What is the CURE for limited DNA? A forensic science course focused on NGS

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Abstract: Course-based undergraduate research (CURE) courses can increase the number and diversity of undergraduate students involved in research projects compared to one-to-one traditional student-faculty research experiences or research internships. Next generation sequencing (NGS) is an emerging method for performing DNA typing for forensic applications. We report upon our development and implementation of a forensic biology CURE course that introduces and employs advanced sequencing methods, including NGS to answer forensic questions, to students.

Keywords: Forensic science, CURE, undergraduate research, teaching methods, diversity, student engagement

Introduction

Several institutions with forensic science programs accredited by the Forensic Science Education Programs Accreditation Commission (FEPAC) offer courses with content on the theory and practice of modern forensic DNA analysis with a focus on short tandem repeat (STR) analysis (1). Traditional STR analysis employs capillary electrophoresis (CE) to separate autosomal DNA (aDNA) STR amplicons following polymerase chain reaction (PCR) amplification of the targeted regions. In cases in which the only recoverable DNA is of very low quantity, the samples are referred to as low template (LT) or trace and an STR profile may be unobtainable. Solutions to the problem of LT DNA include reanalyzing the sample multiple times and reporting the consensus profile or concentrating the sample to obtain the best profile possible. Alternatively, more sensitive methods for DNA typing can be used.

Massively parallel sequencing (MPS) or next generation sequencing (NGS) offers a solution to the low template problem. Also PCR-based, NGS is more sensitive than traditional DNA typing methods. Additionally, more loci are targeted and shorter amplicons are produced with NGS DNA typing kits for forensic use. For example, using the Verogen ForenSeq™ Signature Prep kit, up to 58 STRs, the amelogenin locus, and 172 single nucleotide polymorphisms (SNPs) are amplified in the same reaction mixture and up to 96 samples can be sequenced simultaneously (2). The SNPs offer additional loci for discrimination as well as phenotypic estimation of hair color, eye color and skin tone and biogeographical ancestry (BGA) estimation. Forensic labs have begun to adopt phenotype and BGA estimation to missing persons casework and to aid in identifying human remains in mass disaster and historical archeology cases (3). The first criminal case employing NGS data leading to a conviction in sexual assault case in the Netherlands was reported in 2019 (4).

CURE is an acronym for course-based undergraduate research experience (5). CURE courses may exhibit one or more of the CURE elements: research activities, discovery, relevance, collaboration, and iteration (5). The elements align to the four highest levels of the revised Bloom's Taxonomy hierarchy of learning which includes the skills remember, understand, apply, analyze, evaluate, and create (6). Traditional courses have been criticized for not drawing and developing sufficient talent to sustain the bioscience workforce pipeline (7,8). Clickers are one approach that has been used to promote active learning in classrooms (8,9). CURE courses represent another approach to active learning and aim to better align how science is taught to how it is done in the workforce. In CURE courses, some or all student instruction on course content is delivered in the form of a research project which students conduct in small groups (10-15). CURE courses have been shown to be able to engage more students and a more diverse student population in research than traditional mentor-mentee models and have been shown to have a significant effect on students' intentions to pursue research-related careers (16).

Depending upon the structure of the CURE course, students develop the research question and methodology to be used, collect and analyze data and report upon the results. At Towson University (TU), we developed a CURE focused on introducing students to research while simultaneously teaching them about advanced sequencing methods including NGS and how they can be used to

answer ancestry and relatedness questions for human remains samples, determining compatible DNA extraction and direct PCR approaches for various samples, investigating the effects of sampling and DNA source from different bones, and investigating the effectiveness of DNA enrichment tools.

TU is a large, public university with a Carnegie classification of Doctoral/ Professional University (DPU). TU offers FEPAC-accredited undergraduate and graduate degree programs focused on forensic science. With a Master of Science in Forensic Science (MSFS) degree program enrolling 50-60 students and approximately 150 declared Forensic Chemistry majors seeking a Bachelor of Science degree serviced primarily by four full-time forensic faculty and a program director, we do not currently have the capacity to offer traditional one-on-one student-faculty research experiences to all of our undergraduate majors and our graduate students who are required to conduct research in accordance with our FEPAC accreditation. In addition, the majority of our students are "non-traditional" in the traditional sense meaning that they are older, reside off-campus and work nearly full-time. Their commuting, class, and work schedules make it difficult to impossible for them to undertake a research project. Our undergraduate Forensic Chemistry majors are required to complete a capstone experience which can be an internship, undergraduate research or a capstone course focused on research, writing and preparation skills. However, the faculty feel strongly that practicing being a scientist in a research lab is the ultimate capstone. Thus, in addition to providing students an opportunity to learn and practice NGS in a regular class setting, an additional goal of the CURE course is to engage more undergraduate students and a more diverse group of students in research.

