TEXAS STATE Soil & Water CONSERVATION BOARD

Texas State Soil and Water Conservation Board
State Nonpoint Source Grant Program
FY 2024 Workplan 24-50

SUMMARY PAGE						
Title of Project	Texas Bacterial Source Tracking Program (FY24 - FY25)					
Project Goals	• Further evaluate, update, and refine the Texas *E. coli* BST Library. • Evaluate a next generation sequencing (NGS) approach for BST and pathogen characterization. • Support Bacterial Source Tracking (BST) analyses throughout Texas. • Integrate BST results with Quantitative Microbial Risk Assessment (QMRA) to assess public health impacts. • Statistically evaluate and analyze metagenomics findings. • Review NGS findings with measured *E. coli* concentrations at sampling sites. • Compare findings of NGS BST and pathogen characterization and its effectiveness for informing future watershed management. • Provide education and outreach regarding BST and NGS. • Assessment and discussion of NGS and QMRA for use in watershed management.					
Project Tasks	(1) Project Administration; (2) Quality Assurance; (3) BST Sample Collection; (4) NGS-based BST Analyses & QMRA; (5) NGS Analyses of Water Samples for Pathogens; (6) BST Method and SOP Refinement; (7) Education and Outreach;					
Measures of Success	• Collection of up to 100 source-specific fecal samples for development of the NGS BST Library • Collection of up to 116 water samples for NGS BST analysis and characterization • Statistical characterization of NGS findings • Evaluation and refinement of the current Texas *E. coli* BST Library • Evaluation of NGS BST and pathogen characterization data • Outreach through website and delivery of NGS BST informational materials and the utility of NGS approaches for watershed managers. • BST and QMRA analysis for Medina River Above Medina Lake Watershed					
Project Type	Implementation ( ); Education ( ); Planning ( ); Assessment (X); Groundwater ( )					
Status of Waterbody on *2022 Texas Integrated Report*	Segment ID 1905	Parameter of Impairment or Concern Bacteria, Fish Community, Habitat	Category 5c, CN, CS			
Project Location (Statewide or Watershed and County)	Statewide, but with BST support in Bandera County.					
Key Project Activities	Hire Staff ( ); Surface Water Quality Monitoring (X); Technical Assistance ( ); Education ( ); Implementation ( ); BMP Effectiveness Monitoring ( ); Demonstration (X); Planning ( ); Modeling ( ); Bacterial Source Tracking (X); Other (X)					
2022 Texas NPS Management Program Reference	• Component 1 – LTG Objectives 1, 2, 3, 6, 8 • Component 1 – STG 1C, 3B • Components 2, 3, 5					
Project Costs	Federal	$510,735	Non-Federal	$0	Total	$510,735
Project Management	• Texas A&M AgriLife Research, Texas Water Resources Institute					
Project Period	February 1, 2024 – January 31, 2026					

Part I – Applicant Information

Applicant							
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Co-Applicant							
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Title	Professor						
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Co-Applicant							
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Project Partners	
Names	Roles & Responsibilities
Texas State Soil and Water Conservation Board (TSSWCB)	Provide state oversight and management of all project activities and ensure coordination of activities with related projects and TCEQ.
Texas A&M AgriLife Research, Texas Water Resources Institute (TWRI)	Project coordination and administration, quality assurance, reporting, and outreach, and water sample collection (Tasks 1, 2, and 3).
Texas A&M AgriLife Research, Department of Soil and Crop Sciences (AgriLife SCSC)	Work in conjunction with UTHealth H SPH to perform all work described in Tasks 2-7.
UTHealth Houston School of Public Health, El Paso Regional Campus (UTHealth H SPH)	Work in conjunction with AgriLife SCSC to perform all work described in Tasks 2-7.
Bandera County River Authority (BCRA)	Water and BST sample collection support (Task 3).

Part II – Project Information

Project Type							
Surface Water	X	Groundwater					
Does the project implement recommendations made in: (a) a completed WPP; (b) an adopted TMDL; (c) an approved TMDL I-Plan; (d) a Comprehensive Conservation and Management Plan developed under CWA §320; (e) the *Texas Coastal NPS Pollution Control Program*; or (f) the *Texas Groundwater Protection Strategy*?			Yes		No	X	
If yes, identify the document.	N/A						
If yes, identify the agency/group that developed and/or approved the document.	N/A		Year Developed	N/A			

Watershed Information				
Watershed or Aquifer Name(s)	Hydrologic Unit Code (12 Digit)	Segment ID	Category on 2022 IR	Size (Acres)
Medina River Above Medina Lake	121003020101-0107;0201-0206; 0301, 0302	1905	5c	341,767

Water Quality Impairment

Describe all known causes (i.e., pollutants of concern) and sources (e.g., agricultural, silvicultural) of water quality impairments or concerns from any of the following sources: *2022 Texas Integrated Report*, Clean Rivers Program Basin Summary/Highlights Reports, or other documented sources.

