Promega

Instructor's Guide

Genomic DNA Purification

Accompanying Laboratory: Genomic DNA Purification

IG004
Printed in USA. 8/08

Genomic DNA Purification Instructor's Guide

Promega

Instructor's Guide

Writers:
Isobel Maciver, PhD
Michele Arduengo, PhD

Laboratory:
Terri Sundquist, MS

Animations:
Sara Klink, MS

www.promega.com

I. About This Resource

I.A. Introduction

Genomic DNA Purification is an instructional unit designed to supplement courses that introduce students to basic molecular biology. The accompanying laboratory reinforces concepts presented in class and may be used as a starting point for student-designed, inquiry-based investigation. The modular nature of this unit allows instructors to adapt individual components for their exisiting courses. Writing assignments are included in this instructor's guide for courses that must meet "writing across the curriculum" or "write to learn" requirements.

Pages of this instructor's guide, such as "Reporting Experimental Results" or the descriptions of writing assignments can be printed and distributed directly to students. Grading rubrics are provided as guidelines for instructors.

Teachers using the material in this instructional unit are asked provide feedback to **michele.arduengo@promega.com** so that we can continue to develop and improve the educational resources available from Promega. Promega products that you are using for teaching laboratories, including the products used in this laboratory, are available at a significant discount for educators in the United States. Visit **www.promega.com/us/trainingsupport** for information on the training support program at Promega.

Note: This instructor's manual is available online only.

I.B. Learning Objectives

Depending upon which parts of this instructional unit are used, students will:

1. become familiar with the basic chemistry of DNA (lecture, animations, laboratory, writing assignments)
2. be able to name steps in DNA isolation (lecture, animations, laboratory)
3. be able to describe purpose of each component/step in DNA isolation procedure (lecture, animations, laboratory)
4. be able to compare/contrast solution- and membrane-based purification methods (lecture, animations, laboratory)
5. gain experience with quantitative methods for measuring DNA yield (lecture, laboratory)
6. perform calculations to determine DNA yield (laboratory)
7. gain historical perspective on the development of our understanding of DNA structure and chemistry (lecture, writing assignments)
8. be able to report experimental data in a standard scientific format (laboratory, writing assignments)
9. improve science writing skills (writing assignments)
10. demonstrate subject mastery (writing assignments)
11. explore theoretical knowledge using the linked laboratory exercise (laboratory, writing assignments)
12. formulate original questions based on experimental data (laboratory, writing assignments)

I.C. Using the Accompanying Laboratory

The laboratory protocol that accompanies this instructional unit has two parts: a solution-based DNA purification protocol and a membrane-based DNA purification protocol. The household "home-brew" lab is designed to get students to think about the role of each step and component in the DNA isolation protocol. Students then can see how these basic principles can be applied to create the more robust and reliable system in the membrane-based purification protocol.

✷ **Inquiry-Based Learning Idea:** After having students complete the "home-brew" DNA isolation protocol, ask them to design their own "commercial" DNA isolation system complete with a user protocol.

This unit provides an instructor's manual for the laboratory as well as a separate student's manual. Both manuals are available as PDF files and can be downloaded and printed for educational use. We have included postlab "thought" questions to foster student inquiry or to serve as prompts for student-designed, inquiry-based laboratory exercises.

II. Writing Assignments

The writing assignments included in this instructor's guide can be used to meet "writing across the curriculum" or "write to learn" requirements for science courses. The laboratory report writing guide can be distributed directly to students as a guide for laboratory reports.

II.A. Reporting Experimental Results

Your laboratory report should include the following components:

Title. Your title should be specific so that a reader can know at a glance what the report is about. Specificity does not necessarily equate with length.

Abstract. Abstracts are short (50 to 100 words) summaries of the work. They often include one sentence to provide context. They state what was tested and why, the system used, and briefly describe the results. They are written as one paragraph, except in some medical literature where structured abstracts are used.

Introduction. A short section that clearly states your question or hypothesis. This section will answer the question "who cares?" by providing context and background that show why your study is important.

Materials and Methods. This section should be written so that someone can duplicate your experiment. It should be brief but informative. Any special steps taken should be explained, but standard details (e.g., how to use a pipettor) should be omitted. Quite often this section is written using the passive voice, because who did the work is not as important as what was done.

Results Section. This part of your report presents the experimental data obtained; it does NOT interpret that data. Quite often this section will use tables and graphs. Make sure that any tables you construct for your data are easily understandable. Label columns and rows. Generally put the known information (context) to the left and the new information to the right.

For example, the sample table below summarizes data from experiments to purify DNA from a variety of organisms. The organisms used are "known" and presented first (on the left); what is new is how much DNA was obtained, and it is presented to the right.

