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Lesson

A CURE for *Salmonella*: A Laboratory Course in Pathogen Microbiology and Genomics

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Abstract

Rapid advances in genomics and bioinformatics, the vast amount of data generated by next-generation sequencing, and the penetration of the '-omics' into many areas of biology have created a need for students with hands-on experience with computational and 'big data' methods. Additionally, laboratory experience in the isolation, identification, and characterization of unknown bacteria is a vital part of a microbiology student's training. This lesson is a course-based undergraduate research experience (CURE) focusing on *Salmonella enterica*, a common and relatively low-virulence foodborne pathogen. In Module 1, students isolate and identify *S. enterica* strains from stream sediment, poultry litter, or other sources. They conduct phenotypic evaluation of antimicrobial resistance (AMR) and can search for plasmids. Isolates' whole genomes may be sequenced by the United States FDA or public health laboratories, typically at no charge. In Module 2, students learn basic methods of genome assembly, analysis, annotation, and comparative genomics. They use easily accessible, primarily web-based tools to assemble their genomes and investigate areas of interest including serotype, AMR genes, and *in silico* evidence of mobile genetic elements. Either module can be used as a standalone learning experience. After course completion, students will be able to isolate and identify *Salmonella* from natural sources, and use computational analysis of microbial genomic data, particularly of the *Enterobacteriaceae*. This lesson offers undergraduate microbiologists a genuine research experience and a real-world microbiology application in genomic epidemiology, as well as a valuable mix of field, laboratory, and computational skills and experiences.

Citation: Jurgensen SK, Harsh J, Herrick JB. 2021. A CURE for *Salmonella*: A Laboratory Course in Pathogen Microbiology and Genomics. *CourseSource*. https://doi.org/10.24918/cs.2021.24

Editor: William Morgan, College of Wooster

Received: 5/19/2020; **Accepted:** 2/24/2021; **Published:** 9/15/2021

Conflict of Interest and Funding Statement: None of the authors has a financial, personal, or professional conflict of interest related to this work.

Supporting Materials: Supporting Files S1. CURE for *Salmonella* – Laboratory safety contract; S2. CURE for *Salmonella* – Pre-enrichment and enrichment media preparation; S3. CURE for *Salmonella* – Field sampling protocol; S4. CURE for *Salmonella* – Lab notebook grading rubric; S5. CURE for *Salmonella* – Pre-enrichment and enrichment inoculation protocol; S6. CURE for *Salmonella* – Plate media preparation; S7. CURE for *Salmonella* – Plating and purification protocol; S8. CURE for *Salmonella* – Miscellaneous test protocols - oxidase, catalase, KOH; S9. CURE for *Salmonella* – invA PCR and gel visualization protocol; S10. CURE for *Salmonella* – Preparing isolates for shipping and freezing; S11. CURE for *Salmonella* – Bioinformatics Lab Guide - Navigating and using GalaxyTrakr and Galaxy; S12. CURE for *Salmonella* – Bioinformatics lab notebook grading rubric; S13. CURE for *Salmonella* – Bioinformatics Lab Guide - *Salmonella* serotyping; S14. CURE for *Salmonella* – Bioinformatics Lab Guide - Assessing & filtering illumina data using *FastQC* and *Trimmomatic*; S15. CURE for *Salmonella* – Bioinformatics Lab Guide - File naming conventions; S16. CURE for *Salmonella* – Bioinformatics Lab Guide - Genome assembly using *SPAdes* and *Shovill*; S17. CURE for *Salmonella* – Bioinformatics Lab Guide - Assessing assembly quality Using *QUAST* and *Bandage*; S18. CURE for *Salmonella* – Bioinformatics Lab Guide - Selecting reference genomes/Ordering and viewing assembled contigs using *Mauve*; S19. CURE for *Salmonella* – Bioinformatics Lab Guide - Gene annotation; S20. CURE for *Salmonella* – Bioinformatics Lab Guide - Antibiotic resistance gene detection; S21. CURE for *Salmonella* – Bioinformatics Lab Guide - Miscellaneous gene and genetic feature detection; S22. CURE for *Salmonella* – Presentation evaluation rubric; S23. CURE for *Salmonella* – Poster evaluation rubric; S24. CURE for *Salmonella* – Sources and description of preexisting survey instruments; S25. CURE for *Salmonella* – Course Overview; S26. CURE for *Salmonella* – Media, Isolation, Sampling; S27. CURE for *Salmonella* – Intro to GalaxyTrakr mini-lecture; S28. CURE for *Salmonella* – Overview of Next-Gen Sequencing and Assembly; and S29. CURE for *Salmonella* – FastQC & Trimmomatic.

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Learning Goals

Students will:

- Describe how whole genome sequences (WGS) of pathogens such as *Salmonella* can be used in genomic epidemiology to track and source outbreak strains.
- Understand how human impact on the environment can influence the evolution of microorganisms (e.g., emerging diseases and the selection of antibiotic resistance).
- Describe the processes of isolation, identification, and characterization of human pathogenic bacteria found in environments such as streams and manure.
- Gain experience collecting microorganisms in the environment.
- Learn how to properly store genomic data and other large scientific datasets in cloud systems designed for this purpose.
- Learn how to assess the quality of whole genome sequencing runs, to assemble microbial genomes, and to use WGS to serotype isolates and determine their phylogenetic relationships to other strains.
- Learn how to use various bioinformatics methods to annotate genes and to study antibiotic resistance, virulence, and mobile genetic elements in pathogens.
- Work in groups on a semester-long project and present their results.
- Gain practice in navigating scientific obstacles
- Learn about potential careers in public health microbiology and bioinformatics.

Learning Objectives: Module 1

Students will:

- Work in groups on a semester-long project and communicate research findings in oral and poster presentations. Maintain a physical lab notebook.
- Learn guidelines for safely handling *Salmonella* and other potential pathogens in a laboratory environment.
- Learn how to make *Salmonella* pre-enrichment and enrichment media.
- Keep a laboratory notebook.
- Collect and record sample metadata using a sampling probe and metadata management application.
- Collect sediment from streams aseptically.
- Use liquid and plate media to enrich and isolate *Salmonella* from stream sediments and other sources..
- Use biochemical tests such as the Gram stain, KOH, oxidase, and catalase tests to identify putative *Salmonella*.
- Verify isolates as *Salmonella enterica* using *Salmonella*-specific PCR and gel electrophoresis or real-time PCR.
- Verify the isolates as *S. enterica* using Enteropluri(TM) tubes, designed for the identification of *Enterobacteriace* (optional).
- Use Kirby-Bauer and/or Sensititre MIC plates to test isolates for their resistance to antibiotics used to treat systemic *Salmonella* infections.
- Freeze isolates in cryotubes for long-term cryostorage
- Ship isolates to the U.S. FDA or other public health laboratories for Illumina whole-genome sequencing.

