 2.

Activity 4 - Conduct a virtual digest of phage genomes downloaded from phages.db. Students will compare virtual digest of phages within the same cluster and across different clusters. This activity will help students understand how to interpret their own results and ultimately select what phage(s) should be submitted for genome sequencing. This activity will make more sense to students after they have completed a digest of their own phage.

Intended Teaching Setting

Course level: Undergraduate students, SEA-PHAGES students

Instructional Setting: In-person classroom, online; individual or groups

Implementation Time Frame: Each activity should be able to be completed in 30-60 minutes. The instructor can choose to use any or all of these activities in their class depending on their needs and time available. Activities 2-3 are meant to introduce students to restriction digestion prior to conducting the procedure in the lab. Activities 1 and 4 can be conducted before, during or after conducting the procedure.

Project Documents

Facilitator document(s):

0 - Facilitator Guide Restriction Enzyme Digest (this document)

Learning activity document(s):

1A - Primary Literature Review

1B - Naming of Restriction Enzymes

1KEY - Primary Literature Review

2 - Paper Restriction Enzyme Activity

2KEY - Paper Restriction Enzyme Activity

3 - Digital Lambda-HindIII Digest

3KEY - Digital Lambda-HindIII Digest

4 - Virtual Digest of Phage Genomes

4KEY - Virtual Digest of Phage Genomes