TEXAS STATE Soil & Water CONSERVATION BOARD

Texas State Soil and Water Conservation Board
State Nonpoint Source Grant Program
FY 2022 Workplan 22-50

SUMMARY PAGE	
Title of Project	Texas Bacterial Source Tracking Program (FY23-FY24)
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) • 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 • Evaluate potential for a host-associated molecular marker for bats • Provide outreach regarding BST • Assessment and discussion of NGS for watershed management • Initiate WPP implementation efforts in Petronila and San Fernando Creek watersheds through watershed coordinator support and engagement activity
Project Tasks	(1) Project Administration; (2) Quality Assurance; (3) BST Sample Collection; (4) NGS-based BST Analyses & QMRA; (5) Next Generation Sequencing Analyses of Water Samples for Pathogens; (6) Library-Independent Marker Development; (7) BST Library Refinement; (8) Education and Outreach; (9) Double Bayou BST Assessment; (10) WPP Implementation
Measures of Success	• Collection of up to 100 source-specific fecal samples for the development of the NGS BST Library • Collection of 48 water samples for NGS BST analysis and pathogen characterization • Statistical characterization of NGS findings • Evaluation of the development of a host-associated bat molecular marker • 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 of the Lavaca River watershed • BST analysis for Double Bayou • Petronila and San Fernando Creeks watershed stakeholders engaged in WPP implementation activity
Project Type	Implementation (x); Education ( ); Planning ( ); Assessment (X); Groundwater ( )

Status of Waterbody on *2020 Texas Integrated Report*	Segment ID 1602 1602B 1602C 2422B 2422D 2203 2204 2492A	Parameter of Impairment or Concern Bacteria Bacteria Depressed Dissolved Oxygen Bacteria, Depressed Dissolved Oxygen Bacteria Bacteria Bacteria Bacteria	Category 5a 5a 5b 5c, 5b 5c 5c 5b 5c
Project Location (Statewide or Watershed and County)	Statewide, but with BST support in Lavaca, De Witt, Jackson, Gonzales, and Fayette counties		
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)		
2017 Texas NPS Management Program Reference	• Component 1 – LTG Objectives 1, 2, 3, 6 • Component 1 – STG 1C • Components 2, 3, 5		
Project Costs	Total | $646,154		
Project Management	• Texas A&M AgriLife Research, Texas Water Resources Institute		
Project Period	May 25, 2022 – May 31, 2024		

Part I – Applicant Information

Applicant							
Project Lead	Lucas Gregory, Ph.D.						
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Co-Applicant							
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Co-Applicant							
Project Lead	Anna Gitter, Ph.D.						
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City	El Paso	County	El Paso	State	TX	Zip Code	79905
Telephone Number	915-975-8530			Fax Number	915-779-2502		

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 (Tasks 1, 2, 3, and 8).
Texas A&M AgriLife Research, Department of Soil and Crop Sciences (AgriLife SCSC)	Work in conjunction with UTSPH EP to perform all work described in Tasks 2-8.
The University of Texas Health Science Center at Houston School of Public Health, El Paso Regional Campus (UTSPH EP)	Work in conjunction with AgriLife SCSC to perform all work described in Tasks 2-8.
Lavaca Navidad River Authority (LNRA)	BST sample collection support (Task 3).
Houston Advance Research Center (HARC)	Water and BST sample collection support (Task 9).
Texas Sea Grant	Lead stakeholder engagement and watershed coordination efforts to implement the Petronila and San Fernando Creek WPP
Texas A&M University Corpus Christi (TAMU CC)	Support stakeholder engagement efforts in the Petronila and San Fernando Creek watershed area

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 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	X	No	
If yes, identify the document.	Lavaca River Watershed Protection Plan Petronila and San Fernando Creek Watershed Protection Plan						

If yes, identify the agency/group that developed and/or approved the document.	Lavaca River Watershed Stakeholders; Texas Water Resources Institute Petronila and San Fernando Creek Watershed Stakeholders; Texas Water Resources Institute	Year Developed	2018 2022