Learning Objectives: Module 2

Students will:

- Work in groups on a semester-long project and communicate research findings in oral and poster presentations. Maintain a group electronic bioinformatics lab notebook.
- Learn how to use GalaxyTrakr and Galaxy for the computational analysis of genomes.
- Learn the use of Open Science Framework for data storage and retrieval.
- Understand the advantages and disadvantages of short- and long-read genome sequencing.
- Learn how to name data and analysis files so they are machine- and human-readable and sortable.
- Learn how and why DNA sequence and assembly data quality is assessed. Use *FastQC* and *Trimmomatic* to analyze and improve the quality of raw reads and assemblies, respectively.
- Learn how and why microbial genomes are assembled and the limitations of short-read sequences for assembly.
- Assemble isolate genomes using *SPAdes* and visualize assemblies using *Bandage*.
- Serotype isolates using *SeqSero* and *SISTR* on GalaxyTrakr.
- Learn how to find and download reference genomes from NCBI.
- Order their assembled contigs and visualize the order using *Mauve*.
- Understand the purpose and process of genome annotation.
- Annotate their isolates' genomes using Prokka and RAST and visualize annotated genomes using a genome browser.
- Determine and compare the antibiotic resistance genotypes and phenotypes of isolates.
- Learn how to find and compare mobile genetic elements – plasmid-specific genes, transposons, integrons, pathogenicity islands, prophages, etc. – in isolates.

INTRODUCTION

Course-based undergraduate research experiences (CUREs) are becoming an increasingly valuable feature of college science teaching (1,2). CUREs have the potential to engage *all* students in authentic research practices by offsetting barriers commonly associated with traditional apprenticeship models (3). CUREs engage students in collaborative, iterative research activities as the students work to collect, analyze, and communicate novel data of broader interest to the community (4, CURENet). Though the nature of these experiences can vary widely as seen in the growing catalogue of published descriptions in bioscience education at the introductory (5-8) and upper division levels (9-11), studies on CURE participation have documented a range of cognitive, affective, behavioral, and psychosocial gains (as reviewed in (12)).

Microbial genomics is a particularly fruitful field in which to focus a CURE, especially considering CURE design features highlighted in the literature (4) as well as how the experience may help science students achieve their educational or career goals (12). Genomics, transcriptomics, and related fields are now of central importance in microbiology, and in the life sciences more broadly. The concept knowledge and technical skills developed in practice with common technologies and tools learned in studying microbial genomes are largely applicable to other organisms. In addition, students gain exposure to and training in data-driven discovery by working with large data sets, which has been deemed key to the preparation of a data-capable workforce.

Common foodborne pathogens are of great interest to the U.S. FDA, CDC, and other public health laboratories and therefore these agencies are often eager to sequence foodborne pathogens at little or no cost to researchers and educators because the data can provide needed context for tracking future outbreaks (13). This urgency combined with the advances in whole genome sequencing (WGS) make this system ideal for the development of a CURE. We have developed a lesson that is part of an ongoing research project in our laboratory. We use *Salmonella enterica* as our model organism in this lesson because it is a foodborne pathogen that infects over 1.3 million Americans every year, causing approximately 420 deaths, and is one of the leading infectious causes of hospitalization in the United States (14, Centers for Disease Control and Prevention). However, *Salmonella* are also relatively much less virulent than other foodborne pathogens of interest to these labs, such as *Listeria* and pathogenic *E. coli* (15), making it more appropriate in an undergraduate lab setting. Additionally, introducing students to ongoing large-scale governmental projects, such as the epidemiological tracking of foodborne illnesses like *Salmonella*, gives students valuable insight into potential career paths that they may otherwise be unaware of. Thus, this lesson may be of particular interest to students interested in pursuing careers in public health or infectious disease.

While its pathogenicity has made *Salmonella* a commonly studied organism, its occurrence in natural environments has not been extensively investigated (16,17). 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 sources and manure (18). We typically sample sediment from agriculturally impacted streams because it potentially harbors a more stable microbial community than water (15). For this CURE, we also worked with several local small-scale and industrial poultry farmers who provided poultry litter from their farms, as *Salmonella* is typically a commensal organism in turkeys and chickens.

Recently-developed high throughput (or "next generation") sequencing methods have made it possible to sequence entire bacterial genomes quickly and affordably (19). As a result, a vast amount of microbial DNA sequence data is being generated. In particular, an abundance of DNA sequence data is being generated by the U.S. FDA, CDC, and U.S. state public health laboratories as part of their recent thrust to use whole genome sequences for epidemiological tracking of pathogens. The CDC has successfully used WGS to identify the source of dozens of foodborne illness outbreaks since its initial implementation in 2013, as well as to uncover new or unknown sources of infection. For example, recently the CDC traced an outbreak of *Salmonella* Javiana to cut fruit produced in New Jersey by using WGS to show that the ill people were infected with genetically similar *Salmonella*, which suggested a single infection source. These high throughput methods allow for higher resolution in typing, distinguishing, and characterizing outbreak strains at the subspecies level because small genetic differences (such as single-nucleotide polymorphisms or SNPs) can be identified. The datasets also constitute a valuable and under-utilized resource for genomic and bioinformatics lessons such as this.

Intended Audience

This two-part CURE can be directed to different student populations and levels. We use the experience in its entirety in an upper-division course at a large master's degree-granting university with biology majors concentrating in microbiology. The course (previously *Bacterial Discovery*, now known as *Laboratory in Bacterial Pathogenomics*) has been offered nearly every term since Spring 2018, and is taught by a faculty member assisted by an upper division undergraduate research student with previous experience in the course. Both modules provide students with experiences and skills similar to those gained from working in a research laboratory, so this course is particularly useful for students who are unable to work in a one-on-one mentored research setting. As the first module – on *Salmonella* isolation and identification – requires a biological safety level (BSL) 2 laboratory space, not every institution may be able to incorporate it into their course. However, the bioinformatics module of the lesson can be adapted and implemented in biology and biotechnology courses for introductory 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 computer programming experience or the 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 approximate time required for each laboratory activity, set up, and out of class time for preparation (as necessary).

Prerequisite Student Knowledge

Module 1 - *Salmonella* Isolation, Isolation, and Identification

For this first module, students should have taken a general or introductory microbiology course with a laboratory to develop 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 *Salmonella* isolation. Thus, this module can be implemented alone in an introductory microbiology laboratory course with the laboratory skills discussed above taught before beginning the module. BSL-2 safety training for all students is required for the implementation of this module since the target organism (*Salmonella*) is a human pathogen, and there is also the possibility that other pathogens (e.g., *Klebsiella pneumoniae* or pathogenic *E. coli*) could be isolated.

