BJHS Themes (2020), **5**, 225–243
doi:10.1017/bjt.2020.5

RESEARCH ARTICLE

Recipes for recombining DNA: A history of *Molecular Cloning: A Laboratory Manual*

Angela N.H. Creager*

Department of History, Princeton University, 129 Dickinson Hall, Princeton, NJ 08544-1174, USA.
*E-mail: creager@princeton.edu

Abstract

Laboratory instructions and recipes are sometimes edited into books with a wide circulation. Even in the late twentieth century, publications of this nature remained influential. For example, protocols from a 1980 summer course on gene cloning at Cold Spring Harbor Laboratory provided the basis for a bestselling laboratory manual by Tom Maniatis, Ed Fritsch and Joe Sambrook. Not only did the *Molecular Cloning: A Laboratory Manual* become a standard reference for molecular biologists (commonly called the 'bible'), but also its recipes and clear instructions made gene cloning and recombinant DNA technologies accessible to non-specialists. Consequently, this laboratory manual contributed to the rapid spread of genetic-engineering techniques throughout the life sciences, as well as in industry. As is often the case with how-to books, however, finding a way to update methods in this rapidly changing field posed a challenge, and various molecular-biology reference books had different ways of dealing with knowledge obsolescence. This paper explores the origins of this manual, its publication history, its reception and its rivals – as well as the more recent migration of such laboratory manuals to the Internet.

The invention of recombinant DNA techniques in the early 1970s consolidated the high-profile focus on molecular genetics, a trend under way since Watson and Crick's double-helical DNA model in 1953. With the tools of genetic engineering, biologists doing research on a wide variety of molecules (including enzymes, hormones, muscle proteins and RNAs, as well as chromosomal DNA), and any organism, could identify and copy the gene containing its 'code' and place that copy in a bacterial cell. At this point, the copied gene could be amplified, sequenced or analysed, or its product (usually a protein) expressed, purified and characterized. Initially, only a few molecular biologists and biochemists had the materials and know-how to do this. Many other life scientists sought this practical knowledge, to bring their labs into the vanguard of gene cloners. Manuals became a key part of this dissemination of expertise.

What did it mean to clone a gene? Simply put, cloning is copying. A gene would be isolated from all of the other genes in a cell, then inserted into a DNA 'cloning vector' that could replicate in a bacterial cell, so that the copied gene could be propagated indefinitely in a culture of the host cell, usually *E. coli*. In seeking to make copies of genes and move them around from organism to organism, biologists were inspired by bacteria,

whose ability to exchange genetic material had been recognized in 1946.[1] It turned out that there were numerous genetic units (dubbed plasmids) that enabled gene exchange among bacteria in the real world.[2] By the 1960s, researchers were using these naturally occurring gene shuttles in microbes to identify, map and characterize bacterial genes.[3]

Unsurprisingly, many biologists were more interested in studying genes found in humans and other 'higher' organisms (*eukaryotes* – plants, animals and fungi – as opposed to the one-celled *prokaryotes*, mostly bacteria). The discovery of bacterial restriction enzymes, which cleave DNA strands at specific base-pair combinations, inspired molecular biologists to attempt to use these as submicroscopic scissors.[4] In principle, if a researcher could identify and locate a particular eukaryotic gene, she could use a restriction enzyme to 'cut' it out of chromosomal DNA, insert it into a circular bacterial plasmid, then introduce the 'recombinant' plasmid into bacteria (see Figure 1).[5] That this could be done with genetic material from an animal was demonstrated first in 1973 by a collaborating group of scientists from the laboratories of Herbert Boyer (University of California, San Francisco – UCSF) and Stanley Cohen (Stanford). They inserted a frog ribosomal RNA gene into a customized bacterial plasmid. Not only was the inserted gene on its plasmid vector taken up and replicated by *E. coli*, but also the foreign DNA was transcribed into the corresponding rRNA product.[6]

However, cloning genes from higher organisms remained immensely challenging, for both intrinsic and extrinsic reasons. Intrinsically, it was technically difficult to locate specific genes in higher organisms. Both the human and mouse genomes, for instance, are 650 times larger than that of *E. coli*, so a given mammalian gene might comprise a ten-millionth of that organism's DNA.[7] The best way to identify the gene of interest was with a matching piece of nucleic acid, obtained by isolating the messenger RNA (mRNA) and using it to generate a DNA copy (cDNA). This was hard enough, but if a nucleic acid probe could not be produced, gene screening was even more arduous: every candidate clone had to be put into a protein expression vector, to search with antibodies or enzyme assay for the identifiable product.[8] Extrinsically, concerns about public-health hazards from genetically engineered pathogens led the National Institutes

1 Joshua Lederberg and Edward L. Tatum, 'Novel genotypes in mixed cultures of biochemical mutants of bacteria', *Cold Spring Harbor Symposia on Quantitative Biology* (1946) 11, pp. 113–14.

2 Joshua Lederberg, 'Cell genetics and hereditary symbiosis', *Physiological Reviews* (1952) 32, pp. 403–30.

3 E.g. E.L. Wollman, F. Jacob and W. Hayes, 'Conjugation and genetic recombination in *Escherichia coli* K-12', *Cold Spring Harbor Symposium on Quantitative Biology* (1956) 21, pp. 141–62.

4 Richard J. Roberts, 'How restriction enzymes became the workhouses of molecular biology', *Proceedings of the National Academy of Sciences of the United States of America* (2005) 102, pp. 5905–8.

5 These plasmids were often modified from naturally occurring extrachromosomal hereditary units in bacteria, often adding antibiotic resistance genes that could be used to screen for copies with the desired DNA.

6 John F. Morrow, Stanley N. Cohen, Annie C.Y. Chang, Herbert W. Boyer, Howard M. Goodman and Robert B. Helling, 'Replication and transcription of eukaryotic DNA in *Escherichia coli*', *Proceedings of the National Academy of Sciences of the United States of America* (1974) 71, pp. 1743–7. Boyer and Cohen filed patents as well as publishing results: Rajendra K. Bera, 'The story of the Boyer–Cohen patents', *Current Science* (2009) 96, pp. 760–3; Doogab Yi, 'Who owns what? Private ownership and the public interest in recombinant DNA technology in the 1970s', *Isis* (2011) 102, pp. 446–74. For background to Boyer and Cohen's work, Harrison Echols, *Operators and Promoters: The Story of Molecular Biology and Its Creators* (ed. Carol A. Gross), Berkeley: University of California Press, 2001, pp. 333–40; Doogab Yi, *The Recombinant University: Genetic Engineering and the Emergence of Stanford Biotechnology*, Chicago: The University of Chicago Press, 2015.

7 S.M. Tilghman, D.C. Tiemeier, F. Polsky, M.H. Edgell, J.G. Seidman, A. Leder, L.W. Enquist, B. Norman and P. Leder, 'Cloning specific segments of the mammalian genome: bacteriophage λ containing mouse globin and surrounding gene sequences', *Proceedings of the National Academy of Sciences of the United States of America* (1977) 74, pp. 4406–10, 4406.