In this paper, we report upon the design of a CURE course focused on NGS including the research projects, student population, class meeting time, assignments, grading, survey results, lessons learned, changes made for the second iteration of the course, and conclusion. CURE courses and experiences have been offered to general chemistry (13), cell biology (10), molecular biology (11), bioinformatics (12), and organismal biology students (14), among others (15). One of us developed and incorporated shorter research-based experiences in our Biochemistry lab and Criminalistics II courses many years ago (17,18) and others have reported CUREs at recent conference symposia (19). To our knowledge, this is the first, full-length report of a CURE course for forensic science students.

Overview of CURE Course Design

Next Generation Sequencing in Forensic Science (3 credits) was taught twice as a special topics course: once each in the spring 2019 and 2020 15-week semesters. In 2019, we scheduled the class around other courses requiring the same lab space and other courses enrolled by upper-level students and held class for 100 minutes each on Wednesday and Thursday mornings.

We designed the course to introduce advanced sequencing techniques including next generation sequencing to our undergraduate and graduate forensic science students. The goals of our CURE course are shown in **TABLE 1**. The students study traditional short tandem repeat (STR) DNA typing methods using capillary electrophoresis (CE) in other courses; the goals of our CURE course included introducing forensic science students to NGS, working with human remains and difficult, low template samples, and improving their report writing and delivery skills. The student learning objectives of the course are listed in **TABLE 2**. They focus on the selection, implementation and reporting of DNA typing results.

TABLE 1 *Course goals*

Goal 1	The fundamental goal of this course is to introduce next generation sequencing using the ForenSeq™ Signature Prep Kit to enhance forensic methodology knowledge, skills, and marketability of students pursuing careers as forensic scientists in forensic laboratories.
Goal 2	An emphasis is placed on applying concepts of autosomal DNA typing to the analysis of human remains and forensic type samples in the law enforcement setting.
Goal 3	Students will learn and exercise problem-solving and troubleshooting skills and to be persistent in the laboratory
Goal 4	Students will integrate human identification concepts and analyze human remains and other samples using next generation sequencing and report the results in a paper suitable for publication in a forensic journal and in poster and oral presentations suitable for a forensic meeting.

TABLE 2 Student leaning objectives

1.	Describe DNA sequencing methods
2.	Select the appropriate DNA sequencing tool for a problem
3.	Summarize process of DNA-based human identification
4.	Design a project to analyze human remains samples and samples from collection devices
5.	Design a project and provide rationale and hypotheses
6.	Judge efficiency of sampling techniques and decide which to use
7.	Employ DNA extraction, quantitation and library preparation techniques and troubleshoot as necessary
8.	Generate sequence data
9.	Create graphs and charts to summarize data
10	Assemble data and results into a research paper suitable for publication in a forensic journal
11.	Prepare and present oral and poster presentations
12.	Reflect on the research project experience

Research Projects and Sample Acquisition

The research in our labs is diverse and includes investigating modifications to improve DNA extraction (20), determine the optimal DNA extraction method for difficult samples (21), testing methods to eliminate DNA contamination, determining the optimal DNA recovery region of long bones (22), developing PCR melt assays to genotype mitochondrial and phenotypic SNPs (and using Sanger sequencing to confirm the results) (23), assaying DNA methylation variation in body fluids using PCR melt assays (24) and pyrosequencing, and applying whole genome amplification to improve DNA typing for degraded and low template samples.

