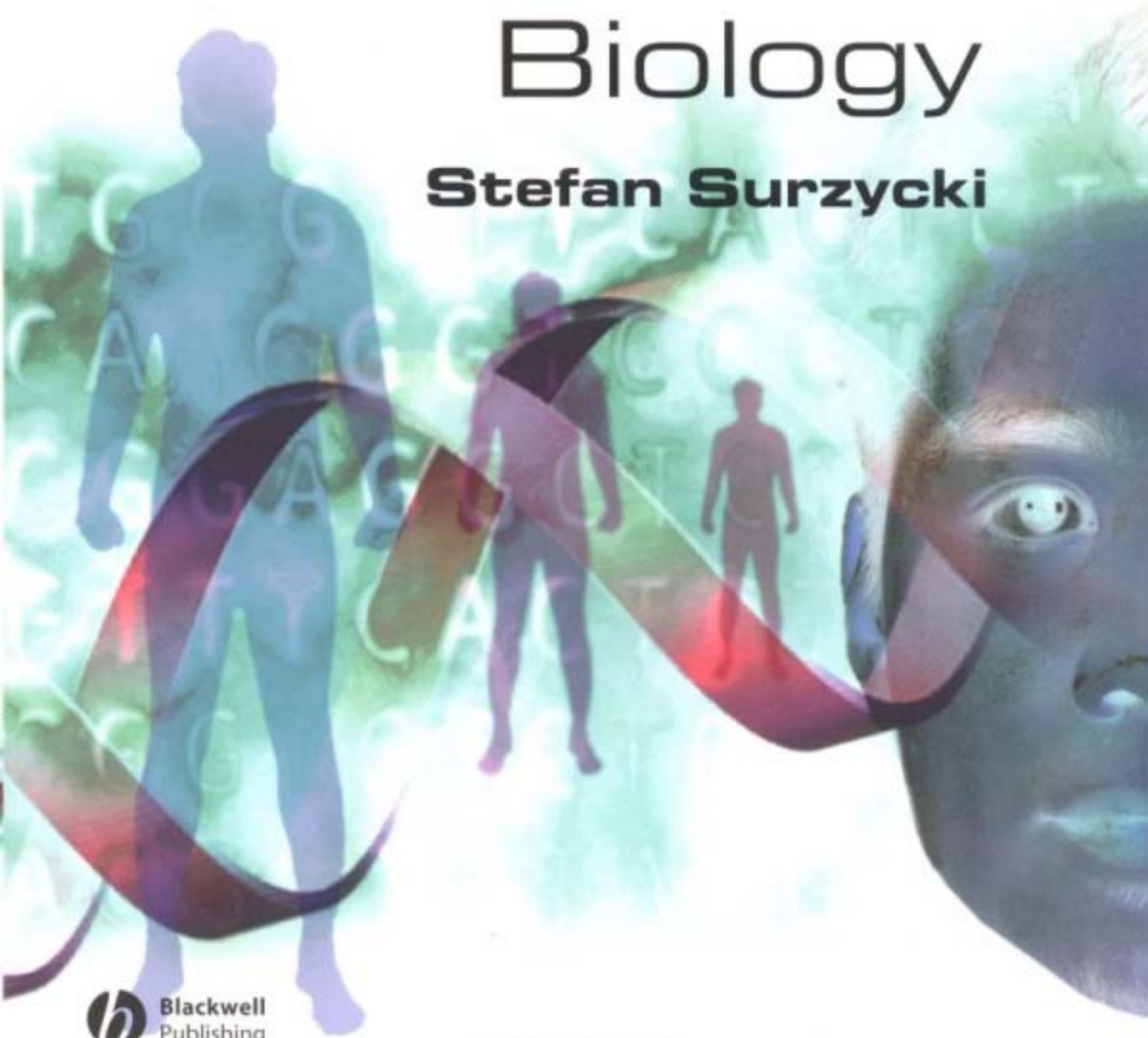
Laboratory Manual

Human Molecular Biology

Stefan Surzycki



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Preface

The recent completion of the human genome-sequencing project is an important development in the history of biological sciences. It will not only promote the understanding of the human genome, but will also profoundly change the discipline of molecular biology and affect medical practices. The human genome is of great interest and is the subject of intensive basic and applied research. The molecular biology techniques used in this research are highly advanced and unique. Learning these techniques will permit students to learn the basic principles of molecular biology and will prepare them to work with the human genome.

These skills are in great demand by biotechnology, forensic laboratories, and pharmaceutical companies. This laboratory manual provides the student with basic experience in and an understanding of cutting-edge techniques in molecular biology. In addition, the experiments described in this manual will provide students with an opportunity for analyzing and studying their own genes.

The goal of this laboratory manual is not only to teach basic molecular biology techniques, but also to convey the excitement of performing experiments and comparing the results to a large body of data collected about the human genome.

The topics of the course revolve around a central theme of analysis of the student's own genome, i.e. its structure and gene expression. These topics include eight exercises.

1. Preparation of genomic DNA. Cheek cells are the source of this DNA. Collecting these cells is a non-invasive procedure that makes it possible to use DNA purification in a classroom situation. The techniques that are used in the course of this experiment are large-scale purification of DNA, spectroscopic analysis of DNA, and determination of DNA concentration and purity.