8 Stephanie Broome and Walter Gilbert, 'Immunological screening method to detect specific translation products', *Proceedings of the National Academy of Sciences of the United States of America* (1978) 75, pp. 2746–9.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

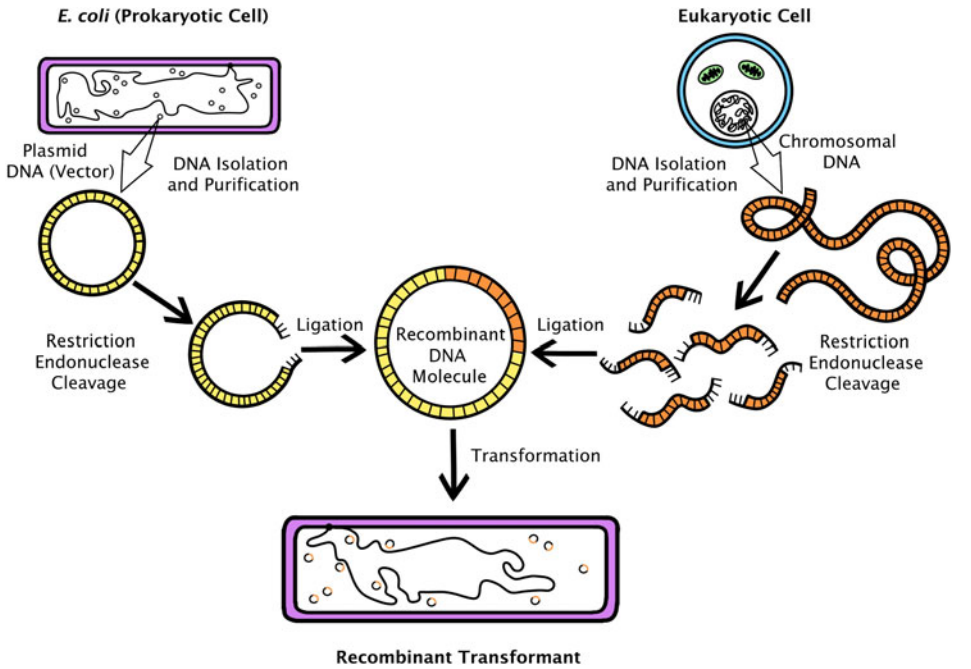

Figure 1. A typical recombinant DNA experiment depicting the cloning of eukaryotic genomic DNA fragments into a plasmid that is transformed into *E. coli*. Drawing by Georgia Creager.

of Health (NIH) to require that recombinant work on higher organisms be done in biocontainment facilities with special ventilation systems and protective clothing, masks and gloves. Few such facilities existed in universities in the 1970s, as compared with military laboratories such as Fort Dietrich, set up for working with biological-warfare agents.[9] The impact on the field was dramatic, because the NIH funded nearly all of the leading academic molecular-biology laboratories in the US.[10]

The first complete mammalian gene, that for mouse globin, was cloned in 1977 by scientists in Philip Leder's laboratory at NIH.[11] Shirley Tilghman, then a postdoc in the lab, recalls going with colleague David Tiemeier into a biocontainment facility with their cumbersome protective gear, and picking out tens of thousands of candidate clones,

9 Complying with the new NIH guidelines brought a wide range of complications, including at the community level. In Cambridge, MA, the city council did not approve Harvard's proposal in 1976 to build a biocontainment facility for its molecular biologists, instead enacting a three-month moratorium on recombinant DNA experiments. See James D. Watson and John Tooze, *The DNA Story: A Documentary History of Gene Cloning*, San Francisco: W.H. Freeman, 1981, pp. 91–135; Sheldon Krimsky, *Genetic Alchemy: The Social History of the Recombinant DNA Controversy*, Cambridge, MA: MIT Press, 1982, pp. 294–311.

10 Susan Wright, *Molecular Politics: Developing British and American Regulatory Policy for Genetic Engineering*, Chicago: The University of Chicago Press, 1994, pp. 160–218; J. Benjamin Hurlbut, 'Remembering the future: science, law, and the legacy of Asilomar', in Sheila Jasanoff and Sang-Hyun Kim (eds.), *Dreamscapes of Modernity: Sociotechnical Imaginaries and the Fabrication of Power*, Chicago: The University of Chicago Press, 2015, pp. 126–51; Melanie Armstrong, *Germ Wars: The Politics of Microbes and America's Landscape of Fear*, Oakland, CA: University of California Press, 2017.

11 Tilghman et al., op. cit. (7); D.C. Tiemeier, S.M. Tilghman and P. Leder, 'Purification and cloning of a mouse ribosomal gene fragment in coliphage lambda', *Gene* (1977) 2, pp. 173–91. The group first cloned part of a ribosomal RNA gene, to test their vector, before cloning the mouse globin gene. The ribosomal RNA gene had the advantage of existing in more than one copy per genome.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

which appeared on Petri dishes of *E. coli* as plaques. Each plaque (a specially constructed lambda vector containing a random bit of mouse DNA) had to be transferred onto a nitrocellulose filter which would then be hybridized to a radioactively labelled globin cDNA probe.[12] They successfully identified the complete beta globin gene on a seven-thousand-base pair fragment of mouse DNA, but Tilghman remarked of the gruelling labour required, 'it was ugly'.[13]

Conditions for would-be genetic engineers improved in 1978, when NIH began relaxing the safety guidelines for recombinant DNA work on higher organisms. This meant that most work with recombinant DNA from higher organisms could be conducted in ordinary labs. Many more biologists wanted to learn these techniques, not only in academia but also in the burgeoning biotechnology industry.[14] One book became a canonical guide to this field, *Molecular Cloning: A Laboratory Manual* by Tom Maniatis, Ed Fritsch and Joe Sambrook, first published in 1982 (Figure 2). Including subsequent editions, this manual sold over 200,000 copies, making it a bestseller in the cottage industry of methods publications.[15] Drawing on both documentary sources and oral histories, this paper examines how protocols, recipes and pragmatic tips for gene cloning were shared, highlighting the role of published manuals as sources of practical knowledge.[16]

Learning how

By the late 1970s, scientists and journalists alike spoke of the 'recombinant revolution'.[17] Most of the excitement revolved around the ability to clone and characterize individual genes from animals and plants, not least humans. The company Genentech, founded in 1976, led the race to clone genes and produce therapeutic proteins such as human insulin.[18] But the bounty was not restricted to the biotech industry. The techniques of genetic engineering had the potential to transform nearly every area of biomedical research. As one neuroscientist expressed it,

> Imagine setting out to purify and characterize all of the proteins in a rat brain: each protein would require a specifically designed purification scheme, perhaps kilogram

12 Lynn Enquist, also working at NIH in Robert Weisberg's laboratory, constructed the lambda vector (λgt*WES*-λB) used in this cloning experiment. D. Tiemeier, L.W. Enquist and P. Leder, 'An improved derivative of a bacteriophage λ EK-2 vector useful in the cloning of DNA molecules', *Nature* (1976) 263, pp. 526–7; Lynn Enquist, interview, 25 June 2018.

13 Shirley M. Tilghman, interview, 25 May 2018. Their group was using two methods after digesting the mouse genome into fragments to enrich the genetic material of interest about two-thousand-fold, making their brute-force screening of candidates more feasible. Otherwise they could have had to screen more than a million possible clones.

14 Nicholas Wade, 'Cloning gold rush turns basic biology into big business', *Science* (1980) 208, pp. 688–92.

15 There is some literal truth to this expression: the same year *Molecular Cloning* appeared, Cold Spring Harbor Laboratory Press moved to Urey Cottage at Cold Spring Harbor Laboratory. Nancy Ford, 'Publications', in Cold Spring Harbor Laboratory, *Annual Report 1983*, Cold Spring Harbor Laboratory Archives, p. 12. On the number of copies sold see Jan A. Witkowski, *The Road to Discovery: A Short History of Cold Spring Harbor Laboratory*, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2016, p. 267.

16 Oral histories were conducted with two authors of *Molecular Cloning: A Laboratory Manual*, participants in the Cold Spring Harbor Laboratory course from which the manual emerged, and other molecular biologists who used this manual in the 1980s. When possible, factual information was checked against documentary sources. Unpublished interview quotes are included with permission.

17 Susan Wright, 'Recombinant DNA technology and its social transformation, 1972–1982', *Osiris* (1986) 2, pp. 302–60.

18 Stephen S. Hall, *Invisible Frontiers: The Race to Synthesize a Human Gene*, New York: Atlantic Monthly Press, 1987; Nicolas Rasmussen, *Gene Jockeys: Life Science and the Rise of Biotech Enterprise*, Baltimore: Johns Hopkins University Press, 2014.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

Figure 2. Photograph of Tom Maniatis, Ed Fritsch and Joe Sambrook, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1982.

