AMERICAN SOCIETY FOR MICROBIOLOGY | JMBE Journal of Microbiology & Biology Education

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| Perspective

How advancements in molecular biology impact education and training

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ABSTRACT Molecular biology, broadly defined as the investigation of complex biomolecules in the laboratory, is a rapidly advancing field and as such the technologies available to investigators are constantly evolving. This constant advancement has obvious advantages because it allows students and researchers to perform more complex experiments in shorter periods of time. One challenge with such a rapidly advancing field is that techniques that had been vital for students to learn how to perform are now not essential for a laboratory scientist. For example, while cloning a gene in the past could have led to a publication and form the bulk of a PhD thesis project, technology has now made this process only a step toward one of these larger goals and can, in many cases, be performed by a company or core facility. As teachers and mentors, it is imperative that we understand that the technologies we teach in the lab and classroom must also evolve to match these advancements. In this perspective, we discuss how the rapid advances in gene synthesis technologies are affecting curriculum and how our classrooms should evolve to ensure our lessons prepare students for the world in which they will do science.

KEYWORDS molecular biology, gene synthesis, DNA sequencing, cloning, laboratory exercise, *in silico*, *in vitro*

Molecular biology research has changed significantly over the past decades (1, 2). The pioneers of these disciplines did not have access to the information or reagents that we take for granted in the lab today. As such, investigations often involved not only researching a biological problem but also generating the reagents and materials necessary to do so. Enzymes and other biological components had to be purified (3, 4) and genes had to be sequenced and cloned (5, 6) before a hypothesis-driven investigation into function could begin in earnest. It was this lack of tools, in part, that led to the development of many of the technologies that we now use routinely in the lab and classroom (7).

From these initial forays into molecular biology, the entrepreneurial spirit led to the development of industries that leveraged both this need for reagents by academic labs but also sought to solve important problems facing humanity (8). These companies supply common reagents at a cost that made buying them a practical decision for students when deciding on a course of investigation. In many cases, students have gone from buying individual reagents to purchasing kits that combine several reagents. These kits are used to perform an assay or purify a particular biomolecule and save valuable time during the course of investigation. This process is ever evolving, as every year more reagents and combinations of reagents meant to save time, money, and increase standardization across experimental processes are developed. A comparison of miniprep kits is provided here as an example (9); however, we as authors are not promoting a particular product or reagent.

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The authors declare no conflict of interest.

Received 17 April 2024
Accepted 4 June 2024
Published 8 July 2024

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DNA manipulation is critical for many molecular biology experiments, and there have been many reagents developed that enable investigators to change DNA accurately and efficiently as required for a particular experiment. Teaching students to leverage reagents that cut (10), amplify (11), link (12), and modify DNA (13, 14) have been critical to student training for decades. Such manipulation has advanced significantly over the past decades as the technology to determine the DNA sequences of organisms advanced (5) and more and more efficient reagents to cut and link pieces of DNA together were developed. As such, investigators have become less and less limited by the practical aspects of DNA construct generation. Relatively recent advances in CRISPR-mediated gene editing technology (15) in combination with gene synthesis (16, 17) have only accelerated the pace of this progress and now allow scientists to manipulate DNA *in vivo* at a scale that was previously impractical.

Many technologies in the biological sciences are developed at cutting-edge research laboratories, and these technologies then spread as they become more user friendly, less costly, and more broadly available. Indeed, gene synthesis has been used to design chromosomes and even entire genomes (18–20). These projects were/are being done by investigators with decades of experience in molecular biology, and these projects had the beneficial complementary effect of lowering the barriers such as cost and availability of gene synthesis for other investigators (16, 17). Indeed, it is now practical for undergraduate students to have constructs synthesized for in class projects (16). As such, synthesis eliminates what can be a significant bottle neck in the research experience. As trainers and investigators, we must consider how this paradigm shift should and could affect our training environments.