Module 2 Bioinformatics

For the second module, students should have a basic knowledge of the characteristics of the bacterial genome and foundational genetic concepts such as the Central Dogma and horizontal gene transfer. No prerequisite knowledge of bioinformatics is required, though many of our students have a basoc introduction to bioinformatics in JMU's CURE-model first-year biology curriculum (5). All tools used in this lesson are freely available online, so only basic computer expertise is required to complete this module.

Prerequisite Teacher Knowledge

Module 1

The instructor should have significant training/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 sampling sources that reliably yield *Salmonella* and be able to recognize *Salmonella* on the selective media. Familiarity with BSL-2 safety protocols is a necessity.

Module 2

This lesson assumes that the instructor has some basic knowledge of microbial genomics. For those who are not familiar with bioinformatics and are interested in incorporating this module into their course, we recommend the excellent paper by Edwards and Holt (20) with its accompanying tutorial. GalaxyTrakr and especially Galaxy provide a variety of tutorials to introduce users to the interface (21,24).

SCIENTIFIC TEACHING THEMES

This lesson was designed and implemented as a semester-long CURE (4,22) comprising two modules (S25. CURE for *Salmonella* – Course Overview). It is a hands-on introduction to laboratory and bioinformatics 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. Most activities are carried out in teams, and students work in small groups to plan and implement their approach to each lab period. We occasionally assign review and other summary readings as pre-class homework, followed by instructor-led group discussions. These whole class discussions also aid in troubleshooting, which are necessary due to the inherent unpredictability of 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 which advanced analyses to pursue based on their interests. Both poster and oral presentations are designed, presented, and evaluated (by the instructor) as a group.

Assessment

Using a backward design approach (23), formative and summative assessments - as well as learning activities - were mapped to course learning objectives (Table 1).

Formative assessments include observations of research-related skills (e.g., molecular techniques, communication), in-class pre-lesson quizzes on the protocols to be completed to ensure that protocols were understood before implementation, short homework assignments to help students gain experience using bioinformatics programs and pipelines, in-class activities, discussions, and maintenance of physical (for Module 1) and electronic (for Module 2) laboratory notebooks.

Summative assessments include homework assignments, group oral and poster presentations evaluated using rubrics focused on understanding of the material (S22. CURE for *Salmonella* – Presentation evaluation rubric and S23. CURE for *Salmonella* – Poster evaluation rubric), and rubric-scored physical and electronic lab notebooks (S4. CURE for *Salmonella* – Lab notebook grading rubric, S12. CURE for *Salmonella* – Bioinformatics lab notebook grading rubric). Detailed information on these assessments is included in the outlines of each weekly lesson below.

Inclusive Teaching

By their general nature, CUREs increase access to authentic research experiences for all students independent of the personal and institutional hurdles that they may face in engaging in an independent research program (3). Students work *collaboratively* throughout the semester as they use authentic microbiology and genomics techniques that closely align with epidemiological investigations conducted by the FDA, CDC, and other public health laboratories. Student research has the potential for discovery and to make contributions to the field (33 and E. Gline, E. Gross, B. Puma, R. Zoldork, M. Thinnes, E. Seracino, and J. B. Herrick. Presented at the Annual Meeting of the American Society for Microbiology, Virginia Branch, 8 to 9 November 2019). Through active engagement in authentic practices via wet lab and/or computer-based activities, students gain an understanding of the nature of "real world" scientific endeavors in the field, and also experience autonomy, project ownership, and how to navigate challenges in a research setting.

Other design features of the CURE lend well to inclusive teaching practices. In studying the socially relevant topics of foodborne pathogens and antibiotic resistance in their own community, this lesson would be broadly meaningful to students of varying backgrounds and professional interests (e.g., medicine, epidemiology, bioinformatics, infectious disease microbiology). Pedagogical approaches implemented in this lesson accommodate a variety of learning styles and ability through demonstrations, mini-lectures, videos, hands-on laboratory and computer-based activities, worksheets, discussions, and optional additional tutorials. Similarly, varying forms of formative and summative assessments are used to measure student learning trajectories and guide instruction.

LESSON PLAN

Module 1

One of the most time-intensive aspects of preparing to incorporate 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 (33). We also use poultry litter because *Salmonella* is typically a commensal organism in the fowl gut. Other environmental sources could include amphibians, 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 (see S3. CURE for *Salmonella* – Field sampling protocol) and we store this and other metadata (date, time, latitude and longitude) using EpiCollect5 which is available as both a mobile application and website. When culturing bacteria (labs 3-5, S5. CURE for *Salmonella* – Pre-enrichment and enrichment inoculation protocol and S7. CURE for *Salmonella* – Plating and purification protocol), all procedures must be conducted in a BSL-2 laboratory space. Safety documentation for students can be found in the supporting file (S1. CURE for *Salmonella* – Laboratory safety contract).

In this module, students prepare their own enrichment and isolation media (S2. CURE for *Salmonella* – Pre-enrichment and enrichment media preparation, S6. CURE for *Salmonella* – Plate media preparation, and S26. CURE for *Salmonella* – Media, Isolation, Sampling), 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 6-8 (S8. CURE for *Salmonella* – Miscellaneous test protocols - oxidase, catalase, KOH). If availability of time or funds are limited, lab 9 may be removed from the module. After lab 10 (S9. CURE for *Salmonella* – invA PCR and gel visualization protocol), all confirmed *Salmonella* isolates were shipped to the Virginia Department of Consolidated Laboratory Services (DCLS) for WGS (S10. CURE for *Salmonella* – Preparing isolates for shipping and freezing). An overview of the *Salmonella* isolation protocol is shown in Figure 1.

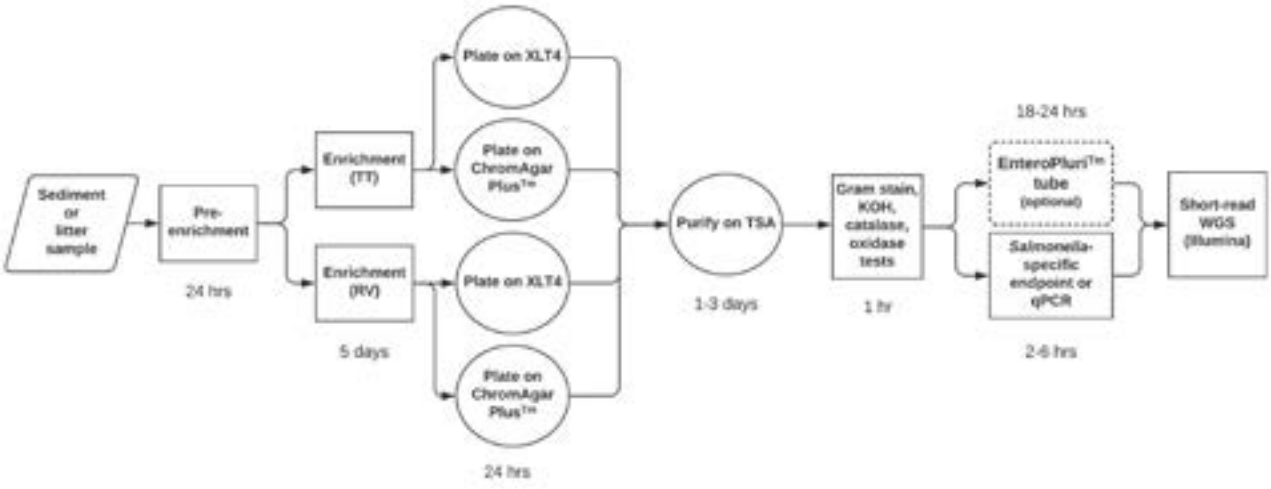

Figure 1. Overview of methodological workflow for the enrichment, isolation & purification, and identification of *Salmonella* used in Module 1. The estimated time expected for each step is indicated.