Watershed Information

Watershed or Aquifer Name(s)	Hydrologic Unit Code (12 Digit)	Segment ID	Category on 2020 IR	Size (Acres)
Lavaca River Watershed	121001010101-0108; 0201-0206; 0301-0305; 0401, 0403, 0404	1602 1602B 1602C	5a 5a 5b	1,125,642
Double Bayou	120402020100	2422B, 2422D	5b, 5c	89,325
Petronila Creek	121102050501-0506; 0601-0608; 0808	2203, 2204	5c, 5b	368,912
San Fernando Creek	121102040101-0109; 0201- 0206; 0301- 0310; 0401-0409	2492A	5c	814,144

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: *2020 Texas Integrated Report,* Clean Rivers Program Basin Summary/Highlights Reports, or other documented sources.

2020 Texas Integrated Report

Impairments and Concerns

Segment 1602: Lavaca River above Tidal

- From a point 806km (5.3 mi) downstream of US 59 in Jackson County to the confluence of Campbell Branch west of Hallettsville in Lavaca County

	Impairment	Category	Year Listed
1602_02	Bacteria	5a	2008
1602_03	Bacteria	5a	2008

Segment 1602B: Rocky Creek

- Perennial stream from the confluence with the Lavaca River upstream to 2.9km upstream of County Rd 364 north west of the City of Shiner

	Impairment	Category	Year Listed
1602B_01	Bacteria	5a	2014
	Concern		
1602B_01	Total phosphorus in water	CS	

Segment 1602C: Lavaca River above Campbell Branch

- From the confluence of Campbell Branch in Hallettsville to approximately 3.4mi upstream of SH 95 in Lavaca Co.

	Impairment	Category	Year Listed
1602C_01	Depressed Dissolved Oxygen	5b	2004
1602C_02	Depressed Dissolved Oxygen	5b	2004

Segment 2422B: Double Bayou West Fork

- From the Trinity Bay confluence to Belton Road in Chambers County

	Impairment	Category	Year Listed
2422B_01	Bacteria	5c	2006
2422B_01	Depressed Dissolved Oxygen	5b	2004
	Concern		
2422B_01	Chlorophyll-a	CS	

Segment 2422D: Double Bayou East Fork

- From the Trinity Bay confluence to a point 2.6 km (1.6 mi) upstream of SH 65

	Impairment	Category	Year Listed
2422D_01	Bacteria	5c	2014

Segment 2203: Petronila Creek Tidal

- From the confluence of Chiltipin Creek in Kleberg County to a point 1 km (0.6 mi) upstream of private road crossing near Laureles Ranch in Kleberg County

	Impairment	Category	Year Listed
2203_01	Bacteria	5c	2010
	Concern		
2203_01	Chlorophyll-a	CS	

Segment 2204: Petronila Creek Above Tidal

- From a point 1 km (0.6 mi) upstream of private road crossing near Laureles Ranch in Kleberg County to the confluence of Agua Dulce and Banquete Creeks in Nueces County

	Impairment	Category	Year Listed
2204_01	Bacteria	5b	2016
	Concern		
2204_01	Chlorophyll-a	CS	
2204_02	Bacteria	5b	2016
	Concern		
2204_02	Chlorophyll-a	CS	

Segment 2492A: San Fernando Creek

- From the Cayo Del Grullo confluence in Kleberg County upstream to the confluence with Chiltipin Creek and San Diego Creek in Jim Wells County

	Impairment	Category	Year Listed
2492A_01	Bacteria	5c	2006
	Concern		
2492A_01	Chlorophyll-a	CS	
2492A_01	Nitrate	CS	

2492A_01 Total Phosphorus CS

2020 Texas Integrated Report

Sources

Lavaca River Above Tidal: Segment ID 1602, AU IDs 1602_02 and 1602_03
E. coli
Point sources: Unknown
Non-point sources: Unknown

Rocky Creek: Segment ID 1602B, AU ID 1602B_01
E. coli
Point sources: Unknown
Non-point sources: Unknown
Total Phosphorus
Point sources: Unknown
Non-point sources: Unknown