A team of investigators from the TU Department of Biological Sciences was awarded a U.S. National Science Foundation (NSF) grant in 2013 that funded the purchase of an Illumina MiSeq instrument. We were awarded grants in 2018 and 2019 from the TU Fisher College of Science and Mathematics Endowment fund that enabled us to upgrade the instrument to a Verogen MiSeq FGx for forensic applications, obtain the Universal Analysis Software (UAS) for performing sequencing and analysis, and participate in a three-day intensive training course. This work was performed under the auspices of, and supported in part by, a Howard Hughes Medical Institute Inclusive Excellence grant to TU. The authors were part of the second cohort of faculty recruited and accepted into the program by competitive application to incorporate CURE experiences into regular courses toward the goals of a structural change throughout TU. Unlike the faculty in cohort 1 who were all members of the Department of Biological Sciences, we are members of the Chemistry Department. Like several of the biologists in cohort 1, our formal training is in biochemistry and molecular biology. With the HHMI program, we participated in 50 hours of professional development during the academic year. The FCSM grants also supplied initial sequencing reagents and consumables for training and teaching purposes. DNA standards and human blood and saliva were purchased from commercial suppliers including Lee Biosolutions (Maryland Heights, MO), Origene (Rockville, MD) and Promega (Madison, WI).

Because of the new capabilities of our lab, we were able to develop a new collaboration with Maryland Department of Transportation (MDOT) Highway Administration anthropologist Dr. Julie Schablitsky and Dr. Dana Kollmann, a TU forensic anthropology professor and former crime scene investigator. Maryland is an old state and the cutting of new roads for development has unearthed unmarked human remains. Relatedly, Dr. Kollmann is frequently asked to analyze teeth and bones excavated from several historic sites including those in Maryland and Virginia. The NGS approach we adopted enables determination of familial relationships and sex typing, differentiation of monozygotic twins, and BGA and phenotype prediction. Currently MDOT and Dr. Kollmann's labs send out their samples for DNA typing to several labs across the country. Dr. Kollmann had also obtained modern bone samples from a body donation facility for teaching purposes. The collaboration provided us with unique samples that our students could incorporate into the CURE projects.

Other items we had acquired included DNA collection devices supplied by companies that were interested in our performance evaluations of their devices, a whole genome amplification kit, and modern teeth we obtained from the University of Maryland Dental School (Baltimore, MD).

Due to the time constraints of the course as well as the lengthy Institutional Review Board (IRB) process, we decided to provide students with options for samples that did not required IRB approval.

CURE Implementation

When we developed and taught the course for the first time, we front-loaded the course with traditional lectures comprising core content; we flipped the timeline in the second iteration and front-loaded the course with lab work. The final timeline is shown in **FIGURE 1**. The lecture content included the history of sequencing and the evolution from first to third (or fourth) generation sequencing, applications of STRs and SNPs for forensic DNA typing, history and practice of forensic DNA typing of STRs and SNPs using CE, DNA typing issues and troubleshooting, and practice of using NGS to sequence forensically relevant samples. These lectures were

followed by lectures on DNA extraction, DNA quantitation, library preparation, creating a sequencing run in the instrument software, instrument maintenance, performing a sequencing run, data analysis using the manufacturer's software and open-source tools, how the software works, and laboratory details and helpful tips for best sequencing performance.

We also scheduled the students to watch pre-recorded forensic NGS seminars by other scientists from a list we provided and planned journal club discussions of NGS papers from the literature. The two journal club sessions focused on the developmental validation of the ForenSeq Signature Prep Kit and results (2) and analysis of human bone and teeth samples remains using NGS and this kit (25). Students earned points by reporting on the seminars in short summaries and participation in journal club. The pre-recorded seminars were assigned on days when the faculty were traveling to conferences and were away from campus.

The students were provided class time to work in groups to develop their research questions, chart the samples they identified that they would need to answer the questions they asked and list the graphs and charts they planned to create from the data. Owing to the course focus on NGS, the students were asked to make their research questions tractable with NGS. They were told to choose samples that they could obtain or that our team had previously collected. They were informed which NGS sequencing kits and instrumentation were available. They were given the opportunity to decide which PCR primer set to use but were required to justify their choice. We provided feedback on all of the proposals. The students were able to revise the proposals prior to submitting them for grading.

Halfway through the course, the students were given a take-home essay mid-term exam that assessed their understanding of the material.

