AC 2012-3929: DNA TO GO: A DO-IT-YOURSELF PCR THERMOCYCLER LAB

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Abstract

There is currently a need for innovative educational experiences that unify and reinforce fundamental principles at the interface between physics, molecular biology, and the chemical sciences. These experiences also empower students by helping them recognize how this knowledge can be applied to develop new products and technologies that benefit society. This presentation describes our efforts to address this need by creating innovative hands-on lab activities that introduce chemical engineering students to molecular biology by challenging them to harness natural convection phenomena to perform DNA replication via the polymerase chain reaction (PCR).

Experimentally, we have constructed convective PCR stations incorporating a simple design for loading and mounting the cylindrical PCR reactor between independently controlled thermal plates. Each station independently interfaces with a Windows-based PC via a USB connection, and is operated by a custom designed software package that enables temperature profiles to be easily input and monitored. A motion analysis microscope enables flow patterns inside the convective PCR reactors to be directly visualized. We have also developed course modules focused on modeling the problem of thermal convection in a fluid layer heated from below (the Rayleigh-Bénard problem) in the context of geometries that could be used to design lava lamps. After the fundamental problem is introduced and connected with the course material, the students are walked through a hands-on CFD exercise, then assigned a problem that gives them an opportunity to explore the effects of varying parameters in the model. Initial feedback has been very positive, as the computer simulations seem to excite student interest because they can actually "see" what they have been learning in the lecture. These capabilities uniquely enable us to connect the theoretical/computational, experimental, and biochemical reaction into a unified experience.

Introduction

Analysis of DNA samples that are only present in very small quantities has become an increasingly critical element in the development of new miniaturized medical diagnostic technologies. But a major challenge to these efforts lies in the design of instrumentation used to perform a key step in the analysis[1]. This step, the polymerase chain reaction (PCR), involves harnessing a sequence of thermally activated biochemical processes to selectively replicate well-defined sub regions within a longer DNA strand. The PCR is incredibly efficient (capable of producing billions of copies) and is also fairly straightforward to perform. Typically, a reagent mixture containing template DNA, primers, dNTPs, thermostable *Taq* polymerase enzyme, and buffering agents is dispensed into plastic reaction tubes or multiwell plates that are then inserted into a programmable thermocycling machine. This instrument has a single function: to repeatedly heat and cool the PCR reagent mixture through about 30 – 40 cycles between temperatures corresponding to *denaturation* of the double-stranded target DNA, *annealing* of primers to complementary locations on the denatured single-stranded fragments, and enzyme catalyzed *extension* to synthesize the complementary strands.

The predominant thermocycler design employed in most laboratories essentially consists of a metal heating block whose temperature is regulated by computer-controlled thermoelectric heaters. Unfortunately, this design is very inefficient because its inherently high heat capacity severely limits the attainable heating and cooling rates and consumes considerable electrical power. These problems are compounded by the low thermal conductivity plastic materials (e.g., polypropylene) used to construct the reaction tubes and plates, resulting in the need to hold the temperature constant at each stage of the reaction for a significant period of time so that the entire reagent volume can equilibrate. As a consequence, it is not uncommon for PCR reactions to require 1–2 hours to complete, imposing severe limitations on achievable throughput.

Harnessing Natural Convection

Thermal convection has emerged as a promising alternative thermocycling approach that has the potential to overcome these limitations[2-9]. Convective flows are an everyday occurrence in a diverse array of settings ranging from the Earth's atmosphere, oceans, and interior, to decorative and colorful lava lamps. Fluid motion is initiated in the same way in each case: a buoyancy driven instability arises when a confined volume of fluid is subjected to a spatial temperature gradient. These same phenomena offer an attractive way to perform PCR thermocycling. By applying a static temperature gradient across an appropriately designed reactor geometry, a continuous circulatory flow can be established that will repeatedly transport PCR reagents through temperature zones associated with the denaturing, annealing, and extension stages of the reaction (**Fig. 1**). Thermocycling can therefore be actuated in a pseudo-isothermal manner by simply holding two opposing surfaces at fixed temperatures, completely eliminating the need to repeatedly heat and cool the instrument.