Lavaca River above Campbell Branch: Segment ID 1602C, AU IDs 1602C_01 and 1602C_02
Dissolved Oxygen
Point sources: Drought Related Impacts
Non-point sources: Unknown

Double Bayou West Fork: Segment ID 2422B, AU ID 2422B_01
Chlorophyll-a
Point sources: Drought Related Impacts
Non-point sources: Rural (Residential Areas)
Enterococcus
Point sources: Drought Related Impacts
Non-point sources: On-Site Treatment Systems; Rural Residential Areas
Dissolved Oxygen
Point sources: Drought Related Impacts
Non-point sources: : On-Site Treatment Systems; Rural (Residential Areas)

Double Bayou East Fork: Segment ID 2422D, AU ID 2422D_01
Enterococcus
Point sources: Drought Related Impacts
Non-point sources: Rural Residential Areas

Petronila Creek Tidal: Segment ID 2203, AU ID 2203_01
Enterococcus
Point sources: Unknown
Non-point sources: Unknown
Chlorophyll-a
Point sources: Unknown
Non-point sources: Unknown

Petronila Creek Above Tidal: Segment ID 2204, AU IDs 2204_01 and 2204_02
E. coli
Point sources: Unknown

Non-point sources: Unknown
Sulfate, Chloride, Total Dissolved Solids
Point sources: Unknown
Non-point sources: Petroleum/Natural Gas Production Activities (Permitted)
Chlorophyll-a
Point sources: Unknown
Non-point sources: Unknown

San Fernando Creek: Segment ID 2492A, AU ID 2492A_01
Chlorophyll-a
Point sources: Municipal Point Source Discharges
Non-point sources: Unknown
Nitrate
Point sources: Municipal Point Source Discharges
Non-point sources: Unknown
Total Phosphorus
Point sources: Municipal Point Source Discharges
Non-point sources: Unknown
E. coli
Point sources: Municipal Point Source Discharges
Non-point sources: Grazing in Riparian or Shoreline Zones; Rangeland grazing; Unrestricted Cattle Access, Wildlife Other Than Waterfowl

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 UTSPH EP 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. 03-20) currently contains 1,912 *E. coli* isolates obtained from 1,653 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 further research has indicated, 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 deeper 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).

Despite its expansiveness, continued development and refinement of the Texas *E. coli* BST Library are needed to further increase its utility. One area of need is the specific detection of feral hogs. The current Texas *E. coli* BST Library includes feral hogs within the "wildlife" category. This is not optimal since feral hogs are not considered to be wildlife by many stakeholders and typically are managed differently than true wildlife species. The continued importance of feral hog sources and inclusion of new feral hog isolates in recent expansions of the Texas *E. coli* BST Library warrants a renewed evaluation of whether a separate feral hog source category can be created for use in watershed projects.

Looking to the future, library independent BST holds much promise. It is already being used to support BST analyses in Texas. However, to improve its ability to address the needs in Texas, further work is needed to develop and evaluate new markers. Bats have been identified by stakeholders as a potential source of contamination in specific watersheds. To aid detection of bat fecal contamination, previous BST projects have targeted addition of *E. coli* from bats during expansions of the Texas *E. coli* BST Library. While this helps with source delineation, detected contamination can only be as described as being from "wildlife" sources using the current library-dependent BST approach. It would be helpful to have a BST marker specific to bats, similar to those specific for humans, poultry, and other sources.

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 provides the opportunity to analyze for a wide array of pathogens that has not been previously possible with traditional microbial techniques. Utilizing NGS to characterize for 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 the 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 human health.

Advances in NGS methods provides 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 will continue to inform watershed stakeholders and provide 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 recent personnel changes at UTSPH EP and TWRI, there is also a near-term need for increased interaction among laboratories to facilitate the transition. To meet the needs of the state, BST analytical capabilities will be maintained at both UTSPH EP and AgriLife SCSC BST laboratories. Financial support will be used to maintain lab personnel at UTSPH EP 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 of pollutant sources, directly evaluating a water body for an array of microbial pathogens provides the potential for rapidly identifying specific exposure risks.

