

# ESSENTIALS OF Molecular Biology

**DAVID FREIFELDER**

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**DAVID FREIFELDER**

University of California, San Diego



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# Preface

In 1983 *Molecular Biology, A Comprehensive Introduction to Prokaryotes and Eukaryotes*, was published—a 979-page book designed for a complete course in molecular biology. I was pleased to receive many favorable comments about the book in the following year. However, many instructors said that the book was too large to require for use in some courses and suggested that I prepare a short version.

In mid-1984 I began the job, cutting and reassembling existing material, but I quickly found this method to be impossible: in the parts I kept, there were too many references to deleted material, and the flow of thought was often interrupted. Thus, it was clear that many paragraphs had to be rewritten, and connecting sentences were needed. While attending to this, I took the opportunity to update existing material, where needed, and added new information in the interest of making a better “short book.” The most advanced material has been omitted, along with descriptions of techniques and some experimental support for the facts. This information will have to be provided by the instructor, or the student can be directed to the more detailed text of the larger book. Thus the reader will occasionally find in this book phrases such as “For more information, see MB, pages 000-000.” MB refers to the first edition of the original book, *Molecular Biology*.

*Molecular Biology* had no exercises and problems for the student; instead a separate problems book, *Problems for Molecular Biology*, was

published. *Essentials of Molecular Biology* contains problems and answers, taken from this book, so a second purchase is not necessary.

I hope the short book will prove to be useful to others as well as to those who requested it.

November, 1984  
San Diego, California

David Freifelder

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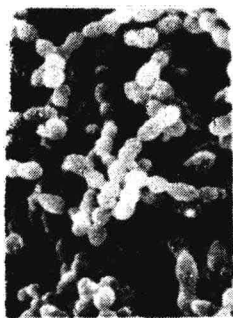
# 1 Systems and Methods of Molecular Biology

The term molecular biology was first used in 1945 by William Astbury, who was referring to the study of the chemical and physical structure of biological macromolecules. By that time, biochemists had discovered many fundamental intracellular chemical reactions, and the importance of specific reactions and of protein structure in defining the numerous properties of cells was appreciated. However, the development of molecular biology had to await the realization that the most productive advances would be made by studying "simple" systems such as bacteria and bacteriophages (bacterial viruses), which yield information about basic biological processes more readily than animal cells. Although bacteria and bacteriophages are themselves quite complicated biologically, they enabled scientists to identify DNA as the molecule that contains most, if not all, of the genetic information of a cell. Following this discovery, the new field of molecular genetics moved ahead rapidly in the late 1950's and early 1960's and provided new concepts at a rate that can be matched only by the development of quantum mechanics in the 1920's. The initial success and the accumulation of an enormous body of information enabled researchers to apply the techniques and powerful logical methods of molecular genetics to the subjects of muscle and nerve function, membrane structure, the mode of action of antibiotics, cellular differentiation and development, immunology, and so forth. Faith in the basic uniformity of life processes was an important factor in this rapid growth. That is, it was believed

that the fundamental biological principles that govern the activity of simple organisms, such as bacteria and viruses, must apply to more complex cells; only the details should vary. This faith has been amply justified by experimental results.

In this book prokaryotes and eukaryotes will be discussed separately and compared and contrasted. Usually prokaryotes will be discussed first because they are simpler. In keeping with this, we begin by describing the properties of bacteria that are important in molecular biological research.

## BACTERIA



Bacteria are free-living unicellular organisms. They have a single chromosome, which is not enclosed in a nucleus (they are prokaryotes), and compared to eukaryotes they are simple in their physical organization. For all practical purposes, a bacterium can be thought of as a solution of several thousand chemicals and a few organized particles enclosed in a rigid cell wall.

