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A cure for salmonella: Engaging students in pathogen microbiology and bioinformatics

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A CURE for *Salmonella*: Engaging Students in Pathogen Microbiology and Bioinformatics

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Preface

Pilot Semester of *Bacterial Discovery*

As a new requirement of the Microbiology concentration of the Biology major, *Bacterial Discovery* (BIO 346) is a Course-based undergraduate research experience (CURE) model laboratory course designed as a part of this project. The course integrates methods developed in the Herrick laboratory to isolate and characterize novel environmental *Salmonella* using laboratory and bioinformatics techniques (Jurgensen and Herrick, 2018). The course was piloted in the spring 2018 semester with Dr. James B. Herrick as the instructor and Sophie Jurgensen serving as a teacher's assistant.

During the spring 2018 semester, a total of seventeen students enrolled in *Bacterial Discovery* (fifteen Biology/Microbiology students and two Health Sciences students). Students collected sediment samples from two sites along Cook's Creek (38.390302N, -78.947585 W and 38.372706 N, -78.934501 W) and one on Muddy Creek (38.467152 N, -78.974999 W) in Rockingham County, Virginia. Poultry litter was provided from three different sources by the instructors: one from a local large-scale turkey farm, and two from small-scale poultry houses.

Students used the Herrick lab protocols to isolate *Salmonella* from a total of 21 sediment and litter samples. After selective enrichments and plating on selective and differential media, coupled with standard microbiological tests (Gram stain, KOH test, etc.), students used PCR to amplify the *Salmonella*-specific *invA* gene as a final confirmation of their isolates. I*nvA* is a virulence gene specific to *Salmonella*; it encodes an invasion gene that allows entry into epithelial cells as part of a Type III secretion system (Galan *et. al*, 1992). This gene is an ideal target in this situation because it has no recorded false-positive results (Yan *et. al*, 2017, Calayag *et. al*, 2017). Students in *Bacterial Discovery* successfully isolated 3 *Salmonella* strains and 9

Escherichia coli strains. The Virginia Department of Consolidated Laboratory Services (DCLS) sequenced each of the *Salmonella* isolates' whole genomes, and is currently processing the *E. coli* for sequencing, although this data will likely not become available during the pilot semester of this course. Further bioinformatic analyses were performed on the raw read sequence files. These analyses included genome assembly, serotyping, virulence gene identification, identification of genomic islands, detection of prophages, and plasmid occurrence. The semester will culminate in student poster presentations at the annual Biosymposium, as well as an in-class oral presentation.

This thesis is formatted as a manuscript to be submitted to CourseSource, an open-access journal of peer-reviewed teaching resources for undergraduate biological sciences (https://www.coursesource.org/). Because the course was not yet completed at the time this thesis was submitted, the manuscript itself is not yet in its final form for submission for publication. We plan to use parts of the Course-based undergraduate research experience survey (Lopatto, 2008), the Undergraduate Scientists: Measuring the Outcomes of Research Experiences from Multiple Perspectives (USMORE) survey (Maltese, Harsh, and Jung, 2017), and the Laboratory Course Assessment survey (LCAS, Corwin *et al.*, 2015) to assess students' perceived learning gains, outcomes, and influence of collaboration, discovery, and iteration on the experience. All of these assessments will be administered at the end of the semester. This study was reviewed by the JMU Institutional Review Board (IRB #18-0508).

Manuscript

A CURE for *Salmonella*: Engaging Students in Pathogen Microbiology and Bioinformatics

Sophie K. Jurgensen and James B. Herrick

Abstract

Advances in Next Generation Sequencing (NGS) technology have generated a vast amount of publicly available genomic data, creating a need for students with training in computational analysis. This laboratory lesson is a course-based undergraduate research experience (CURE) focusing on environmental *Salmonella*, a common foodborne pathogen that is of great interest to public health laboratories but is relatively less virulent than most other such pathogens. As discovery is a central tenet of CUREs, students isolate novel *Salmonella enterica* and related strains from stream sediment, poultry litter, or other sources in the first half of the lesson (Module 1). They also conduct phenotypic detection of antimicrobial resistance and large plasmids. Isolate genomes may be sequenced by the FDA or public health laboratories (ours were sequenced by the Virginia Department of Consolidated Laboratory Services at no charge). The second half of the lesson (Module 2) involves the bioinformatic analysis of this sequence data. Students use easily accessible, primarily web-based tools such as GalaxyTrakr and Enterobase to assemble their genomes and investigate areas of interest including serotyping, identification of antibiotic resistance genes and genomic islands, and evidence of plasmids. After completion of this course, students should be able to demonstrate skills in the isolation and identification of *Salmonella* from natural sources, as well as skills necessary for computational analysis of microbial genomic data, particularly of members of the *Enterobactericaeae*. While this course consists of two modules, one focusing on laboratory skills and the other bioinformatics, either could be used as a standalone module.