At the undergraduate level, molecular biology techniques are principally learned in one of two environments: (i) laboratory classes/CURES and (ii) undergraduate research projects (21). The continued evolution and development of cheap and reliable gene synthesis technologies have the potential to and in many ways already has drastically changed the way we must approach molecular biology education (17). Purchasing DNA constructs as opposed to cloning them yourself can be cheaper and more convenient, but what effects do these protocol shifts have on student understanding of the underlying science? One could argue it is no longer necessary for students to learn to clone in the laboratory *in vitro*. Adding nucleotides, enzymes, buffer, and template DNA to a tube to generate a construct could be considered an inefficient skill to teach in class or make part of an independent research project. The process of cloning a DNA construct or plasmid, especially those that require multiple steps, can often be done more simply through gene synthesis.

From an educational perspective, this leads to an interesting challenge. To design a DNA construct that will be made by gene synthesis, the designer must understand the experiment they would like to perform and the underlying tenants of the central dogma of molecular biology (22, 23). The incorrect placement of a stop codon or a shift in reading frame can have devastating effects on construct function, and thus, it is imperative that students understand the principles that underly the constructs they are designing. One of the best ways for students to learn these concepts is to spend time cloning a construct (24). The potential for failure can sharpen the mind and make students appreciate why and how they were generating a construct. Furthermore, while cloning in the lab can require multiple steps to all go correctly, the process is often separated into multiple smaller steps many with a high likelihood of success. Successfully performing restriction digestion or PCR can be important for a student, as it helps them gain confidence even if this is only a small part of a larger goal (25). Gene synthesis technologies can eliminate some of these smaller achievements and require students to design a construct potentially without ever having performed the underlying molecular biology experiments necessary to generate such a construct. It is in essence asking someone to design a part for a car after having read the owner's manual, but without ever having seen or driven a car before, not impossible but potentially confusing.

CALL TO ACTION

As gene synthesis technologies are now more readily available, how should an undergraduate training environment in the classroom look like to prepare students so they will thrive in a workplace leveraging these technologies. One could continue to train students by having them clone by hand, at least initially, so they might more fully understand the rationale behind the design of their constructs. One drawback of this is the obvious time and expense required. In addition, the technology being taught could be considered outdated, and this may not be in the student's best interest moving forward (Fig. 1). In addition, student-driven cloning projects, especially in undergraduate classes, can be challenging to organize depending upon how often a class meets and the number of students in the class. However, the process of successfully performing an experiment in a lab class can be a powerful motivator and having to physically perform a cloning project can require students to engage in some higher-order thinking skills.

A second possibility is shifting classroom activities away from traditional wet lab cloning projects and towards more *in silico* cloning projects. In these types of exercises, students can design constructs that could be later used in activities in a faculty member's research lab or classroom experiments. Depending upon the student's experience level, they could be taught how to design a variety of constructs of varying complexity. This would benefit students by providing the opportunity to participate in more robust construct design than could practically be done in a wet lab. For instance, codon optimization (26), using alternative genetic codes (27), generation of constructs with dozens of fragments are impractical for students to physically do in the lab without gene synthesis. However, many programs used to design such constructs are available for free to academics, and as such the cost of designing such constructs is generally not

Type	Pros	Cons
In vitro	• Better understanding of the rationale behind construct design • Force students to engage in higher level thinking and problem-solving skills • Students feel more accomplished upon completion	• Time and Cost • Technology might be outdated by the time students reach the job market • Student projects are challenging to organize depending upon the class size
In silico	• Constructs can be designed that will be used down the road by investigators and students • Students can gain experience designing various types of constructs and constructs with greater complexity • Has the potential to be more appropriate to train students for future careers	• Potential loss of understanding of underlying principles of construct design and molecular biology • May provide less chances to develop problem-solving skills • Lose experience with low stakes hands-on lab work

FIG 1 Pros and cons for different modes of molecular biology training.

prohibitive even for undergraduate classes (28, 29). Design of more complex constructs would require students to engage in distinct sets of thinking skills from traditional *in vitro* experiments, and the skills learned from these exercises may suit the students better in their future careers (Fig. 1).

