INTRODUCTION

High-throughput screening (HTS) has become a common method for drug discovery in the pharmaceutical and research laboratory. It relies on automation to quickly assess a large array of compounds for cellular, biochemical, or genetic activity. High-throughput screening is a powerful tool used for discovering drug-receptor interactions, enzymes, and other targets. While these assays are typically performed using robotics, they can be run on a smaller scale using microtiter plates and instrumentation capable of analyzing directed outputs. Despite its versatility and widespread use in industry and research, HTS is underutilized in undergraduate laboratories (1).

Integrating inquiry-based research experiences into science courses can enhance student understanding of the scientific process as a way of learning about the natural world (2–6). This type of experience is particularly powerful when students have the opportunity to design their own research question (2, 3). We created an open, inquiry-based laboratory exercise exploiting a small-scale HTS assay using the budding yeast S. cerevisiae (7, 8). This exercise not only introduces students to data collection and analysis techniques but also equips them with skills to help prepare them for 21st century jobs. In this report, we describe a student-led laboratory module in which participants use HTS methods to generate and investigate the effects of a genotoxic agent on yeast growth. University students are given the model system and background information on HTS methods. They then research the literature, create hypotheses, and design and generate growth curves of S. cerevisiae using a microtiter plate reader. After treatment with their insult of choice, students analyze their data using Microsoft Excel.

This lab module presents an ideal opportunity for students in college/university biochemistry or cell biology courses, or high school honors biology/chemistry courses, to learn about the process of science in a manageable and cost-effective manner. Furthermore, discussion questions engage students in critical dissection of biological pathways and experimental procedures. Using active learning in this manner has been shown to increase student retention and understanding of the material (9).

PROCEDURE

Materials and methods

A detailed list of materials, methods, and equipment is available in the Teacher Preparation Notes (Appendix I). The non-pathogenic S. cerevisiae strains wild-type HAO and UV-sensitive (G948-1/C, α, rad1 rad18 phr1 ura3) are available from Carolina Biological Supply Company (Burlington, NC).

Intended audience

This lab module is appropriate for students in college/university or in high school honors courses. At High Point University (HPU), this module was used as an open inquiry activity in a cancer biology course for junior and senior students. At the high school level, this module was used as a structured inquiry activity with junior and senior students as part of their chemistry curriculum (8).

Experiments

Before implementation of this module, HPU students are given S. cerevisiae as a model organism and taught HTS methodologies. Groups of three to four students discuss which variables that may influence yeast growth they will test...
in their experiments. Once the insult(s) are chosen, students spend 30 to 60 minutes searching the scientific literature for background information to formulate a testable hypothesis, design an experiment to test their hypothesis, and analyze and communicate their results in written form. The details of the experimental design as formulated by students at HPU who chose to test the effects of microwave radiation on yeast growth are described below (Fig. 1A). Other experiments were implemented using insults such as UV radiation (Fig. 1B) and the growth inhibitor rapamycin (Fig. 1C). Detailed protocols generated by university students for these growth insults are found in Appendix 3 and Appendix 4, respectively. Furthermore, these protocols were used as structured inquiry activities at a high school during science outreach events.

Briefly, student groups label sterile microcentrifuge tubes for each of the following experimental conditions: control (no radiation), 5 seconds, 30 seconds, and 60 seconds (microwave radiation exposure times) (Table 2, Appendix 1). Twenty-four hours prior to lab implementation, *S. cerevisiae* cultures are grown in yeast extract peptone dextrose (YEPD) medium, incubated with shaking for aeration at 30°C and 120 rpm, and standardized to 0.5 OD$_{600}$/mL for the day of the experiment (10). The cell suspensions are mixed to homogeneity, and 200 μL of the sample is added into each of the four labeled microcentrifuge tubes. Each tube is microwaved individually for the designated amount of time on high power using a standard 1100-W microwave. Students must uncap each tube before microwaving to avoid the tube exploding under pressure and use a microwave-safe rack or plate.

After microwave radiation exposure, students pipette 50 to 100 μL of yeast cell suspension from each treatment, including the control, into the appropriate A-row wells of a 96-well microtiter plate (Fig. 1, Appendix 2). Students fill every well on the plate (B1 through H12) with 100 μL of YEPD medium. If available, students may use a multichannel pipettor for this purpose. Yeast suspensions are serially diluted 1:10 from row A to row H within the same column by taking 10 μL from the preceding well and pipetting it onto the next well until row H is reached. At row H, 10 μL of YEPD medium from each well is discarded. The final medium volume in wells B1 through H12 should be 100 μL (Fig. 1, Appendix 2).

Once yeast cells have been appropriately diluted, the 96-well microtiter plate can be placed in a plate reader to monitor cellular growth at OD$_{600}$ nm at 30°C continuously for the next 40 to 48 hours.

After completion of their experiments, students generate a graph of their growth curves with standard deviation in Microsoft Excel. Representative HPU student-generated data are shown in Figures 1A–C. Students discuss the results of their findings and compare them with their earlier hypotheses. Detailed instructions on using Microsoft Excel to plot growth curves are found in Appendices 2–4.

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**FIGURE 1.** Various insults decrease yeast growth. (A) Growth curves of *S. cerevisiae* treated for various times with microwave radiation. (B) Representative growth curves of wild-type *S. cerevisiae* and the mutant UV-sensitive strain treated with or without sunlight for various times. (C) Growth curves of *S. cerevisiae* treated for various times with 0.05 μg/mL, 0.1 μg/mL or 0.2 μg/mL of rapamycin. Results are representative of university students’ data. Averages ± STD of experiments performed in triplicate are shown.
CONCLUSIONS

This activity is an effective way to introduce high school students and undergraduates to HTS methods, reinforcing student understanding of cell biology and biochemistry. Furthermore, students have opportunities to engage in critical thinking activities and the scientific process.

SUPPLEMENTAL MATERIALS

- Appendix 1: Teacher materials and preparation notes for HTS yeast-growth experiments
- Appendix 2: Student handout/protocol—Assessing the effects of microwave radiation on yeast growth
- Appendix 3: Student handout/protocol—Assessing the effects of UV radiation on yeast growth
- Appendix 4: Student handout/protocol—Assessing the effects of rapamycin on yeast growth

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