Scientific Teaching Context

Learning goals

Students will experience an authentic faculty-led research experience in the classroom that can produce original and potentially publishable data on the comparative genomics of environmental *Salmonella enterica* and related members of the *Enterobacteriaceae*. The students are thus an integral part of the research team for this broad project. Exposure to advanced microbiological laboratory techniques as well as bioinformatics tools will allow students to develop their skills on real genomic and phenotypic data that they have helped to generate. They will also learn how these techniques are currently being used in epidemiology to track infectious disease outbreaks.

Learning objectives

1. Understand how whole genome sequencing is used in epidemiological tracking of foodborne bacterial pathogens.
2. Learn how comparative genomics methods can be used to study antibiotic resistance, virulence, and mobile genetic elements in pathogenic bacteria.
3. Prepare media and reagents used in the isolation, identification, and characterization of *Salmonella enterica* and related members of the *Enterobacteriaceae* from environmental sources.
4. Safely handle human pathogenic bacteria in a Biosafety Level 2 laboratory environment.
5. Isolate *S. enterica* from stream sediments, poultry litter, etc.
6. Identify and characterize isolates using microbiological and molecular techniques.
7. Assemble isolates' whole genome sequences and assess sequencing and assembly quality.
8. Type isolates using multiple methods and determine their phylogenetic relationship to other *S. enterica* strains.
9. Determine and compare antibiotic resistance genotypes and phenotypes of isolates.
10. Investigate the occurrence of mobile genetic elements – plasmid-specific genes, transposons, integrons, pathogenicity islands, prophages, etc. – in isolates.
11. Work in groups throughout the lesson to prepare and present data in poster and oral formats.

Introduction

Introduction

The most current and complete method of characterizing individual bacterial isolates is whole-genome sequencing (WGS), where the entire bacterial chromosome is sequenced. WGS of bacteria has become increasingly employed due to its relatively low cost for information received. Sanger sequencing methods have been used for decades to sequence single genes and even whole bacterial genomes, although at a high monetary and time cost. More recently-developed high throughput (or "next generation") sequencing methods have made it possible to sequence entire bacterial genomes quickly and affordably (Goodwin, McPherson, and McCombie, 2016). These higher throughput methods allow for higher resolution in typing, distinguishing, and characterizing bacteria on the subspecies level because even subtle genetic differences (such as single-nucleotide polymorphisms or SNPs) can be identified. The U.S. Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), and state public health laboratories have long conducted extensive investigations when outbreaks of foodborne pathogens occur in order to discover the origins of the outbreak. The advent of WGS has made it possible for these agencies to vastly expand their knowledge of known pathogens for epidemiological tracking. WGS provides the finest currently available level of classification and identification possible for tracking potential pathogens and their outbreaks.

Common foodborne pathogens are of great interest to the FDA and other public health laboratories such as the Virginia Department of Consolidated Laboratory Services (DCLS); both agencies are eager to sequence *Salmonella* at no cost and with a relatively short turnaround time. This urgency combined with the advances in WGS make this system ideal for the development of a Course-based Undergraduate Research Experience. We developed a lesson that stems from

and contributes to an ongoing research project at James Madison University. We use *Salmonella enterica* as our model organism in this lesson because it is a foodborne pathogen that infects over one million Americans every year, causing approximately 378 deaths, and is one of the leading infectious causes of hospitalization in the United States (Scalla *et al.*, 2011). However, *Salmonella* is also relatively less virulent than other foodborne pathogens of interest to these labs, such as *Listeria* and pathogenic *E. coli* (Bell *et al*., 2015). This problem is an authentic one and authentic experience is a central tenant for CUREs.

While the isolation of *Salmonella* from clinical and food sources is relatively common, information on *Salmonella* isolated from environmental sources such as freshwater is still relatively uncommon. Interestingly, *Salmonella* are more easily isolated from fresh water and sediment samples than from feces (Bell *et al,* 2015). While its pathogenicity has made *Salmonella* a commonly studied organism epidemiologically, its occurrence environmentally has not been sufficiently investigated. Pathogens have traditionally been studied in the context of human infection and food, with less regard to their potential environmental reservoirs. These potential reservoirs include reptiles, fresh waters, and manure (Burgess *et al.*, 2015). We used sediment from agriculturally impacted streams because it potentially harbors a more stable microbial community than water (Bell *et al.*, 2015). For this CURE, we worked with several local small-scale and industrial poultry farmers who provided poultry litter from their farms, as *Salmonella* is normally a commensal organism in turkeys and chickens.

