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Primer Design for Reverse Transcription-Quantitative PCR Simulation: an Online Lab Exercise Using a SARS-CoV-2 Model

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INTRODUCTION

The technique of PCR is foundational to both research and diagnostic testing. It is routinely used to detect infections from pathogens, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (1), and can be used in multiplex reactions to discriminate between respiratory viral infection caused by influenza A or influenza B viruses or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (https://www.cdc.gov/coronavirus/2019-ncov/lab/multiplex.html). Most students learn the principles of PCR in introductory biology classes, but they are often unclear about how PCR primers are designed and why they amplify specific pieces of DNA (2). During the 2019 coronavirus disease pandemic, most laboratory courses were taught online. This exercise was designed to help students design primers that specifically detected SARS-CoV-2 using easily available online bioinformatic tools. Students tested their primers in simulated PCR experiments to identify potential false positives from related coronaviruses and false-negative test results from SARS-CoV-2 variants. Several previous papers have described primer design activities for upper-level, honors, or graduate students; those activities were aimed at helping those students analyze PCR results while dispelling misconceptions (3–6). Here, we describe a relatively simple online lab for students in introductory biology classes that can be completed in one session using NCBI Primer-BLAST and a virtual PCR tool (https://www.bioinformatics.org/sms2/pcr_products.html), both of which are openly available. This exercise addresses a core concept from the ASM Recommended Curriculum Guidelines for Undergraduate Microbiology Education (i.e., mutations and horizontal gene transfer, with the immense variety of microenvironments, have selected for a huge diversity of microorganisms [https://asm.org/Guideline/ASM-Curriculum-Guidelines-for-Undergraduate-Microb]) and two from an ASM committee's Microbiology in Nursing and Allied Health (MINAH) Undergraduate Curriculum Guidelines (7): (i) understanding mechanisms that impact pathogen evolution (i.e., vertical and horizontal genetic variation, mutations, recombination, etc.) is central to limiting pathogen evolution, as are (ii) a variety of methods are used to identify infectious agents, as well as several lab skills and competencies described in both sets of guidelines.

Learning goals

This exercise is designed for small groups of students in an online or "dry lab" session. It can be a stand-alone module or used in preparation for either an in-class PCR experiment or further exploration of bioinformatics tools. It is appropriate for undergraduates enrolled in introductory biology, genetics, or microbiology courses as well as advanced high school students. With minor modifications, it can also be used with more advanced students. At the conclusion of this exercise, students will be able to: (i) describe the difference between PCR and reverse transcription-PCR (RT-PCR), (ii) design PCR primers using NCBI Primer-BLAST, (iii) conduct virtual PCR experiments, and (iv) evaluate the quality and specificity of PCR primers that may be used in research or diagnostic tests.

Safety issues

No safety issues were identified.

PROCEDURE

The supplemental materials include Appendix SA, a worksheet we give to students that describes how to complete the exercise and contains critical thinking questions

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based on the procedure and results. Appendix SB contains the worksheet answers. In our class, students perform the following activities:

1. Learn about PCR in a lecture entitled "Molecular Microbiology" that features the inventor of PCR, Kari Mullis, and describes different types of PCR assays, including endpoint versus quantitative PCR (qPCR) and standard PCR versus RT-PCR. Depending on the context of the lab exercise, a mini-lab lecture could include a comparison of PCR assays and enzyme-linked immunosorbent assays that detect either antigen or antibodies. This is followed with a discussion of when PCR tests are most appropriate. For example, why would PCR be the most appropriate test to detect *Chlamydia trachomatis* or *Neisseria gonorrhoeae*? What additional step would be necessary to detect sequences from RNA viruses like Ebola virus or SARS-CoV2?
2. Meet in online groups (or in person) to complete the worksheet. Students first use the SARS-CoV-2 Wuhan sequence to generate PCR primers. They are asked to choose one set of primers based on parameters such as GC content. This helps students review the difference between G/C and A/T pairing. Primers are chosen using the Primer-BLAST database, which helps eliminate potential primers that could bind to other sequences in the database. The GenBank "Features" helps students identify if they will amplify a gene or an untranslated region. This is a good opportunity for students to review the concept of a gene. Students can relate to the importance of reliability from a test for SARS-CoV-2, which they have likely taken more than once. After designing their own set of PCR primers, students perform virtual PCR assays (https://www.bioinformatics.org/sms2/pcr_products.html) to look for false positives using their primers as well as primers designed by the Centers for Disease Control and Prevention (CDC), using sequences of the related coronaviruses OC43, HKU1, SARS, and Middle East respiratory syndrome virus as templates. They look for false negatives using the sequences of SARS-CoV-2 variants linked on the worksheet and another they identify themselves. At the end of the virtual PCR, they can choose to keep their primers or try another pair.
3. Debrief in a second session (optional). Students may want to share their results and go over answers to the worksheets. This could be followed by an actual PCR experiment or an additional bioinformatics activity, such as aligning diverse coronavirus sequences or related SARS-CoV-2 variants using tools like Clustal Omega (https://www.ebi.ac.uk/Tools/msa/).

CONCLUSION

Students are motivated to learn about issues like SARS-CoV-2 that affect their daily lives. We have used this exercise for many semesters in microbiology classes (majors and allied health) at two universities. Nearly all student groups have been able to complete the worksheet with minimum instructor assistance. Students who completed the lab reported to have more confidence in bioinformatics work when asked in a lab survey. While most students scored high in this exercise, we suggest that the instructor expound more on the concept of false positives and negatives, as that seemed to be a point of confusion to most students. This activity could also be easily adapted to detect other microorganisms. It fills a need for more online activities as teaching modalities have shifted. It also serves as a low-cost alternative to traditional PCR in the laboratory.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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