The remainder of the sessions were focused on collecting and preparing samples for DNA extraction, performing DNA extraction, quantifying the extracted DNA using quantitative PCR, performing library preparation steps, sequencing the samples, analyzing the results, preparing graphs and tables, and preparing written, poster and oral reports. The group submitted reports were graded using rubrics. The groups were not graded on the data obtained but their critical analysis and interpretation of the data and communication of the results.

In lieu of a final exam, students presented their oral and poster presentations during the last week of the course and anonymously responded to surveys and reflection questions we prepared. Students presented their posters at a multi-department CURE poster session.

The individual assessments included the pre-recorded seminar summaries, journal club participation, mid-term exam and end-of-semester survey responses. The remainder of the assessments were group work. Responding to the surveys was voluntary and students were told that they were not required to answer any question and their grades would not be affected.

The course has been renamed and approved by the TU department and university curriculum committees as FRSC 422/622 Advanced Sequencing Methods and will continue to be offered annually under the new course numbers.

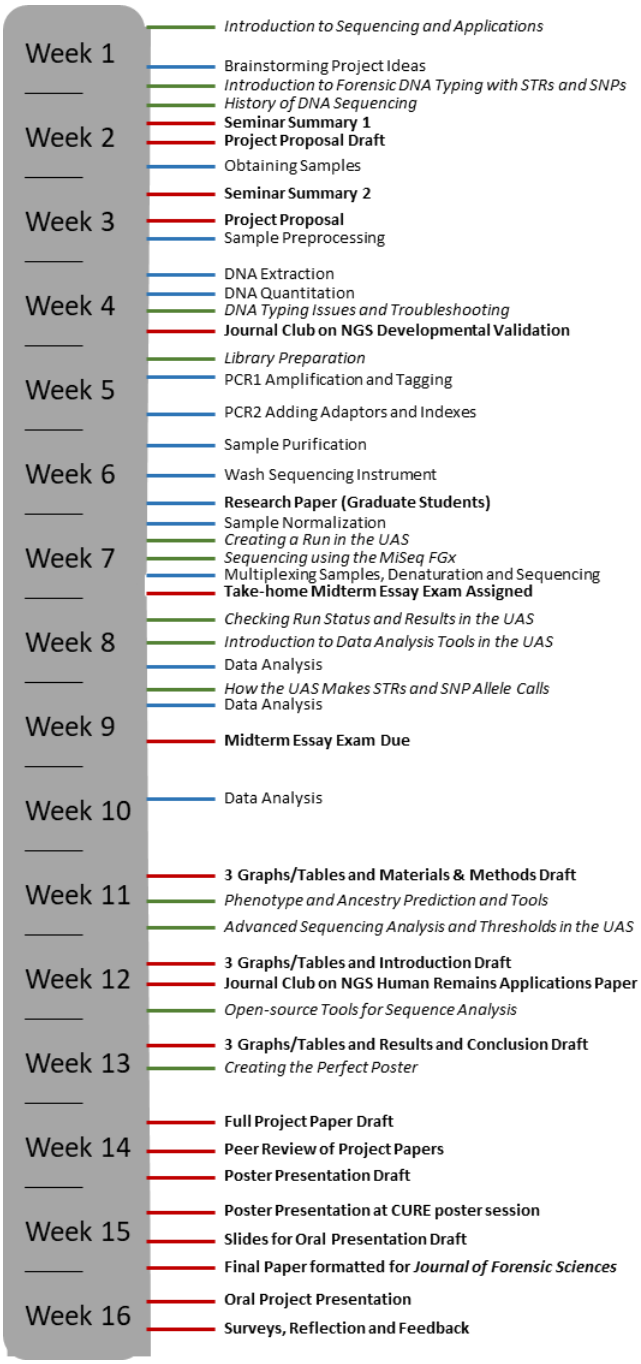

FIGURE 1 *Chromosomal course mapping of CURE weekly timeline. The bands for the lecture, lab and graded activities are denoted with the green, blue and red bands, respectively.*

Student Population and Groups for Projects

Our spring 2019 class was enrolled by 8 graduate students and 2 undergraduate students. The demographics of the course were as follows: 90% female, 10% male, 80% Caucasian, and 20% African-American. All of the students had at least limited undergraduate or graduate research experience. Both undergraduates had conducted undergraduate research and all of the graduate students had defended project proposals for their research projects the previous spring.

