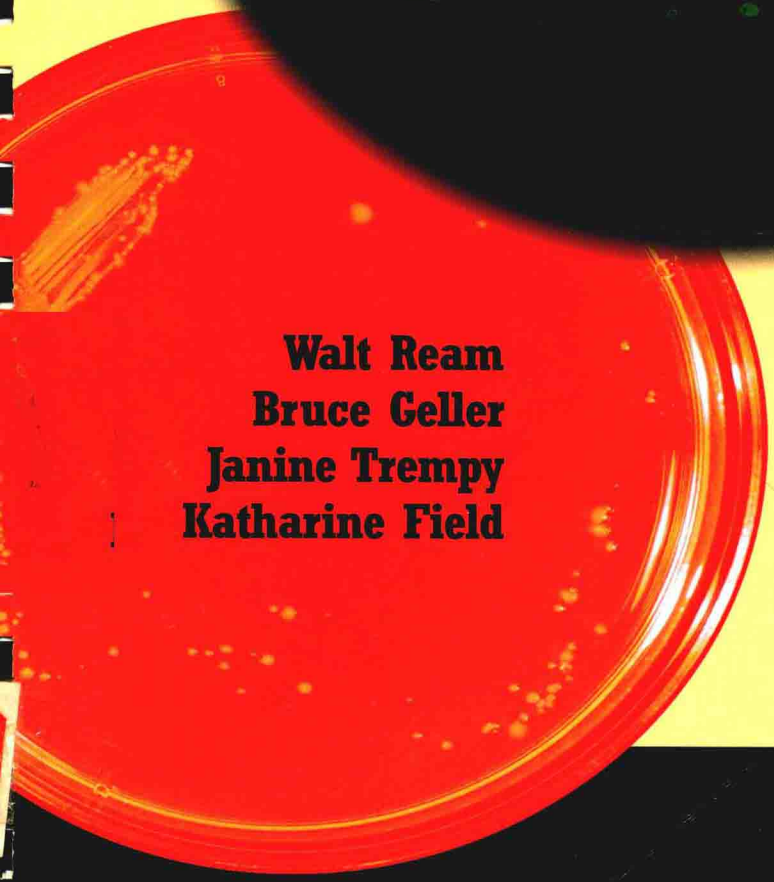
Molecular Microbiology Laboratory

A Writing-Intensive Course



Walt Ream
Bruce Geller
Janine Trempy
Katharine Field

MOLECULAR MICROBIOLOGY LABORATORY

A WRITING-INTENSIVE COURSE

Walt Ream, Bruce Geller, Janine Trempy, and Katharine Field

Department of Microbiology
Oregon State University
Corvallis, Oregon

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REQUIRED AND SUGGESTED READINGS

Introduction

I. WRITING-INTENSIVE COURSE

A. Goals

This 10-week course is designed to teach undergraduate students molecular biology techniques commonly used in the life sciences and to develop the students' scientific writing skills.

B. Means

The course contains four units that introduce procedures most life scientists will encounter during their careers. In the first unit, students prepare plasmid DNA, construct a restriction map of the plasmid, and transform it into *Escherichia coli*. The plasmid contains a luciferase reporter gene, which introduces the concept of reporter genes through firsthand experience. In the second unit, students express, purify, and analyze an affinity-tagged protein. The third unit requires intellectual input from students, who will isolate bacteria from environments that they choose. Each student will select one unknown bacterium to culture, examine by light microscopy, and identify by DNA sequence analysis. During this experiment students learn to isolate genomic DNA, perform a polymerase chain reaction (PCR), purify PCR products, and analyze DNA sequence data. The fourth unit teaches students to perform Southern blots and to prepare hybridization probes. The methods students use in this course are basic techniques that introduce the fundamental principles of molecular biology.

This is also a writing-intensive course. The manual contains a general discussion of scientific writing and critical reading, and it includes detailed instructions for preparation and peer review of lab reports. Additional writing exercises based upon journal articles accompany each experimental unit. The studies in these articles

employ the techniques used in the laboratory exercises. By evaluating these papers, students reinforce their understanding of the technology. Students see how diverse authors report their findings and how formats differ from one journal to another. They also discover that all scientific papers share several essential components. Lectures based on the book "How to Write and Publish a Scientific Paper," by Robert Day, discuss each section of a scientific paper in detail. To improve their copyediting skills, students read and discuss "Line by Line," an outstanding manual written by a copyeditor, Claire Kehrwald Cook. Thus, to build their writing skills and enhance their understanding of molecular microbiology, students compose and revise lab reports, edit their peers' reports, critique journal articles, and study writing manuals.