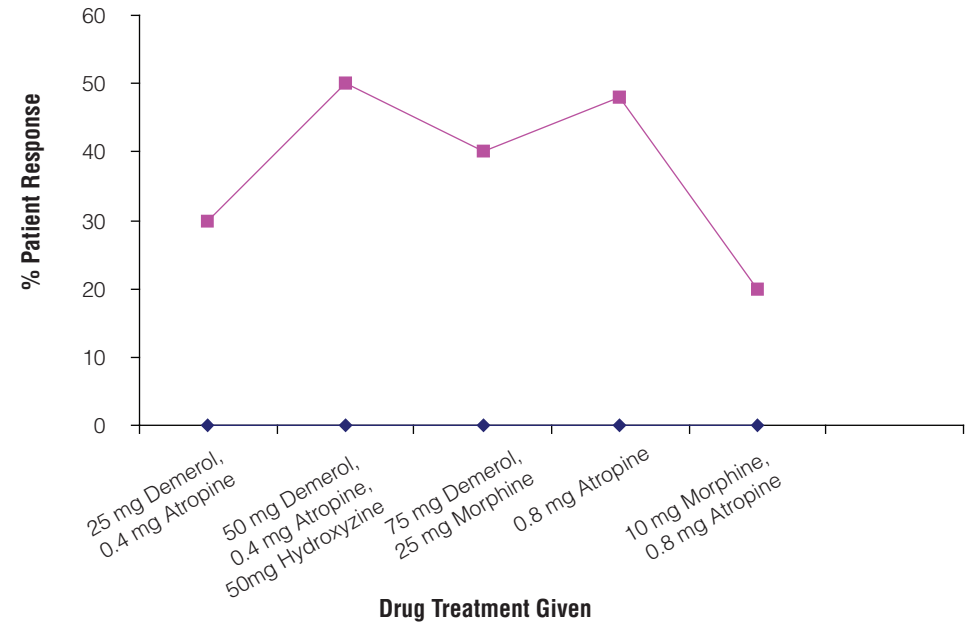
Table 1. Quantity of DNA Purified from Various Sample Types Using the Automated Purification Method.

Organism (Tissue)	Starting Material	DNA Yield (µg)
D. melanogaster (whole fly)	5 flies	1.52
Artemia fransciscana (whole shrimp)	1 shrimp, 3–4 mm	1.72
C. elegans (whole worm)	50,000 worms	0.08

Graphs are used to illustrate trends in the data that are not immediately obvious when you put your data in a table format. Do not include graphs in your laboratory report merely for the sake of including a graph. Make sure that the graph, tells an important story or shows a trend that you cannot call to your reader's attention by any other medium.

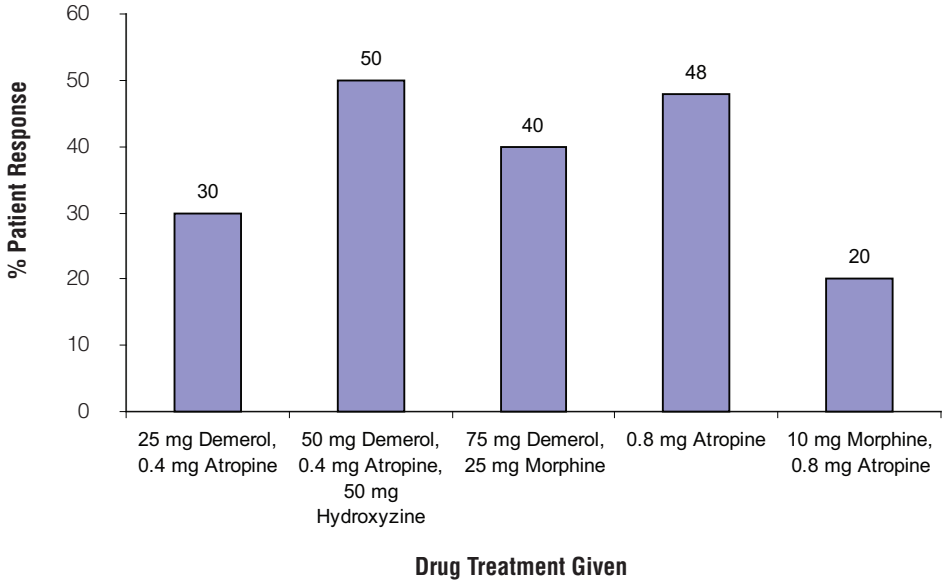
When plotting data to create graphs, make sure that you are representing your data correctly. For instance, do not plot categorical data as a continuous line. Consider the example below where categories (drug treatment) are plotted as points connected by lines:

A.