> quantities of starting material, and the determination of each primary amino-acid sequence would be complex and time-consuming. To do this for each of the many thousands of proteins in a mammalian brain would be a daunting task indeed! Yet a few micrograms of rat brain mRNA contain molecules coding for every protein in the brain: by means of recombinant DNA techniques we can use the information encoded in each mRNA to investigate not only the structure of the corresponding protein but also its genetic regulation ... This simple cloning procedure is the essence of recombinant DNA technology.[19]

For biologists new to genetic engineering, assembling the needed materials required effort and expense. An array of specialized reagents, enzymes and plasmids began to be sold by companies such as New England Biolabs, which issued their first catalog in 1975. As mentioned earlier, finding a specific gene in chromosomal DNA was a major hurdle. The method used by Tilghman and colleagues to clone the first mouse gene effectively used a purification approach, fractionating the chromosomal DNA and then searching for the desired gene. Another approach was to construct a stable collection of DNA fragments from a particular organism, which could be screened for any individual gene. Such a collection was dubbed a genomic 'library'. Tom Maniatis at Caltech, in collaboration with Arg Efstratiadis and others at Harvard, pioneered the methods for

19 Robert J. Milner, 'Recombinant DNA strategies and techniques', *Trends in Neurosciences* (1982) 5, pp. 297–300, 297.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

creating such a library and cloning genes from it.[20] He made his organism-specific libraries available to other researchers, even though each library was a biologically limited resource.[21] When Ed Fritsch, at that time a postdoc in Maniatis's lab, succeeded in developing a library of human DNA, it was reported in the *Boston Globe*, and researchers from many institutions began requesting it.[22]

Even if one had all the materials at hand, however, reliable protocols and bench know-how could be just as challenging to procure. Even the cognoscenti struggled to keep up with new knowledge. As one of the cloners of the first mouse gene put it, 'There was a lot of sharing "tricks" back then – it was pre-email of course. We all had folders or file boxes or notes stuck in notebooks of the various techniques that came as letters, phone messages, or notes from meeting talks.'[23] One key resource became available in 1979: volume 68 of the serial *Methods in Enzymology*, edited by Raymond Wu, was on *Recombinant DNA*.[24] Since 1955, *Methods in Enzymology* had provided standard protocols for biomedical researchers. This volume brought together the innovators of many of the key techniques for cloning genes. It provided a definitive set of methods, from their originators, for trained biochemists.

For DNA novices, courses were developed to teach recombinant techniques. In the autumn of 1979, Raymond L. Rodriguez, Robert C. Tait and other colleagues at the Department of Genetics at University of California, Davis offered a ten-week course entitled Advanced Molecular Genetics Laboratory. Rodriguez had been a pioneer in the field, having worked in Herbert Boyer's UCSF group that designed the most widely used plasmid cloning vector of that era.[25] His Davis course enrolled both undergraduate and graduate students, but there was, in addition, 'a heavy demand for copies of laboratory handouts and protocols'. This inspired the publication of those in a course manual, *Recombinant DNA Techniques: An Introduction*, in 1983.[26] As it turned out, while the Davis course may have been the first to offer training in recombinant DNA techniques, its book was not the first entry onto the market.

Cold Spring Harbor Laboratory had been offering summer courses on new laboratory techniques since the 1940s. One popular course, Advanced Bacterial Genetics, already offered researchers a chance to learn how to identify, map and copy genes from microbes. These courses resulted in several important manuals for bacterial genetics, making the

20 Tom Maniatis, Ross C. Hardison, Elizabeth Lacy, Joyce Lauer, Catherine O'Connell, Diana Quon, Gek Kee Sim and Argiris Efstratiadis, 'The isolation of structural genes from libraries of eucaryotic DNA', *Cell* (1978) 15, pp. 687–701. There were two kinds of library that simplified gene cloning: (1) a 'cDNA library' of cloned copies of every mRNA in a cell, and (2) a genomic DNA library, created from chromosomal DNA, not mRNA. Maniatis was involved in developing both.

21 Ron Davis at Stanford also made libraries and shared them. Echols, op. cit. (6), p. 346, lauds the non-proprietary shipping of these 'libraries … in a test tube' to labs worldwide.

22 Robert Cooke, 'Biologists start full "library" of human genes', *Boston Globe*, 22 December 1978, p. 7; Richard M. Lawn, Edward F. Fritsch, Richard C. Parker, Geoffrey Blake and Tom Maniatis, 'The isolation and characterization of linked δ- and β-globin genes from a cloned library of human DNA', *Cell* (1978) 15, pp. 1157–74. On the sharing of the library, Edward Fritsch, interview, 6 September 2018.

23 Lynn Enquist, personal communication, 23 December 2019.

24 Raymond Wu (ed.), *Recombinant DNA*, *Methods in Enzymology* (1979) 68.

25 The plasmid was called pBR322 after Bolivar and Rodriguez. Francisco Bolivar, Raymond L. Rodriguez, Patricia J. Greene, Mary C. Betlach, Herbert L. Heyneker, Herbert W. Boyer, Jorge H. Crosa and Stanley Falkow, 'Construction and characterization of new cloning vehicle. II. A multipurpose cloning system', *Gene* (1977) 2, pp. 95–113.

26 Raymond L. Rodriguez and Robert C. Tait, *Recombinant DNA Techniques: An Introduction*, Reading: Addison-Wesley Publishing Company, 1983, p. xvii.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

instruction available to many more scientists than could come to Long Island.[27] Since 1969, molecular genetic techniques for higher organisms were taught in an Animal (Tumor) Virus Course.[28] Nancy Hopkins co-taught the version of this course in 1979, but the enrolment was relatively low, in part because tumour virus know-how was available in 'most academic centers'.[29] She argued that what was most needed at CSHL was a course on molecular cloning of eukaryotic genes, and that it had to involve Maniatis, whose lab was leading the development of recombinant DNA methods.[30] Maniatis, agreeing to teach it, recruited his postdoc Ed Fritsch to join as an instructor; Hopkins remained on board as the third member of the team that first year. Fritsch would put together all of the reagents and supplies for the course and adapted protocols from the Maniatis lab and the literature – no small task.[31] Helen Donis-Keller and Catherine O'Connell served as course assistants.[32]

When CSHL advertised the postgraduate training course, Molecular Cloning of Eukaryotic Genes, 172 people applied for the sixteen spots. Thus even before it began, it immediately became the most popular course ever offered at CSHL.[33] Among the sixteen students selected for 1980, Robert Waterston would go on to a renowned career mapping the genome of a model worm (*C. elegans*) and, ultimately, playing a leadership role in the Human Genome Project.[34] Happily for the historian, he also kept his immaculately organized course notebook, as did Steve Goodbourn, now a renowned British virologist who took the course the second year.[35] Like other CSHL summer courses, the three-week schedule consisted of laboratory sessions and lectures by leading scientists in the field – in this case, twenty-one lectures (about half at 9 a.m., the others at 8 p.m.). Days were reserved for benchwork. Situated on a beautiful stretch on the coast of Long Island, Cold Spring Harbor Laboratory had a summer camp feel.

Both Hopkins and Donis-Keller recall that the night before the first class, just after they had finished setting up the teaching laboratory, there was a fire that filled the entire room

27 J.H. Miller, *Experiments in Molecular Genetics*, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1972; Ronald W. Davis, David Botstein and John R. Roth, *Advanced Bacterial Genetics: A Manual for Genetic Engineering*, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1980; Thomas J. Silhavy, Michael L. Berman and Lynn W. Enquist, *Experiments with Gene Fusions*, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1984.

28 From 1969 to 1976, CSHL had two consecutive postgraduate courses, one on Animal Viruses and the other on Tumor Viruses (or, in later years, Immunogenetics and Tumor Immunology). In 1977 Animal Viruses was replaced by Oncogenic Viruses; in 1979 this became RNA Tumor Viruses. Cold Spring Harbor Laboratory, *Annual Report 1968–Annual Report 1979*, Cold Spring Harbor Laboratory Archives.

29 James D. Watson, 'Director's report', in Cold Spring Harbor Laboratory, *Annual Report 1980*, Cold Spring Harbor Laboratory Archives, pp. 6–18, 13.

