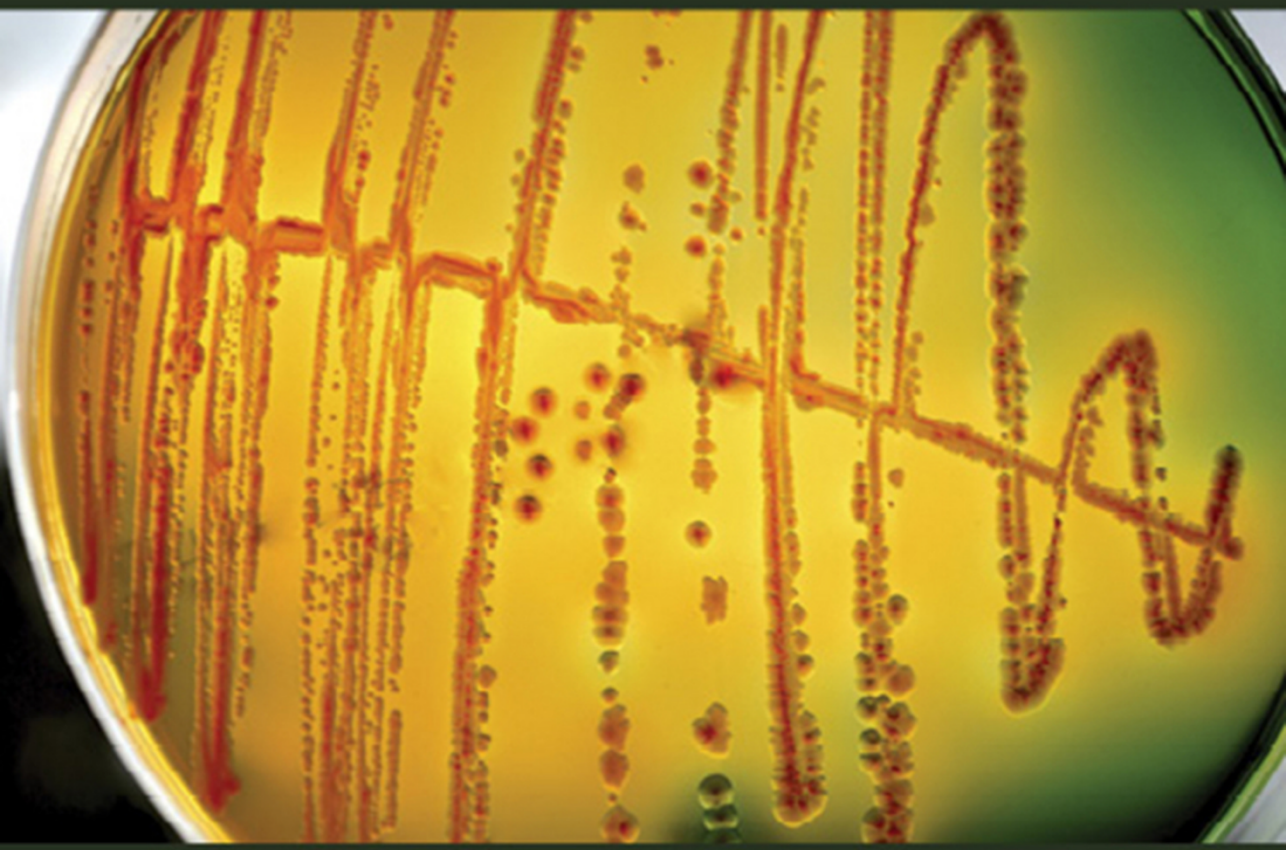
Marcy A. Kelly with contributions from Pryce L. Haddix

The Fundamentals of SCIENTIFIC RESEARCH

An Introductory Laboratory Manual



WILEY Blackwell

The Fundamentals of Scientific Research

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An Introductory Laboratory Manual

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The journey to develop this manual was linked to my personal journey from the time of its inception to its completion. There is one single person that has been my inspirational rock and source of enduring support during this endeavor. I dedicate this work to my husband, Thomas Kelly.

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Preface

In 2011, major stakeholders in the life sciences published a document entitled "Vision and Change in Undergraduate Education: A Call to Action" (AAAS, 2011). This document proposes a new way to teach undergraduate life sciences majors. The report suggests that undergraduate educators directly engage their students in the process of science by providing them with authentic experiences that mimic what we do as professional scientists. They postulated that biological content and the skills commonly associated with successful scientists can be introduced to students through meaningful evaluations of real biological phenomena.