A third potential possibility, and what might be the most beneficial for students given the current environment, is some combination of *in vitro* and *in silico* experimentation. *In vitro* cloning experiments supplemented with *in silico* exercises that complement the *in vitro* exercises to provide a broad learning experience for students. One challenging aspect of teaching molecular biology in the classroom is that physical exercises often have incubations and breaks when students can work on other activities. During this time, students would have the opportunity to complete *in silico* assignments that complement or supplement the *in vitro* exercises being performed (Fig. 2). In supplementing a wet lab experience with robust *in silico* construct design students will gain an appreciation for generating a construct at the bench but also have the opportunity to learn how to design more complex constructs (Fig. 2). Furthermore, because *in silico* experiments require limited capital expense, the designs students and instructors could work on are limited only by the imagination of the group.

For instance, in our upper-level molecular biology class at an R2 research institution that caters to seniors and graduate students, we find cloning short segments of DNA into a vector provides students with opportunities to practice a variety of core skills including pipetting, dilution, and DNA concentration determination that will serve them well regardless of their interests. Building DNA constructs is also an important concept to understand as it is core to many aspects of assay development. This practice though, can take multiple class periods/weeks, does not always lead to successful generation of plasmid on the initial try, and can lead to class periods with incubation steps or "orphan steps" such as setting up an overnight culture that while required do not have a logical companion lab activity to fill the rest of the class period. This unused time provides students with the opportunity to engage in alternative types of lab learning. During these steps, we initially have students design *in silico* the construct that they are designing *in vitro*. While this is a straightforward exercise, it requires the students to understand base pairing, restriction digestion, and DNA polarity key concepts of

	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8	Class 9	Class 10
in vitro	Miniprep plasmid DNA for cloning	PCR Amplify small (300-1000bp) piece of DNA using provided primers	Restriction enzyme digest of miniprepped plasmid vector	Resolve PCR product and cut plasmid on agarose gel	Phosphorylate PCR product CIP treatment of digested plasmid	Ligate PCR product into cut vector	Transform the ligated plasmid into competent cells Plate the transformation cells on selective media	Select transformed colonies and inoculate in liquid selective media	Miniprep plasmids and measure quality and quantity using a spectrophotometer	Digest plasmids and run gel to identify correct clones Alternatively sequence plasmids
***in vitro* time estimate**	Full class period	~30 min setup ~1hr thermocycler	~15 min digest setup ~ overnight incubation	~20 min gel preparation ~1hr electrophoresis and imaging	~20 min setup ~1hr incubation	~15 min setup ~1hr incubation	Full class period with multiple 10 min - 1hr incubations	~15 min	Full class period	~15 min ~-1hr electrophoresis and imaging
in silico		Download/ register for software such as Benchling or APE Begin navigating the software and examining its features	BLAST scavenger hunt: Provide students a minimally-labeled plasmid and have students use BLAST to identify sequence features	Import a "simple gene" of interest into software and practice manipulating it i.e., inserting restrictions sites	Import a mammalian gene, remove introns leaving only the ORF design primers to amplify single exons or entire gene. Codon optimize for a bacterial expression system.	Continue with tasks from Day 5	Provide students with a plasmid sequenc e of a bacterial expression vector and have them add their gene into it. Students should also label and characterize all features of the plasmid.	Students continue with task from Day 7		Student's swap the promotor in their expression vector and/or add a codon optimized GFP tag.

FIG 2 Potential timeline for incorporation of *in vitro* and *in silico* experiences in class. The timeline is based on an in-person lab class that meets for 1.5–2 h per class period. Lecture time can be incorporated into the plan as needed based upon student needs and prior experiences. *In silico* experiences are recommended to be carried out during *in vitro* downtime such as incubation periods.

molecular biology. This can be done before *in vitro* cloning begins as well. Once these constructs have been designed, one could order them from a vendor so that they arrive before the end of the semester. The number of constructs ordered depends on budget, but it is not necessary to order a different construct for every student, even if *in silico* they are all cloning a different construct. Once these constructs are ordered, we move on to more complex *in silico* cloning projects such as, building *de novo* a construct to express and target a protein to a certain portion of a cell in a particular organism that the students perform while they attempt the simpler *in vitro* project in class. An example of a potential classroom flow chart for this is provided in Fig. 2.