These data are better represented by a bar chart (see below).

B.



You could argue, of course, that the data presented above do not show any particular trend and might best be presented in a table.

II.A. Reporting Experimental Results (continued)

When writing the text portion of your results section, draw your reader's attention to the key patterns or trends in the data, but do not recreate your graphs in words. When you write your results section, make sure that you know what you want your reader to notice in your data.

Avoid the temptation to sum things up by saying the experiment "worked" or "didn't work". The data may not be what you expected, but if the experiment was performed correctly with all of the appropriate controls, you have results that should be presented.

Discussion Section. Here is where you will interpret your results against the backdrop of the original hypothesis or question you set out to investigate. Your discussion can be built around the following questions:

1. What were the predictions of your original hypothesis (or expectations in a descriptive study)?
2. How did the results you obtained compare to your original predictions or expectations?
3. How might you explain any unexpected results? Be sure to include information about how your positive and negative controls performed.
4. Can you suggest ways to test your explanations?
5. Do your results raise any questions for future study?

Literature Cited. This section lists all of the papers or communication that you are using to support your statements in the introduction and discussion sections. There are some standards for using references in scientific writing:

1. Do not use footnotes within the text. You can cite sources using a numerical method, where each source is assigned a number based on the first place in the paper where the source is used. Or, you can use the "author, date" format within the text and alphabetize your list of references at the end of the paper. Your instructor will tell you which style to use.
2. You can cite information from a lecture, presentation or conversation as "personal communication". You should have permission of the person you are citing before you do so.
3. Make sure that you have read the ENTIRE paper if you cite it. Do not read the abstract from a PubMed search and then cite the paper without reading the actual paper.
4. If you are citing an online source, do your homework. Who is the author or sponsor of the web site? Do they have the credentials necessary to be an authoritative voice on the subject? When was the information last updated?
5. Although *Wikipedia* is an excellent starting point for most research, it should not be a primary source for your work.

Style. Good science writing, like any good writing, must communicate an idea to the reader clearly, which means that the writer needs to use good grammar, use language correctly and proofread the work. Good science writing is judged by the readers' responses to the writing. Can the reader easily find needed information? Can the reader recall the message of the text? Can the reader make a decision or take an appropriate action after reading the text?

There are many excellent resources for writers. One of the best is the writing center at your college or university. Most writers also have one or two key style guides on hand for reference as they write.

Below are some style guides that are useful for writers in the life science and medical fields. Your instructor may assign a specific style for you to follow. If not, use one of the style guides below to guide you with your writing.

Science Writing

Council of Science Editors, Style Manual Committee. 2006. *Scientific style and format: The CSE manual for authors, editors and publishers*. 7th ed. Reston (VA): The Council.

Dodd, J.S., ed. 1997. *The ACS style guide*. 2nd ed. Washington (DC): American Chemical Society.

Iverson, C. *et al.* 2007. *AMA manual of style: A guide for authors and editor*s. 10th ed. New York (NY): Oxford University Press.

General Writing Style

Sabin, W.A. 2004. *The Gregg Reference Manual*. 10th ed. New York (NY): McGraw-Hill Irwin.

Sabin, W.A. 2008. *The Gregg Reference Manual, online*. 10th ed. New York (NY): McGraw-Hill Irwin. http://highered.mcgraw-hill.com/sites/0073545430/information_center_view0/ (accessed 7/27/2007)

Strunk, W. and White, E.B. 1979. *The Elements of Style*. 4th ed. Boston (MA): Allyn and Bacon.

II.B. Book Reviews and Discussion

Scientists involved in the elucidation of DNA structure and science historians have written extensively about the events, time period and personalities involved in this research. Three books are available that present the story of the elucidation of DNA structure from slightly different viewpoints:

What Mad Pursuit by Francis Crick

The Double Helix by James Watson

Rosalind Franklin and DNA by Anne Sayre

Assignment

Divide your students into three groups, assigning each group one of the three books above. Ask the students in each group to read their assigned book and analyze it. Have each group present their book to the rest of the class.

Suggested activities:

Have each create a timeline of dates, places, people and events important to the elucidation of the DNA structure. After all three timelines are presented to the entire class, allow the students to debate the events from the point of view of the author of the book that they read. Are there differences in the way the various authors describe the events?

Have each group tell the "story" of DNA to the rest of the class in a dramatic format from the point of view of the author of their book. After each group has told its story, ask each member of the class to write a nonbiased summary of the events. Use this opportunity to hold a discussion about objectivity in science. Is it possible to be completely objective in scientific research? How does the scientific method contribute to or hinder objectivity? Did Watson and Crick use the scientific method of inquiry when they elucidated the structure of DNA? If not, what type of inquiry did they use?