The most traditional pathway for training students in research is the apprentice-mentor model, where students learn under the direct supervision of an advisor (Brownell *et al.*, 2011). However, the number of students desiring such mentoring exceeds the supply of research mentors, available space, and money in many institutions (Brownell *et. al*, 2015). A well-

researched alternative to one-on-one mentoring is a course-based undergraduate research experience (CURE), in which students in small classes (fewer than 50 students) engage in authentic research under the supervision of an expert (Bakshi *et. al*, 2016). These differ from 'cookbook' style laboratory classes in that students are given freedom in defining procedures and analyzing data (Brownell and Kloser, 2015). Potentially publishable data may be produced by student analyses. Students enrolled in CUREs experience authentic research via common processes such as discovery, iteration, and collaboration in a broadly relevant research context; in this lesson, students are an integral part of the scientific process. Collaboration is a particular focus of this CURE, as students work in teams throughout the lesson from designing their sampling scheme to presenting their results. CUREs typically produce highly engaged, confident students who feel they are more capable of continuing their STEM education than their counterparts in more traditional courses (Brownell *et. al*, 2015). Additionally, these students show progress in their views of science as creative and process-based relative to their peers in traditional courses (Auchincloss *et al*., 2014). CUREs represent an educational paradigm shift geared toward authentic research experiences for STEM students. According to a survey of 118 institutions, in 1993 only 10% of colleges used what was perceived as an inquiry-based laboratory curriculum while over 70% of colleges reported using inquiry-based laboratory instruction in 2005 (Brownell and Kloser, 2015). Resources for teaching these courses are available through groups such as CUREnet (https://curenet.cns.utexas.edu/).

CUREs, however, can be difficult to design and implement because the course outcomes by definition are often unpredictable. This challenge can be addressed by implementing a backwards design model, where instructors first identify desired learning outcomes, then develop assessment methods, and finally plan the course activities to achieve the desired results (Pelaez,

Anderson, and Postlethwait, 2015; Sanders *et. al*, 2016). This lesson follows such a model, building from a continuing research project in our research laboratory.

Intended Audience

This lesson could be directed to different student populations and levels. We have incorporated it into an upper-division course (*Bacterial Discovery*, BIO 346) at JMU, intended for a biology major concentrating in microbiology. Because the first module requires a BSL-2 lab space, not every college or university may be able to incorporate it into their course. However, the bioinformatics module of the course could potentially be implemented in some form into a course for even non-major underclassmen through graduate level students. In order for the material to be accessible to novices in bioinformatics, this module exclusively uses freely available online tools that do not require any use of the command line.

Required Learning Time

This laboratory lesson is divided into two modules, each taking roughly 8 weeks of a 16-week semester. We taught the course in twice weekly 90-minute lab periods as a standalone laboratory course (with no required lectures). Our microbiology laboratory spaces can accommodate up to 24 students, and we currently offer one section of this course per semester. The Teaching Timeline (Table 1) includes the time required for each laboratory activity, set up, and out of class time (as necessary).

Prerequisite Student Knowledge

Module 1

The prerequisite student knowledge depends on the course level and focus. In a course employing this first (microbiology) module, students should have taken a general or introductory microbiology course with laboratory and have sufficient skills in culture maintenance, basic diagnostic biochemical tests, and common isolation methods. However, if module 2 is not being used, there would be sufficient time to teach these concepts before isolating the *Salmonella*. BSL-2 safety training for all students is required for the implementation of this module as the target organism (*Salmonella*) is a human pathogen, and there is the possibility that other, unknown pathogens could be isolated.

Module 2

In courses using only the second (bioinformatics) module, students should have a basic knowledge of the characteristics of the bacterial genome and basic genetic concepts such as the Central Dogma and horizontal gene transfer. No pre-requisite knowledge of bioinformatics is required. All tools used in this lesson are freely available online, so only basic computer expertise is required to complete this module.

Pre-requisite Instructor Knowledge

Module 1

This lesson assumes that the instructor has significant training and experience in microbiology, as well as some familiarity with field sampling and working with environmental samples in the laboratory, before implementing this lesson. We recommend that the lesson be piloted before full implementation so the instructor can become familiar with how to, for example, recognize *Salmonella* on the selective media, and identify sampling sources and sites expected to reliably yield *Salmonella*. Familiarity with BSL-2 safety protocols is a necessity.