The description we provide above is an example of how we have adjusted a single class to recent advancements in gene synthesis technology. However, classes do not exist in vacuums and instead are parts of intentional curriculum allowing students to build skill sets and a knowledge base over several semesters. Multi-year/semester approaches should be considered when developing exercises, making sure to provide suitable progression and review as students move through a curriculum. For instance, hands on cloning techniques could be introduced in introductory courses and more *in silico* methods could be applied in the advanced courses if this is deemed practical for an institution. The size of the class and student experience will all play roles in the determination of what is appropriate. In addition, it is critical that we appreciate that student career goals are not all the same, and thus their needs in training can vary. A student who wishes to go to medical school, for instance, may have different motivations than one that wishes to enter the work force after graduation or proceed to graduate school. Variation in exercise type and better understanding of the student's career goals can help guide exercise development. In summary, the widespread adoption of gene synthesis techniques in professional settings should fundamentally change the way we approach molecular biology training. It is critical as educators; we are mindful of such advancements and adapt our classrooms to keep pace. Both classroom instruction and personal mentorship of junior scientists by senior personnel play critical roles in laboratory skill development. The extensive adoption of gene synthesis technology will continue to change the nature of this instruction, and such evolution is critical if we are to best prepare students for the workforce they will enter.

ACKNOWLEDGMENTS

This work was supported by NIH grant 1R15AI130950-02 to D.A.B.

The authors would like to thank Drs. Eric (VJ) Rubenstein and Gennifer Mager for thoughtful comments on the manuscript.