30 Nancy Hopkins, interview, 7 September 2018. As Hopkins remembers it, she made this case about a cloning course to Joe Sambrook, who had her approach Maniatis about teaching it, which he agreed to do. Maniatis recalls being asked by Watson. Tom Maniatis, 'Tom Maniatis on writing and science: molecular cloning', Oral History Collection, Cold Spring Harbor Digital Archives, 22 March 2003, http://library.cshl.edu/oralhistory/interview/james-d-watson/writer/writing-and-science-molecular-cloning, accessed 2 January 2020.

31 Fritsch interview, op. cit. (22).

32 Tom Maniatis, interview, 25 October 2016.

33 Watson, op. cit. (29), p. 13.

34 Kathryn Maxson Jones, Rachel A. Ankeny and Robert Cook-Deegan, 'The Bermuda triangle: the pragmatics, policies, and principles for data sharing in the history of the Human Genome Project', *Journal of the History of Biology* (2018) 51, pp. 693–805.

35 Bob Waterston and Steve Goodbourn kindly loaned me their notebooks; both are now deposited in the Cold Spring Harbor Laboratory Archives. Whereas Waterston's notebook consists mainly of his notes and handouts from the lectures, Goodbourn's includes photocopied protocols and some experimental notes and results. For using lab notebooks as historical sources, Frederic L. Holmes, Jürgen Renn and Hans-Jörg Rheinberger (eds.), *Reworking the Bench: Research Notebooks in the History of Science*, Dordrecht: Kluwer Academic Publishers, 2003.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

with smoke, ignited by frayed wires in a piece of equipment.[36] They stayed late into the night cleaning up the mess. As Donis-Keller recalls of the rest of the course, while the lectures were great, it was a lot of trouble to get the experiments working, even with the expertise of the instructors.[37] The students were supposed to learn how to clone like the pros, construct a library in lambda phage, screen plaques with radioactively labelled nucleic acid probes and make a cDNA clone from messenger RNA, among other techniques. Likely reflecting the precedent of the tumour virology course, there were a number of lectures on that field's exemplars, such as SV40, adenovirus, RNA retroviruses and oncogenes. In contrast to these lectures on animal viruses, the laboratory exercises focused on cloning and manipulating eukaryotic DNA.[38]

A student in the 1981 summer course (who had reapplied after not being selected in 1980), Gert-Jan van Ommen, remarked that socializing was a key part of what made the course so successful – amidst the long hours in the labs were breaks to swim in the Banbury pool, play volleyball games and attend barbeques (see Figure 3).[39] But it was the information conveyed in the course, going far beyond what was available in the published literature, that made the experience so valuable. Van Ommen recalls that 'we were taught lots of tricks like cDNA synthesis, lambda cloning, making packaging mix, and cosmid cloning'.[40] Upon returning to the Netherlands, Van Ommen wrote up an extensive report 'on all the techniques and tricks I learned there', and sent it around the molecular-biology community in the Netherlands. 'People were really over the moon with the report.'[41]

From course to book

Watson saw the opportunity to make this cloning know-how available to a wider base of users through publication.[42] Issuing an instructional guide on gene cloning from Cold Spring Harbor Laboratory would further consolidate the institution's reputation for being at the vanguard of molecular biology – and there was already a tradition from the Advanced Bacterial Genetics summer school of publishing course manuals as books.[43] Watson wanted Maniatis anchoring the team of authors, not only as an instructor but also on account of his renown in the world of cloning. But Maniatis had recently moved to Caltech, where he was busy with chairing an NIH study section, teaching and running his own lab. He only agreed to prepare a manual based on the course if he had significant help.[44] Watson persuaded Joe Sambrook, a talented and combative British tumour virologist at the lab, to join the effort. Although Sambrook never served as a formal instructor for the summer course, he was a leading research scientist at

36 Hopkins interview, op. cit. (30); Helen Donis-Keller, interview, 8 September 2018.

37 Donis-Keller interview, op. cit. (36).

38 Robert Waterston, CSH Molecular Cloning notebook, 1980; Steve Goodbourn, CSH course notes, 1981.

39 Gert-Jan van Ommen, interview, 12 July 2018.

40 Van Ommen interview, op. cit. (39).

41 Van Ommen interview, op. cit. (39). Van Ommen was more advanced than many students taking the course; he even brought along to CSHL the DNA, from a goat with an inborn genetic thyroid disease, from which he hoped to clone the thyroglobulin gene – an effort at which he succeeded. Goodbourn was also more advanced, and the two of them were labmates at the course.

42 'Tom Maniatis: gene expression, cloning and beyond', 16 November 2004, www.mcb.harvard.edu/archive/tom-maniatis-gene-expression-cloning-and-beyond, accessed 2 January 2020.

43 The course-based generation of manuals at CSHL has continued, notably with the 'Mouse Book' in 1986. Brigid Hogan, Franklin Costantini and Elizabeth Lacy, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1986; Dmitriy Myelnikov, 'Transforming mice: technique and communication in the making of transgenic animals', PhD dissertation, University of Cambridge, 2015, p. 176.

44 Maniatis interview, op. cit. (32); Maniatis, op. cit. (30).

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

Figure 3. Instructors and students working in the lab during the Molecular Cloning of Eukaryotic Genes course at Cold Spring Harbor Laboratory, summer 1981. The lack of lab coats reflects the casual atmosphere. There were more men than women students in the 1981 course, but almost equal numbers in 1980. Seated is Tom Maniatis; the man in the striped red shirt is Ed Fritsch. Photo courtesy of Gert-Jan van Ommen.

CSHL and a superb writer.[45] Fritsch, who was beginning a tenure-track faculty position at Michigan State University, remained centrally involved in the project.[46] The writing was already under way before Maniatis and Fritsch taught the course a second time with Doug Engels, in the summer of 1981.[47]

None of the authors imagined the impact the 'Maniatis manual', as it came to be known, would have when it came out in 1982. For the second edition, Sambrook took a lead role and became first author, but the book's nickname stuck, irritating him.[48] Nancy Hopkins feels she should have been invited to participate as an author, having taught the 1980 course, though Fritsch and Maniatis are puzzled as to why she did not raise this at the time.[49] As the Preface makes clear, there were in fact a number of contributors who were not listed as authors. Maniatis, Fritsch and Sambrook thanked

45 James D. Watson, 'James D. Watson on Joe M. Sambrook', Oral History Collection, Cold Spring Harbor Digital Archives, http://library.cshl.edu/oralhistory/interview/scientific-experience/molecular-biologists/j-sambrook, accessed 2 January 2020. Maniatis met Sambrook when doing his cloning work at CSHL in the 1970s. On Sambrook's research see Gregory J. Morgan, *Cancer Virus Hunters: How Tumor Virology Influenced a Century of Biology and Medicine*, under review, Johns Hopkins University Press.

46 Maniatis interview, op. cit. (32). After teaching twice with Maniatis, Fritsch taught the course for one more year, in 1982.

47 T. Maniatis, E. Fritsch and J. Sambrook, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1982, p. iii.

48 Sambrook never expressed this frustration directly to Maniatis and Fritsch. Personal communication, 13 January 2020.

49 Nancy Hopkins, personal communication, 1 July 2018; Hopkins interview, op. cit. (30). As Hopkins observes, she might not have agreed to become an author even if asked – writing the manual was an enormous task, and cloning technology was not her area of expertise.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

individual scientists (e.g. Brian Seed, Doug Melton) for providing particular protocols or for supplying the anthology of methods for a chapter (Nina Irwin).[50] In addition, Maniatis, Fritsch and Sambrook provided full references for methods adapted from the published literature.[51] Attribution was harder to ascertain for other protocols. As they stated,

> We have tried to give credit at appropriate places in the text to the people who originally developed the procedures presented here, but in many cases tracing a particular method to its undisputed roots has proved to be impossible. We therefore wish to apologize – and to express gratitude – to those we have been unable to acknowledge for an idea, procedure, or recipe. Our major function has been to compile, to verify, and, we hope to clarify; less frequently we have introduced modifications, and only in rare instances have we devised new protocols.[52]