Module 2

This lesson assumes that the instructor has some knowledge of bioinformatics. For instructors who are not familiar with bioinformatics and are interested in incorporating this module into their course, working through our provided materials should be sufficient to understand the concepts addressed, but reading cited peer-reviewed literature is recommended. GalaxyTrakr and especially Galaxy provide a variety of tutorials to introduce users to the interface and available tools and offer extensive explanations of provided tools (https://usegalaxy.org). Instructors and students may request GalaxyTrakr accounts for themselves (https://galaxytrakr.org/). Enterobase also offers users explanations on provided tools via a Wiki manual (https://enterobase.warwick.ac.uk/). Instructors and students may sign up for Enterobase accounts without any special permissions from developers, whereas requests must be made for GalaxyTrakr accounts. The Center for Genomic Epidemiology's website (http://www.genomicepidemiology.org/) does not require users to create accounts.

Scientific Teaching Themes

This lesson is implemented as a course-based undergraduate research experience (CURE) (Bakshi *et. al*, 2016, Brownell *et. al*, 2015). It is a hands-on introduction to laboratory and bioinformatic techniques that encourages students to work in teams to produce and analyze genomic and other data with real-world applications. The main goal of this lesson is to produce students who are knowledgeable and confident in their abilities to work at the bench and with a computer on a project that they initiate, carry out, and conclude within the timescale of the course.

Active Learning

This laboratory lesson uses multiple approaches to engage students in active learning. There is very little mere observation or passive acquisition of knowledge in either of these lesson modules. Most activities are carried out in teams, and students work in small groups to plan and implement their approach to each lab period. We assign review and other summary readings as pre-class homework, followed by instructor-led group discussions. These whole class discussions also aid in troubleshooting. These troubleshooting situations are inevitable due to the inherent unpredictability of authentic research, as well as student errors. Because students follow the research process from sample collection through isolation of target organism to genomic data analysis, there is significant project ownership inherent in the lesson. In Module 1, students must make real-time decisions about the outcomes of each procedure and determine their next steps as a group. In Module 2, students follow developed tutorials at their own pace and decide as a group what analyses to pursue based on their interests. Both poster and oral presentations are designed, presented, and evaluated as a group.

Assessment

In our implementation, students completed short quizzes on the protocols to be carried out during lab periods to ensure that protocols were read and understood before being implemented. To assess overall knowledge of the project, our students presented posters with their groups at the annual departmental symposium and were scored by the instructors based on the included rubrics (Supplementary File S1). Students also completed an oral presentation in class and completed laboratory notebooks (both traditional lab books for Module 1, and electronic notebooks for Module 2) which were assessed for content knowledge and clarity as final evaluation opportunities (S2, S3). Learning objectives were aligned with lesson activities and assessment instruments (Table 1). We used sections of the CURE, USMORE, and LCAS surveys to assess students' perceived learning gains, outcomes, and influence of collaboration, discovery, and iteration on the experience (S4).

Inclusive Teaching

In general, CUREs are a way for students who otherwise may not be able to participate in research due to extracurricular activities access to the knowledge and skills required of research. The CURE we have developed is readily adaptable to student populations at a variety of levels and institutions. This lesson as a whole is designed for implementation at the 300 or 400 level for biology/microbiology/allied health students at a four-year institution. However, Module 2 could be modified and implemented as a standalone online module at the community college or even high school level as the tools used are mainly web-based and thus accessible with an internet connection. Doing so would make the lesson more accessible to non-traditional students,

although access to a computer is still required. While the lesson focuses entirely on *Salmonella*, nearly all aspects of Module 2 could be applied to other organisms including relatively more virulent pathogens that instructors or students may be interested in studying but do not have the appropriate facilities to work with at the bench.

Students work together in small groups throughout the lesson, encouraging cooperation in overcoming obstacles during both modules. Students learn to balance their "ideal" experimental design with feasibility and access, specifically related to accessing streams and poultry litter sources in Module 1 and available genomic data and analysis tools in Module 2. Discussion throughout the lesson emphasizes the importance of environmental reservoirs of pathogenic organisms and their connection to the dissemination of antibiotic resistance, as well as how research conducted by students directly applies to vital epidemiological investigations conducted by the FDA, CDC, and state public health laboratories. Additionally, pedagogical approaches implemented in this lesson accommodate a variety of learning styles and ability through demonstrations, mini-lectures, videos, hands-on activities, worksheets, discussions, and optional additional tutorials.

