Teaching DNA Structure and Function on a Shoestring Budget

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The experimental evidence, that enabled scientists to identify deoxyribonucleic acid (DNA) as the genetic material (Avery et al., 1944; Hershey and Chase, 1952), and enabled Watson and Crick (1953) to create their model for the structure of DNA, can be demonstrated in the classroom in a variety of ways. Much of the research into the nature and function of the genetic material is possible naturally through mutations, defined as heritable changes in the sequence of nucleotides in the DNA. Now, however, changes in the DNA structure can be generated in the classroom laboratory using a variety of molecular techniques (Cohen, 1975). A laboratory-generated technique called splicing makes possible the ability to cut and to ligate together DNAs from a variety of different types of organisms. Through the process of splicing, a change has been created in the DNA that will result in a new material that has the ability to create new life forms capable of destroying human-produced materials and to alter life so that not only is the present affected, but the future as well (Miller, 1992; Murray, 1991).

Many high school and undergraduate biology laboratory teachers find themselves operating on a shoestring budget and are always searching for ways to provide some form of a hands-on experience to students when teaching DNA structure and function. Examples such as gene mutation, mutation frequency, DNA replication (DNA synthesis) and transcription (RNA synthesis), messenger RNA translation (protein synthesis), and genetic engineering are classical and modern-day concepts in which DNA structure and function can be taught. The two exercises described below demonstrate how inexpensive, cost-saving items, and sometimes basic household products, may be used to teach certain aspects and concepts in DNA structure and function.

Exercise 1: DNA Structure, Function, and Regulation

Purpose: After completion of Exercise 1, students should be able to: (1) describe or diagram the basic chemical structure of a single-stranded and double-stranded nucleic acid strand and distinguish chemically between DNA and varieties of RNA; (2) give the basic sequences of one strand of DNA and predict that of a complementary strand of DNA; (3) point out degeneracy, redundancy, and the flexibility of the genetic code using a standard genetic code table that is provided; (4) calculate mutation frequencies following one, two, or three rounds of cell division (DNA replication); and (5) draw a diagram illustrating an operon, a group of contiguous, coordinately controlled genes, and describe how it functions.

Introduction: The experimental evidence, that enabled scientists to identify DNA as the genetic material, and enabled Watson and Crick to create their model for the structure of DNA, are discussed in this exercise. The processes by which information encoded in the DNA is decoded and expressed in the cell are also discussed. Much of the research into the nature and function of the genetic material is possible through mutations, defined as heritable changes in the sequence of

nucleotides in the DNA. Students will be introduced to the mechanisms of gene regulation in both eukaryotic and prokaryotic cells.

Diagrams: models of DNA; models of nucleotides showing double and triple bonds; illustrations representing repression, induction, and constitutive synthesis of the lactose operon; and genetic code table. *Equipment:* small portable typewriters or personal computers with keyboards for typing letters, words, and sentences that are used to represent codes, codons, nucleotides, amino acid sequences, and mutation frequencies. *Supplies:* plastic snap beads of varying lengths and colors to illustrate nucleotide, amino acid sequences, and mutation sites.

Directions: Students will complete the DNA replication and transcription, and RNA translation of a given nucleotide sequence using the genetic code table, nucleotide sequence, and DNA models provided. Students are given 1-minute, 2-minute, and 3-minute time periods to type from the nucleotide sequence of a single-stranded DNA: (1) the daughter DNA molecule (double-stranded) after one replication cycle; (2) the RNA transcript; and (3) the amino acid sequence of the polypeptide (protein) chain formed after translation of messenger RNA transcript. In the 1-, 2-, or 3-minute specific time periods, point mutations (base substitution, addition, or deletion of a letter or space on the message) can be illustrated using a simple sentence to be typed in specific 1-, 2-, or 3-minute time periods, such as "the guest are now here". That represents the normal or single-stranded parent DNA molecule. The following examples show the single-stranded daughter DNA molecule after a base substitution or a base addition mutation.

Base substitution: "the guest are not here"
Base addition: "the guest are nowhere"

A period (.) or an "x" can be used to create nonsense or missense mutations.