In short, manuals raise the same problems of credit as other compilations, such as atlases and databases.[53] Though the authors (especially Maniatis and Fritsch) had personally developed key methods in the book, their role as authors of the manual made them stand-ins for many other scientists who had pioneered techniques. Not surprisingly, researchers began citing the manual rather than the original literature.[54] Sometimes, methods acquire an eponymous name, such as Maxam–Gilbert sequencing or Southern blotting. But for most methods, the successful circulation (and adaptation) of a lab recipe or method subverted conventional notions of authorship and credit.[55]

That said, *Molecular Cloning* achieved a distinctive authorial voice, one aimed at the novice, but not the amateur. The three authors explain in the Preface that because 'the manual was originally written to serve as a guide to those who had little experience in molecular cloning, it contains much basic material'.[56] Through chapter introductions that functioned in some respects like a textbook, the manual communicated enough about the science behind the recipes that users could troubleshoot the problems they encountered.[57] For instance, the first chapter on plasmids provided general information about these cloning vectors and genetic maps of the most commonly used ones, and outlined the three most popular methods of inserting a gene. As the authors note, 'In principle, cloning in plasmid vectors is very straightforward'.[58] They then go on to enumerate the usual complications, and include a special section covering 'Problems in cloning large DNA fragments in plasmids'.[59] This leads to sections on vectors used to handle larger

50 Maniatis, Fritsch and Sambrook, op. cit. (47), p. iv.

51 Of course, neither the scientists thanked in the Preface nor those who had published the original methods received royalties from the manual, even when attribution was maintained.

52 Maniatis, Fritsch and Sambrook, op. cit. (47), p. iii.

53 Bruno J. Strasser, 'Collecting, comparing, and computing sequences: the making of Margaret O. Dayhoff's *Atlas of Protein Sequence and Structure*, 1954–65', *Journal of the History of Biology* (2010) 43, pp. 623–60, 644; Bruno J. Strasser, 'The experimenter's museum: GenBank, natural history, and the moral economies of biomedicine,' *Isis* (2011) 102, pp. 60–96.

54 For one early example: Lance S. Davidow, Diane Apostolakos, Michele M. O'Donnell, Alan R. Proctor, David M. Ogrydziak, Rod A. Wing, Irene Stasko and John R. DeZeeuw, 'Integrative transformation of the yeast *Yarrowia lipolytica*', *Current Genetics* (1985) 10, pp. 39–48.

55 For more on protocols as a genre of collective or quasi-anonymous scientific text see Hans-Jörg Rheinberger, '"Discourses of circumstance": a note on the author in science', in Mario Biagioli and Peter Galison (eds.), *Scientific Authorship: Credit and Intellectual Property in Science*, New York: Routledge, 2003, pp. 309–23, 318.

56 Maniatis, Fritsch and Sambrook, op. cit. (47), p. iii.

57 Michael S. Levine, interview, 13 September 2016; Jan Witkowski, personal communication, 31 March 2018.

58 Maniatis, Fritsch and Sambrook, op. cit. (47), p. 11.

59 Maniatis, Fritsch and Sambrook, op. cit. (47), p. 14.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

pieces of DNA. A table lays out which of the four vectors was best for various experimental goals, such as cloning large DNA fragments, sequencing DNA or expressing foreign genes in *E. coli*.[60] This chapter repeats, and expands upon, material presented in the 1980 CSHL summer course.[61] Each of the other eleven chapters similarly instructs the reader on the relevant biology as well as giving practical advice in selecting methods. The text is also clear on omissions, such as 'enzymes that find occasional use in molecular cloning but that are not necessary to carry out any of the procedures described in this manual'.[62] By contrast, chapters in the *Methods in Enzymology* volume 68 on *Recombinant DNA*, which also provided protocols, tended to be more like review articles in their comprehensive coverage. Readers might need to consult chapters by several different authors to understand the alternative strategies available for reaching their experimental goal.[63]

The chapters of *Molecular Cloning* are organized around specific materials or procedures (e.g. Chapter 4, 'Enzymes used in molecular cloning'; Chapter 7, 'Synthesis and cloning of cDNA'). The overall sequence of information reflects the key choices involved in identifying and manipulating DNA, in the general order of steps required for cloning a gene. The second chapter, 'Propagation and maintenance of bacterial strains and viruses', provides basic instruction on isolating single colonies of bacteria and verifying strains through genetic markers, as might be taught in a microbiology course, as well as techniques more specific to molecular cloning, such as large-scale preparation of the vector bacteriophage lambda. This combination of instruction on basic lab methods with more specialized techniques distinguished *Molecular Cloning* from both textbooks and methods papers in the scientific literature.

The book includes numerous drawings showing how to perform various procedures, such as recovering purified bacteriophage from a caesium chloride gradient, or how to pour an electrophoresis gel, in both cases showing where and how to position the hands (see Figure 4).[64] Integrated into the text are hundreds of tables and diagrams, including detailed genetic maps, as well as sample photographs of results. A well-furnished molecular biology lab of the time relied on a local machine shop to fabricate gel electrophoresis tanks; the manual included all the needed information in engineering drafting, with complete measurements.[65] Not all materials had to be custom-made, of course, and the manual mentioned many lab supplies by brand, such as Kodak X-Omat AR film, Whatman-52 filter paper, Sigma Type-III sodium salt DNA, and Dowex XG-8 mixed-bed resin. In addition, the authors included specific tips on how to keep costs down on restriction enzymes, the most expensive commercial reagents for gene cloning.[66]

As a physical object, the 545-page manual brought together text, photographs, tables and diagrams, as well as countless technical symbols and some equations. The editing and layout of such a complicated publication must have been laborious, but the final product is clear and easy to read. Notably, the design is much more sophisticated and pleasing than that of the Advanced Bacterial Genetics manual that Cold Spring Harbor brought out just two years prior, which looked more like a print-out than a book, lacking any integration of images into the text.[67] *Molecular Cloning* did borrow one feature of that earlier manual,

60 Maniatis, Fritsch and Sambrook, op. cit. (47), p. 54.

61 Waterston, op. cit. (38), notes on 'Overview of plasmids,' 30 June 1980.

62 Maniatis, Fritsch and Sambrook, op. cit. (47), p. 107.

63 J.C. Wotton, 'Review of *Methods in Enzymology*, vol. 65, *Nucleic Acids* and *Molecular Biology*, and vol. 68, *Recombinant DNA*', *Heredity* (1981) 46, pp. 142–4.

64 Maniatis, Fritsch and Sambrook, op. cit. (47), pp. 105, 154.

65 Maniatis, Fritsch and Sambrook, op. cit. (47), pp. 154–5.

66 Maniatis, Fritsch and Sambrook, op. cit. (47), p. 108.

67 Davis, Botstein and Roth, op. cit. (27). This is in contrast to the CSHL's Advanced Bacterial Genetics manual by Miller, op. cit. (27), which is professionally produced, including colour images.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