Assessment Tip:

Group activities can be difficult to grade because not all members contribute the same amount of work or talent to the group. Teamwork requires that the individuals on a team be able to work independently and reliably on their portions of the project. To avoid giving the same grade to all members of the group, regardless of contribution, there are several things you can do:

1. Have each member of the group evaluate the other members' contribution to the project. Use these anonymous evaluations to assign a percentage of the grade.
2. Test each group member on the facts of the book that was read, and include this test score in the assigned grade.
3. Ask each group member to write a short paper or analysis of the assigned book and include the grade in the final evaluation of the group work.
4. Have the group log the activities and assigned task of the group members, including whether the task was completed on time to the satisfaction of the other members of the group. Use this chart to assign a "contribution" portion of the grade.

II.C. Bioethics Case Study: Patenting Biological Material: Who Owns Your Cells?

Background

Once we identified DNA as the genetic material and solved its biochemical structure, our research questions turned to understanding how DNA determined the physical traits of an organism. Scientists used the biochemical properties of DNA to isolate it and to sequence it. In 1978, Boyer and Cohen showed that using naturally occurring enzymes as molecular tools, scientists could manipulate small pieces of DNA. Soon researchers were studying the function of single genes in cells.

The Human Genome Diversity Project (HGDP) builds even further on these early studies to take molecular biology to the level of entire populations. This project is described as an "international anthropology project that seeks to study the genetic richness of the entire human species" (as cited in 1). The goals of HGDP are to:

a. increase our understanding of human history of identity,

b. understand genetic and environmental factors involved in predisposition and resistance to disease (genetic epidemiology)

c. encourage development of local laboratories where genetic samples will be collected, stored and analyzed. (1)

Previous research based in linquistics suggests that the world contains approximately 5,000 population groups. HGDP would like to study at least 10% of these groups. Some of these will be larger western populations, but HGDP would like to include as many small, indigenous populations as possible.

All population genetics studies must be designed to fit within the auspices of international human rights law that recognizes the inherent dignity of all members of the human family. Additionally, the studies must be conducted with respect for the ethical norms of the culture in which the studies are being conducted, and extra precautions must be taken to protect the rights of vulnerable populations.

As with all human research studies, informed consent must be obtained from the indvidual subjects. However, HGDP presents an additional level of difficulty because obtaining informed consent from a population or community is more difficult. How is that obtained?

The importance of informed consent for obtaining individual genetic information is obvious. An individual has a right to be assured that genetic information will not be used later to deny health insurance or employment or to discriminate in other ways. Informed consent from an individual from an isolated indigenous population is more difficult. Can that individual truly understand what it means to donate a tissue or blood sample for research, especially for the longer term? Can a western-trained scientist adequately communicate with that individual? (2)

What about a obtaining informed consent from a community? Who has the authority to give such consent? Can an oppressive, dictatorial government of a third-world country truly speak for a marginalized indigenous population? What if a community shows a high risk for a genetic disease? Could an insurance company start denying insurance based on a person's ancestry or ethnic background based on the results on a population genetics study?

Members of small indigenous populations are distrustful of projects like HGDP, viewing them as projects in which these small, "endangered" populations are exploited

for their rare genetic material, seen as a commodity, but not valued as individuals with inherent dignity. These groups are also asking when they will begin to see the benefits promised by participating in what, for them, is extremely invasive testing and disruptive to their lifestyles. They wonder if the motivation of HGDP is truly to add to the scientific body of knowledge, especially since there is a history of natural products and knowledge obtained from indigenous populations being used for profit in the developed world, and rarely are members of the indigenous population included in the research development or analysis.

His Heart Belongs to the Jungle, But His DNA Belongs *To*?

About 260 Hagahai hunter-horticulturists live along the Yuat River deep in the Shrader Mountains of New Guinea. Mosquitoes carrying malaria are endemic to this area, and for several decades the Hagahai have suffered an increasing number of deaths among their children. In the late 1980s they asked for outside help, and a researcher from the Papua New Guinea Institute of Medical Research responded.

In 1989, 24 blood samples were collected from Hagahai men and women. The researcher said that she was collecting the blood "to see a 'binitang'–an insect–in their blood" (7). She said she assured them "it would not make them sick, but could help others" (7).