II. SCHEDULE

Day	Laboratory	Lecture	In-class writing	Hand in	Read
1		Introduction; how to write lab reports and proposals			Manual 1–43; Day Ch. 1–10
2	Purify plasmid; restriction	Restriction enzymes and mapping	Rewrite sentences	Flow Chart 1	Kragelund *et al.*, 1997
3	Agarose gel; transform	Transformation; reporter genes	Restriction mapping problems		Day Ch. 13–15 and 32–35; Day Append. 3–4
3+1	Examine plates				
4		Affinity-tagged protein purification	Peer review Report 1, critique Kragelund *et al.*, 1997	Report 1 draft	Bush *et al.*, 1991
5	Lyse cells; bind Ni resin	Lysozyme		Lab Report 1, Flow chart 2	Cook Ch. 1
6	SDS–PAGE	SDS–PAGE			Cook Ch. 2
7		How to read a journal article	Peer review Report 2, critique Bush *et al.*, 1991	Report 2 draft	Cook Ch. 3
8	Isolate bacteria		Peer review proposal	Proposal draft	Cook Ch. 4
8+1	Examine plates and streak				

9	Gram stain; microscopy; inoculate broth	PCR; rRNA-based phylogeny	Describe colonies	Lab Report 2	Cook Ch. 5
10	Prepare genomic DNA; freeze cultures	DNA purification		Flow Chart 3	Borneman and Triplett, 1997
11	PCR	Primer stock preparation	Write abstract for Borneman and Triplett, 1997		Rappé *et al.*, 1998
12	Purify PCR product	DNA sequencing; using GenBank		Proposal	*Nature* Editorial, 1996
13	Agarose gel and template preparation	Review	Sample problems		Review questions
14			Test		
15	Restriction; agarose gel	Southern blots; probes	Edit sequences	Flow Chart 4	Salyers, 1996
16	Blot gel; prepare probe		Peer review Report 3	Report 3 draft	Bengtsson, 1997
17	Hybridization		Peer review Editorial	Lab Report 3, Editorial draft	
18	Wash and develop blots		Discuss GMO (genetically modified organism) papers	Editorial	
19		Engineered crops	Peer review Report 4	Report 4 draft	
20		Summary		Lab report 4	

III. ATTENDANCE AND GRADING POLICIES

Attendance is **mandatory**. Each unexcused absence will result in a 5% deduction from your final grade. More than two absences will result in an Incomplete. Arrival more than 15 minutes late will count as half an absence.

Requests for an excused absence will be considered on a case-by-case basis, but exercises cannot be rescheduled. Students with an excused absence must complete all missed assignments.

A. Grading

Final Grade

A/A–	= 90–100% of top score
B+/B/B–	= 80–89%
C+/C/C–	= 65–79%
D	= 50–65%
F	= below 50%

Lab reports	= 20% each × 4 = 80%
Test	= 20%

IV. LABORATORY RULES

You must prepare a flow chart prior to each experiment. You may not begin an experiment without a completed flow chart, which is due at the start of class. Feel free to ask questions when you do not understand the instructions or the principles involved.

You must have a rubber pipette bulb, a lab coat, and safety glasses. **Lab coats and protective eye wear are REQUIRED for the experiments that use phenol.** Please do not wear shorts or sandals because phenol causes severe chemical burns when it contacts skin; wash with water to remove phenol.

Assume that all bacteria you use may cause disease. Observe the following safety rules at all times:

1. **Do not pipette by mouth.**
2. **Wear a laboratory coat and safety glasses.**
3. **Do not eat, drink, or chew gum in the laboratory.**
4. **Disinfect your bench surface before and after you work.**
5. **Insert pipette into the rubber bulb gently** to avoid breaking the pipette, which could cut your hand.
6. Disinfect contaminated equipment and surfaces.
7. Place used liquid cultures, supernatants, and glassware in autoclave containers. Discard contaminated plates and plasticware (tips and tubes) in autoclave bags. Discard organic solvents (phenol and chloroform) in waste containers.
8. **Wash your hands after you finish working.**

V. FLOW CHARTS

Prepare a flow chart in ink (not pencil) prior to each experiment and include it in your lab report. You may not participate in the laboratory exercise without a flow chart.

A flow chart outlines each procedure step by step and guides you through the experiment. If you modify a procedure during the course of an experiment, note these changes on the flow chart. Record observations on a separate page as you work.

Flow charts contain words, symbols, diagrams, and arrows. Begin your flow chart by listing the first step of the procedure. Use an arrow to connect the first step to the second, and so forth. The arrows indicate major procedural steps and direct your attention to the next task. The steps taken to proceed from one intermediate to the next are listed beside each arrow. A sample flow chart appears on the next page. Can you understand the experiment by reading the flow chart?