2022 Texas Integrated Report

Impairments and Concerns

Segment **1905**: Medina River Above Medina Lake

- From a point immediately upstream of the confluence of Red Bluff Creek in Bandera County to the confluence of the North Prong Medina River and the West Prong Medina River in Bandera County

Assessment Unit	***Impairment***	***Category***	***Year Listed***
1905_01	Bacteria	5c	2020

Assessment Unit	***Concern***	***Category***
1905_01	Fish Community	CN
1905_01	Habitat	CN
1905_02	Fish Community	CN

Potential Sources of Impairments or Concerns

Medina River Above Medina Lake: Segment ID 1905, AU ID 1905_01

E. coli

Point sources: Unknown
Non-point sources: Unknown

Fish Community

Point sources: Unknown
Non-point sources: Impacts from Hydro structure Flow Regulation/Modification; Rural (Residential Areas)

Habitat
Point sources: Unknown
Non-point sources: Unknown

Medina River Above Medina Lake: Segment ID 1905, AU ID 1905_02
Fish Community
Point sources: Unknown
Non-point sources: Unknown

Project Narrative

Problem/Need Statement

Bacteria continues to remain the number one cause of water quality impairments in the state of Texas. Numerous approaches have been applied to evaluate bacteria sources in streams and rivers to develop effective watershed management practices. Evaluating water quality integrity for contact recreation (and shellfish harvesting) has been dependent on measuring fecal indicator bacteria (FIB), specifically *Escherichia coli* (*E. coli*) and *Enterococcus* species. Bacteria source tracking (BST) has been identified as a valuable tool for identifying the different sources of fecal pollution, therefore informing the development of watershed plans, TMDLs and other strategies for addressing the impairments. Comprehensive BST has been completed by UTHealth H SPH and AgriLife SCSC in numerous watersheds throughout Texas with support provided by the TSSWCB. As a result of these joint efforts over the last decade, the Texas *E. coli* BST Library (ver. 04-22) currently contains 1,942 *E. coli* isolates obtained from 1,775 different domestic sewage, wildlife, livestock, and pet fecal samples.

While comprehensive BST projects have been completed in watersheds across Texas and provide considerable value to planners working to prioritize implementation, methodological limitations exist for traditional library-dependent BST. The use of FIB has been integral in assessing exposure risks for fecal pollution, but as recent research has suggested, there are limitations to solely relying on these indicators. Measuring for *E. coli* and *Enterococcus* species in recreational and shellfish harvesting waters remains the primary approach for assessing bacteria contamination and health risks in water bodies; however, advances in molecular technology with next generation sequencing (NGS) provides another tool to assess the presence of various fecal contaminants in a water body.

Early applications of NGS were limited by the lack of taxonomic resolution of pathogens due to short DNA read lengths (approximately 60 base pairs). In recent years, sequence read lengths have continued to increase, therefore providing increased confidence in the classification of NGS-sequences to the bacterial species level (Tan et al., 2015). Further work applying NGS techniques to evaluate bacterial communities impacted by different land uses and water quality has indicated that the genera and species of pathogen sequences has varied according to land use and FIB concentrations (Nshimyimana et al., 2015).

NGS techniques have been used to assess the biodiversity of aquatic habitats, but more recently, used in water microbiology to supplement water quality monitoring efforts. These techniques provide the opportunity to simultaneously test for the presence of various pathogenic targets (e.g., bacteria, protozoa, viruses) without the need to culture specific organisms in the lab (Hamner et al., 2019; Ji et al., 2020). In addition, some newer NGS sequencing platforms are field-portable and capable of generating near real-time results thus opening possible applications for source identification in water bodies. Multiple studies have demonstrated the potential for NGS-based approaches to be used for BST and help provide a clearer understanding of the fecal sources impacting a water body (Raza et al., 2021; Unno et al., 2018). In a brief overview, NGS methods involve four key steps that include DNA isolation from the environmental sample, library preparation, sequencing, and bioinformatic data analysis. This approach is not limited by requiring a pre-selected list of microbes that require being identified by traditional culture-based, immunoassay, microscopy or PCR-based analyses (Miller et al., 2013). NGS techniques permit the DNA sequence-based characterization of a wide array of microorganisms that may be present in a water body (Hamner et al., 2019).

Quantitative microbial risk assessment (QMRA) is a valuable tool that can integrate BST results and estimate potential human health risks in recreational waters. Using BST data and QMRA is supported by the U.S. Environmental Protection Agency's recommendation to assess water quality based on health risks (U.S. EPA, 2012). Efforts to delineate QMRA outputs to inform policy and best management practices can increase the utility of BST work. Finally, continued outreach and technology transfer is needed to expand awareness and understanding of BST, foster dialogue and collaboration, and bring water resource managers up to speed on advances in BST technologies, methodologies, applications, and results.

The ability to screen water samples for genetic sequences relating to waterborne pathogens assists in identifying potential human health risks and provides a preliminary characterization and distribution of pathogens in water bodies influenced by different pollutant sources. Current efforts to measure water quality and exposure risks using FIB requires inferring about potential sources of fecal pollution and if pathogens may exist. Advances in NGS methods provide the opportunity to analyze a wide array of pathogens that has not been previously possible with traditional microbial techniques. Utilizing NGS to characterize microbial pathogens instead of relying on FIB enumeration provides a direct identification of microorganisms that could be a risk for human health. Such information is imperative for watershed managers striving to identify management practices that reduce human exposure and therefore health risk, to pathogens in recreational waters. Further, direct detection of pathogens can potentially prioritize sites for targeted management, therefore implementing funds and efforts that may provide the greatest protection for public health.