The blood samples were later used to study human T-cell leukemia virus, HTLV at the National Institutes of Health (NIH) in the United States, and several papers were published that included data from HTLV-1 detected in these samples. Additionally, U.S. Patent 5,397,696 was filed, naming researchers at the NIH and Papua New Guinea Institute of Medical Research as inventors. The patent covered "a human T-cell line (PNG-1)...and the infecting virus." The utility of the invention described in the patent was that the cell line or DNA could be used to develop a diagnostic tool or vaccine for certain kinds of leukemia. The cell line was derived from a 20-year-old male healthy Hagahai donor from the 1989 expedition. Neither the donor nor the government of New Guinea was informed of the patent or named as a beneficiary. (3–8)

The patent was challenged in US court. The challengers argued that the biological material belonged to the donor and that the researchers could not patent it. The inventors countered that the donor had voluntarily given the sample, and indeed others had argued in the past in the US "that once separated from the body the cells or DNA no longer shares a relationship with the body that it was taken from" (as cited in 6).

However the United Nations had responded to a previous attempt to patent DNA from an indigenous person by drafting language that included human genetic material as cultural property that indigenous populations are entitled to control.

Research to travel to remote locations and work on rare diseases is extremely expensive. One way to finance such efforts is through commercialization. Some argue that researchers need to be able to patent findings that come out of such research and commercialize research findings whenever possible in order to continue to bring the highest quality research possible to all parts of the globe. Eventually, these patents will result in low-cost medicines and disease prevention that will benefit all communities.

Although this particular research project was not part of HGDP, the decisions made here will influence how indigenous populations view such global research projects as HGDP and the guidelines that are drafted for HGDP researchers.

So, should the patent be allowed? If so under what conditions?

II.C. Bioethics Case Study: Patenting Biological Material: Who Owns Your Cells?

References

1. Leng, C.H. *et al*. (1995) Bioethics and Human Population Genetics Research. United Nations Educational, Scientific and Cultural Organization. (15 November)
2. Marks, B.A. and Steinberg, K.K. (2002) The ethics of access to online genetic databases: private or public? *Am. J. Pharmacogenomics* **2**, 207–12.
3. Thomas, S.M., Hopkins, M.M. and Brady, M. (2002) Shares in the human genome—the future of patenting DNA. *Nature Biotechnology* **20**, 1185–8.
4. Butler, D. (1995) Genetic diversity proposal fails to impress international ethics panel. *Nature* **377**, 373.
5. Macer, D. *et al*. (1996) Unesco and population genetics. *Nature* **379**, 11.
6. Whelan, M.L. (2007) What, if any, are the ethical obligations of the U.S. Patent Office? A closer look at the biological sampling of indigenous groups. *Duke Law and Technology Review* **14**.
7. U.S. Patent on tribesman's blood raises ethical questions. (1996) Associated Press. April 20. Goroka, Papua New Guinea.
8. Yahagihara, R. *et al*. (1991) Characterization of a variant of human T-lymphotrophic virus type I isolated from a health member of a remote, recently contacted group in Papua New Guinea. *Proc. Natl. Acad. Sci. USA* **88**, 1446–50.

II.C. Bioethics Case Study: Patenting Biological Material: Who Owns Your Cells?

Discussion Guide

1. State the problem. What is the decision that needs to be made?
2. State the facts. What do we know? What do we not know?

   a. Information about Hagahai
   b. Samples were collected
   c. Samples were used
   d. Unclear how informed consent was obtained

3. Who are the stakeholders in the decision? In what ways are the stakeholders affected? Who is most affected by the decision?

   a. Researchers
   b. Hagahai
   c. US Government
   d. New Guinea Government
   e. UN
   f. HGDP
   g. all indigenous peoples

4. What are the values involved in making the decision?

   a. confidentiality/privacy
   b. nonmaleficence
   c. individual's right to know
   d. greater good
   e. economics/profits
   f. societal costs
   g. community privacy
   h. human dignity
   i. value of life

5. Propose solutions (options) available to the decision maker.
6. Create criteria for making a decision. Rank those criteria. Based on those criteria, what would your decision be?

   The answers provided to some of the questions listed here are merely for example and are not meant to be an all-inclusive list of possibilities.

   These questions can be used to guide the entire class in discussion of the case, or they can be used by small groups of students working on the case. Alternatively, students can prepare a position paper supporting a particular plan of action using these questions as a guide for their writing.

   Students can discuss this case by role play of the various participants or they can have a pro/con debate.

VIII. Suppliers and Ordering Information

Ordering Information

Product	Size	Cat.#
Wizard® SV Genomic DNA Purification System	50 preps	A2360
	250 preps	A2361

Promega Training Support Program: Discounts for Educators in the United States

To order Promega products for your teaching laboratory (U.S. only) at a significant educational discount, visit: **www.promega.com/us/trainingsupport/default.htm**