Module 2

If instructors wish to use module 2 as a follow up to module 1, instructors should contact their local state or other public health laboratory or the FDA Whole Genome Sequencing (WGS) Program to determine whether they will accept samples and expedite sequencing to ensure a turn-around time short enough for a semester-long lab. Some 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. If the delay proves to be too long for a single semester course, genomes of strains isolated in a previous semester could be used for analysis.

Alternatively, module 2 can be taught as a standalone set of lessons using as raw material the vast number of recently-sequenced *Salmonella* genomes available from the NCBI Sequence Read Archive (SRA). These can be downloaded within GalaxyTrakr using the accession (SRR) number as explained in S11. CURE for *Salmonella* – Bioinformatics Lab Guide - Navigating and using GalaxyTrakr and Galaxy. Accession numbers can be selected from the NCBI site in a number of ways, for example via the Taxonomy Browser; choose strain and then click on "SRA Experiments") or via GenomeTrakr BioProjects.

This module requires preparation to ensure that student-generated files are well organized and analyses are easy to find and use. We recommend the use of Open Science Framework (OSF), an open source data management platform, to access and store genomic data and analysis files. We also use Google Docs student electronic lab notebooks (template; S12. CURE for *Salmonella* – Bioinformatics lab notebook grading rubric). We have created a public OSF page to function as a living repository of protocols, templates, and instructions to be used for both modules of this course. 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, although this isn't a requirement for its use.

For the majority of analyses in this lesson we use GalaxyTrakr (S11. CURE for *Salmonella* – Bioinformatics Lab Guide - Navigating and using GalaxyTrakr and Galaxy, S27. CURE for *Salmonella* – Intro to GalaxyTrakr mini-lecture), which is a specific implementation of Galaxy, an open, web-based platform for computational tools used to analyze genomic data. GalaxyTrakr (24) includes a relatively limited number of tools specific to microbial (and especially foodborne pathogen) genomics, including most of those used in this module, which may make it easier for students to use. GalaxyTrakr tools used in this module include *SeqSero, SISTR, FastQC, Trimmomatic, SPAdes, Shovill, QUAST, PROKKA,* and *Abricate* (Supporting Files S13, S14, S16, S17, S19, S20, and S29). Other web-based multi-tool platforms we use include PATRIC for gene annotation using RAST (S19. CURE for *Salmonella* – Bioinformatics Lab Guide - Gene annotation) and the Center for Genomic Epidemiology for a number of tools (S21. CURE for *Salmonella* – Bioinformatics Lab Guide - Miscellaneous gene and genetic feature detection). Although not described in this lesson, Enterobase may also be a useful resource, particularly for multilocus sequence typing of strains. Individual web-based programs for gene feature identification include *INTEGRAL* and *PHASTER* (S21. CURE for *Salmonella* – Bioinformatics Lab Guide - Miscellaneous gene and genetic feature detection). Standalone programs that require installation on the computers used for computational analysis are *Mauve* (Supporting File S18. CURE for *Salmonella* – Bioinformatics Lab Guide - Selecting reference genomes/Ordering and viewing assembled contigs using *Mauve*), Bandage (S17. CURE for *Salmonella* – Bioinformatics Lab Guide - Assessing assembly quality Using QUAST and Bandage), and *Artemis* (S19. CURE for *Salmonella* – Bioinformatics Lab Guide - Gene annotation). A general overview of the computational tools used in this lesson can be seen in Figure 2.

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 one of the authors' (JBH) research lab at James Madison University. These sites were therefore relatively well characterized with respect to their potential as

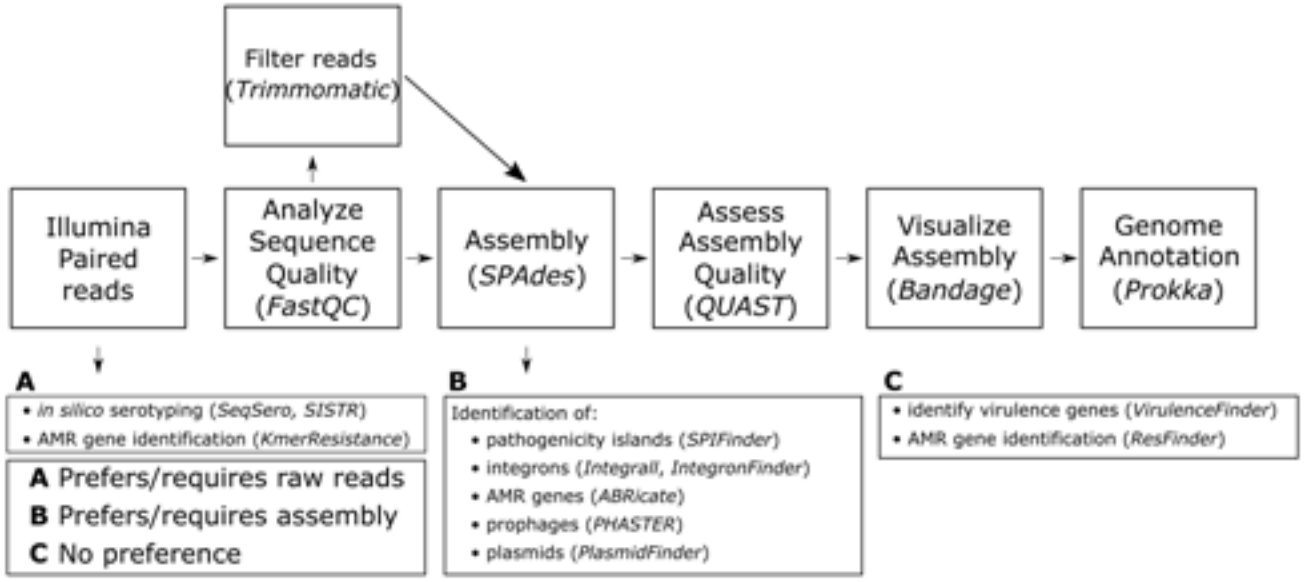

Figure 2. Overview of computational workflow used in Module 2, as well as potential tools for further identification of genes and genome regions. A, tools that require or function best with raw reads (*fastq* format) as input; B, tools that require or function best with an assembly file (*fasta* format) as input; C, tools that can take either raw reads or assemblies equally well as input. AMR = antimicrobial resistance.

reservoirs of *Salmonella*. We also obtained poultry litter samples from local large-scale industrial and small-scale poultry farms and were successful in isolating *Salmonella* from some but not all of these. Since these sample sources are not available to all institutions, other sample sources (e.g., amphibians, reptiles, eggs, and raw poultry meats) could potentially be utilized.