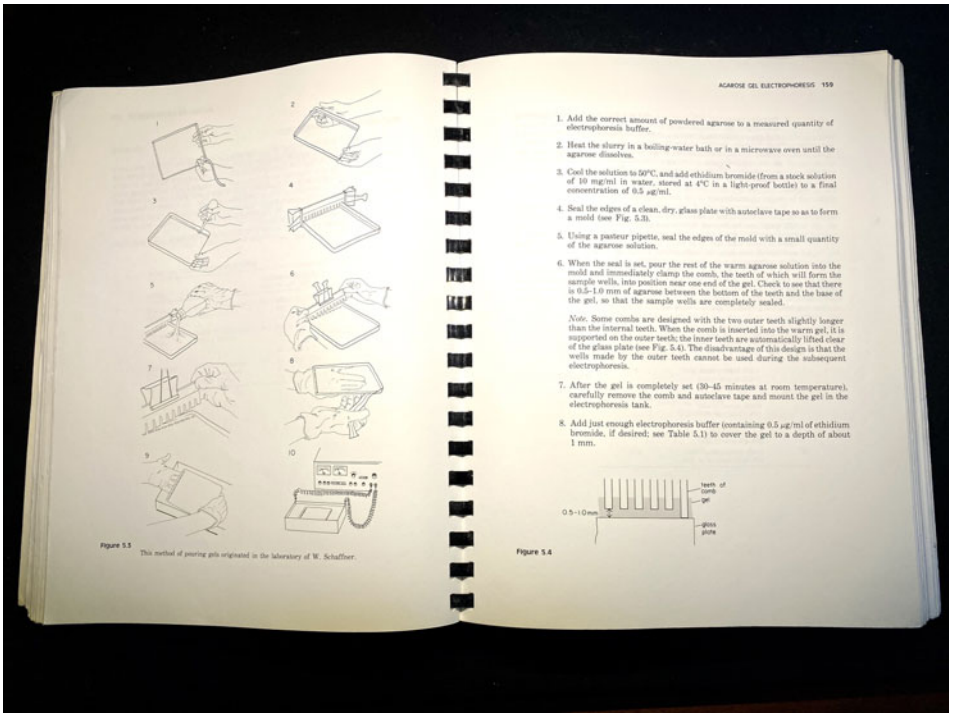

Figure 4. Instructions and diagram on how to pour an agarose gel for electrophoresis. In this set-up apparatus, agarose is used to separate a mixed population of nucleic acid fragments by length (in base pairs). Ethidium bromide is added to the gel to make the DNA visible under ultraviolet light. These descriptions include tips on the 'combs' that create the wells for DNA samples. *Molecular Cloning*, op. cit., pp. 158–9.

though: a plastic ring-comb binding (see Figures 2 and 4).[68] As David Crotty observed, some users complained that this binding served to 'deliberately get the book to fall apart forcing you to buy a new copy'.[69] The advantage, however, was that the manual could be laid flat on a laboratory bench, a feature that the majority of users praised. The much-expanded second edition of *Molecular Cloning* appeared as three thick volumes in 1989; the plastic ring-comb binding remained.[70]

Sales and rivals

Just as Watson expected, *Molecular Cloning* met a strong demand. There were orders for more than five thousand copies before the publication date.[71] Consequently, the press

68 Stephanie Radner, Yong Li, Mary Manglapus and William J. Brunken, 'Joy of cloning: updated recipes', *Trends in Neuroscience* (2002) 25, pp. 594–5.

69 David Crotty, '*Molecular Cloning* (AKA Maniatis, AKA The Bible) at 25', 22 October 2007, *Bench Marks*, https://cshbenchmarks.wordpress.com/2007/10/22/molecular-cloning-aka-maniatis-aka-the-bible-at-25, accessed 2 January 2019.

70 J. Sambrook, E.F. Fritsch and T. Maniatis, *Molecular Cloning: A Laboratory Manual*, 2nd edn, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989. As Karen-Beth Scholthof has noted, you could also easily lay the manual flat on a photocopy machine.

71 James D. Watson, 'Report', in Cold Spring Harbor Laboratory, *Annual Report 1982*, Cold Spring Harbor Laboratory Archives, pp. 5–16, 12.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

sold 5,113 copies the first month of its appearance, July 1982.[72] Sales remained robust that autumn, and in 1983 Cold Spring Harbor Laboratory Press sold more copies of *Molecular Cloning* than all of its other titles together (37,337 versus 24,234). The book went through four printings during its first six months, and three more were issued the following year. In explaining the surprisingly high sales number, the *Annual Report 1982* points to the manual's 'rapid adoption by many, many university courses'.[73]

To be clear, students were not the only ones driving sales; working biologists were also buying the book in droves.[74] As a reviewer for the British Society for Developmental Biology put it, 'no laboratory with any serious interest in molecular biology of development and their [*sic*] cloning should be without it'.[75] The testimony of historian of science Nick Hopwood confirms this view:

> When I worked in developmental biology between 1986 and 1991 only two books had permanent places on my bench. The first, 'Maniatis', was a manual of the molecular cloning methods that our Cambridge laboratory was using to identify genes that specify muscle development in the South African clawed frog ... I know the ring-bound recipes of Tom Maniatis *et al.* inside out.[76]

The reliability of the protocols was a major reason for the book's popularity. As one admirer put it, 'Just like the cookbooks of Betty Crocker and Fannie Farmer, the molecular cloning manual was chock full of recipes that worked'.[77] Another reviewer proclaimed, 'In our laboratory, mirabile dictu, the procedures in this manual nearly always work'.[78]

The trustworthiness of *Molecular Cloning*, however, did not solve the problem of keeping abreast of new and improved methods. Some researchers complained that the manual was out of date as soon as it was published.[79] Plans for a second edition, scheduled for 1984, were under way, but it was deferred for five more years as the first edition went through sixteen printings.[80] The rapid pace of change in molecular biology was blamed for the difficulty in updating the book. To take the most prominent example, the development of polymerase-chain reaction (PCR) by Kary Mullis and others at Cetus Corporation in 1985 made it possible to amplify a piece of DNA in a test tube, transforming cloning methods.[81] Thereafter, Crotty contended, a new *Molecular Cloning* manual without it would be 'pointless'.[82]

72 Memorandum from Susan Gensel to Jim Watson, 10 December 1982, re: sales at the American Society for Cell Biology meeting, James D. Watson Collection, Cold Spring Harbor Laboratory Archives, Record Group III, Series 4 (hereafter Watson Papers).

73 Watson, op. cit. (71), p. 12.

74 One can see this from sales at professional meetings: memorandum from Gensel to Watson, op. cit. (72). At that meeting *Molecular Cloning* sold eighty-three copies, and all the other CSHL Press sales together, twenty-two titles in all, made up 102 copies.

75 British Society for Developmental Biology Newsletter VII, October 1982, review of *Molecular Cloning: A Laboratory Manual*, copy in Watson Papers.

76 Nick Hopwood, 'Visual standards and disciplinary change: normal plates, tables and stages in embryology', *History of Science* (2005) 43, pp. 239–303, 239.

77 Steven L. McKnight, 'Pure genes, pure genius', *Cell* (2012) 150, pp. 1100–2, 1102. For more on *Molecular Cloning* and 'cookbook technologies' see Joan Fujimura, *Crafting Science: A Sociohistory of the Quest for the Genetics of Cancer*, Cambridge, MA: Harvard University Press, 1996, pp. 84–6.

78 George McCorkle, 'Review of *Molecular Cloning: A Laboratory Manual*', *American Scientist* (1983) 71, p. 418; also quoted in Crotty, op. cit. (69).

79 Crotty, op. cit. (69).

80 Mala Mazzullo, Cold Spring Harbor Laboratory Press, personal communication, 3 April 2019.

81 Paul Rabinow, *Making PCR: A Story of Biotechnology*, Chicago: The University of Chicago Press, 1996.

82 Crotty, op. cit. (69).

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

When the second edition of *Molecular Cloning* appeared in 1989, with the authors now listed as Sambrook, Fritsch and Maniatis, it was received just as enthusiastically as the first.[83] As a reviewer in *Nature* put it,

> Few molecular biologists welcome publication of any of the many protocol books that promise to be the single source for their laboratory methods. For the most part, such laboratory methods fall far short of this goal. So why the excitement surrounding the long-awaited second edition of the classic guide, *Molecular Cloning*, which first appeared in 1982? The original version immediately filled the need for an anthology of laboratory procedures pertinent to the emerging field of recombinant DNA. With the 545-page spiral-bound paperback in hand, virtually any experimentalist could make a stab at cloning and have a reasonable expectation of success.[84]

Both the first and second editions were reviewed in other languages, and translations appeared. A Russian translation of the first edition appeared in 1984; Chinese translations of the first edition came out in 1987 and of the second in 1992. Maniatis was told by travelling scientists in the early 1990s that 'every lab they go to in China has the cloning manual on their desk'.[85]

Reflecting its canonical status, *Molecular Cloning* was generally referred to by its users as the 'bible'.[86] Extending this common metaphor, one biochemist made reference to 'those who daily worship the Cold Spring Harbor idol'.[87] But the deity had rivals. Its main competitor in the first decade was *Current Protocols in Molecular Biology*, introduced in 1987 by a group of researchers based at Massachusetts General Hospital.[88] Sarah Greene was the original publisher, but the series was soon bought by Wiley. Rather than being written by three authors, this manual was produced by an entire team of scientists, who contributed individual pieces on various techniques. In addition, *Current Protocols* had a very different way of dealing with the rapid growth (and obsolescence) of techniques – the book was designed to be expanded via subscription. Through a quarterly update service, subscribers received supplements to insert into the original loose-leaf binder, which was separated into sections by preprinted dividers (see Figure 5). This meant that the table of contents also needed frequent updating. Five thick binders were published in the original series. The first volume covered most of the topics and methods in *Molecular Cloning*.