Advances in NGS methods provide the opportunity to further evaluate and expand the Texas BST Library, as well survey water quality for potential pathogens. Findings from this work can be utilized to evaluate the appropriateness of NGS techniques for water quality management. Continued support of the Texas BST Infrastructure project is imperative for watershed managers striving to identify management practices that reduce pollutant sources and minimize human health risks in Texas water bodies. Continued BST application across Texas remains a valuable tool to inform watershed stakeholders in watershed planning and implementation efforts. Other engagement activity regarding bacteria sources and feasible management efforts is also needed to promote and support WPP implementation.

References

Ji, P., Aw, T. G., Van Bonn, W., & Rose, J. B. 2020. Evaluation of a portable nanopore-based sequencer for detection of viruses in water. *Journal of Virological Methods*, *278*, 113805.

Hamner, S., Brown, B. L., Hasan, N. A., Franklin, M. J., Doyle, J., Eggers, M. J., Colwell, R.R., & Ford, T. E. 2019. Metagenomic profiling of microbial pathogens in the little Bighorn river, Montana. *International Journal of Environmental Research and Public Health*, *16*(7), 1097.

Miller, R. R., Montoya, V., Gardy, J. L., Patrick, D. M., & Tang, P. 2013. Metagenomics for pathogen detection in public health. *Genome medicine*, *5*(9), 81.

Nshimyimana, J. P., Freedman, A. J. E., Shanahan, P., Chua, L. C. H., & Thompson, J. R. 2015. "Variation of Bacterial Communities and Pathogen Taxa as a Function of Land Use and Water Quality in an Urban Tropical Catchment of Singapore" in *Proceedings of the 115th General Meeting of American Society for Microbiology*, New Orleans.

Raza, S., J. Kim, M.J. Sadowsky, & T. Unno. 2021. Microbial source tracking using metagenomics and other new technologies. *Journal of Microbiology, 59*, 259-269.

Tan, B., Ng, C. M., Nshimyimana, J. P., Loh, L. L., Gin, K. Y. H., & Thompson, J. R. 2015. Next-generation sequencing (NGS) for assessment of microbial water quality: current progress, challenges, and future opportunities. *Frontiers in microbiology*, *6*, 1027.

Unno, T., C. Staley, C.M. Brown, D. Han, M.J. Sadowsky, and H.-G., Hur. 2018. Fecal pollution: new trends and challenges in microbial source tracking using next-generation sequencing. *Environmental Microbiology, 20*, 3132-3140.

U.S.EPA. 2012. Recreational Water Quality Criteria. Office of Water, United States Environmental Protection Agency: Washington, D.C., USA.

Project Narrative

General Project Description (Include Project Location Map)

Continued interest in BST among state agencies, river authorities, and stakeholder groups across Texas emphasizes the necessity of maintaining statewide BST analytical infrastructure. Advances in BST science and methodology remain an important component of the state BST analytical infrastructure and program. This includes needed maintenance and repairs of analytical equipment, and continued support, training, and retention of skilled personnel to facilitate using novel NGS techniques. With personnel changes at UTHealth H SPH, AgriLife SCSC, and TWRI, there is a need for increased interaction among entities to facilitate the transition. To meet the needs of the state, BST analytical capabilities will be maintained at both UTHealth H SPH and AgriLife SCSC BST laboratories. Financial support will be used to maintain lab personnel at UTHealth H SPH and AgriLife SCSC, continue refinement and evaluation of the Texas *E. coli* BST library, continue work on marker development and evaluation, and support targeted NGS BST analysis. Utilizing NGS techniques to screen water bodies for bacterial pathogens also provides opportunities to better assess the influence of different fecal sources on the distribution of specific microbial pathogens in surface waters, therefore informing watershed management practices. While measuring water quality for FIB and BST informs pollutant sources, directly evaluating a water body for an array of microbial pathogens provides the potential for rapidly identifying specific exposure risks.