Our isolation protocol is based on the FDA Bacteriological analytical manual (BAM) protocol for *Salmonella* enrichment and isolation (25). We have modified the protocol, however, to include a 5-day rather than a 24-hour enrichment period, which we have found to substantially improve recovery from sediments. We have also replaced Bismuth Sulfide agar with ChromAgar Plus plate medium, as we have found that less-experienced students can more easily distinguish putative *Salmonella* on this medium. We continue to use XLT-4 agar medium; however, its use is optional. Included in Table 1 (Labs 6-8) are links to manufacturer's guides to the expected appearance of *Salmonella* on CHROMagarPlus and XLT4 plate media. Other selective and differential media such as Brilliant Green Agar, Hektoen Enteric Agar, or Xylose Lysine Deoxycholate Agar have been successfully used by other labs for isolating *Salmonella* strains from various sources (26).

This CURE can be readily adapted to meet the needs of the student population and institutional resources at hand. In particular, Module 2 could be modified and implemented as a standalone online research experience. Students could rely on sequences from instructor-isolated strains or existing genomes readily available through the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) database. Here, freely accessible web-based bioinformatics tools (described in Table 1) could permit non-traditional or remote students as well as those at institutions without BSL-2 labs to engage in whole-genome research. Furthermore, while the lesson focuses entirely on *Salmonella*, nearly all aspects of Module 2 could be applied to other organisms including 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. Large numbers of genomes are being sequenced from, for example, *Escherichia/Shigella, Clostridiodes, Vibrio, Yersinia, Helicobacter,* and *Moraxella*, with sequence data publicly available.

In Module 2, challenges are mainly related to a lack of student exposure to bioinformatics before taking the course. We used tools with user-friendly web-based and other 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 welcome to use our site and materials freely as templates.

Another challenge of Module 2 may be the acquisition of sequence data and the turnaround time of isolate sequencing. We sent isolates to the Virginia DCLS for whole-genome sequencing about halfway through the semester, after Module 1 Lab 12, so that sequence data would be available for Module 2. The DCLS turnaround is typically only two or three weeks. If this rapid turnaround isn't possible, the students could analyze sequence data from isolates from a previous semester. While we are waiting for sequence data, we typically do antibiotic resistance phenotyping (Module 1, Lab 13). Other advanced microbiological tests and protocols for verifying and characterizing *Salmonella* (e.g., SIM, TSI, Voges-Proskauer tests; native plasmid preps; MICs, etc.) might also be performed. We also introduce the first few labs of Module 2 using raw read (SRR) files available from the NCBI Short Read Archive (SRA). If only Module 2 is to be utilized, there are thousands of freely available short-read sequences of *Salmonella* and other bacterial pathogens available in the NCBI short read archive. The NCBI Pathogen Browser might be of interest to students as it displays phylogenetic relationships, sources and other metadata of not only *Salmonella* but a number of bacterial pathogens (and even *Candida*).

Lesson Effectiveness

To complement course activities, student self-report data were gathered using pre- and post-surveys to assess the effectiveness and impact of the experience (S24. CURE for *Salmonella* – Sources and description of preexisting survey instruments). Here, Likert-type items were adopted from existing validated instruments (27-30) as well as open response prompts designed for this study to gather data on self-perceived cognitive and non-cognitive outcomes and perceptions of course design and instruction. Data were collected and aggregately analyzed from two semesters (Fall 2018 and Spring 2019) after a pilot semester of implementation (Spring 2018). All procedures were performed in accordance with the university's institutional review board guidelines.

Students (n=35; all biology majors and upperclassmen, 75% female, 51% White, 31% first-generation) reported notable gains in various research-related cognitive and non-cognitive outcomes. Overall, students indicated that the course increased their confidence in being able to complete tasks individually and/or teaching others how to complete tasks using microbiology (100% of students), molecular biology (100%), and bioinformatics (94.29%) research techniques. More specifically, as recorded by 5-point Likert-type items, > 80% of students self-rated higher levels of confidence after the course in research-related outcomes including designing a research study, employing basic and advanced technical skills, working collaboratively, troubleshooting problems, using scientific literature to guide research, understanding relevant concepts knowledge, and communication with research mentors and faculty. Development of these skills were also commonly cited by students in response to an open-ended question of how the CURE may contribute to their academic and career goals - as represented in the following quote: "*[this class] significantly helped me learn…how to analyze DNA sequences, and learn how to use specific databases to analyze and interpret particular data to discover what the exact genes represent*" (Student A). These self-perceived gains were corroborated through direct measures such as observed *in situ* performance of technical practices, conceptual knowledge, and the communication of findings in oral and poster presentation format as well as in-class quizzes, homework assignments, and rubric-critiqued lab notebooks.

Multiple positive indicators drawn from student survey responses collected over the two semesters also support the instructional effectiveness of the CURE design. First, 91% of students reported that the course met or exceeded their initial expectations as represented by the following quotes: "*I didn't expect to learn so many new techniques that I can now add to my CV and I am happy that I was able to participate in research as a class since some semesters, I didn't have time to dedicate to a research lab*" (Student B) and "*My initial expectations were this class was going to another biology class that would just teach us the methods, and approach and possibly an analysis of data; however, it gave me so much more than just the standard knowledge. It gave me actually a way to feel important that my work was done and was significant, that I am a real scientist*" (Student C). For those few students (n=3) that reported that their experience was less than what was initially expected, each identified anticipating more wet lab work (rather than computational). Next, all students agreed with the statement that the course was a good way to learn the process of science, reflecting the incorporation of CURE design features recommended in the literature (4). Here, responding to a series of vetted Likert-scale items (31,29), students rated high levels of project ownership of content (4.42 [SD=0.87], on a 5-point scale) as well as collaboration (22.57 [SD=3.40], on a 6-24 point scale), discovery (26.34 [SD=5.32], on a 5-30 point scale), and iteration (28.94 [SD=4.45], on a 6-36 point scale). These results were echoed and expounded on by students in response to open-ended prompts about course characteristics that they learned or that they enjoyed, such as "*I also surprisingly think that failing at getting Salmonella in our first round and having to start over completely from the beginning was helpful, because it really did show me that research is sometimes starting over and figuring it out all over again*" (Student D), "*In comparison to my other lab classes, we had free reign in the direction of our research that really made me excited*" (Student E), and "*We worked together to do everything ourselves! From the field work to the genome analysis. My group was awesome!*" (Student F). In addition, students regularly highlighted the value of the CURE in exposing them to authentic research practices: "*The fact that we went through the entire scientific process, from collecting data to analyzing results. There are not many classes that would allow you to do that*" (Student G). Importantly, several students that had been engaged in independent research found the CURE comparable to that experience: "*I enjoyed the independence of this class. As a member of a different research lab, I found this class to possess a similar environment to a research lab. This class is a valuable experience to those who have not been exposed to research"* (Student H). Finally, students reported discussing their research (i.e., networking) with family (78%), friends (95%), other on-campus students (97%) and faculty (62%) not affiliated with the CURE, and other off-campus students (70%).

