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Restriction Enzyme Digestion Exercise – An In-class Activity

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INTRODUCTION

Understanding the concepts of molecular biology and then applying those concepts to laboratory experiments can be challenging to entry-level students. In order to facilitate the topics of restriction enzyme digestion and the generation of compatible ends in the process of gene cloning, an in-class activity was designed. This restriction enzyme digestion exercise, designed for an introductory undergraduate course in genetics, molecular biology and molecular diagnostics, can be utilized in either a lecture or laboratory setting. Students are provided with information on enzyme discovery and origin, sticky, blunt and compatible ends, base-cutters, isoschizomers and isocaudomers (1) . Students then review the components required for restriction enzyme digestion setup, such as DNA concentration, buffer volume and compatibility and multi-enzyme digestions (1) . Upon completion of the theory review, students participate in this classroom activity where scissors and paper replace restriction enzymes and DNA, providing a visual learning experience.

PROCEDURE

Students, working individually or in small groups, select a 4′ by 8″ piece of contact paper from the board displaying a written gene sequence (Fig. 1). This "paper gene" contains a written DNA sequence corresponding to the nucleotides found upstream and downstream of a gene of interest, and containing several restriction enzyme sites found within the gene. Additionally, students are given a list of all the restriction enzymes utilized in this activity. Lastly, also placed on the board, is a contact paper representation of a plasmid cloning vector, displaying a multiple cloning site (MCS) containing Kpn I, Dra I, Eco RI, Nru I, Mun I, Sma I, Hind III, Xma I, Bgl II, and Bam HI. The DNA sequence of each restriction endonuclease present in the "paper plasmid" MCS is also present on a contact paper sequence placed on the board. Once each group has selected a paper gene, they review possible enzyme combinations and choose the correct enzyme(s) to digest both the upstream and downstream "paper gene" sequences, as well as the correct enzyme(s) to digest the "paper plasmid MCS." The ultimate goal of these digestions is for the students to generate compatible ends.

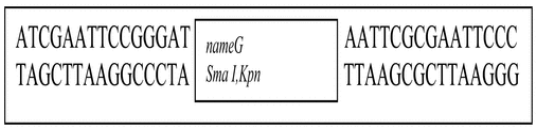

FIGURE 1 . Diagram of paper gene DNA sequence

Students review restriction enzyme catalogs to determine compatibility and digestion conditions required for each enzyme digestion. This information is recorded on worksheets, helping the students organize information and explain their choices. The worksheet is later given to the instructor for review. When selecting the correct enzyme(s) students must remember not to choose an enzyme found within their gene of interest, as this would result in digestion of that gene, and they must ensure they do not choose enzymes that will not generate compatible ends. Once combinations are determined, students cut the "paper gene" and "paper plasmid MCS" sequences with scissors in a manner that mimics RE digestion of DNA. Each group then places their cut "paper gene" and "paper plasmid MCS" sequences on the board, discussing their enzyme choices. If correctly designed, students demonstrate their compatible ends by displaying the correct base pairing of the cut paper gene DNA sequence with cut paper plasmid MCS DNA, resulting in successful cloning of the gene into the plasmid.

In the planning of the activity, through the design of the upstream and downstream paper DNA sequences, a range of difficulties to challenge the students are employed (Fig. 2). Some paper genes require the use of a single enzyme while others are designed to be more challenging; for example, requiring the use of isocaudomers. This requires students to critically think about the best enzyme and reaction conditions to choose that would produce a successful digestion.

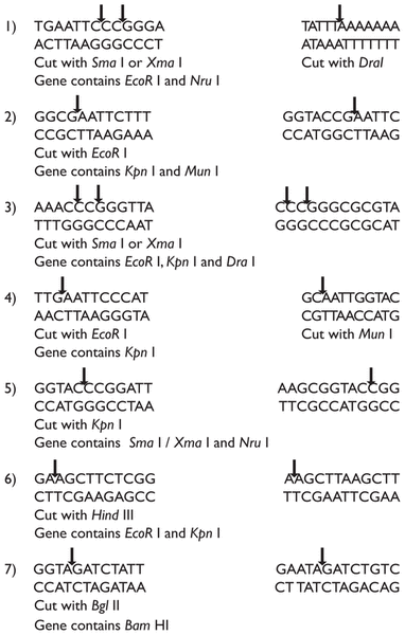

FIGURE 2 . Paper DNA sequence

The goal of this activity is to reinforce the theory of restriction endonuclease digestion and gene cloning, and to encourage critical thinking about the problem posed in order to successfully generate compatible ends from the gene selected. This activity, which has been used for several semesters, is very well-received, generating student comments such as "really enjoyed the in-class activities and I feel like they helped me better understand the material", and "the exercise was very appropriate and very helpful in understanding the rather complex material".

REFERENCES

1 . **Greene, J.J. and V.B.R. Rao (eds).** 1998. Recombinant DNA Principles and Methodologies. 1st ed. Marcel Dekker, Inc., New York.

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