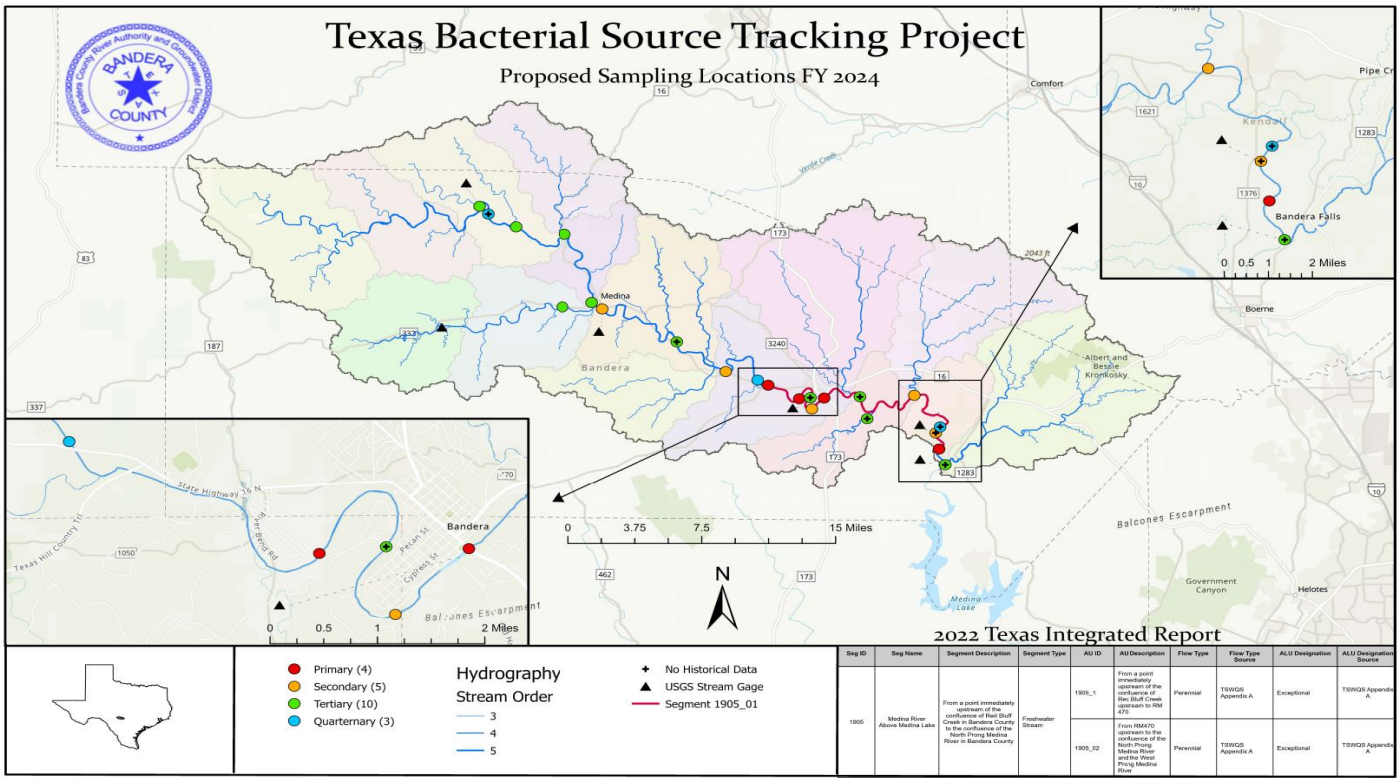

Figure 1. Medina River Above Medina Lake Watershed and proposed sampling sites for the Texas Bacterial Source Tracking Project.

There are two parallel aims to this project which include: 1) conduct BST analyses using NGS techniques for water samples gathered from up to 9 chosen sampling sites in the Medina River Above Medina Lake Watershed and 2) conduct NGS analyses for water samples gathered at the up to 9 chosen sampling sites to provide an overview of potential pathogens present. Water samples will be collected over 12 months to provide an overview of different fecal sources impacting these water bodies. Additionally, four storm samples will be collected at two sampling sites during this period. Grab samples will be collected concurrently to measure for *E. coli*, which will be incorporated into the metagenomics analysis and evaluation of NGS techniques as a potential tool for the watershed management toolbox. AgriLife SCSC personnel will work with Bandera County River Authority to 1) filter collected water samples to collect microbial biomass, 2) extract microbial DNA, and 3) conduct metagenomic sequencing using NGS

technology. Generated data will be compared against sequence data from known-source samples also collected in this project to identify the sources of fecal microorganisms. In consultation with stakeholders, up to 10 potential sources of fecal contamination in the watershed will be identified. From each of these sources, 10 unique samples will be collected (up to 100 total known-source samples) and sequenced as described above to generate a known-source microbiome sequence library. Bioinformatics will then be used to compare NGS data from water samples against the known-source NGS data for source determination. Generated data will also be compared against publicly available genomic databases to identify the presence of pathogenic microorganisms. AgriLife SCSC will work with the Texas A&M Institute for Genome Sciences and Society core facility or comparable laboratory for sequence analysis and bioinformatics training needed to interpret the metagenomics data for a water quality management context. Findings from the study will be evaluated for application to watershed management and how information can be translated to the stakeholder level.

The proposed project will represent continued use of NGS-based approaches for watershed source delineation in the Texas BST Program. This approach will be a valuable addition to the BST toolbox, complementing current library-independent tools. The second aim of using NGS techniques for pathogenic microorganism detection will complement source tracking efforts by attempting to directly identify pathogens of public health concern. Comparing findings from these two aims will improve the utilization and interpretation of NGS-based work for future water management. Further, it has the potential to provide information similar to that obtained using culture-based, library-dependent approaches, but at substantially lower cost due to rapid advances in sequencing technologies.

The project will also include continued development and refinement of the Texas *E. coli* BST Library, specifically to evaluate the delineation of feral hogs. Existing DNA fingerprints of feral hogs in the library will be evaluated to determine if a four-way split of source classes, including human, domestic animals, wildlife, and feral hogs is feasible.

BST results, from previous studies and this one, will be integrated into the QMRA framework to not only inform of human health risks associated with contact recreation, but also assist in informing watershed management practices. The QMRA will follow methods described in Haas et al. (2014). Estimated risk outputs will be evaluated and the feasibility of recommendations for incorporating QMRA into future watershed management across the state of Texas will be developed.

All NGS data generated from both watersheds will be deposited in the National Center for Biotechnology Information GenBank database and will be a valuable asset to other water quality projects. Discussing and sharing findings from this novel project are critical towards advancing watershed management science and water quality protection. TWRI, AgriLife SCSC, and UTHealth H SPH will develop materials concerning the project and the application of the science and distribute the information to water resource managers, natural resources agencies, universities, and other stakeholders. TWRI will include information on the project in its publications. A final report will be developed that describes the findings of this study and its application for watershed management.

Reference

Haas, C.N., Rose, J.B., & Gerba, C.P. 2014. Quantitative microbial risk assessment. John Wiley & Sons.