Lesson Plan

Module 1

One of the most time-intensive parts of incorporating this module into a course may be identifying likely sources for *Salmonella*. We use agriculturally-impacted local streams because we previously found that they were a source for *Salmonella* in our area. We also use poultry litter because *Salmonella* is typically a commensal organism in the fowl gut. Other environmental sources could include reptiles, food, or other manures. Relative proximity to your institution should of course also be a factor when choosing sampling sites. We use a YSI Professional Plus multiparameter instrument (SKU 6050000) to collect water temperature, pressure, salinity, and conductivity data at stream sample sites and store this and other metadata (date, time, latitude and longitude) using EpiCollect5 (https://five.epicollect.net/) which is available as both a mobile application and website (S5). When culturing bacteria beginning in lab 3, all procedures in this Module must be conducted in a BSL-2 laboratory space (S6). Safety documentation for students can be found in supplementary file S7.

In this module, students prepare their own enrichment and isolation media, although the media can of course simply be provided. We provided trypticase soy agar (TSA) and broth (TSB) for students to maintain cultures and to grow liquid cultures to prepare for labs 7-9. After lab 6, all our confirmed *Salmonella* isolates were sent to the Virginia Department of Consolidated Laboratory Services (DCLS) for WGS. Before teaching this lesson, instructors should contact state public health laboratories or the FDA Whole Genome Sequencing (WGS) Program (https://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/default.htm) to ensure that they will accept samples and expedite sequencing to ensure a turn-around

time short enough for a semester-long lab. If availability of time or funds are limiting, labs 9 and 10 may be removed from the module. Many state public health laboratories are particularly interested in receiving such samples because they must meet established quotas for WGS of common pathogens as a means of contributing to governmental databases. While we used a professional connection to establish our relationship with the DCLS, other state health labs have similar quotas and should be willing to establish similar relationships. We reached out to Oxford Nanopore to request a sequencing kit at a reduced cost, and suggest that instructors do the same if they wish to use a MinION® in their course. Oxford Nanopore provided our pilot semester with a sequencing kit at no cost, although we were required to purchase a flow cell separately.

Module 2

This module requires extensive preparation to ensure that student-generated files are well organized and analyses are easy to find and use. We recommend use of the Open Science Framework (OSF, https://osf.io), an open source data management platform, to access and store genomic data and analysis files, as well as student electronic lab notebooks. We have created an OSF page to function as a living repository of protocols, templates, and instructions to be used for both modules of this course (Jurgensen and Herrick, 2018). If your institution does not already use OSF, you can work with the Center for Open Science to create a dedicated institutional OSF landing page so that students can use their university sign-in credentials to connect to the OSF.

We use GalaxyTrakr, which is a limited implementation of Galaxy – an open, web-based platform for computational tools used to analyze genomic data – for the majority of student analyses in this lesson. Other web-based platforms we use include Enterobase (http://enterobase.warwick.ac.uk/) and the website of the Center for Genomic Epidemiology

(http://www.genomicepidemiology.org/). A general overview of tools used and where they may be found can be seen in Figure 1. Instructors should familiarize themselves with these platforms before beginning instruction.

Teaching Discussion

Challenges in Implementation

A crucial step in developing Module 1 was choosing appropriate sample sources and sites. We used sediment from agriculturally-impacted streams in the Shenandoah Valley that have been regularly sampled in our research lab at James Madison University. These sites are relatively well characterized with respect to their potential as reservoirs of *Salmonella*. We also contacted farmers at local large-scale industrial and small-scale poultry farmers to obtain poultry litter as samples for students. Since these types of sample sites are not available to all institutions, other sample types such as food, pet birds or reptiles should be considered.

Another challenge for Module 1 can be getting sufficient recovery of *Salmonella* from the first round of sampling. In our pilot semester, 6 student teams created 28 pre-enrichments from a total of 3 stream sites and 3 litter sites, yet they only successfully isolated 3 *Salmonella* strains. Students isolated 9 *Escherichia coli* strains as confirmed by Enterotube testing. These results contrasted with those typically obtained by undergraduates in our research lab, where *Salmonella* is routinely isolated from the same sites. Identification of *Salmonella*-like morphology on BS and XLT-4 agars can be difficult at first as *Salmonella* may exhibit atypical morphology and other members of the *Enterobacteriaceae* may appear similar to *Salmonella*. In a typical semester, there is sufficient time for students who are unsuccessful in isolating *Salmonella* to repeat labs 2-6 with minimal additional direction by the instructor. Other selective and differential media such as Brilliant Green agar, Hektoen Enteric agar, Xylose Lysine Deoxycholate agar, or CHROMagar Salmonella can also be used to increase the proportion of *Salmonella* isolated. The pilot semester of our CURE did not utilize these additional media because they were not necessary for our research lab students: less-advanced students, however,

may struggle to correctly identify *Salmonella* on selected media. Additionally, increasing the number of samples taken per student may increase recovery.