Maintaining the three-ring binders of *Current Protocols* as supplements arrived was inconvenient for users. Karen-Beth Scholthof, who was a postdoc in plant virology in Berkeley at the time, was responsible for updating the *Current Protocols* binders in the lab she worked in:

> Your writing about *Current Protocols* reminded me of what a terrible system it was. I was assigned to add the quarterly updates (and remove the 'old' pages). This was such a hassle – having to take the entire binder apart to insert the new pages.

83 Sambrook, Fritsch and Maniatis, op. cit. (70). This edition was in three volumes.

84 Stuart Orkin, 'By the book', *Nature* (1990) 343, pp. 604–5, 604.

85 Mike Fortun interview with Tom Maniatis, February 1992, as quoted in Fujimura, *Crafting Science*, p. 84.

86 David Crotty found the first such reference in a *New York Newsday* piece from 1984, which I have not been able to recover. Crotty, op. cit. (69). One prominent reviewer commented, 'This book is omnipresent in molecular biology laboratories and is utilized to the point where it is frequently referred to as "The Bible".' Kevin Struhl, 'Cloning cookbook for the laboratory', *Nature* (1985) 316, p. 222.

87 S.J.W. Busby, 'Comprehensive cloning', *Trends in Genetics* (1988) 4, p. 352.

88 The Harvard-affiliated editors were Frederick Ausubel, Robert Kingston, Jonathan Seidman and Kevin Struhl.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

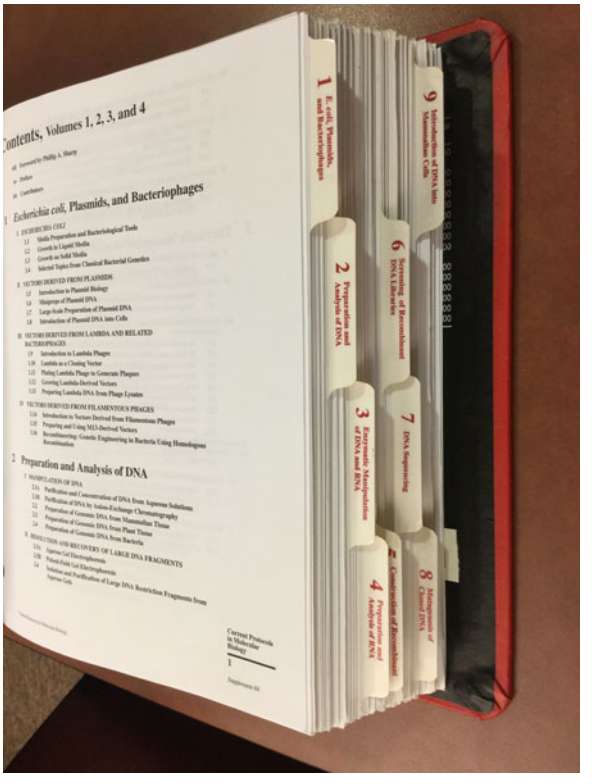

Figure 5. Volume I of *Current Protocols in Molecular Biology* opened to reveal how the protocols are organized by dividers in each three-ring binder. The topics in this volume are similar to those covered in *Molecular Cloning*. Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A. Smith and Kevin Struhl (eds.), *Current Protocols in Molecular Biology*, 5 vols., New York: John Wiley & Sons, 1987.

> The spiral-bound Maniatis was so much more user-friendly and the group annotations made it a kind of living document for the lab.[89]

Given the unwieldiness of the *Current Protocols* loose-leaf format, in 1989 Wiley published *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*. This single-volume work was bound as a traditional text, with wide pages in a format that would prop open easily on the back of a lab bench. That same year the second edition of *Molecular Cloning* appeared, and more than seven thousand scientific articles cited the title that year alone. From 1991 to 2000, editions of *Molecular Cloning* were being cited over ten thousand times per year; by comparison, *Current Protocols in Molecular Biology* peaked at 2,520 citations in 1998.[90]

There were many other manuals besides the rivals *Molecular Cloning* and *Current Protocols* – some geared towards students, with discrete lab exercises, and some to more advanced practitioners aiming to incorporate new techniques (e.g. *PCR Cloning Protocols*).[91] Others sought a place on the bench as essential reference tools. Bernard Perbal's *A Practical Guide to Molecular Cloning* combined up-to-date protocols with useful information on commercially available enzymes and cloning vectors.[92] In effect, this

89 Karen-Beth G. Scholthof, personal communication, 5 May 2018.

90 Citation analysis done using the Cited Reference search (with the appropriate abbreviations) on Web of Science Core Collection.

91 Bruce A. White (ed.), *PCR Cloning Protocols: From Molecular Cloning to Genetic Engineering*, Totowa: Humana, 1997. This was published as vol. 67 of *Methods in Molecular Biology*.

92 Bernard V. Perbal, *A Practical Guide to Molecular Cloning*, New York: Wiley, 1984 (2nd edn 1988).

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

guide compiled what was available, but scattered, in free handbooks from companies such as Amersham, Boehringer and Pharmacia, as well as listing equipment needed for molecular biology and safety guidelines. Along similar lines, Terence A. Brown at the Department of Biomolecular Sciences at Manchester issued a book called *Molecular Biology Labfax*, a 'compendium of essential information – on genotypes, reagents, enzymes, reaction conditions, cloning vectors, and suchlike – that is needed to plan and carry out molecular biology research'. As the author explained,

> Some of this information is already available in cloning manuals, catalogues and possibly on pieces of paper kept somewhere safe, but tracking down exactly what you need to know takes time and can be a frustrating experience. With molecular biology becoming an interesting sophisticated science, an acute need has arisen for a *databook* to complement the traditional cloning manuals.[93]

The notion of a 'databook' stands in an odd relationship to database, and the updating of such hard-copy publications attests to how slowly search engines came to replace other information-hunting methods.

The true test of any such reference work, however useful, was to become indispensable. As one reviewer remarked of *Labfax*,

> To use books such as these to the best advantage, you need to spend a long time becoming familiar with them and, frankly, life is too short to master every book. I suspect that most mortals learn to feel their way around just one or two of these massive works and then use them over and over again: my favourites are *Molecular Cloning: A Laboratory Manual* and the 1989 Pharmacia Molecular Biology Catalogue (which, incidentally, is much better organized than the current catalogue!). Of course, Labfax contains a lot more facts than either of my two favourite works, but are they facts that I am ever going to want to know?[94]

This comment crystallized the task facing publishers of compendia on cloning: how to determine exactly the current information most needed by biologists, in a format they would be happy to pay for, more than once as editions became obsolete?

The challenge of updating was more easily accommodated by the growth of multimedia technologies in the 1990s. By its third edition in 2001, *Molecular Cloning: A Laboratory Manual* came with a CD-ROM 'Lab Book'. It also had an associated website, MolecularCloning.com, with the book's protocols and 'discussion space for those asking questions or making alterations to techniques'.[95] However, the site did not have a 'mechanism for continuously adding updated new material', which was considered a major drawback given the technical feasibility of doing this online.[96] But who was to be the maintainer of up-to-date knowledge? The authors? The readers? In reality, there was not much community participation in the manual's online forum. Efforts by other publishers to create 'yet another "myspace for biologists"' also met with disappointment.[97]

93 T.A. Brown (ed.), *Molecular Biology Labfax*, Oxford: BIOS Scientific Publishers, 1991, p. v, emphasis added.

94 Steve Busby, 'Drowning by numbers: review of *Molecular Biology Labfax*, edited by T.A. Brown', *Trends in Genetics* (1992) 8, p. 77.