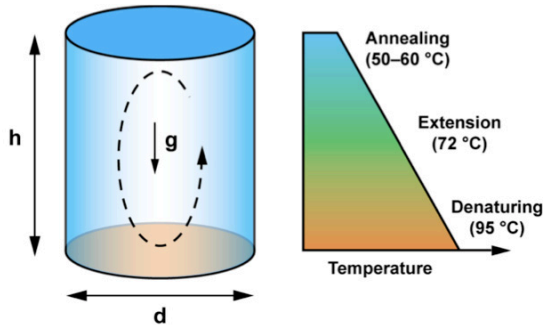

Fig. 1. Thermal convection in a cylindrical chamber whose top and bottom surfaces are maintained at different fixed temperatures. If the temperature at the bottom surface is higher than at the top, a vertical density gradient is established within the enclosed fluid that is capable of generating a circulatory flow pattern. With the right choice of geometric parameters (height h and diameter d), the convective flow field can be harnessed to actuate PCR thermocycling when the top and bottom surfaces are manintained near annealing and denaturing temperatures, respectively (gravity acts vertically downward).

Optimal design of convective thermocyclers involves choosing the correct reactor geometry that will generate a circulatory flow capable of transporting reagents through the key temperatures involved in the PCR process. The geometric parameters that can be varied in the cylindrical reactors considered here are the height (h) and diameter (d), or equivalently the aspect ratio (h/d). We have explored the 3-D flow fields inside convective PCR reactors over a range of different aspect ratios using computational fluid dynamics (CFD), and found that unexpectedly complex patterns can arise. More importantly, our analysis has uncovered a subset of these complex flow fields that significantly accelerate the reaction[12]. Extremely rapid DNA amplification times (under 10 min) are achievable in reactors designed to generate these flows (**Fig. 2**).

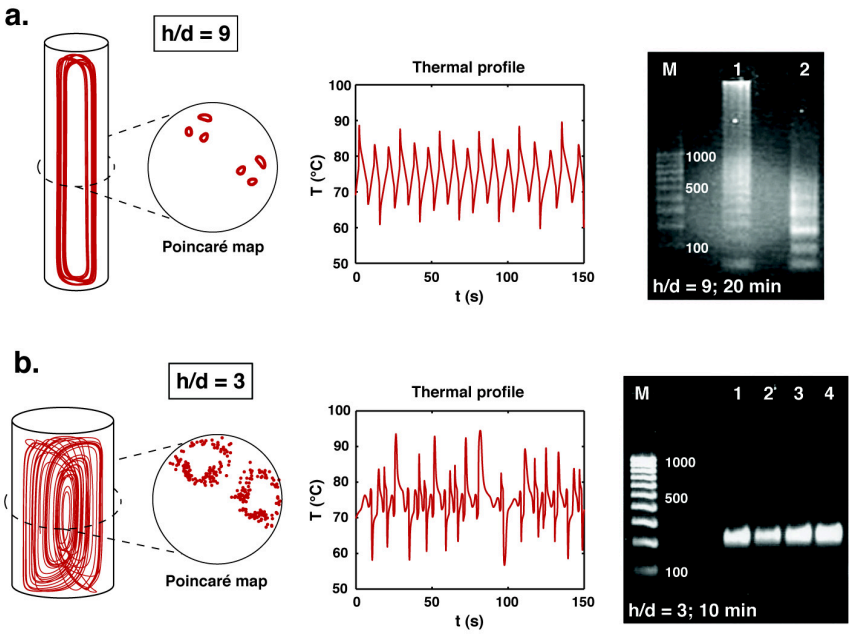

Fig. 2. Illustration of different flow fields emerging under PCR conditions, as evident in simulations at **(a)** *h/d* = 9 (38.2 µL reactor volume) and **(b)** *h/d* = 3 (18.5 µL reactor vikyne) with *T* = 53 and 96 °C at the top and bottom surfaces, respectively. In a tall narrow reactor (*h/d* = 9), reactions must run 20 min before visible PCR products are evident, whereas strong products are evident after only 10 min in a shorter wider cylinder (*h/d* = 3).

PCR Thermocycler Design Lab

Inspired by the conceptual simplicity of the convective thermocycler design and its unique ability to combine fundamental concepts in transport phenomena and biochemistry, we have formulated a laboratory exercise that walks students through the process of designing, building, and operating convective PCR thermocyclers. We envision that this work will create an educational experience suitable for integration into our chemical engineering transport sequence, with instrumentation to allow four groups of 4-5 students to perform the laboratory exercises simultaneously. But we have also divided the instructional content into three sections so that the emphasis of the course can be tailored to a range of target audiences (e.g., students with strong biochemistry background versus students with strong transport backgrounds).