The goal of the experiments in this laboratory manual is to introduce undergraduate students to the process of science through a guided introductory laboratory experience. Highlights of this manual include a semester-long experience working with one organism using experiments directed toward a single unifying goal, pre- and postlaboratory assignments and laboratory reports aimed to enhance the students' analytical, critical thinking and scientific writing skills, and experience using common laboratory equipment and techniques similar to what are used in the professional setting.

Specifically, the laboratory curriculum centers on studying *Serratia marcescens*. *S. marcescens* is a Gram-negative bacterium that is unique in that it produces a red pigment, prodigiosin, at high cell density. It has been demonstrated that prodigiosin has several interesting properties; it is antimicrobial, it has been shown to induce apoptosis in cancer cells, and it has been demonstrated that it has potential to act as an immunosuppressant (reviewed in Khanafari *et al.*, 2006). There are currently many research laboratories that are attempting to enhance the production of prodigiosin because of these unique properties. The overarching goal of the laboratory course described in this manual is to have the students learn about

the organism so that they can generate and initially characterize mutants of the organism that overproduce the pigment.

The laboratory manual breaks down the laboratory course into three separate modules. For the first module, the students familiarize themselves with common laboratory equipment and techniques. For the second module, the students begin to work with and appreciate *S. marcescens* by performing growth curves and Lowry protein assays, quantifying prodigiosin and ATP production, and performing complementation studies to understand the biochemical pathway responsible for prodigiosin production. They learn how to employ Microsoft Excel to prepare and present their data in graphic format and how to use specific calculations to convert their data into meaningful numbers that can be compared across experiments. The third module requires that the students employ UV mutagenesis to generate hyperpigmented mutants of *S. marcescens* for further characterization. They use experimental data and protocols they learned during the first and second modules of the course to help them develop their own hypotheses and experimental protocols and to help them analyze their data.

For each laboratory session, students are required to answer prelaboratory questions that are designed to probe their understanding of the required prelaboratory reading materials (which includes the experimental background and protocol for that session and, in some cases, relevant primary scientific literature related to the experiments they will be performing in the laboratory). The questions also guide the students through the development of hypotheses and predictions. Following each laboratory, the students are required to answer a series of postlaboratory questions to guide them through the presentation of their data, analysis of their data, and placing of their data into the context of the primary literature. They are also asked to review their initial hypotheses and predictions to determine if their conclusions support their initial assertions. If their conclusions do not support their initial assertions, the students are asked to provide possible explanations as to why they think their conclusions did not agree with their hypotheses. The pre-laboratory and postlaboratory questions were designed to assist the students with

the preparation of two formal laboratory reports after the second and third modules. The format for the reports is similar to that of primary scientific literature.

This laboratory manual seeks to introduce introductory undergraduate life sciences majors to an environment that fosters the development of scientific curiosity, creativity, and the critical thinking and communication skills required for success in the scientific disciplines. The laboratory techniques and skills that they will master through the exercises presented herein will provide the students with a strong foundation in the practice and process of science. All of these, in turn, should enable the students to persist and succeed as life sciences majors.

Acknowledgments

This work would not be possible if it was not for the students, faculty, and staff in the Department of Biology and Health Sciences–NYC. The feedback and support provided during the development of this work have been immeasurable and greatly appreciated.

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com\go\kelly\fundamentals

The website includes:

- Instructor's Companion to the Lab Manual

Introduction

As life scientists, we are uniquely positioned to use our inquisitive natures to ask questions about the world around us. We have developed a systematic method to address the questions we pose. This method, the scientific method, is the major tenet behind what we do. The scientific method is as follows:

(1) Make an observation.
(2) Ask a question based upon your observation.
(3) Develop a hypothesis.
(4) Develop experiments to test your hypothesis.
(5) Collect and analyze experimental data.
(6) Confirm hypothesis or develop and test a new hypothesis.

You have probably memorized these steps at some point during your academic career, but have you ever *really* put them to the test to answer a biological question that has not yet been addressed? If not, you will gain practical experience with scientific method in this laboratory. If you have practiced the scientific method in the past, this laboratory will help you hone your skills.

Throughout the semester, you are going to be studying a single organism, *Serratia marcescens*. *S. marcescens* is a bacterium. Bacteria are considered prokaryotic organisms and have features commonly associated with prokaryotic cells. *S. marcescens* is classified as a Gram-negative bacterium because of the structure of its cell wall. Gram-negative bacteria have thin cell walls with phospholipid bilayers on both sides of the cell wall.

S. marcescens is unique among bacteria because it produces a red pigment called prodigiosin. Although the exact biological role of the pigment with respect to the organism is unknown, several laboratories throughout the world are studying the pigment and its activities. We are going to perform our own experiments in this laboratory