2. DNA fingerprinting using multi-locus analysis with a human variable number tandem repeat probe. Students use their own DNA for this analysis. In this procedure students learn the techniques of Southern blot transfer, preparation of non-radioactive probes, hybridization, and chemiluminescent autoradiography. Use of a non-radioactive probe removes the difficulties

of working with radioactive materials in the class environment. It also eliminates the problem of disposing of a large quantity of radioactive waste that will invariably be generated when working with a large class. Moreover, the non-radioactive procedure is a more advanced technique that has recently been finding general acceptance in basic research and industry.

3. DNA fingerprinting with a single-locus probe. This technique is used in standard forensic analysis. The probe used is the standard forensic D2S44 probe. It represents a tandem repeat region that is present on human chromosome 2. Students will learn methods of forensic profiling and analyze data using a fixed bins database of allele frequencies prepared for this probe by the FBI.

4. Linkage disequilibrium analysis using the DNA markers *Alu* CD4 and the TTTTC repeat. This experiment is based on the paper of Tishkoff et al. (1996) (see the reference section in Chapter 4). The authors introduced this innovative technique in determining a common and recent African origin for all non-African human populations. Analysis of the data consists of the calculation of linkage disequilibrium for the entire class. The results are compared to the disequilibrium found in different world populations. During the course of this experiment, students learn how to perform PCR (polymerase chain reactions), use the thermal cycler, and analyze products using high-resolution agarose gel electrophoresis.

5. Sequencing of human DNA using an ABI capillary sequencer and Big Dye technology. The goal of this experiment is to sequence human DNA using the same procedures employed in large sequencing projects. Students prepare their DNA for sequencing using the random sequencing strategy used by the Human Genome Project. The techniques used in the course of this experiment are preparation of a random sequencing library by nebulization, cloning DNA fragments into a sequencing plasmid, transformation of *Escherichia coli* cells by electroporation, preparation of plasmid DNA for sequencing, and PCR cycle sequencing.

6. Computer analysis of sequencing data. Students carry out local (Basic Local Alignment Search Tool or BLAST) and global alignment analysis using their own sequencing data. They analyze direct and inverted repeats in their DNA by dot plots and learn how to use various databases available for the analysis of human DNA sequences (ESTSDB, ALUDB, ICRDB, etc.).

7. Determination of human telomere length. Telomere length is a reflection of the "mitotic clock" of normal somatic cells and is therefore age dependent. In the course of this experiment, students determine the telomere length of their DNA. The techniques used are multi-enzyme digestion of genomic DNA, turbo-blot transfer, hybridization using an oligonucleotide probe, and computer determination of average telomere length.

8. Analysis of the expression of the β-actin gene in human cheek cells. This determination is carried out using single-tube RT-PCR. Students carry out isolation of total RNA from cheek cells, determine its purity and

concentration, perform RT-PCR reactions, and analyze the results by gel electrophoresis.

The manual is an outgrowth of a semester course taught each year to undergraduate students at Indiana University. Each of the eight experiments constitutes an integrated unit performed in one or more laboratory sessions. The laboratory sessions are designed to meet twice a week for 4 hours and are designed for a limit of 20 students per class. Occasionally students (or instructors) will need to spend additional time in the laboratory in order to finish experiments or to collect results. These times are indicated in the outline for each procedure. The descriptions of the laboratory procedures assume that students will perform all the steps of the procedure. However, at the discretion of the instructor, pre-preparing some materials (e.g. preparation of labeled probes, preparation of plasmids for sequencing, etc.) can reduce the session times and session numbers.

In this manual I try to go beyond cookbook recipes for each technique. The description of each technique includes an overview of its general importance, historical background, and theoretical basis for each step. This is done in the hope that students will acquire enough of an understanding of the theoretical mechanisms that they will be able to go on to design their own modifications and methods.

All of the procedures in this book have been used extensively in the teaching of undergraduate laboratories and passed the ultimate test for "working" in the hands of several generations of undergraduates. The descriptions of each step in the protocols are very specific and detailed as to how to carry them out. These instructions may appear to be overly detailed, but they have been developed because of years of experience teaching undergraduates and trying to ensure that the experiments work in inexperienced hands the first time they are performed. In addition, technical tips for carrying out each procedure are incorporated into the text.

In the course preparation I make extensive use of commercially available kits. There are several reasons for their use. First, kits save enormous time in preparation and afford substantial savings in the cost of reagents. Frequently the cost of individual reagents necessary for preparing a laboratory for a large class exceeds the cost of the kit. Second, when using reagents from supply companies, the expertise of their technical support is only a World Wide Web page or telephone call away.

In the manual, I also recommend the use of some instruments for class use. This is generally guided by the usefulness of this instrument in a classroom environment, as well as cost itself.

Laboratory Safety

Anybody using this manual should be familiar with and should follow laboratory safety procedures. Instructors should be familiar with all national,

state, local, and university regulations and practices. This is particularly important when disposing of waste (e.g. ethidium bromide, phenol, etc.) and working with human cells. Students should use this manual under instructor supervision. Using some instruments, such as an electrophoresis power supply or high-speed centrifuge, without knowledge of the instrument and proper training or supervision can be very dangerous.

In addition, the description of each experiment includes a section on safety precautions. Before performing any procedure, students should make themselves familiar with its content.

Acknowledgments

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