First, students are introducd to the molecular biology concepts related to mechanics and applications of PCR in genomic analysis. Reference to DNA analysis in the context of forensics (e.g. crime scene investigations) and infectious disease survielance helps students connect the concepts with real world applications and identify how this set of tools can be applied in a variety of important and relevant ways. Presentation of this topic culminates in a lab exercise where students perform their own PCR reactions using conventional thermocycling instruments and analyize the results using agarose gel electrophoresis. This material is designed to dovetail with the biology requirement we have instituted in a recent update of our undergraduate chemical engineering curriculum to ensure that sutdents possess a suitable fundamental background in this area.

Next, students learn the fundamental aspects of buoyancy driven convective flows. This content is designed to integrate with previous coursework in the transport sequence. The importance of the dimensionless Rayleigh number, a parameter that expresses the ratio of destabilizing buoyant forces to the restoring effects of viscous and thermal diffusion, is highlighted using the design of lava lamps as a relatable illustrative example. Once the mathematical framework has been established, fluid properties and reaction conditions associated with a typical PCR process are introduced and students are asked to evaluate reactor geometries suitable for thermocycling. This section culminates with a hands-on lab where students apply a 3D computational fluid dynamics (CFD) model we have developed using STAR CCM+ software to evaluate a series of reactor designs by performing flow and heat transfer analysis, estimation of thermal residence times, and quantification of reaction product yields. I

Finally, the physics and biochemistry fundamentals introduced in the previous two course components are combined in a hands-on design project. Students construct reactor geometries based on their calculations and use them to first perform simple flow visualization experiments by imaging the motion of an aqueous suspension of fluorescent latex microspheres inside the reactors. Students then use their designs to perform PCR amplification of DNA in parallel with controls run in a conventional thermocycler. Results are analyzed by agarose gel electrophoresis.

We have partnered with Lynntech, Inc. (a local technology development firm), to commission 5 dedicated convective PCR stations (**Fig. 3a-c**). These stations incorporate a simple design for loading and mounting the cylindrical PCR reactor between independently controlled thermal plates. Each station independently interfaces with a Windows-based PC via a USB connection, and is operated by a custom designed software package that enables temperature profiles to be easily input and monitored. Course materials including operation and lab manual have also been produced. A Keyence motion analysis microscope enables the patterns inside the convective PCR reactors to be directly visualized and recorded (**Fig. 3d-e**). This capability connects the theoretical/computational, experimental (flow field), and biochemical reaction into a unified experience.

We have applied this lab module for the first time in two different contexts an undergraduate chemical core course (CHEN 304 - Fluid Mechanics Operations) and a graduate elective survey of advanced biotechnology methods (BIOT 602 - Biotechnology Principles and Techniques II). Overall, feedback from the course assessment was overwhelmingly positive. In the undergraduate adaptation, we distributed pre- and post-assessment assignments focused on the transport phenomena concepts and integration of chemical reactions localized within distinct thermal zones. This assessment revealed that the CFD simulations seem to particularly excite student interest because it allowed them to actually "see" what they have been learning in the lecture. This component also addresses broader student feedback we have received from recent graduating senior exit interviews where a desire for increased exposure to simulation tools was expressed. A more quantitative assessment is ongoing this semester and results will be reported as soon as they are available.

In the graduate adaptation, emphasis was placed more on the molecular biology component, consistent with the students' backgrounds. Additionally, because these students were part of a cohort enrolled in a professional science master's program in biotechnology, we tailored the