In Module 2, challenges are mainly related to a lack of student exposure to bioinformatics prior to taking the course. We used tutorials on DNA sequencing and bioinformatics from various sources including Oxford Nanopore, Galaxy, and GalaxyTrakr to introduce students to new concepts and interfaces (S8). We used tools with user-friendly graphic interfaces to reduce student intimidation. Data analyses were disseminated and stored on the Open Science Framework (OSF). We created a Project for the overall course, made a Component Project for the semester, and had students "fork" this so each group had their own page to edit. OSF allows for easy "templating" of projects and instructors are very welcome to use our site (Jurgensen and Herrick, 2018) and materials freely as templates.

Another challenge of Module 2 may be the acquisition of sequence data for student use. We sent isolates for WGS after lab 6 so that sequence data would be available for Module 2. This may be a limiting factor of the course. If few *Salmonella* are recovered during Module 1, sequencing data will not be available in time. If only Module 2 is to be utilized, there are thousands of freely available short-read sequences of *Salmonella* available through the FDA GenomeTrakr Project (https://www.ncbi.nlm.nih.gov/bioproject/183844) in the NCBI short read archive, so students may access and analyze data from *Salmonella* (or other bacteria) that they did not isolate themselves (https://www.ncbi.nlm.nih.gov/sra). We assigned each student one strain to analyze and had them work in teams to check each other's work.

Assessment of Student Learning

We used qualitative and quantitative assessment approaches to assess achievement of learning goals as well as student comfort with the CURE structure. In class, we gave students short quizzes with both low and high-cognitive order questions as incentive to read lab protocols before the lab period and short homework assignments to help students gain confidence in using bioinformatics programs and pipelines. Individual students were also graded on their physical and electronic lab notebooks using a detailed rubric (S3). The more heavily weighted assignments of the course – oral and poster presentations – were completed as group assignments. Both of these presentations had qualitative rubrics with a focus on student confidence and understanding of the material (S1, S2).

Figures and Tables

Figure 1.

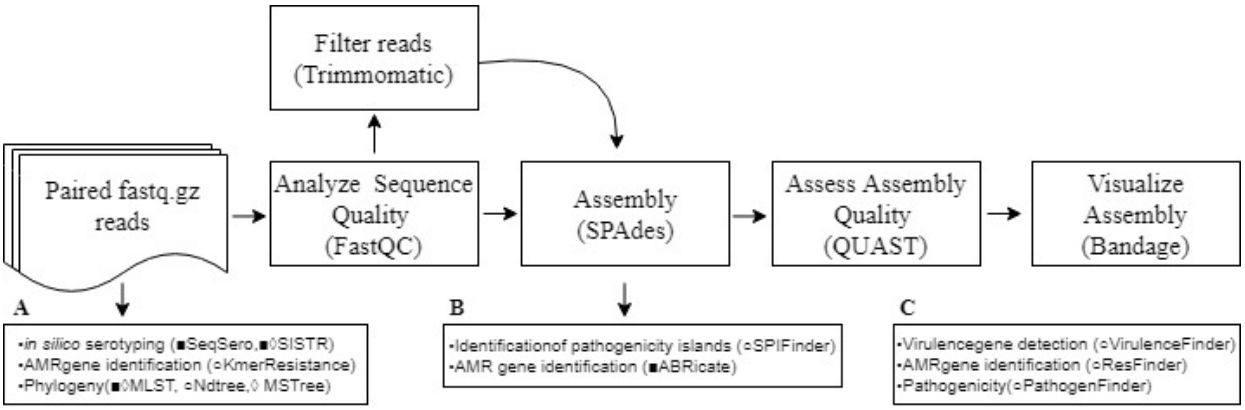

Figure 1. Post-sequencinganalysis pipeline. Assemblies were created with trimmed/untrimmed reads using GalaxyTrakrtools. After evaluation using QUAST and Bandage, the highest quality assemblywas saved (see methods for details). A/BAnalyses that require or prefer raw reads or assemblies, respectively. CAnalyses that have no preference for input. ■ Tool found onGalaxyTrakr(https://galaxytrakr.org/)○ tool found on the Center for Genomic Epidemiology website (http://www.genomicepidemiology.org/) ◊ tool found on Enterobase(http://enterobase.warwick.ac.uk/).