Tasks, Objectives and Schedules				
Task 1	Project Administration			
Costs	$14,403			
Objective	To effectively administer, coordinate, and monitor all work performed under this project including technical and financial supervision, and preparation of status reports.			
Subtask 1.1	TWRI will prepare electronic quarterly progress reports (QPRs) for submission to the TSSWCB. QPRs shall document all activities performed within a quarter and shall be submitted by the 1st of December, March, June and September. QPRs shall be distributed to all Project Partners.			
	Start Date	Month 1	Completion Date	Month 24
Subtask 1.2	TWRI will perform accounting functions for project funds and will submit appropriate Reimbursement Forms to TSSWCB at least quarterly.			
	Start Date	Month 1	Completion Date	Month 24
Subtask 1.3	TWRI will host coordination meetings or conference calls, at least quarterly, with Project Partners to discuss project activities, project schedule, communication needs, deliverables, and other requirements. TWRI will develop lists of action items needed following each project coordination meeting and distribute to project personnel.			
	Start Date	Month 1	Completion Date	Month 24
Subtask 1.4	TWRI will develop a Final Report with Project Partners that summarizes activities completed and conclusions reached during the project and discusses the extent to which project goals and measures of success have been achieved.			
	Start Date	Month 1	Completion Date	Month 24
Deliverables	• QPRs in electronic format • Reimbursement Forms and necessary documentation in electronic or hard copy format • Final Report in electronic and hard copy formats			

Tasks, Objectives and Schedules				
Task 2	Quality Assurance			
Costs	$5,107			
Objective	To develop data quality objectives (DQOs) and quality assurance/control (QA/QC) activities to ensure data of known and acceptable quality are generated through this project.			
Subtask 2.1	TWRI will develop a QAPP for activities in Task #3-7 consistent with the most recent versions of *EPA Requirements for Quality Assurance Project Plans (QA/R-5)* and the *TSSWCB Environmental Data Quality Management Plan*. All monitoring procedures and methods prescribed in the QAPP shall be consistent with the guidelines detailed in the *TCEQ Surface Water Quality Monitoring Procedures, Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment, and Tissue (RG-415)* and *Volume 2: Methods for Collecting and Analyzing Biological Assemblage and Habitat Data (RG-416)*. [Consistency with Title 30, Chapter 25 of the Texas Administrative Code, *Environmental Testing Laboratory Accreditation and Certification*, which describes Texas' approach to implementing the National Environmental Laboratory Accreditation Conference (NELAC) standards, shall be required where applicable.]			
	Start Date	Month 1	Completion Date	Month 24
Subtask 2.2	TWRI, AgriLife SCSC, UTHealth H SPH, and BCRA will implement the approved QAPP. TWRI will submit revisions and necessary amendments to the QAPP as needed.			
	Start Date	Month 1	Completion Date	Month 24
Deliverables	• QAPP approved by TSSWCB and EPA in electronic format • Approved revisions and amendments to QAPP, as needed • Data of known and acceptable quality as reported through Task #3-7			

Tasks, Objectives and Schedules				
Task 3	BST Sample Collection			
Costs	$103,679			
Objective	To use NGS-based approaches for BST to characterize fecal source contributions and pathogens in the Medina River Above Medina Lake Watershed through the collection of approximately 100 known source fecal samples and 116 water samples.			
Subtask 3.1	TWRI will work with UTHealth H SPH, AgriLife SCSC and BCRA to develop a targeted list of needed species for fecal sample collection and plan for their collection and delivery.			
	Start Date	Month 2	Completion Date	Month 4
Subtask 3.2	BCRA will collect up to 100 fecal samples, which will include 10 fecal samples per 10 specific fecal sources, from the watershed in accordance with the plan developed in Subtask 4.1 and work closely with AgriLife SCSC to coordinate delivery of the samples. BCRA will communicate with a select group of organizations, agencies and businesses in the watershed to arrange and resolve any access concerns and gather input to improve geographic targeting of sample collection. BCRA will coordinate closely with TWRI, UTHealth H SPH and AgriLife SCSC to ensure sample delivery adheres to established QA/QC procedures. A known source sample data set will be finalized after completion of the field work and included in the project final report.			
	Start Date	Month 4	Completion Date	Month 24
Subtask 3.3	BCRA will collect monthly grab samples from up to 9 selected monitoring sites in the Medina River Above Medina Lake Watershed. BCRA/TWRI will coordinate preparation and delivery of samples to AgriLife SCSC for processing. Collected water samples will be also analyzed for *E. coli* and incorporated into the overall NGS BST and pathogen characterization study.			
	Start Date	Month 4	Completion Date	Month 16
Deliverables	• Proposed list of up to 10 needed species recommended for fecal sample collection • MS Excel summary data sheets cataloging known source samples collected • Water samples collected and delivered to AgriLife SCSC • Highlights of work performed in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 4	NGS-based BST Analyses & QMRA			
Costs	$132,791			
Objective	To use NGS-based BST to characterize fecal source contributions in the Medina River Above Medina Lake Watershed to expand the Texas BST toolbox through the analysis of approximately 100 known source fecal samples and up to 120 water samples. BST results to be utilized in a QMRA to evaluate human health risks in Medina River Above Medina Lake.			
Subtask 4.1	UTHealth H SPH and AgriLife SCSC will maintain BST analytical equipment (e.g., RiboPrinter) and general laboratory equipment to support BST analyses. This includes securing maintenance contracts, replacement parts and expendable supplies.			
	Start Date	Month 1	Completion Date	Month 24
Subtask 4.2	UTHealth H SPH and AgriLife SCSC will retain (or hire) lab personnel, students and/or Postdoctoral Research Associates to maintain laboratory operating capacities and technical expertise to conduct BST studies across the state.			
	Start Date	Month 1	Completion Date	Month 24
Subtask 4.3	UTHealth H SPH and AgriLife SCSC will perform targeted BST analysis to support future WPP implementation efforts in Medina River Above Medina Lake Watershed. BST analyses will be performed on monthly samples for up to 9 sites (i.e., 12 months x 9 sites = 108 samples) and on four storm event samples at two sites (8 additional samples) in the Medina River Above Medina Lake Watershed for a total of approximately 116 samples.			
	Start Date	Month 4	Completion Date	Month 24