The students self-selected into groups focusing on one of four projects based upon their interests and the available samples. The result was two groups of two students and two groups of three students.

Course Outcomes and Student Work

TABLE 3 lists the project titles of the four 2019 projects. The students evaluated body fluid collection devices, whole genome amplification (WGA), and modern and historic bone and teeth human remains samples. The students sought to determine if they could obtain STR and SNP DNA profiles from the extracted DNA, if WGA pretreatment improved NGS profile success, which collection device performed best with different DNA extraction methods, if consensus profiles could be obtained with LT samples, and if ancestry and phenotype characteristics could be predicted using NGS.

TABLE 3 *Titles of group project reports in spring 2019*

Group 1	Comparison Study of DNA Profiles: Historical and Modern Bones using NGS and CE
Group 2	Taking a Bite out of Forensic Profiling: The Utilization of Next Generation Sequencing on Three Different Ages of Teeth
Group 3	Evaluation of SwabSqueezer, EasiCollect+™, and FTA card performance using next generation sequencing
Group 4	Evaluation of Whole Genome Amplification using Capillary Electrophoresis and Next-Generation Sequencing Methods

Overall, the students performed all of the laboratory work with interest and care and produced high quality reports. We encountered some issues implementing the library preparation steps in a class setting. For example, the bead purification steps need to be performed quickly on a few samples at a time and novices in groups performed the steps relatively slowly. Better outcomes were obtained when one, more experienced student performed these steps independently. Based upon their previous lab experiences working with purchased body fluid and standard samples, the students expected higher quality sequence data from the samples and found the sequencing data to be frustrating to analyze. But overall, the data reflected the expectations of the faculty for standard DNA and degraded and low template samples. All of the groups' lab work led to DNA sequence data and all students gained experience with NGS data analysis.

The groups working with human remains samples obtained partial profiles for most of the samples and were able to predict the phenotype and ancestry for the modern bone and teeth samples and some of the historic bone and teeth samples and reported the results to our community partners. Full profiles from 3.0 mm punches of dried blood were obtained using the SwabSqueezer, EasiCollect+™ and FTA card collection devices. DNA was extracted using the manual QIAamp DNA Investigator Kit and EZ1 DNA Investigator Kit using the BioRobot EZ1 and "Tip-Dance" protocol for FTA cards (Qiagen, Germantown, MD) using the manufacturer's protocols. The students captured a strength of NGS in detecting loci with sequence variations (**TABLE 4**).

TABLE 4 *Sequence variation (red) detected using NGS reported by collection device project group for a blood sample punched from the SwabSqueezer device*

Locus	Allele	Reads	Sequence
D2S1338	20	492	TGCCTGCCTGCCTGCCTGCC TGCCT**T**CCTTCCTTCCTTCCT TCCTTCCTTCCTTCCTTCCTT CCTTCCTTCCTTCCTTCC
		490	TGCCTGCCTGCCTGCCTGCC TGCCT**G**CCTTCCTTCCTTCCT TCCTTCCTTCCTTCCTTCCTT CCTTCCTTCCTTCCTTCC

WGA treatment improved DNA profile success as shown in the Venn diagram comparison of recovery of alleles for a DNA standard using NGS before WGA but after one minute of sonication at 18% and post-WGA using the REPLI-G kit (Qiagen, Germantown, MD) using the manufacturer's instructions (**FIGURE 2**).

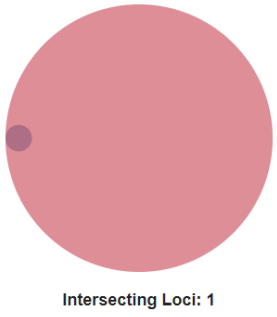

FIGURE 2 *Venn diagram comparison from UAS of typed STR loci for a degraded sample (1) and following WGA (102) using ForenSeq™*

Student Presentations

The student groups prepared and presented their oral presentations to forensic faculty, the Forensic Program Director and Director of the Forensic Laboratory Section at the Baltimore Police Department in 2019 and a Crime Scene Technician for Baltimore Police Department with a background in DNA typing in 2020. The presentations were well received by all parties.