Table 1

Scientific Concepts	Learning Objectives Addressed	Activity	Assessment	Suggestions/Tips
MODULE 1				
Lab 1: Introduction to *Salmonella*, course overview, lab safety, and pre-survey (60 min, 1 day)				
Introduction to classical microbiological techniques and bioinformatic analyses to be performed during the course.	**1, 2**	• Mini lecture (S9): Introduction to classic enrichment and isolation techniques, specific media used, previous data collected, goals of course (40 min) • Class discussion of potential poultry litter sites, determine who will use what sample types and why (20 min)	None	• If possible, form student groups during this lab period.
Lab 2: Student media preparation (90 min, 1 day)				
Introduction to preparation of *Salmonella* isolation and enrichment media (FDA Bacteriological Analytical Manual)	**3**	• Quiz (10 min) • Prepare all media used for isolation except Bismuth Sulfite (BS) agar (80 min)	Quiz	• BS agar is only selective for 48 hours, so instructor should make this the day before Lab 4. • Provide 2 L flasks to avoid boiling over agar-containing media. • Media may be prepared in advance by instructor, but we believe media preparation is an important skill for microbiology students.
Lab 3: Sediment/poultry litter sample collection and processing (>90 min, 2 days)				
Sample collection and processing.	**3, 5**	• Sample collection: students collect sediment samples and record metadata in EpiCollect5 (S5) at a variety of sites (>90 min, Day 1) • Sample processing: inoculate pre-enrichments and begin incubation (10 min, Day 1) • Sample processing: inoculate enrichments after 24 hrs, begin incubation (15 min, Day 2)	None	• This lab period may go over time depending on the number of sampling sites and their distance from your institution. • We provided poultry litter, and allowed students to bring in litter if they had connections to farmers.

Scientific Concepts	Learning Objectives Addressed	Activity	Assessment	Suggestions/Tips
Lab 4: Plating from enrichments (90 min, 2 days)				
Aseptic technique, sample processing, selective growth.	**4, 5, 6**	• Quiz (10 min) • Day 1: plate from enrichments (30 min) • Day 2: students decide which colonies have *Salmonella*-like morphology and plate onto different selective media (50min)	Quiz	• BS agar is only selective for 48 hours, so instructor should make this the day before Plating Day 2. • Instructor should become familiar with *Salmonella* colony morphology on agar before class as they can be difficult to identify. • Students may have time to complete another task on Day 1.
Lab 5: Diagnostic biochemical tests (>90 minutes. 1 day)				
Characterization of isolates using classical microbiological techniques	**4, 5, 6**	• Quiz (10 min) • Biochemical tests to characterize isolates: Gram stain (20 min), Oxidase test (5 min), TSI agar slants (5 min) • Tests that vary for *Salmonella* to differentiate isolates: Citrate agar (5 min), Catalase test (5 min) • Plate on non-selective media (15-25 min) • Interpret results (15 min)	Quiz	• Students may require more than one lab period to complete tests depending on how many isolates they have. • Students may struggle to manage time efficiently if not given instructions on which order to perform tests. • TSI and citrate agars should be interpreted after 24 hours.
Lab 6: *invA* PCR and gel electrophoresis (180 minutes, 2 days)				
PCR, gel electrophoresis	**4, 5**	• Quiz (10 min) • Students set up colony PCR using *invA* primers (60min, Day 1) • Run PCR protocol (300 min, Day 1) • Students load and run gels (85 min, Day 2) • Visualization and interpretation of individual results (10-15 min, Day 2)	Quiz	• Students may mix sample, loading dye, and buffer in PCR tubes or on parafilm strips.