Subtask 4.4	AgriLife SCSC and UTHealth H SPH, in connection with the Texas A&M Institute for Genome Sciences and Society core facility, will determine water sample source contributions through bioinformatics (e.g., SourceTracker) evaluation of water sample NGS data against known-source NGS data.			
	Start Date	Month 4	Completion Date	Month 24
Subtask 4.5	AgriLife SCSC will deposit NGS data in a publicly available database (GenBank).			
	Start Date	Month 4	Completion Date	Month 24
Subtask 4.6	UTHealth H SPH and AgriLife SCSC will evaluate differences in BST data and findings between the Medina River Above Medina Lake and other comparable watersheds in the state with traditional library-dependent BST results.			
	Start Date	Month 4	Completion Date	Month 24
Subtask 4.7	UTHealth H SPH and AgriLife SCSC will integrate the BST results from the project into a quantitative microbial risk assessment (QMRA) to evaluate the public health significance of the project's data.			
	Start Date	Month 4	Completion Date	Month 24
Deliverables	• BST analyses for the Medina River Above Medina Lake Watershed • QMRA analysis integrating BST results for the Medina River Above Medina Lake Watershed • Discussion of all findings included in final report • Highlights of work performed included in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 5	NGS Analyses of Water Samples for Pathogens			
Costs	$127,684			
Objective	Evaluate water samples for the presence of pathogens using NGS-based metagenomic DNA sequencing. Interpret data in context for watershed management and assess the potential of the technology and analyses for future watershed planning. Interpret data in context of potential pollutant sources and human health risk.			
Subtask 5.1	AgriLife SCSC will perform metagenomics sequencing on DNA from the approximately 116 extracted water samples (Task 4.3) using MinION sequencing platform. With bioinformatics support from the Texas A&M Institute for Genome Sciences and Society core facility, sequence data will be compared against publicly available genomics databases (e.g., GenBank) for identification of detected organisms with a focus on human pathogens.			
	Start Date	Month 4	Completion Date	Month 24
Subtask 5.2	TWRI and AgriLife SCSC will compare metagenomics data, *E. coli* grab sample data, water quality parameters, and land use information to determine any trends or unique findings pertinent to watershed management efforts.			
	Start Date	Month 4	Completion Date	Month 24
Subtask 5.3	AgriLife SCSC will evaluate findings for potential areas for further research concerning the use of NGS technology and watershed management.			
	Start Date	Month 4	Completion Date	Month 24
Subtask 5.4	TWRI and AgriLife SCSC will collaborate with other experts in the field of NGS techniques to better understand the potential of the science for advancing water quality management approaches in Texas.			
	Start Date	Month 4	Completion Date	Month 24
Subtask 5.5	AgriLife SCSC will deposit NGS data in a publicly available database (GenBank).			
	Start Date	Month 20	Completion Date	Month 24
Deliverables	• Highlights of work performed will be included in QPRs and the Final Report			

Tasks, Objectives and Schedules				
Task 6	BST Method and SOP Refinement			
Costs	$96,427			
Objective	Evaluate and refine the statewide *E. coli* BST Library.			
Subtask 6.1	Support BST across Texas through continued development, updating and implementation of statewide BST template-SOPs for ERIC-PCR, RiboPrinting and *Bacteroidales* PCR along with coordination amongst other entities conducting BST in the state to standardize methodologies employed. Consider development of SOPs and best practices for additional methods such as NGS-based BST approaches.			
	Start Date	Month 4	Completion Date	Month 24
Deliverables	• Updated/new SOPs • Highlights of work performed included in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 7	Education and Outreach			
Costs	$30,644			
Objective	Provide continued education and outreach regarding BST and its application through improving the statewide knowledge base regarding current BST practices, scientific advances, improvements in the application of BST and incorporating information from other areas of the nation into the BST approaches used in Texas. Outreach will also include discussion of scientific advances and opportunities of applying NGS technology to assess water quality and pollutant sources in water bodies in Texas.			
Subtask 7.1	TWRI will host and maintain the http://texasbst.tamu.edu website to disseminate educational materials, project updates, science updates, notify readers about educational opportunities and other outreach efforts to advance the science and application of BST in Texas and nationally.			
	Start Date	Month 1	Completion Date	Month 24
Subtask 7.2	TWRI, UTHealth H SPH and AgriLife SCSC will promote the use of and provide resources on BST. TWRI, UTHealth H SPH and AgriLife SCSC will develop and distribute informational material via social media platforms (e.g., Facebook and Twitter) and in print (e.g., tri-folds and handouts) that can be used to 1) discuss the appropriate application of BST in identifying fecal contamination sources and 2) promote the analytical laboratory capability of public BST labs that the state has invested in. As appropriate, TWRI will include information about BST in general, and this project specifically, in the *txH2O* magazine and *Conservation Matters* e-mail newsletter. Additionally, TWRI will coordinate a state of the science conference (Task 8) Finally, TWRI, UTHealth H SPH and AgriLife SCSC will periodically meet with natural resource agencies, public and private laboratories, and other researchers/academia to advance the general knowledge and understanding of BST and appropriate methodologies and SOPs for use of BST in Texas.			
	Start Date	Month 1	Completion Date	Month 24
Deliverables	• Summaries of outreach efforts included in QPRs and Final Report			