Alternative implementations

As stated previously, for Module 1 if environmental *Salmonella* sources are unavailable, sources such as the feces of captive amphibians and reptiles (especially turtles and snakes), backyard poultry, or raw eggs or poultry meat from the grocery may be utilized. Also, other members of the *Enterobacteriaceae* may be easier than *Salmonella* to isolate in some areas. *E. coli* in particular is often found in fecal-contaminated environmental waters and sediments. Protocols for the isolation and identification of *E. coli* are readily available (32). However, state and federal labs may be unwilling to provide short-read sequencing of these for free, since their main interest is in the pathogenic E. coli strains.

If *only* Module 2 is to be utilized, there are thousands of freely available short-read sequences of *Salmonella* and other bacterial pathogens available in the NCBI short read archive. The NCBI Pathogen Browser might also be of interest to students as it displays phylogenetic relationships, sources and other metadata of a number of bacterial and fungal pathogens.

Conclusion

This lesson offers students an authentic research experience, valuable skills in pathogen microbiology and genomics, and results that are potentially of use to the wider community of microbiologists, particularly those studying the distribution and genomic epidemiology of foodborne pathogens. Students ask important questions such as 'Are there *Salmonella* in this stream or fecal sample?', 'Based on their serotypes, what is the potential for human infection from these isolates?', 'Are these *Salmonella* related to strains isolated from similar environments or regions?', 'Do they exhibit antibiotic resistance and, if so,

what is the genetic basis of the resistance?', and so forth. They learn methods and approaches in field sampling, enrichment and culturing, identification and characterization, and the safe handling of pathogens. Students learn how to assemble, annotate, and analyze microbial genomes. Additionally, students learn to work in teams to solve problems and to present their work orally and in poster form. The results students gather – particularly the genome sequences of their isolates – are of genuine value to genomic epidemiologists at the CDC, the FDA, and other public health labs who seek to track outbreaks in real time (for example, we were directly contacted by the CDC about one of our isolates that was potentially linked to a *Salmonella* outbreak in poultry). Some of the results of this study have been published (33).

Students are also prepared for careers in public health and infectious disease. For example, a number of our students have already gone on to jobs or to graduate programs in public health, epidemiology, and infectious disease. This lesson provides students a powerful mix of field, wet lab, genomic and bioinformatics experiences and skills not typically seen in an undergraduate microbiology curriculum. In one student's words, this lesson "*gave me so much more than just the standard knowledge. It gave me ... a way to feel important, that my work was done and was significant, that I am a real scientist*."

SUPPORTING MATERIALS

Module 1

- S1. CURE for *Salmonella* – Laboratory safety contract
- S2. CURE for *Salmonella* – Pre-enrichment and enrichment media preparation
- S3. CURE for *Salmonella* – Field sampling protocol
- S4. CURE for *Salmonella* – Lab notebook grading rubric
- S5. CURE for *Salmonella* – Pre-enrichment and enrichment inoculation protocol
- S6. CURE for *Salmonella* – Plate media preparation
- S7. CURE for *Salmonella* – Plating and purification protocol
- S8. CURE for *Salmonella* – Miscellaneous test protocols - oxidase, catalase, KOH
- S9. CURE for *Salmonella* – invA PCR and gel visualization protocol
- S10. CURE for *Salmonella* – Preparing isolates for shipping and freezing

Module 2

- S11. CURE for *Salmonella* – Bioinformatics Lab Guide - Navigating and using GalaxyTrakr and Galaxy
- S12. CURE for *Salmonella* – Bioinformatics lab notebook grading rubric
- S13. CURE for *Salmonella* – Bioinformatics Lab Guide - *Salmonella* serotyping
- S14. CURE for *Salmonella* – Bioinformatics Lab Guide - Assessing & filtering illumina data using FastQC and Trimmomatic
- S15. CURE for *Salmonella* – Bioinformatics Lab Guide - File naming conventions
- S16. CURE for *Salmonella* – Bioinformatics Lab Guide - Genome assembly using SPAdes and Shovill
- S17. CURE for *Salmonella* – Bioinformatics Lab Guide - Assessing assembly quality Using QUAST and Bandage
- S18. CURE for *Salmonella* – Bioinformatics Lab Guide - Selecting reference genomes/Ordering and viewing assembled contigs using Mauve
- S19. CURE for *Salmonella* – Bioinformatics Lab Guide - Gene annotation
- S20. CURE for *Salmonella* – Bioinformatics Lab Guide - Antibiotic resistance gene detection
- S21. CURE for *Salmonella* – Bioinformatics Lab Guide - Miscellaneous gene and genetic feature detection
- S22. CURE for *Salmonella* – Presentation evaluation rubric
- S23. CURE for *Salmonella* – Poster evaluation rubric

Miscellaneous

- S24. CURE for *Salmonella* – Sources and description of preexisting survey instruments
- S25. CURE for *Salmonella* – Course Overview
- S26. CURE for *Salmonella* – Media, Isolation, Sampling
- S27. CURE for *Salmonella* – Intro to GalaxyTrakr mini-lecture
- S28. CURE for *Salmonella* – Overview of Next-Gen Sequencing and Assembly
- S29. CURE for *Salmonella* – FastQC & Trimmomatic

ACKNOWLEDGMENTS

This development and implementation of this course would not have been possible without the assistance of Dr. Rebecca Bell and Dr. Marc Allard of the U.S. FDA and especially Dr. Lauren Turner and her team at the Virginia Department of Consolidated Laboratory Services. We also acknowledge former members of the Herrick laboratory, especially Charles Holmes II and Curtis Kapsak, for their assistance in the development and optimization of many of the methods outlined here. The authors wish to acknowledge the financial support of the Madison Trust and the JMU Department of Biology in the development and implementation of this course. Lastly, we express our appreciation for the students of Bio 346 Bacterial Discovery who helped with not only the scientific aspects but also the assessment of the course.