The four student groups also presented posters in the CURE Poster Session held outside of the regular class time on the last Friday of the semester in May 2019. Two student groups presented the poster presentations at the 2019 Mid-Atlantic Regional Meeting (MARM) of the American Chemical Society (ACS) in Catonsville, Maryland on June 1, 2019.

Surveys and Student Feedback

There are several "off-the-shelf" assessment instruments that faculty could use to assess their CUREs; several are tabulated in a paper by Shortlidge and Brownell (26). Student attitudes and reflections about the course were assessed using the Student Assessment of Learning Gains (SALG) survey (27), Laboratory Course Assessment Survey (LCAS) (28), and Project Ownership Survey (POS) (29) instruments and open-ended questions we created that were more closely aligned with our CURE. The SALG, POS and LCAS instruments were adopted by the HHMI leadership team to evaluate the grant outcomes. These published tools have been demonstrated to be valid and reliable. We used them to evaluate student outcomes and improve our teaching. As suggested by Ohland et al., faculty can incorporate self- and peer evaluations, and analyze them to improve their courses (30).

We conducted the surveys during the final exam week of the course. The students were provided a Microsoft Word document to edit on a computer and instructed not to change the font. A sign in sheet was placed in the Chemistry Department office. Upon completing the survey and printing the forms, the students turned in the surveys to the office anonymously and signed the sheet. All students were given full credit for the survey points when the surveys were returned.

Lessons Learned

The lessons learned about student perception from our open-ended survey questions were apparent during class. We asked the ten students (8 GRAD / 2 UGRAD and all female) in the first iteration of the course about their thoughts on several aspects of the course design. On the topic of ideal class size, all of the students felt that the ideal enrollment for the course should be twelve students and four students suggested six as an ideal number. Separately, we asked how many students should be the maximum number of students. The majority of the students indicated that the maximum number of students for the course should be eight although a couple felt 15-16 was acceptable. Regarding optimal group size, a strong majority indicated two students while a few felt groups of three were acceptable.

While the class met for approximately four hours a week in 2019, the majority of the students responded that more class time was needed and one student responded that 10-12 hours a week were needed. Four students found four hours acceptable and the majority of students felt that 5-6 hours were needed. The student who felt that 10-12 hours were needed may have been conflating class time with Masters research time since the NGS projects were heavily scaffolded on samples procured for student research projects in the first iteration. All of the students indicated that there were not enough lab hours but that there were sufficient lecture hours.

All of the students reported that the number of assessments was about right but one felt there could be an additional assignment before the midterm. All of the students reported they understood NGS after the course. After one iteration of working with the ForenSeq kit for library preparation and the MiSeq FGx for sequencing, seven of the students reported confidence with the procedure and three said they would need more runs or help with the protocol. Our population was students pursuing forensic degrees but when asked if they would prefer a general NGS research or ForenSeq-focused course, the results were split evenly. The majority of students felt the best feature of the course was the hand-on experience with the forensic library preparation kit and sequencing instrument although one of the students found the data interpretation portion to be the best part and two students responded that the research report and presentation were the best part.

NGS is also being used to analyze mitochondrial DNA in the forensic setting. When asked if mitochondrial DNA analysis be added to the course, almost all of the students responded that it should be a separate course.

The majority of students responded that their least favorite part of the course was the time they needed to put in out of class time. Other responses include having the take-home midterm over spring break, the group project, having three students in a group, too few hours for analysis, the course structure (perceived lack thereof), and the undergraduates did not like working with the graduate students. One student did not like having the faculty co-teach the course. The majority of students felt more time should be allocated to the lab and group work while a few felt the course should allocate more time to theory.

Changes for Second Iteration

Based on the survey results and student feedback we received, we instituted several changes for the second

iteration of the course. The first change was to the course meeting times and contact hours. In 2020, after scheduling around existing courses and the students' schedules, we were able to arrange class time on Monday and Wednesday afternoons totaling six hours a week. Students indicated in their feedback last year that they disliked when class ran over or they had to spend a lot of time out of class.