Project Goals (Expand from Summary Page)
• Further evaluate, update and refine the Texas BST capacity through continued personnel support, and operation and maintenance of analytical infrastructure at public BST laboratories. • Provide personnel training for NGS methodologies and interpretation of metagenomics data for BST analysis and watershed management. • Support BST across Texas through continued development, updating and implementation of statewide BST template-SOPs for ERIC-PCR, RiboPrinting and *Bacteroidales* PCR along with coordination amongst other entities conducting BST in the state to standardize methodologies employed.

- Evaluate integration of BST results and QMRA to develop human health risk estimates for measured water quality scenarios.
- Initiate development of metagenomics database for pathogen sequences identified in water bodies impacted by different fecal sources and statistically assess metagenomics findings for relevance in identifying contributing bacteria sources compared to traditional BST methodologies.
- Characterize pathogens through NGS techniques to identify exposures risks and how these findings compare to measured bacteria concentrations and inform efforts to reduce bacteria pollution in water bodies.
- Continue information delivery regarding BST and NGS activities in Texas describing the use, capabilities, and applicability of BST, NGS, and other services provided by the state-supported analytical labs to local, state, and national stakeholder audiences.
- Assess potential for NGS techniques to expand BST assessment capacity in future watershed management.

Measures of Success (Expand from Summary Page)

- Continued personnel training and skill development with BST and NGS methods.
- Updated BST template-SOPs for ERIC-PCR, RiboPrinting and *Bacteroidales* PCR ensuring that template-SOPs include current methods, technologies, and approaches.
- Maintain the necessary level of training of AgriLife SCSC and UTHealth H SPH personnel.
- Continued operation and maintenance of BST analytical equipment and support of personnel needs to sustain operating capability and expand the use of BST applications statewide.
- Targeted BST supporting watershed planning and implementation efforts in the Medina River Above Medina Lake Watershed.
- Analysis of the presence and distribution of pathogens in water samples collected from sites in Medina River Above Medina Lake Watershed.
- Development of the NGS BST Library through the analysis of approximately 100 fecal samples collected by BCRA.
- BST analysis of water samples for Medina River Above Medina Lake
- Evaluation of bioinformatics data from NGS methods to characterize fecal sources and pathogens in the Medina River Above Medina Lake Watershed.
- Evaluation and refinement of the Texas *E. coli* BST Library to potentially identify feral hogs as a source-specific class.
- QMRA integrating BST data to assess associated human health risks in recreational waters.
- Continued outreach through a BST state of the science website (http://texasbst.tamu.edu/) that serves as a repository for collected/produced BST information and source of BST related materials, updates, meeting announcements for educational opportunities. The website will be updated to include information regarding BST applications utilizing NGS technology.
- Continued outreach through delivery of BST informational materials describing the state of the science, applicability, usefulness, and analytical capabilities of state-supported BST laboratories to water resource professionals across the state and nation.
- Evaluation of the use of NGS techniques for BST in Texas watersheds to assist with watershed management approaches to protect water quality and human health.

***2022 Texas NPS Management Program* Reference (Expand from Summary Page)**
Components, Goals, and Objectives
Component 1 – Explicit short- and long-term goals, objectives, and strategies to restore and protect surface… water.
LTG 1 – Objective 1 – Focus … available resources in watersheds and aquifers identified as impacted by NPS pollution.
LTG 1– Objective 2 – Support the implementation of state, regional, and local programs to prevent NPS pollution through assessment…
LTG 1– Objective 3 – Support the implementation of state, regional, and local programs to reduce NPS pollution, such as the implementation of strategies defined in TMDL I-Plans, [and] WPPs…
LTG 1– Objective 6 – Develop partnerships … to facilitate collective, cooperative approaches to manage NPS pollution.
LTG 1– Objective 8 – Enhance public participation and outreach by providing forums for citizens and industry to contribute their ideas and concerns…
Short-Term Goal One – Data Collection and Assessment – Objective C – Conduct special studies to determine sources of NPS pollution and gain information to target… BMP implementation.
Short-Term Goal Three – Education – Objective B – Administer programs to educate citizens about water quality and their potential role in causing nonpoint source pollution.
Component 2 – Working partnerships and linkages to appropriate State, interstate, Tribal, regional, and local entities, private sector groups, and Federal agencies.
Component 3 – Combination of statewide nonpoint source programs and on-the-ground projects achieve water quality benefits…
Component 5 – …Progressively address these identified waters by conducting more detailed watershed assessments…