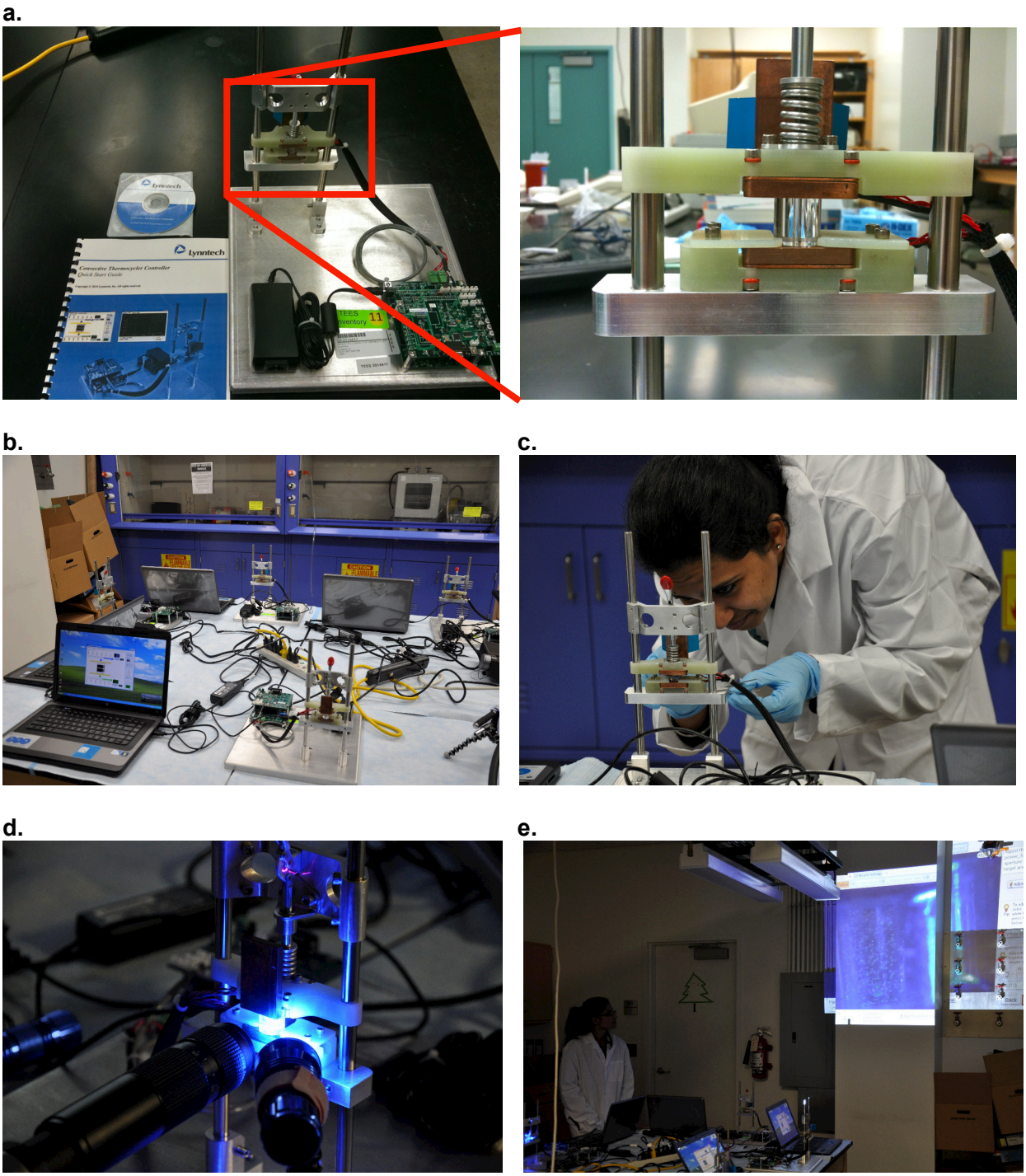

Fig. 3. **(a)** Custom-built convective PCR stations commissioned for this project (the cylindrical transparent reactor is shown clamped between upper and lower heating plates in the expanded view on the right). The setup includes complete documentation and software. **(b)** Overview of 5 workstation setup. **(c)** Student loading the cylindrical convective reactor into the heating device. **(c)** The Keyence motion analysis microscope enables the flow field inside the cylindrical convective PCR reactors to be directly visualized. **(e)** The microscope's video image was projected on the wall to reveal the internal flow patterns.

assignments toward a scenario of evaluating new technology (as might occur in a company). Prior to beginning the lab experiment, the concept of convective PCR was presented in a lecture and reinforced by a video demonstration[13]. Since the students were experienced with PCR through prior coursework, they were therefore asked to critically evaluate performance (in terms of raw speed, sensitivity, specificity, etc.) and practicality from a product and end-user standpoint (in terms of operator input, sample and instrument preparation, turn-around time between runs, cost of components and consumables, etc.) in comparison with traditional instruments. They were also asked to make recommendations about what improvements could be made to make this device competitive with existing technology[14]. An excerpt from the assessment assignment is provided below.