Scientific Concepts	Learning Objectives Addressed	Activity	Assessment	Suggestions/Tips
Lab 7: Sensititre MIC assays (60 min, 2 days)				
Antibiotic resistance phenotyping	**4, 9**	• Quiz (10 min) • Inoculate plates (45 min) • Interpret results (15 min)	Quiz	• We recommend using Gram negative NF or NARMS Sensititre plates for *Salmonella*, but Kirby-Bauer could be conducted instead. • Results must be read in 24 hours.
Lab 8: Plasmid prep (>90 min, 2 days)				
Plasmid DNA extraction, gel electrophoresis	**4, 6, 10**	• Perform plasmid mini-prep (>90 min, Day 1) • Students load and run gels (90 min, Day 2) • Visualization and interpretation of results (10-15 min, Day 2)	None	• This lab period may require additional time, as the protocol is quite involved. • This protocol may be difficult for students with less experience carrying out molecular biology protocols. • Instructor should provide cell cultures with known plasmids as positive controls. • Provide students with background information on plasmids in addition to the lab protocol. • We ran this gel for students due to time constraints.
Lab 9: Total genome DNA prep (90 min, 1 day)				
DNA extraction for Nanopore sequencing	**4**	• Students choose which of their isolates to extract DNA from (10-15 min) • Extract DNA from isolates (60-75 min)	None	• We chose to only extract DNA from the samples we planned to run on the MinION.
Lab 10: MinION sequencing (>90 min, 1 day)				
Introduction to DNA sequencing technologies	**1**	• Brief introduction to NGS technologies with an emphasis on Illumina and Oxford Nanopore technologies (15 min) • MinION library prep and sequencing (time varies depending on kit used)	None	• MinION sequencing library prep may take >2 hours, so this may need to be done outside of regular lab period. • We used the MinION Rapid Sequencing Kit (SQK-RAD004) • MinION may run for up to 48 hours.

Scientific Concepts	Learning Objectives Addressed	Activity	Assessment	Suggestions/Tips
MODULE 2				
Lab 11: Assessing sequence quality (60 min, 1 day)				
Working with sequence data	**1, 7,**	• Introduction to GalaxyTrakr and associated tools (30 min) • Bioinformatics activity 1 (30 min)	Bioinformatics homework assignments 1 and 2 (S10)	• Instructor should explain the purpose of the tools in addition to how to use them. • This lab can be overlapped with Module 1 if desired.
Lab 12: Genome assembly and quality (>75 min, 1 Day)				
Working with sequence data, genome assembly	**1, 7, 10**	• Introduction to bacterial genome assembly (>30 min, Figure 1) • Students import data into GalaxyTrakr and run assembly (15 min) • Students interpret assembly quality (30 min)	Bioinformatics homework 3 (S10)	• Instructor should prepare an assembly for demonstration beforehand as assemblies through GalaxyTrakr may take hours. • We ran assemblies using trimmed and untrimmed reads as described in lesson plan to teach students different strategies for optimizing assemblies. • This lab can be overlapped with Module 1 if desired.
Lab 13: Typing, phylogenetics, and variant analysis (90 min, 1 day)				
Working with sequence data, typing *S. enterica*	**1, 8, 9, 10**	• Mini lecture on SNP and MLST analysis (30min) • Students serotype their strains (15min) • Students use cgMLST (Enterobase), SNP analysis, and construct phylogenies using their sample genomes	Bioinformatics homework 4 (S10)	• Instructor should prepare a cgMLST analysis and SNP phylogeny for demonstration before class.
Lab 14: Resistance gene detection using ABRicate in GalaxyTrakr (90 min, 1 day)				
Working with sequence data	**1, 9, 10**	• Mini lecture on antibiotic resistance dissemination and genotypic detection (30 min) • Students use ABRICATE (GalaxyTrakr) to detect resistance genes in their sample genomes	Bioinformatics homework 4 (S10)	• Instructor should prepare an example ABRicate analysis before class. • Students should compare their genotypic and phenotypic data.

Scientific Concepts	Learning Objectives Addressed	Activity	Assessment	Suggestions/Tips
Lab 15: Poster and oral presentations (>300 min, >2 days)				
Poster presentation, oral presentation, scientific communication, group work	**11**	• Mini lecture on preparing effective poster and oral presentations (20 min) • Students work in groups to prepare poster and oral presentations (120 min, Day 1/2) • Students give oral presentations (60 min) • Students give poster presentations (60 min)	Oral presentation grading rubric (S2), Poster presentation grading rubric (S1)	• We provided students with model posters. • Presentations may be given in class, at departmental symposia, etc. • We administered post-survey on the last lab period (S10).

Supplementary materials

All supplementary files are available on our OSF repository, which can be accessed via this link:

https://osf.io/5p8dc/?view_only=0520e8492e0a4d81bc3c7f70b6121e62

S1: Poster presentation rubric

S2: Oral presentation rubric

S3: Traditional and online laboratory notebook rubrics

S4: Post-survey assessment materials

S5: EpiCollect5 metadata collection form

S6: Module 1 protocols

S7: Safety documentation for Module 1

S8: Sequencing technologies tutorials

S9: In-class mini lectures

S10: Bioinformatics lab guides and homework assignments

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