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REFERENCES

1. Shapiro JA. 2009. Revisiting the central dogma in the 21st century. Ann N Y Acad Sci 1178:6–28. https://doi.org/10.1111/j.1749-6632.2009.04990.x
2. Thieffry D, Sarkar S. 1998. Forty years under the central dogma. Trends Biochem Sci 23:312–316. https://doi.org/10.1016/s0968-0004(98)01244-4
3. Bickle TA, Pirrotta V, Imber R. 1977. A simple, general procedure for purifying restriction endonucleases. Nucleic Acids Res 4:2561–2572. https://doi.org/10.1093/nar/4.8.2561
4. Pirrotta V, Bickle TA. 1980. General purification schemes for restriction endonucleases. Methods Enzymol 65:89–95. https://doi.org/10.1016/s0076-6879(80)65013-7
5. Heather JM, Chain B. 2016. The sequence of sequencers: the history of sequencing DNA. Genomics 107:1–8. https://doi.org/10.1016/j.ygeno.2015.11.003
6. França LTC, Carrilho E, Kist TBL. 2002. A review of DNA sequencing techniques. Q Rev Biophys 35:169–200. https://doi.org/10.1017/s0033583502003797
7. Schultz C. 2007. Molecular tools for cell and systems biology. HFSP J 1:230–248. https://doi.org/10.2976/1.2812442
8. Gupta V, Sengupta M, Prakash J, Tripathy BC. 2016. An introduction to biotechnology, p 1–21. In Basic and applied aspects of biotechnology
9. Pronobis MI, Deuitch N, Peifer M. 2016. The Miraprep: a protocol that uses a Miniprep kit and provides Maxiprep yields. PLoS One 11:e0160509. https://doi.org/10.1371/journal.pone.0160509
10. Loenen WAM, Dryden DTF, Raleigh EA, Wilson GG, Murray NE. 2014. Highlights of the DNA cutters: a short history of the restriction enzymes. Nucleic Acids Res 42:3–19. https://doi.org/10.1093/nar/gkt990
11. Ishino S, Ishino Y. 2014. DNA polymerases as useful reagents for biotechnology - the history of developmental research in the field. Front Microbiol 5:465. https://doi.org/10.3389/fmicb.2014.00465
12. Shuman S. 2009. DNA ligases: progress and prospects. J Biol Chem 284:17365–17369. https://doi.org/10.1074/jbc.R900017200
13. Eastberg JH, Pelletier J, Stoddard BL. 2004. Recognition of DNA substrates by T4 bacteriophage polynucleotide kinase. Nucleic Acids Res 32:653–660. https://doi.org/10.1093/nar/gkh212
14. Green MR, Sambrook J. 2020. Alkaline phosphatase. Cold Spring Harb Protoc 2020:100768. https://doi.org/10.1101/pdb.top100768
15. Adli M. 2018. The CRISPR tool kit for genome editing and beyond. Nat Commun 9:1911. https://doi.org/10.1038/s41467-018-04252-2
16. Hoose A, Vellacott R, Storch M, Freemont PS, Ryadnov MG. 2023. DNA synthesis technologies to close the gene writing gap. Nat Rev Chem 7:144–161. https://doi.org/10.1038/s41570-022-00456-9
17. Hughes RA, Ellington AD. 2017. Synthetic DNA synthesis and assembly: putting the synthetic in synthetic biology. Cold Spring Harb Perspect Biol 9:a023812. https://doi.org/10.1101/cshperspect.a023812
18. Venter JC, Glass JI, Hutchison CA, Vashee S. 2022. Synthetic chromosomes, genomes, viruses, and cells. Cell 185:2708–2724. https://doi.org/10.1016/j.cell.2022.06.046
19. Dai J, Yang H, Pretorius IS, Cai Y, Shen CY, Chang M, Yuan Y. 2023. A spotlight on global collaboration in the Sc2.0 yeast consortium. Cell Genom 3:100441. https://doi.org/10.1016/j.xgen.2023.100441
20. Beattie KL, Logsdon NJ, Anderson RS, Espinosa-Lara JM, Maldonado-Rodriguez R, Frost JD. 1988. Gene synthesis technology: recent developments and future prospects. Biotechnol Appl Biochem 10:510–521. https://doi.org/10.1111/j.1470-8744.1988.tb00040.x
21. Kuh GD. 2008. Excerpt from high-impact educational practices: what they are, who has access to them, and why they matter, Vol. 14, p 28–29. Vol. 14. Association of American Colleges and Universities.
22. Wright LK, Fisk JN, Newman DL. 2014. DNA → RNA: what do students think the arrow means? CBE Life Sci Educ 13:338–348. https://doi.org/10.1187/cbe.cbe-13-09-0188
23. Crick F. 1970. Central dogma of molecular biology. Nature 227:561–563. https://doi.org/10.1038/227561a0
24. Lau JM, Robinson DL. 2009. Effectiveness of a cloning and sequencing exercise on student learning with subsequent publication in the National Center for Biotechnology Information Genbank. CBE Life Sci Educ 8:326–337. https://doi.org/10.1187/cbe.09-05-0036
25. Wang JTH, Schembri MA, Ramakrishna M, Sagulenko E, Fuerst JA. 2012. Immersing undergraduate students in the research experience. Biochem Molecular Bio Educ 40:37–45. https://doi.org/10.1002/bmb.20572
26. Hanson G, Coller J. 2018. Codon optimality, bias and usage in translation and mRNA decay. Nat Rev Mol Cell Biol 19:20–30. https://doi.org/10.1038/nrm.2017.91
27. Kollmar M, Mühlhausen S. 2017. Nuclear codon reassignments in the genomics era and mechanisms behind their evolution. Bioessays 39. https://doi.org/10.1002/bies.201600221
28. Davis MW, Jorgensen EM. 2022. ApE, a plasmid editor: a freely available DNA manipulation and visualization program. Front Bioinform 2:818619. https://doi.org/10.3389/fbinf.2022.818619
29. Benchling [biology software]. 2024. https://benchling.com.