The second change was to the schedule. Based upon the feedback we received from the students last spring, we reorganized the schedule to begin the laboratory portion of the course earlier as reflected in **FIGURE 1**. After presenting introductory lecture material about forensics, applications of forensic analysis, the history of sequencing and different approaches to next generation sequencing, we engaged the students in a discussion of their interests during the first week. We were able to do this, in part, because of the extra class time we scheduled. During the second week of the course, the students drafted their research proposals and submitted them for feedback. By the end of the third week of the course, all students had clear project plans and approved projects. Upon considering their interests, three groups were formed with students self-selecting based upon their interests from the three projects that had the highest interest. The groups consisted of two graduate students, one graduate student and one undergraduate student and two undergraduates. Based on the feedback from the 2019 cohort, there were no groups of three. We immediately led the students through the steps leading to sequencing. We employed signposting and the just in time (JIT) teaching approach and taught pre-lab content just before the laboratory sessions and data analysis content when the students had data to analyze. Adding additional class time and scheduling a longer day (four hour) for lab activities enabled us to complete sample prep and library prep activities during class time and the students were ready to begin sequencing during the week before spring break. Students were also able to perform multiple iterations of some steps including pipetting, making dilutions, preparing master mixes, and performing PCR, purification and normalization steps and able to redo steps to correct errors or recheck analyses.

The third change was to the prerequisites. We removed the minimum GPA requirement. In the spring of 2020, the course composition was all female including three graduate students and three undergraduate students with 33% identifying as African American and 67% as Caucasian. The graduate students had all completed FRSC 620 Forensic DNA Analysis and had completed or were co-enrolled in FRSC 621 Advanced DNA Analysis. The undergraduate students were all seniors majoring in Chemistry and had completed General Biology. Most of the students had also completed FRSC 420 Forensic Body Fluid Analysis, BIOL 309 Genetics, and BIOL 409 Cellular and Molecular Biology. Because of these changes, we were able to attract a more diverse group in terms of more students who had not had the opportunity to conduct a research project and minority demographics. While three of the students had previous research experience, this was the first research experience for two undergraduates and one graduate student. We were able to meet one of the goals of the HHMI grant: to get more students, including students from minority groups, involved in research.

The fourth change we made was to the due date for the midterm exam. While the students still received the midterm exam prior to spring break, they were given two weeks instead of one to complete the exam so they did not have to work on it over break.

The fifth change was that we included more intentional mentoring. In 2019, we guided the students toward the final paper including graphs and charts by making suggestions for graphics and pointing the students to journal articles that included different and novel approaches to analyzing similar data. We established an intermediate deadline by which the paper draft was due. Each paper was read by peers and the faculty and the groups were provided suggestions for improvement. However, all of the deadlines for the final paper, oral presentation and poster presentation arrived within the last week of the course. Also based upon student feedback, in spring 2020, we carefully planned the schedule to guide the students through the process of creating graphs and charts and writing the paper over a month time frame, but we added weekly deadlines using our course management system with a small amount of associated points for achieving the intermediate goals. Students were able to upload their work for a grade and we were able to provide feedback on their work electronically. The paper, poster and final presentation deadlines were set so that one major product was due each week in the final three weeks of the course, thus avoiding the due date traffic jam that we encountered last spring. We were accommodating in regards to network and internet complications and making deadlines around other courses. We added concrete lessons on how to create the perfect poster and oral presentation tips.

Arguably the two biggest changes that occurred during the spring 2020 iteration were not planned. After we loaded the students DNA samples for sequencing, the instrument failed mid-run. Although we immediately identified the problem and received a quote to repair the instrument, another challenge presented itself. The SARS-CoV-2 (COVID-19) virus caused our campus leadership to close the university campus for all but essential functions and ordered all face-to-face classes online. These events impeded travel and fast instrument repair and forced us to implement our plan b solution. With the instrument down and other campuses in our region also closed, we were not able to sequence the samples the students prepared in class during the semester. Our students installed a VPN application that enabled them to access our NGS data server off-campus. With their accounts to login to the software, they were able to access all of the data our

research students and 2019 class had collected. We provided the students with the sample details for all of the previously sequenced samples and asked them to develop a new "project" based upon the existing data. Because we were all working remotely from our homes, the students each chose a new project and worked individually to produce their papers, posters and oral presentations. NIST scientists provided several advanced webinars that we offered to our students as additional learning opportunities.