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Table 1A. Recommended course activity timeline for A CURE for *Salmonella* Module 1: Wet Lab. "Labs" in both modules, refers to distinct activities to be completed, not to laboratory days. Multiple labs could be done on one day (and the reverse may occur, as well: one lab could be extended over multiple days).

Activity	Description	Estimated Time	Notes
Lab 1			
Course overview, lab safety, class safety and pre-course survey	• Course overview & objectives • Course business: syllabus, schedule, etc. • Safe practices in a BSL-2 laboratory • Assessment pre-course survey	90 min	• The latest versions of these protocols and more can be found https://osf.io/5p8dc/?view_only=ac974486316e4942b5e0783db4fb597e • Safety guidelines can be found here: https://www.asmscience.org/content/journal/jmbe/10.1128/jmbe.v14i1.531 • Safety contract located in supporting file S1. CURE for Salmonella – Laboratory safety contract. A sample course overview presentation can be found in S25. CURE for Salmonella – Course Overview • Pre-course survey administered (See S24. CURE for Salmonella – Sources and description of preexisting survey instruments). • If possible, form student groups during this lab period.
Lab 2			
Preparation for field sampling	• Overview of media prep and sampling • Sampling protocol • EpiCollect 5 overview • Students prepare pre-enrichments and enrichments • Keeping a lab notebook	90 min	• S2. CURE for Salmonella – Pre-enrichment and enrichment media preparation. • S3. CURE for Salmonella – Field sampling protocol • A sample mini-lecture presentation on media, isolation, and sampling can be found in S26. CURE for Salmonella – Media, Isolation, Sampling.pptx • Epicollect5 is a downloadable app for iOS or Android. • We use a simple Google Doc electronic lab notebook template. Our classroom has iPads for accessing lab books. Our students keep these as individuals rather than teams. • S4. CURE for Salmonella – Lab notebook grading rubric
Lab 3			
Sample collection and processing	• Sample collection: class (with instructor) collects sediment samples and records metadata in EpiCollect5 from a variety of sites • Sample processing: inoculate pre-enrichments and begin incubation	90 min	• This lab period may go over time depending on the number of sampling sites and the distance from your institution. • At this step, we can also provide poultry litter for students to sub-sample, and allow students to bring in litter if they have connections to farmers. • The pre-enrichments need to incubate for 24 hours before inoculating the enrichments. Since our lab meets MW, we typically sample on Monday and have the students come in on their own and inoculate their pre-enrichments the next day.
Lab 4			
Inoculation of enrichment media	Inoculate enrichments	15-30 min	• S5. CURE for Salmonella – Pre-enrichment and enrichment inoculation protocol • Enrichments should incubate for 5 days.
Lab 5			
Plating from enrichments	Streak plate onto CHROMagar Plus and XLT-4 plates	15 min	• Recipes for the preparation of plating media can be found in supporting file S6. CURE for Salmonella – Plate media preparation • S7. CURE for Salmonella – Plating and purification protocol • We use CHROMagar Salmonella Plus because it's somewhat easier for students to distinguish the mauve, putative Salmonella colonies but standard CHROMagar Salmonella works fine, also.

Activity	Description	Estimated Time	Notes
Lab 6-8			
Isolation and characterization of isolates using classical microbiological techniques	• Plate and purify on non-selective media • Biochemical tests to characterize isolates: Gram stain, oxidase test, catalase test, KOH test	Varies, typically 3 or more 60-90 minute lab periods depending on the number of isolates	• The expected appearance of Salmonella on CHROMagarPlus and XLT4 plates are shown and described in the CHROMagar Salmonella Plus Instructions for Use page and the Hardy Diagnostics Instructions for Use page, respectively. • Salmonella are Gram neg., oxidase negative rods. They are typically catalase positive. • Students can use Gram stain and oxidase/catalase tests to guide their choice of which colonies to purify but definitive tests should be done after purification. • S8. CURE for Salmonella – Miscellaneous test protocols - oxidase, catalase, KOH • Students will require multiple labs to purify and test their isolates. This process can be sped up considerably if they are able to come in outside of class to streak plates, etc.
Lab 9			
Enteropluri tube identification (optional)	Inoculate and read Enteropluri tubes	5-15 min (inoculation), 45-60 min (reading)	• Other, individual test media such as the TSI agar and citrate tests can be used to ID Salmonella and to teach students the use of these media for identification of Enterobacteriaceae. • The Enteropluri tube manufacturer's protocol (Becton Dickinson, Franklin Lakes, NJ) can be downloaded. The code book is available online. • Enteropluri tubes should be incubated 18-24 hours. In our MW course schedule, we show the students how to inoculate the tubes on Monday, then have them inoculate them on their own on Tuesday. We read the tubes together on Wednesday as interpretation isn't always straightforward.
Lab 10			
PCR	PCR using *invA* primers	60-90 min	• S9. CURE for Salmonella – invA PCR and gel visualization protocol • More recently, we have successfully used an alternative, real-time PCR Salmonella detection kit produced by Norgen Biotek (Thorold, ON Canada). We have found it to be both faster (only requiring a single day, since there is no gel to run) and more reliable than end-point PCR.
Lab 11			
PCR agarose gel electrophoresis	• Load and run PCR gels • Visualization and interpretation of individual results	60-90 min	
Lab 12			
Prep strains for shipping, freezing	• Prepare and inoculate agar stabs for shipping • Prepare and inoculate cryotubes for freezing at -80° C	15-20 min	S10. CURE for Salmonella – Preparing isolates for shipping and freezing
Lab 13			
Kirby-Bauer testing	Typical Kirby-Bauer protocol on Mueller-Hinton plates	20-30 min (should be read in 16-24 hrs)	• Antibiotics used to treat systemic Salmonella infections include chloramphenicol, ceftriaxone, sulfamethoxazole/ trimethoprim, ampicillin, ciprofloxacin and bacitracin. • MICs can be determined for isolates showing resistance to one or more antibiotics. For calculating MICs we have used the Gram negative NF or the NARMS Sensititre plates for Salmonella (ThermoFisher). Sensititre results must be read in 24 hours.

Table 1B. Recommended course activity timeline for A CURE for *Salmonella* Module 2: Genomics. "Labs" in both module 1 and 2, refers to distinct activities to be completed, not to laboratory days. Multiple labs could be done on one day (and the reverse may occur, as well: one lab could be extended over multiple days).