There are two parallel aims to this project which include: 1) conduct BST analyses using NGS techniques for water samples gathered from four sampling sites in the Lavaca River watershed and 2) conduct NGS analyses for water samples gathered at the four 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. 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 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 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 the first 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.

To initiate development of a bat-specific BST marker, we also propose to use NGS sequencing of the microbiome in 50 bat fecal samples to identify unique organisms and sequences in bat samples. Bioinformatics will be used, in connection with Texas A&M Institute for Genome Sciences and Society core facility, to analyze and compare the sequence data against those in publicly available genomic databases. From identified unique sequences, prospective PCR markers will be developed. Prospective markers will be evaluated against the bat samples collected in this project along with the non-target Lavaca River samples (that will also be collected as part of this project). Additional archived fecal samples from previous BST projects may also be evaluated as needed to supplement and expand potential sources.

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. Stakeholders have expressed strong interest in the possibility of a feral hog source class.

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.

Furthermore, NGS data generated from this project 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 UTSPH EP 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.

Traditional ERIC-RP BST analysis will also be conducted on water samples collected in the Double Bayou watershed. Samples will be collected from two sites monthly for a one year period with two additional storm samples collected per site for a total of 20 samples during this period. Analysis results will supply desired information to watershed stakeholders to be used in prioritizing future WPP implementation activity.

An additional task will also support initiation of WPP implementation activity in the Petronila and San Fernando Creek watersheds. Complementary projects have completed the process to develop a WPP and a separate project is about to begin that will perform BST in these and the larger Baffin Bay watershed. This project will support watershed stakeholder engagement efforts by providing resources for a watershed coordinator to meet with stakeholders, provide education and outreach opportunities, and facilitate continued discussions regarding WPP implementation.

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	$19,385			
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 January, April, July and October. QPRs shall be distributed to all Project Partners.			
	Start Date	Month 1	Completion Date	Month 25
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 25
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 25
Subtask 1.4	TWRI will develop a Final Report 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 25
Deliverables	• QPRs in electronic format • Reimbursement Forms and necessary documentation in hard copy format • Final Report in electronic and hard copy formats			

Tasks, Objectives and Schedules				
Task 2	Quality Assurance			
Costs	$6,462			
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 Tasks 3-6 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 25
Subtask 2.2	TWRI, AgriLife SCSC, UTSPH EP, and LNRA will implement the approved QAPP. TWRI, AgriLife SCSC, UTSPH EP, and LNRA will submit revisions and necessary amendments to the QAPP as needed.			
	Start Date	Month 1	Completion Date	Month 25
Deliverables	• QAPP approved by TSSWCB and EPA in both electronic and hard copy formats • Approved revisions and amendments to QAPP, as needed • Data of known and acceptable quality as reported through Tasks 3-6			

Tasks, Objectives and Schedules				
Task 3	NGS-based BST Analyses & QMRA			
Costs	$164,769			
Objective	To use NGS-based BST to characterize fecal source contributions in the Lavaca River watershed and expand the Texas BST toolbox through the analysis of approximately 100 known source fecal samples and 48 water samples. BST results to be utilized in a QMRA to evaluate human health risks.			
Subtask 3.1	UTSPH EP 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 25
Subtask 3.2	UTSPH EP 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 25
Subtask 3.3	UTSPH EP and AgriLife SCSC will perform targeted BST analysis to support WPP implementation efforts in the Lavaca River watershed. BST analyses will be performed on monthly samples from 4 sites (i.e., 12 months x 4 sites = 48 total samples) in the Lavaca River watershed.			
	Start Date	Month 4	Completion Date	Month 25
Subtask 3.4	AgriLife SCSC and UTSPH EP, 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 25
Subtask 3.5	AgriLife SCSC will deposit NGS data in a publicly available database (GenBank).			
	Start Date	Month 4	Completion Date	Month 25