A final change was to the assignments and grading. The graduate students were required to write an 8-10 page review-style research paper on a NGS-related topic of their choosing. A student enrolled in spring 2019 suggested that we add an additional assignment prior to the midterm. This change was supported by our department curriculum committee. Further differentiation of the undergraduate and graduate courses was required for the new graduate course to be approved and formally be adopted into the curriculum and catalog.

Finally, we offered a separate course in spring 2020 focused on advanced sequencing, including NGS, of mtDNA which was enrolled by one graduate student in a pilot iteration.

Discussion

Both times, the CURE course met our student learning goals. We facilitated the students being able to uncover science and be a part of research advancing science rather than feed the student expectation that the faculty will cover the science for them in a lecture. Throughout the course, we used signposting to convey transparently progress and completion of tasks in traditional labs and incorporated into the course. We showed feedback loops demonstrating repeats through experiment when we had to prepare samples again or repeat a step. Upon seeing the NGS results in 2019, the students were (understandably) deflated. For many of them, it was the first time in their college experience that their experiments resulted in incomplete or poor data. Upon seeing their reaction, we immediately pivoted to our second journal club paper (25) discussion which highlighted another lab's challenges with similar samples and data. The students struggled but we, the faculty, were there to assure and lead them; in education this is referred to as the zone of proximal development. The class employed a student-centered approach model and we functioned as mentors or the guides-on the side rather than the sage(s)-on-the-stage. The students produced very high quality presentations of their research in oral, poster and written form as assessed by the course faculty, program director, and external evaluator. Two of the student posters were presented at the Mid-Atlantic Regional Meeting (MARM) of the American Chemical Society in May 2019.

Although the results of our surveys were student perceptions from a small population, we made several changes in 2020 that improved the course. In 2020, we added additional class time to achieve the learning goals. We limited our lectures during the first half of the semester to essential background and theory and the rest of our lectures were short pre-lab type lectures before laboratory and analysis steps. The student-centered approach was most apparent when we only saw our students virtually. We developed a framework to help them to achieve the course goals and guided them through course materials and instituted intermediate deadlines to complete the course products. We created and provided instructions for how to create graphs and charts and download the data that were available to view (and re-view) asynchronously. The students reported upon the data, were provided feedback and closed the loop with the revisions they made prior to submitting their final products.

The most important trait for faculty teaching CURE courses is flexibility. The demands of unpredictable lab work require the faculty to be flexible and be able to pivot and readjust. The course also offers many opportunities for the faculty to model how they respond to failures or issues in laboratory procedures. While we co-taught our CURE course, other faculty employ graduate assistants or undergraduate learning assistants to help with the demands of the course. The change to remote teaching mid-semester in spring 2020 required patience and understanding from our students and us as we all learned to teach and learn using the new online medium.

The students in the course represented our student population in forensic courses which is majority female and our Master of Science in Forensic Science Program which is approximately 95% female. As many of our 2019 students were second year graduate students and senior undergraduate students, the majority had been able to find a research experience. We were inclusive in encouraging students from diverse backgrounds to enroll in the course and served three students (50% of the 2020 class and 18.75% overall) who had not been able to conduct research thus meeting our goal of introducing more students to research. The course and its content has impacted our continuing students in an unexpected way. Several students have developed Master's research projects incorporating NGS data, studies of DNA recovery and profiles of human remains, and studies of NGS thresholds that would not have been possible without the new capability and what they learned in the course. At least three of our former students interviewed for jobs based in part upon their NGS experience.

The CURE course was an elective for our graduate and undergraduate students. Upon adoption by the undergraduate and graduate programs, we expect the enrollment and number of students we reach will increase.

Conclusion

This CURE course offered TU students an opportunity to learn the theory and practice of NGS technology as it is

currently being applied to forensic science and exposure to research in a regular course setting. Although the number of students the courses have reached so far has been small, the students reported that they enjoyed the experience and a couple were offered positions based upon their NGS experience in the course. As faculty, we are pleased that it is making the students marketable. The CURE approach proved successful for students to learn NGS and how to conduct research. The course has been added to the curriculum as a required course for all of our majors in the DNA track in our Master of Science in Forensic Science and Bachelor of Science in Forensic Chemistry programs. Students in other FEPAC-accredited forensic programs would also benefit from instruction on MPS / NGS in a CURE format and our aim is that other faculty will find the framework convenient to employ or adopt to teach NGS at their institutions.

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