

## Chapter 20: Biotechnology

### 1. Define recombinant DNA, biotechnology, and genetic engineering.

Recombinant DNA is formed when segments of DNA from two different sources, often different species, are combined *in vitro*. Biotechnology is the manipulation of organisms or their components to make useful products. This includes genetic engineering, the direct manipulation of genes for practical purposes.

### 2. What is a plasmid?

In 1935, Stanley crystallized the tobacco mosaic virus (TMV), confirming Beijerinck's concept. Subsequently, many viruses could be seen with an electron microscope.

### 3. What is gene cloning?

Gene cloning is the production of multiple copies of a single gene.

### 4. Explain the steps in gene cloning.

First, the gene is inserted into a plasmid, which is then put into a bacterial cell. Next, the host cell is grown in culture to form a clone of cells containing the "cloned" gene of interest. This is used for basic research and various applications, such as heart attack therapy and toxic waste cleanup.

### 5. Explain the steps involved in forming recombinant DNA.

First, a restriction enzyme cuts the sugar-phosphate backbones at specific points on the restriction site. Next, a DNA fragment from another source is added. Base pairing of sticky ends produces various combinations. Finally, DNA ligase seals the strands.

### 6. What is a cloning vector?

Wild-type *Neurospora* has modest food requirements. It can grow in the laboratory on a simple solution of inorganic salts, glucose, and the vitamin biotin, incorporated into agar, a support medium. From this minimal medium, the mold cells use their metabolic pathways to produce all the other molecules they need. Mutating the wild type allowed Beadle and Tatum to identify metabolic defects.

### 7a. Explain why the plasmid is engineered with *amp<sup>r</sup>* and *lacZ*.

The gene *amp<sup>r</sup>* makes *E. coli* cells resistant to the antibiotic ampicillin, while *lacZ* encodes the enzyme  $\beta$ -galactosidase, which hydrolyzes lactose. This enzyme can also hydrolyze a similar synthetic molecule called X-gal to form a blue product. The plasmid contains only one copy of the restriction site recognized by the restriction enzyme, and that site is within the *lacZ* gene. Both the plasmid and the hummingbird DNA are cut with the same restriction enzyme before the fragments are mixed together, allowing base pairing between their complementary sticky ends as their sugar-phosphate backbones are covalently bonded by DNA ligase. The DNA mixture is then added to bacteria that have a mutation in the *lacZ* gene on their own chromosome, making them unable to hydrolyze lactose or X-gal.

### 7b. After transformation has occurred, why are some colonies blue?

Under suitable experimental conditions, the cells take up foreign DNA by transformation. Plating out all the bacteria on solid nutrient medium containing ampicillin allows researchers to distinguish the cells that have taken up plasmids, whether recombinant or not, from the other cells. Only cells with a plasmid will reproduce under these conditions because only they have the *amp<sup>r</sup>* gene conferring resistance to the ampicillin in the medium. Moreover, the presence of X-gal in the medium allows researchers to distinguish bacteria colonies with recombinant plasmids from those with nonrecombinant plasmids. Colonies containing nonrecombinant plasmids have the *lacZ* gene intact and will produce functional  $\beta$ -galactosidase. These colonies will be blue because the enzyme hydrolyzes the X-gal in the medium, forming a blue product. In contrast, no functional  $\beta$ -galactosidase is produced in colonies containing recombinant plasmids with foreign DNA inserted into the *lacZ* gene; these colonies will therefore be white.

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**RM Cervero**



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