



Recombinant DNA Genetic Transformation

Designed and Tested by Teachers
for Teachers

Thank you for your purchase. Science teachers know that abstract concepts are difficult for most students to grasp. Hundreds of teachers at the high school and college level have found this kit to greatly enhance learning because it provides a visual foundation for the fundamental concepts of genetic transformation. It was developed in response to the finding that my students were performing lab exercises without sufficient understanding, despite using expensive equipment. I discovered students had misconceptions about the principals of transformation and could not interpret results of gel electrophoresis. When I began looking for something to help, I didn't find exactly what I was looking for. I'm confident that this kit can assist you while teaching recombinant DNA technology in the classroom or laboratory, with or without expensive laboratory equipment.

Using this manual

Use this manual in combination with the CD in your kit. The CD will make specific references to this manual.

I. Background Information for teachers (CD slides 6-20)

These slides provide a simplified scenario of human insulin production using E. Coli bacteria. I recommend you use this scenario to demonstrate "real world" application. There are many more applications in medicine and agriculture that you may wish to add or supplant.

II. Introduction to antibiotic resistant plasmids (CD slides 21-37)

Naturally occurring antibiotic resistant genes found in bacterial plasmids serve as an excellent model for teaching transformation technology. Bacteria that possess antibiotic resistant genes can survive on media containing the antibiotic, while others cannot. This provides phenotypic evidence that can be readily observed. In fact, scientists often attach antibiotic resistant genes to genes of interest. Bacteria containing plasmids with the gene of interest will grow in antibiotic media. Bacteria that did not uptake the gene will not survive. This provides scientists with a tool for sorting.

Two plasmids that provide antibiotic resistance are used in the kit. One provides resistance to Ampicillin, the other to Kanamycin.

Kanamycin halts protein synthesis in non-resistant bacteria cells by binding to the ribosome. The KAN gene produces a protein (phosphotransferase) that adds phosphate groups to the kanamycin molecule. The kamamycin/phosphate molecule is not able to bind to the ribosome.

Ampicillin interferes with cell wall synthesis in non-resistant bacteria. In normal cell wall biosynthesis, an enzyme (transpeptidase) removes an alinine from the cell wall structure. The antibiotic ampicillin mimics the transpeptidase receptor site, making it unavailable to form cell walls. New bacteria cells cannot develop.

The AMP gene contained in resistant bacteria produce a protein called B- Lactamase. B-Lactamase cleaves the site on the antibiotic that mimics the transpeptidase receptor. This leaves the ampicillin ineffective and allows transpeptidase to resume normal cell wall synthesis.

Be sure to ask questions that will ensure your student understand that:

- Plasmids are bacterial DNA in a circular form.
- Plasmids contain genes.
- Antibiotic resistance genes allow the bacteria to survive on media containing antibiotics.

- The AMP *genotype* produces a *phenotype* of resistance to ampicillin.
- The KAN *genotype* produces a *phenotype* of resistance to kanamycin.

III. Introduction to restriction enzymes (CD slides 38-55)

What are restriction enzymes?

Restriction endonucleases are enzymes that attack and cut internal regions of the DNA of an invading bacteriophage (virus that invades bacteria). They provide the bacteria with protection from invading virus. Think of them as part of a bacterial immune system. A bacteria is able to protect its own DNA from being cut by the enzyme using a process called methylation. Methylated DNA cannot be cut by the restriction enzyme.

What are recognition sequences?

Scientists began to isolate more and more restriction enzymes from bacteria. They found that some enzymes cut DNA randomly and were of little value in rDNA technology. Later, enzymes that cut in a precise and predictable manner were found. These enzymes recognize specific nucleic acid sequences and cut within the recognition sequence. The recognition sequence for *Bam H I* is G/GATCC and for *Hin D III* it is A/AGCTT.

These recognition sites are palindromes (the sequence read in the 5' to 3' direction is the same when read in the 3'to 5' direction on the complementary strand.)

What are “sticky ends”?

“Sticky ends” are unpaired nucleotides remaining attached to a DNA fragment following a digest by certain restriction enzymes. Enzymes that leave sticky ends are ideal for recombinant DNA transformations. Note that when two plasmids are cut with the same restriction enzymes, “sticky end” nucleotides are complementary. This allows a fragment from one plasmid to be able to “splice” into the other.

IV. Possible Recombinant Plasmids (CD slides 56-61)

Once plasmids are “digested” using the kits two restriction enzymes, four DNA fragments are produced. When mixed under proper conditions, these fragments will re-combine. Complementary base pairing will occur at the “stick ends”. Different combinations of fragments will result in a variety of new plasmids. At this point you are limited to the use of the four fragments. Students need to understand that:

- Some fragment combinations are not possible because of base pairing rules at the sticky ends.
- Fragments can re-combine to form the original plasmids.
- It is possible for the fragment containing the AMP gene and the fragment containing the KAN gene to recombine.
- If the above fragments combine a new phenotype (dual antibiotic resistance) as well as a new genotype will be produced.