BOOK REVIEW

Inefficient translation and replication

Human Molecular Biology: Laboratory Manual

S Surzycki

Blackwell Science Ltd., Oxford; 2003. 229 pp.

£49.95, paperback, ringbound. ISBN 0-632-04676-7

Heredity (2003) 91, 438–439. doi:10.1038/sj.hdy.6800324

Reviewed by JB Wolf

With human genomics at the forefront of forensics, medical diagnostics, and pharmaceutical research, an educated biology student should have an appreciation for the principles and applications of nucleic acid analyses and how these techniques are used to further unravel the mysteries of our health, evolution and diversity. Although specialized knowledge and equipment are required, experiments illustrating some basic molecular concepts can be adapted for use in a college classroom. The Human Molecular Biology laboratory manual provides a sequence of experiments covering a wide range of techniques. The manual is a detailed and comprehensive laboratory text designed for senior undergraduate students with extensive background knowledge in genetics and molecular biology.

In each of the experiments, the students isolate and use their own chromosomal DNA and RNA for experiments involving genotype analysis, DNA fingerprinting, random gene sequencing, and gene expression. By requiring students to use their own DNA and RNA as the starting material, any concerns about human pathogens are alleviated and the students can immediately see the relevance of their efforts to the real world. The experiments are written in a very detailed fashion with appropriate safety and hazard information given prominence in the chapters. In addition, the extensive practical tips included in each procedure are designed to ensure experimental success, which apparently was achieved when these experiments were used in laboratory classes at Indiana University. A well-written laboratory manual should have sufficient information to allow an instructor to reproduce these experiments elsewhere. Despite the extensive detail in the procedures written for the students, the manual lacks critical information for the instructor for replication. My primary concerns are the lack of sufficient information about necessary equipment and supplies and some inaccuracies in the procedures.

The first set of experiments involves the fingerprinting of DNA isolated from student cheek cells using a Southern blot to detect mini-satellite sequences in the first experiment, and a single-locus polymorphism in the second. In both of these experiments, the ability to generate the labeled probe, a reagent vital to the success of each procedure, is questionable. For the multilocus probe, the preparation of the digoxigenin (DIG)-labeled oligonucleotides is described in Table 2.2 on p. 51. The final concentrations of five of the reagents used in the reaction are either not expressed as concentrations and/or are incorrect. For example, the final concentration of

nucleotides needed for the labeling reaction should be 200 μ M, not 200 mM (a mistake repeated in Tables 4.3, 4.4, and 8.1). For the single-locus probe, the manual says that the instructor will supply the labeled probe, but the list of equipment and supplies needed for this experiment in the appendix has a reagent no longer supplied by the vendor. In addition, according to the vendor's catalog and web page, they no longer stock any other alkaline-phosphatase-labeled oligonucleotides, nor, as a quick Google search revealed, does anyone else. A custom-labeled probe could be ordered, but the sequence needed was not provided. Alternative genotyping probes are available, but these use different loci, different labels, and, in many cases, are not designed for Southern blots, making the experiments as written unusable. The experiments just described are designed to extend over nine laboratory periods and clearly constitute a major expenditure of time, effort, and supplies. Glaring inaccuracies in the key procedural steps would make any laboratory instructor wary.

In addition to having the necessary reagents, the ability to replicate experiments entails having the equipment to carry out the procedures. Air cyclers for PCR, nebulizers requiring an argon gas source, and electroporators are not often found in a typical undergraduate laboratory (the air cycler was also omitted from the list of equipment needed). Alternative procedures using more common and, in some cases, less expensive means would make the experiments more universally applicable. Additionally omitted from the equipment list are some absolutely essential items including micropipettors, micro-centrifuges, vortexes, and bacterial growth chambers, items that the author must assume are readily available but inclusion of a comprehensive list would facilitate planning a budget and setting up a laboratory for a new course. In fact, a useful addition would be an accompanying teacher's manual containing more extensive setup and purchasing options and alternative procedures.

The best set of experiments described in the book are in Chapters 5 and 6 in which the students prepare a library using their own DNA, pick some random clones for sequencing, and then compare them to DNA sequences in the public databases. This set of experiments covers many different molecular and bacteriological techniques; the results for each student pair will be varied but interesting; and the interface of biology and computer analysis is emphasized. However, I would likely substitute restriction digestion for nebulizing, substitute an isopropanol precipitation step for the more expensive column purification of DNA following cycle sequencing, modify the procedure to use chemically competent cells for the transformation, and assure the students that the white colonies on their transformation plates are in fact, Lac⁻, not 'gal⁻'.

A background section that details, quite extensively, the theory behind each of the methods to be used, prefaces each experiment. These include the principles of electrophoresis, hybridization kinetics, and theoretical predictions of maximum ligation efficiencies. Although commendable in their thoroughness, the voluminous

mathematical formulas overshadow the practical and the empirical nature of the methods and their development. And, in some cases, insufficient information is provided on important steps. For example, despite extensive background on hybridization kinetics in Chapter 2, no information is provided on the antibody/enzyme-conjugate detection step. Also, even though the probe labeling reaction involves a PCR step, this technique isn't described until Chapter 4.

While a heroic effort to provide a much-needed undergraduate laboratory guide to modern molecular

biology, the current edition is in need of extensive editing and fact checking, inclusion of alternate procedures for more commonly available equipment, and an expanded teacher preparation section. The ability to replicate accurately is essential to experimental success.

JB Wolf

*Department of Biological Sciences,
University of Maryland Baltimore County, 1000 Hilltop Circle,
Baltimore, MD 21250, USA*