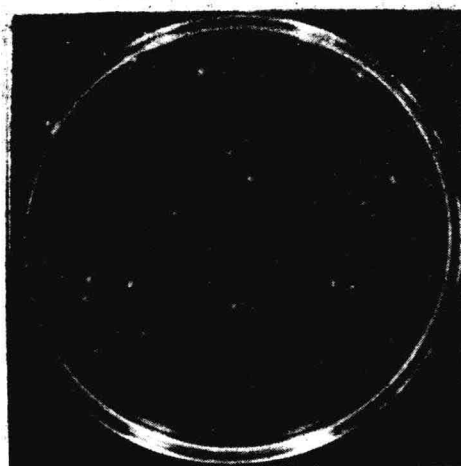
Bacteria have many features that make them suitable objects for the study of fundamental biological processes. For example, they are grown easily and rapidly and, compared to cells in multicellular organisms, they are relatively simple in their needs. The bacterium that has served the field of molecular biology best is *Escherichia coli* (usually referred to as *E. coli*), which divides every 20 minutes at 37°C under optimal conditions. Thus, a single cell becomes  $10^9$  bacteria in about 20 hours. Bacteria can be grown in a **liquid growth medium** or on a solid surface. A population growing in a liquid medium is called a bacterial **culture**. If the liquid is a complex extract of biological material, it is called a **broth**. If the growth medium is a simple mixture containing no organic compounds other than a carbon source such as a sugar, it is called a **minimal medium**. A typical minimal medium contains each of the ions  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{NH}_4^+$ ,  $\text{Cl}^-$ ,  $\text{HPO}_4^{2-}$ , and  $\text{SO}_4^{2-}$ , and a source of carbon such as glucose, glycerol, or lactate. If a bacterium can grow in a minimal medium—that is, if it can synthesize *all* necessary organic substances such as amino acids, vitamins, and lipids—the bacterium is said to be a **prototroph**. If any organic substances other than a carbon source must be added for growth to occur, the bacterium is termed an **auxotroph**. For example, if the amino acid leucine is required in the growth medium, the bacterium is a leucine auxotroph; the genetic symbol for such a bacterium is  $\text{Leu}^-$ . A prototroph would be indicated  $\text{Leu}^+$ . Bacteria are frequently grown on solid surfaces. The earliest surface used for growing bacteria was a slice of raw potato. This was later replaced by media solidified by gelatin. Because many bacteria



excrete enzymes that digest gelatin, an inert gelling agent was sought. **Agar**, which is a gelling agent obtained from a variety of seaweed and used extensively as a thickening agent in Japanese cuisine, is resistant to bacterial enzymes and has been universally used. A solid growth medium is called a nutrient agar, if the liquid medium is a broth, or a minimal agar, if a minimal medium is gelled. Solid media are typically placed in a **petri dish**. In lab jargon a petri dish containing a solid medium is called a **plate** and the act of depositing bacteria on the agar surface is called **plating**.

A bacterium growing on an agar surface divides. Since most bacteria are not very motile on a solid surface, the progeny bacteria remain very near the location of the original bacterium. The number of progeny increases so much that a visible cluster of bacteria appears. This cluster is called a **bacterial colony** (Figure 1-1). Colony formation allows one to determine the number of bacteria in a culture. For instance, if 100 cells are plated, 100 colonies will appear the next day.

Plating is a method for determining if a bacterium is an auxotroph. This is done in the following way. Minimal agar and nutrient agar plates are prepared. Several hundred bacteria are plated on each plate and the plates are incubated overnight in an oven. Several hundred colonies are subsequently found on the nutrient agar because it contains so many substances that it can satisfy the requirements of nearly any bacterium. If colonies are also found on the minimal agar, the bacterium is a prototroph; if no colonies are found, it is an auxotroph and some required substance is not present in the minimal agar. Minimal plates are then prepared with various supplements. If the bacterium is a leucine auxotroph, the addition of leucine alone will



**Figure 1-1**  
A petri dish containing colonies of *E. coli*  
grown on agar. (Courtesy of Robert Haynes.)