Given the single-stranded DNA strand below and the following gene expression hierarchy: (1) show the replication (double-stranded daughter DNA molecules); (2) show the mRNA strand; (3) show the transfer RNAs, tRNAs (anticodons), required for amino acid peptide bond elongation; and (4) show amino acid sequence of the polypeptide formed in the messenger RNA translation product. Upon completing the exercise from each level of gene expression: (1) determine the nature of the mutations; (2) the mutation rates in base pairs per minute or amino acid substitutions per minute; and (3) the mutation frequencies in base or base pair changes and amino acid substitutions in units per molecule (e.g., one amino acid substitution per polypeptide).

Single-stranded parent DNA strand: TACCGTTTGAGCGGGCCCAAAGTGAATGGCATTAAA

Exercise 2: Gene Manipulation Through Recombinant DNA Techniques

Purpose: After completing Exercise 2, students will be able to: (1) describe the primary techniques utilized in recombinant DNA experiments; (2) summarize the problems involved in cloning a single gene; (3) describe the action of restriction endonucleases and their specific function in recombinant DNA experiments; and (4) identify the role of plasmids, circular DNAs that replicate independently of the cell's chromosomes, in recombinant DNA experiments.

Introduction: A laboratory-generated technique called splicing makes possible the ability to cut and to ligate (join) together DNAs from a variety of different types of organisms. Through the process of splicing, a change has been created in the DNA that will result in a new material that has the ability to create new life forms capable of destroying human-produced materials and to alter life so that not only is the present affected, but the future as well. In addition to splicing, DNAs can be chemically synthesized and transferred into different types of cells in which the genes may even

function under appropriate conditions. DNA is currently being used to diagnose the potential for the development of certain diseases and for the medical treatment of certain diseases (Miller, 1992). Much of the above is possible due to recombinant DNA technology.

A vital key in the development of recombinant DNA technology was the discovery of restriction endonucleases and their importance as chemical scalpels (Cohen, 1975). The importance of these enzymes as tools in recombinant DNA technology lies in their specificity for a particular substrate. Each enzyme attacks a specific sequence of nucleotide bases in the DNA double helix. Some DNA restriction endonucleases attack symmetrical (palindromic) sequences composed of four to seven nucleotides while other enzymes attack asymmetrical sequences of four to five nucleotides long. The enzyme cuts the DNA into pieces called "resection fragments". In this exercise students are introduced to the rationale for the procedures and molecules used in DNA technology.

Diagrams: restriction endonuclease maps of various plasmid DNA cloning vectors (pBR322) and restriction endonuclease charts showing restriction recognition sites. *Supplies:* plastic snap beads of varying length and colors to illustrate vector (plasmid) DNA, insert (cloned) DNA, antibiotic-resistant DNA, restriction enzyme sites, and recombinant DNA molecules.

Directions: Below are two nucleotide sequences of double-stranded DNA and a list of restriction endonucleases and their recognition sequence and site. Determine the restriction endonuclease that will cut *both* pieces of DNA and indicate the sites of attack and the number of fragments produced.

DNA sequence:

1. CCAGTCGTTAACGAATTCGTCGACGTCGAC
   GGTCAGCAATTGCTTAAGCAGCTGCAGCTG
2. ACGGGTTAACCCAATGGATCCCAAGTTAACGGTACC
   TGCCCAATTGGGTTACCTAGGGTTCAATTGCCATGG

Restriction endonucleases*	Recognition site**
Eco RI	G/AATTC
Bam HI	G/GATCC
Hpa I	GTT/AAC
Sal I	G/TCGAC

* Each enzyme recognizes a symmetrical or palindromic sequence
** The "/" indicates the cut site

Make a chain of: 10 black snap beads, 6 red snap beads, 4 green snap beads, 3 blue snap beads, and 2 yellow snap beads. Form a circle (plasmid) by connecting these five snap bead chains together. The green snap bead chain = insert DNA; the red snap bead chain = antibiotic-resistant DNA; the blue snap bead chain = origin of DNA replication (ori); the yellow snap bead chain = restriction sites; and the black snap bead chain = vector DNA. Now, describe the structure and function of your recombinant DNA molecule.

Finally, construct hypothetical, but logical, models of recombinant DNA molecules using different numbers and combinations of colored snap bead chains. After Exercise 2 has been completed, answer the following questions: (1) Are your DNA molecules recombinant? (2) Are your recombinant DNA molecules logically constructed? (3) Will your recombinant DNA molecules replicate? (4) Do your recombinant DNA molecules demonstrate unique cloning

strategies? (5) Can your recombinant DNA molecule be easily screened and selected if present in a recombinant DNA library (gene bank)?

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