During this laboratory exercise, you had an opportunity to evaluate a new method for performing PCR thermocycling. It is likely that you may encounter similar experiences in the future, where you are presented with a new technology and asked to assess the extent to which it may (or may not) be commercially feasible. In this assignment, you are asked to draw on your biotechnology expertise and evaluate the convective thermocycling platform.

1. **Initial impression.** Describe your initial impression of this technology based on hearing about it in the lecture (*before* attempting it in the lab). What advantages and disadvantages did you foresee? What questions did you have in your mind about the technology?

2. **Using the instrument.** Briefly discuss your experience in operating the instrument (sample loading, software operation, etc.). In your opinion, would an average lab technician be able to operate a system like this? Why or why not?

3. **Performance.** Compare the performance of the instrument with conventional PCR thermocyclers. Would you characterize it as better or worse than the conventional instrument (and by how much)? Briefly explain your answer.

4. **Final impression.** Having completed the lab, describe how your impressions of convective PCR technology have changed. Describe how using the device was similar or different than you expected. What areas are the most promising? What aspects could be improved?

5. **Future applications.** In what markets or application areas (if any) could you envision this instrument being used? Briefly explain. What barriers could you foresee to entry into these markets?

Please briefly answer the three additional questions below about the lab experience. These questions are intended to help us improve the lab for future students.

1. What do you think the instructor intended for you to learn from the convective PCR laboratory?

2. How would you explain this laboratory experience to an undergraduate student?

3. When you close your eyes and picture this lab experiment, what do you see?

Comparison of the students' impressions about the convective PCR approach before and after completing the laboratory exercise were evaluated. Initial impressions conveyed skepticism because the concept was in a research stage, as opposed to a commercial product. Some comments included "I was skeptical about the convective PCR technology when I first heard about it. If it was so advantageous, why was it not already in the market?"; "It did not occur to me that convection currents could be used for PCR. The major question in my mind is why has this technology not been commercialized yet?"; and "It was tough to imagine that PCR can be done in such less time."

After completing the lab, the students' impressions focused on the simplicity of operating the device. It should be noted, however, that these perspectives were shaped by the student's prior hands-on knowledge of performing PCR in a molecular biology lab setting. Comments along these lines included "This is a great lab to take, but you have to know and understand to take away this lab's implications. If you have never mixed together a PCR reaction and waited on a thermocycler, then you won't understand how significant this technology is." and "I see the entire practical lab experience in front of me. The concept and the steps are clear to me which is a testament to the simplicity of the instrument." It was also evident that being able to actually see the internal flow field within the reaction chamber using fluorescent bead tracers effectively conveyed the operating principles of the device, as expressed in comments like "It was simply amazing to actually see the movement of the sample based on different aspect ratios." and "One specific thing that comes to my mind when I think about the experience is the circulation of the fluid within the cell shown with the help of fluorescent beads."

These responses helped catalyze a discussion about issues involved in successful commercialization of basic research. This is a process that involves multiple steps on a myriad of levels, each with its own considerations. Often research that appears promising in the lab is not ultimately successful as a commercial product for reasons that have nothing to do with the scientific or technical innovation. The students (and instructor) came away with an increased awareness of these issues, and a greater focus on this aspect is planned in future labs targeted at this audience.

Some challenges were encountered in tailoring the content to the appropriate audience, and adjusting the scope of the hands-on activities to fit the time constraints of the allotted class time. We dealt with this by performing some rinsing and preparation steps prior to the beginning of the lab. This needs to be considered, however, when evaluating student comments about the device operation. Some timing issues also arise when students simultaneously run reactions using different reactor designs in which the reaction is performed for different lengths of time. It is desirable to have all students begin their reactions simultaneously to ensure synchronized timing, especially with a larger class size. The video introduction to convective PCR was helpful because students can watch it additional times outside of class to help familiarize them with the fundamental concepts[13]. The lab protocol incorporates downtime during the gel electrophoresis analysis that can be used to demonstrate the flow patterns inside the reactors using fluorescent beads. Alternatively if time is short, the gel separations can be performed by the instructor and results returned to the students at the next class period.

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