

DNA Electrophoresis

By Dale Hancock

Experiment Overview

DNA electrophoresis is a fundamental technique used in Molecular Biology to separate DNA fragments by size. In this experiment first year students digest lambda DNA with an unknown restriction enzyme (one of a possible 4) and separate the products by agarose gel electrophoresis. Based on the number and size of the fragments obtained they can identify their unknown enzyme. The sites on lambda for all 4 possible unknown restriction enzymes are provided so the expected fragment sizes for each possible digest can be predicted.

Learning Experience

ASELL surveys carried out in 2010 identified this lab as one with a "mixed" response (~50% favourite, ~50% disliked the most) from MBLG1001 (normal) students but a very popular lab with the advanced students. While the content is vital fundamental knowledge for molecular biology students, I feel it could be better presented with the normal student in mind.

Aims and Objectives

Experimental Aim: To identify an unknown restriction enzyme by estimating the number and size of the DNA fragments after lambda DNA digestion.

Objectives:

To give the student some experience in the techniques currently used in molecular biology; agarose gel electrophoresis, ethidium bromide staining, restriction enzyme digestion.

- To predict the migration of DNA restriction fragments on an agarose gel based on restriction site data.
- To further develop student data handling and Excel skills.

Level of Experiment

First year undergraduate

Keyword Descriptions of the Experiment

Domain

Molecular Biology

Specific Descriptors

Restriction digestion, DNA electrophoresis

Course Context

This lab is either the 4th or 5th out of 6 labs for the semester so towards the second half of the course.

Prerequisite Knowledge and Skills

A basic understanding of the structure of nucleic acids, the properties of restriction enzymes and bacteriophages such as lambda is required. This will be covered in lectures prior to students doing this lab. Students need to be proficient at using automatic pipettes and familiar with working with small volumes.

Time Required to Complete

Prior to Lab: none

In Laboratory: 4 hours

After Laboratory: 1 - 2 hours

Experiment History

This experiment has been running for a number of years either in first year (MBLG1) or in second year MBLG (prior to the introduction of the first year course). We have added the virtual digest over the last 2 years. Originally the virtual digest was to run with a commercial website but the website seemed to crash every time a large number of students accessed it to do the assignment so we have provided the students with the lambda restriction sites now.

Comments

Other plasmids and cloning vectors could be used, double digests or even restriction mapping.