Activity	Description	Estimated Time	Notes
Lab 1			
Introduction to whole-genome sequencing & assembly, using GalaxyTrakr	• Intro to WGS • GalaxyTrakr registration • Intro to GalaxyTrakr and Galaxy • Import sample raw fastq data to GalaxyTrakr from the NCBI SRA database • Keeping a bioinformatics lab notebook	45 min	• We introduce the history and general concepts of whole genome sequencing and assembly. See S28. CURE for *Salmonella* – Overview of Next-Gen Sequencing and Assembly.pptx • Register for GalaxyTrakr at https://account.galaxytrakr.org/Account/Register. • The instructor can guide the students through the GalaxyTrakr interface on the website. Students can also take the simple user tutorials on the main page. • A sample presentation introducing GalaxyTrakr and Galaxy is found in S27. CURE for *Salmonella* – Intro to GalaxyTrakr mini-lecture. • S11. CURE for *Salmonella* – Bioinformatics Lab Guide - Navigating and using GalaxyTrakr and Galaxy • A Google Docs template for the bioinformatics lab notebook can be found at https://docs.google.com/document/d/1T7ZKtNEapD-IIG_glnoZaz00rDus4FpyV5mdGh-j0zE/edit?usp=sharing • S12. CURE for *Salmonella* – Bioinformatics lab notebook grading rubric
Lab 2			
In silico serotyping of *Salmonella*	• Explanation of *Salmonella* systematics (See Figure 1. General overview of the current classification of *Salmonella enterica*) • In-class serotyping of imported SRA genome using SeqSero in GalaxyTrakr	10 min	• S13. CURE for *Salmonella* – Bioinformatics Lab Guide - *Salmonella* serotyping • Assignment: assign students one or more *Salmonella* genomes from NCBI's Short Read Archive (SRA) to serotype. (Note: these can be used as practice for subsequent analyses in module 2).
Lab 3			
Assessing assembly quality, filtering and trimming reads using *FastQC* and *Trimmomatic* on GalaxyTrakr	• *FastQC* analysis of raw sequencing read files (GalaxyTrakr) • Filtering of raw sequencing read files using *Trimmomatic* (GalaxyTrakr)	30-60 min	• S14. CURE for *Salmonella* – Bioinformatics Lab Guide - Assessing & filtering Illumina data using *FastQC* and Trimmomatic • Assignment: students submit partial *FastQC* outputs for both pre-trimmed and post-trimmed reads from their imported genome (and any others assigned). • A presentation on using *FASTQC* and Trimmomatic can be found in S29. CURE for *Salmonella* – *FastQC* & *Trimmomatic*
Lab 4			
Naming files, genome assembly using SPAdes, assessing assembly quality using QUAST and Bandage.	• How to name files so they are machine- and human-readable and -sortable. • Assembly of sample genomes using SPAdes (and Shovill; GalaxyTrakr) • How to make a new history and move data library files in GalaxyTrakr • Checking assembly quality using QUAST (GalaxyTrakr) • Sequencing and assembly quality standards • Visualizing an assembly using Bandage	45-60 min (not including run time)	• S15. CURE for *Salmonella* – Bioinformatics Lab Guide - File naming conventions • S16. CURE for *Salmonella* – Bioinformatics Lab Guide - Genome assembly using SPAdes and Shovill • S17. CURE for *Salmonella* – Bioinformatics Lab Guide - Assessing assembly quality Using QUAST and Bandage • Bandage is a free standalone program that can be downloaded, installed, and run locally on student computers. Alternatively, it is now also available in GalaxyTrakr and Galaxy • Instructor should prepare an assembly for demonstration beforehand as assemblies through GalaxyTrakr may take an hour or more • If desired, students can learn how to use the free service Open Science Framework for organizing and storing data and analysis files

Activity	Description	Estimated Time	Notes
Lab 5			
Ordering contigs to a reference, read mapping, using *Mauve*	• Finding and downloading a suitable reference genome • Ordering assembly contigs using *Mauve* • Viewing the ordered contigs in *Mauve* • Using *Mauve*: Searching for annotated features, assembly metrics	60 min	• This lab is optional, as no subsequent analyses require the use of an assembly with ordered contigs. • *Mauve* is a free standalone program that will need to be downloaded, installed, and run locally on student computers. • S18. CURE for *Salmonella* – Bioinformatics Lab Guide - Selecting reference genomes/Ordering and viewing assembled contigs using *Mauve*
Lab 6			
Annotation 1: Whole genome using Prokka and RAST; visualization using Artemis	• Explain or demo the meaning of "genome annotation". • Students use Prokka (GalaxyTrakr) and/or RAST (https://www.patricbrc.org/) to annotate their sample genomes • Visualization of annotated genomes in Artemis	30-45 min	• S19. CURE for *Salmonella* – Bioinformatics Lab Guide - Gene annotation • Genome browsers such as UGene, the Integrative Genomics Viewer JBrowse (as implemented in Galaxy), and the UCSC Microbial Genome Browser can be used in place of Artemis.
Lab 7			
Annotation 2: Antibiotic resistance genes	• Background info (e.g., a mini-lecture) on antimicrobials and their classes, as needed • Students use Abricate (GalaxyTrakr) and KmerResistance (Center for Genomic Epidemiology) to detect resistance genes	30-45 min	• S20. CURE for *Salmonella* – Bioinformatics Lab Guide - Antibiotic resistance gene detection • Instructor should prepare an example ABRicate analysis before class. • Students using both modules should compare their genotypic and phenotypic data. If they find genes suggesting resistance they haven't tested for, they could do an additional Kirby-Bauer test.
Lab 8			
Annotation 3: Miscellaneous genes (genomic islands, virulence genes, mobile elements, etc.)	Background info (e.g., a mini-lecture) on virulence factors, *Salmonella* pathogenicity and other islands, and mobile elements (plasmids, phages, transposons, integrons) as needed	30 min to ?	• S21. CURE for *Salmonella* – Bioinformatics Lab Guide - Miscellaneous gene and genetic feature detection • bacWGSTdb is an easy-to-use all-in-one website which can provide – from an uploaded assembly fasta file – a list of virulence and resistance genes, plasmid replicons, and MLST typing, as well as phylogenetic relationships with close relatives • Enterobase is an integrated software environment for comparative genomics of *Salmonella* and other pathogens. It's particularly useful for identifying global population structures and phylogenetic relationships of these bacteria. However, the learning curve can be quite steep. Enterobase uses raw read data as input • At this point in the module, we instruct students to follow their interests with regard to analyzing and understanding the genomes of their isolates. Questions they might pursue could concern pathogenicity, resistance, population structure, genomic epidemiology, mobile elements, etc.
Lab 9			
Poster presentation, oral presentation, scientific communication, group work	• Explain (e.g., in a mini-lecture) how to prepare effective poster and oral presentations. • Students work in groups to prepare poster and oral presentations • Students give oral presentations • Students give poster presentations	Multiple days	• We provide students with model posters • Presentations may be given in class, at departmental symposia, etc. • We administered a post-survey in the last lab period • S22. CURE for *Salmonella* – Presentation evaluation rubric • S23. CURE for *Salmonella* – Poster evaluation rubric