Part III – Financial Information

Budget Summary		
Category	Total	
Personnel	$	135,221
Fringe Benefits	$	55,027
Travel	$	4,192
Equipment	$	0
Supplies	$	12,540
Contractual	$	197,728
Construction		
Other	$	39,410
Total Direct Costs	$	444,118
Indirect Costs (≤ 15%)	$	66,617
Total Project Costs	$	510,735

Budget Justification (Federal)		
Category	Total Amount	Justification
Personnel	$ 135,221	• TWRI PI: $108,524 @ 0.48 mo. (2% per year) – $4,615 • SCSC Co-PI: $152,249 @ 0.5mo. (4.17% per year) – $12,878 • TBD TWRI Project Specialist: $75,040 @ 0.75 mo. (6.25% per year) – $9,521 • TBD TWRI Research Associate: $54,600 @ 4 months (16.67% per year) – $18,475 • TBD TWRI Research Assistant: $47,250 @ 1 months (4.17% per year) – $3,997 • SCSC TBD Post-Doc: $52,000 @ 19.5 months (average 81.25% per year) - $85,735 *named positions are budgeted with a 5% annual pay increase in Year 1 and 3% in subsequent years; TBD positions and graduate students are budgeted with a 3% pay increase in years after year 1 *(Salary estimates are based on average monthly percent effort for the entire contract. Actual percent effort may vary more or less than estimated between months; but in aggregate, will not exceed total effort estimates for the entire project.) *cell phone allowances for project calls/emails during & after business hours & travel are occasionally factored into salaries & fringe, but again, will not exceed overall dollar amount.
Fringe Benefits	$ 55,027	Fringe for faculty and staff is calculated at 19.7% salary plus $1,033 per month. Fringe benefits for eligible students is calculated at 10.7% salary plus $564 per month. *(Fringe benefits estimates are based on salary the estimates listed. Actual fringe benefits will vary between months coinciding with percent effort variations; but in aggregate, will not exceed the overall estimated total.)

Travel	$ 4,192	• SCSC Travel to state meetings & El Paso: $800
		• SCSC Travel to national meetings: $1,000
		• TWRI Travel to Bandera for stakeholder engagement and sample retrieval
		  o Lodging @ state rate for 2 trips per year for 2 people: $784
		  o Per diem @ state rate for 2 trips per year for 2 people: $708
		  o Mileage @ state rate: $225 * 2 trips per year: $900

Equipment	$ 0	N/A
Supplies	$ 12,540	• SCSC Lab filtration and DNA extraction: $4,900 • SCSC Routine water samples E. coli Filtration supplies.: $840 • SCSC Riboprinter supplies: $5,500 • SCSC Miscellaneous project supplies: $1,000 • TWRI General Field Supplies: $100 • TWRI Computer Peripherals (keyboard, mouse, dock, etc.): $200
Contractual*	$ 197,728	UTHealth SPH: $160,430 Bandera County River Authority: $37,298
Construction	$ 0	N/A
Other	$ 39,410	• TWRI Communication Services: $1,200 • TWRI Website Fee: $3,960 • SCSC BST DNA sequencing: $21,600 • Metagenomic DNA sequencing: $10,000 • SCSC Bioinformatics services: $1,000 • SCSC general maintenance on equipment: $500 • SCSC Hazardous waste disposal fees: $100 • SCSC Sample shipment to El Paso and sequencing lab: $650 • SCSC Conference Registration: $400

Indirect	$ 66,617	Per the RFP requirements, indirect costs are limited at 15% of total direct costs. $444,118 Total Direct Costs * 15% = $66,617

Contractual Budget Justification – Bandera County River Authority		
Category	Total Amount	Justification
Personnel	$ 23,304	• 5% time of Aquatic Ecologist and Field Operations Manager at $34/h and 5% time of administrative staff at $43/h.
Fringe Benefits	$ 5,360	Based on actual fringe benefit costs at 23% of salaries.
Travel	$ 3,895	Twelve routine samples at nine sites, four storm samples at two sites, four overnight trips to TAMU (Standard 2024 GSA Rate for lodging, meals, and incidentals), and three attendees at the State of the Science (BST) Conference.
Equipment	$ 0	N/A
Supplies	$ 1,483	Field and lab supplies (bottles, Whirlpaks, sample collection materials, etc.)
Contractual*	$ 0	N/A
Construction	$ 0	N/A
Other	$ 0	N/A
Indirect	$ 3,256	IDC rate is 10% of modified total direct costs

Contractual Budget Justification-UTHealth-Houston School of Public Health		
Category	Total Amount	Justification
Personnel	$ 93,018	• El Paso PI (Gitter): $104,000 at 2.40 months ($21,424) • El Paso Lab Manager (Monserrat): $43,443 at 19.2 months ($71,594)
Fringe Benefits	$ 31,986	• El Paso PI (Gitter): 29% of personnel ($6,212) • El Paso Lab Manager (Monserrat): 36% of personnel ($25,774)
Travel	$ 2,000	Round-trip travel to Texas A&M University in College Station, Texas for cross-training for lab analyses, meetings to discuss the project.
Equipment	$ 0	N/A
Supplies	$ 5,000	Misc. laboratory supplies
Contractual*	$ 0	N/A
Construction	$ 0	N/A
Other	$ 7,500	Refrigerator and general maintenance (Biological Safety Cabinets, freezers, Riboprinter, and refrigerators)
Indirect	$ 20,926	15% Total Direct Costs