95 Crotty, op. cit. (69).

96 Crotty, op. cit. (69).

97 Crotty, op. cit. (69). In this respect, *Molecular Cloning* seems different from feminist health manuals for which readers volunteered their own perspectives and knowledge. See Wendy Kline, '"Please include this in your book": readers respond to *Our Bodies, Ourselves*', *Bulletin of the History of Medicine* (2005) 79, pp. 81–110.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

By contrast, ongoing maintenance of methods, recipes and protocols was vibrant at the level of individual laboratories. In fact, the more manuals and handbooks expanded to include more information, the greater was the need for abstracting the most essential instructions. In this sense authors of manuals face the same dilemma as those of handbooks, which tend to grow fatter with each edition. In response, CSHL Press issued a single volume called *The Condensed Protocols from Molecular Cloning: A Laboratory Manual*.[98] Some laboratories created their own solutions. As Karen-Beth Scholthof recounts about *Molecular Cloning*, 'After it went to three volumes (Sambrook, although we continued to refer to it as Maniatis), we started formally writing and sharing lab recipes for common protocols. I also had an index card/recipe box with my favorite concoctions and cheat sheets for various calculations.'[99] At Washington University, Helen Donis-Keller developed a customized laboratory manual, specifically modelled on *Molecular Cloning*, with the methods and protocols her group relied upon.[100] As she commented,

> We found that even though protocols were published by other labs or in the literature, we needed to test them ourselves and prepare updated standardized methods for use by everyone in the lab. The amounts of the various reagents we typically used might be different from the published protocols as well or we might have found that a different reagent than that specified might be more appropriate for our purposes. I really wanted everyone to work from the same set of protocols to maintain consistency and in order to make troubleshooting easier when things went wrong.[101]

Her in-house manual, while ensuring uniformity in methods in a large (forty-person) lab, also made sure that know-how was not lost when people left. Each protocol was formatted in the same way, with the name of the person who wrote and tested it, the date, instructions, time needed, any special reagents or equipment required, and, at the end, the sources (which included, for example, *Molecular Cloning*).[102] As the annotations of 'NOT DONE' in the table of contents suggest, a customized manual of this sort was usable as a work-in-progress, updated and reimagined as needed.

The original challenge that had prompted *Molecular Cloning*, the need for instructions on how to copy genes from unsequenced chromosomal DNA, was simplified by the Human Genome Project, which was funded by the US Congress in 1988.[103] By the early 2000s, the full genomic sequences of several organisms were online and publicly available to researchers; biologists could retrieve their genes of interest more easily, and the comparisons available through sequence data inspired a new generation of *in silico* rather than *in vitro* experiments.[104] To be sure, *Molecular Cloning* expanded its reach for the age of genomics, and remains a relevant resource. But the massive shift of reference works to the Internet – including serials like *Methods in Molecular Biology*, started in 1983 – made

98 Joseph Sambrook and David Russell, *The Condensed Protocols from Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 2006.

99 Scholthof, op. cit. (89).

100 Donis-Keller interview, op. cit. (36); and Helen Donis-Keller, personal communication, 28 September 2018.

101 Helen Donis-Keller, personal communication, 28 December 2018.

102 Helen Donis-Keller, personal communication, 28 September 2018.

103 For a recent overview of the large literature on the Human Genome Project see Jones, Ankeny and Cook-Deegan, op. cit. (34).

104 Hallam Stevens, *Life out of Sequence: A Data-Driven History of Bioinformatics*, Chicago: The University of Chicago Press, 2013.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

the publication of manuals, and the release of new editions, less eventful.[105] In addition, the publication of protocols has also migrated back to journal publication, now also online. As Crotty notes, 'The lessons learned from MolecularCloning.com led to *CSH Protocols*. We realized that if we were going to continuously add new material, we had to think of the project as a journal, and give it a full editorial staff'.[106] This new journal (and many other similar titles, such as *Nature Methods*) effectively moved the development and dissemination of molecular biology protocols back to the research literature, a full circle after the gene cloning manual's success.

Conclusions

Even among scientific methods, the history of gene cloning is unusual. This set of techniques went in the short space of ten years from being considered dangerous and in need of biocontainment to becoming adopted in laboratories throughout the world. There are many reasons for this radical and rapid shift, not least the politics of deregulation and the massive infusion of venture capital into biotechnology. But changing motivations for the spread of knowledge cannot explain *how* the practices spread so quickly, through a generation of technical workers, most of whom had completed their formal education. Manuals and other sources of how-to knowledge provided guidance to countless life scientists and technicians who mastered these cutting-edge techniques.

Among the manuals available, *Molecular Cloning* succeeded remarkably in becoming an indispensable element of the biology lab, often figured as the genetic 'kitchen'. As one enthusiastic reviewer of the third edition, which was published in 2000, put it,

> In every kitchen there is at least one indispensable cookbook. Sambrook and Russell's *Molecular Cloning: A Laboratory Manual* fills the same niche in the laboratory. Like its kitchen counterparts (e.g. Rombeck's *Joy of Cooking*) Sambrook's *Molecular Cloning* has information to help both the inexperienced and advanced user.[107]

These comparisons are even more apt when one considers some of the supplies in molecular biology labs. Since the 1950s, bacterial geneticists had been using blenders to disrupt bacterial infection or mating. Methods developed in the 1970s for identifying genes using radioactively labelled nucleic acid probes employed 'Seal-a-Meal' bags for the hybridization step.[108] Carnation non-fat dry milk became the blocking agent of choice for Western blots.[109] There are countless other such mundane connections, though of course the traffic between lab chemistry and cooking is hardly new.[110]

Yet seeing the manual as one's standby cookbook for gene cloning obscures a key difference, namely the rapid pace of change in DNA methods that molecular biologists faced in the 1980s. Of course, researchers had and have clever strategies for exploiting trustworthy protocols while also trying to master the latest methods. But there remains a

105 Although the first volume of *Methods in Molecular Biology* appeared in 1983, citations of its many volumes (now over two thousand) took off in the 2000s, as assessed by searches on Web of Science Core Collection.

106 Crotty, op. cit. (69).

107 Radner et al., op. cit. (68).

108 Maniatis, Fristch and Sambrook, op. cit. (47), p. 587.

109 Karen-Beth G. Scholthof, personal communication, 21 May 2019.

110 Ho Ping-Yü and Joseph Needham, 'The laboratory equipment of early medieval Chinese alchemists', *Ambix* (1959) 7, 58–112; Elizabeth Spiller, 'Recipes for knowledge: maker's knowledge traditions, Paracelsian recipes, and the invention of the cookbook', in Joan Fitzpatrick (ed.), *Renaissance Food from Rabelais to Shakespeare*, London: Ashgate, 2009, pp. 55–72; Elaine Leong, *Recipes and Everyday Knowledge: Medicine, Science, and the Household in Early Modern England*, Chicago: The University of Chicago Press, 2018.

https://doi.org/10.1017/bjt.2020.5 Published online by Cambridge University Press

central trade-off in laboratory how-to literature between reliability and state-of-the-art technique. During the height of the recombinant revolution, *Molecular Cloning* seemed able to provide both. But in biology, even the bible has to contend with obsolescence.

Acknowledgements. I thank Tom Maniatis, Ed Fritsch, Nancy Hopkins, Helen Donis-Keller, Gert-Jan van Ommen, Bob Waterston, Steve Goodbourn, Shirley Tilghman, Mike Levine, Karen Scholthof, Lynn Enquist and Arnold Levine for their generosity with time, information, materials and corrections; Kat Maxson Jones and Gina Surita for assistance with research and editing; and Jan Witkowski, Alex Gann, Marta Hanson, Mathias Grote and two referees for helpful suggestions. Gert-Jan von Ommen and Nancy Hopkins have allowed copies of their wonderful personal photographs from the 1980 and 1981 summer courses to be deposited in Cold Spring Harbor Laboratory Archives, along with the notebooks of Bob Waterston and Steve Goodbourn.

Cite this article: Creager ANH (2020). Recipes for recombining DNA: A history of *Molecular Cloning: A Laboratory Manual*. *BJHS Themes* **5**, 225–243. https://doi.org/10.1017/bjt.2020.5