Subtask 3.6	UTSPH EP and AgriLife SCSC will evaluate differences in BST data and findings between the Lavaca River watershed and other comparable watersheds in the state with traditional library-dependent BST results.			
	Start Date	Month 4	Completion Date	Month 25
Subtask 3.7	UTSPH EP and AgriLife SCSC will integrate the BST results from the project into a quantitative microbial risk assessment to evaluate the human health significance of the project's data.			
	Start Date	Month 4	Completion Date	Month 25
Deliverables	• BST analyses for the Lavaca River watershed • QMRA analysis integrating BST results • Discussion of findings included in final report • Highlights of work performed included in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 4	BST Sample Collection			
Costs	$64,615			
Objective	To use NGS-based approaches for BST to characterize fecal source contributions and pathogens in the Lavaca River watershed through the collection of approximately 100 known source fecal samples and 48 water samples.			
Subtask 4.1	TWRI will work with UTSPH EP, AgriLife SCSC and LNRA 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 4.2	LNRA 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. LNRA 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. LNRA will coordinate closely with TWRI, UTSPH EP 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 25
Subtask 4.3	LNRA will collect monthly grab samples from 4 selected monitoring sites in the Lavaca River watershed. LNRA/TWRI will coordinate 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 5	Next Generation Sequencing Analyses of Water Samples for Pathogens			
Costs	$103,384			
Objective	Evaluate water samples for the presence of pathogens using NGS-based 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 48 extracted water samples (Task 3.3) 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 25
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 25
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 25
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 25
Subtask 5.5	AgriLife SCSC will deposit NGS data in a publicly available database (GenBank).			
	Start Date	Month 20	Completion Date	Month 25
Deliverables	• Highlights of work performed will be included in QPRs and the Final Report			

Tasks, Objectives and Schedules				
Task 6	Library-Independent Marker Development			
Costs	$103,385			
Objective	Develop and refine library-independent markers.			
Subtask 6.1	TWRI will collect approximately 50 bat fecal samples and deliver them to AgriLife SCSC.			
	Start Date	Month 4	Completion Date	Month 12
Subtask 6.2	AgriLife SCSC will extract DNA from bat fecal samples and sequence the bat fecal microbiome using 16S rRNA gene-based NGS at the Texas A&M Institute for Genome Sciences and Society core facility. Sequence data will be deposited in a publicly available database (GeneBank).			
	Start Date	Month 4	Completion Date	Month 18
Subtask 6.3	AgriLife SCSC, in connection with the Texas A&M Institute for Genome Sciences and Society core facility, will analyze sequence data and compared against those in publicly available genomic databases to identified unique, bat-specific sequences.			
	Start Date	Month 4	Completion Date	Month 22
Subtask 6.4	From these sequences, prospective PCR markers will be developed and evaluated for specificity and sensitivity using the bat samples collected in Subtask 5.1 along with the known-source samples collected from the Lavaca River watershed in Subtask 4.2. Additional archived fecal samples from previous BST projects may also be evaluated as needed to supplement specific source categories.			
	Start Date	Month 4	Completion Date	Month 22

Subtask 6.5	As funding allows, AgriLife SCSC and UTSPH EP will use the best available bacterial indicators to evaluate and further develop/refine source-specific bacterial PCR markers using known source fecal material. AgriLife SCSC and UTSPH EP efforts will focus on evaluating additional library-independent PCR markers (e.g., HF183 human marker) for the Texas BST toolbox.			
	Start Date	Month 4	Completion Date	Month 25
Deliverables	• Highlights of work performed included in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 7	BST Library Refinement			
Costs	$64,615			
Objective	Evaluate and refine the statewide *E. coli* BST Library.			
Subtask 7.1	UTSPH EP and AgriLife SCSC will collaborate to evaluate the geographical and temporal stability, composition, average rates of correct classification (accuracy), diversity of source specific isolates, and further development and refinement needs of the Texas *E. coli* BST library.			
	Start Date	Month 4	Completion Date	Month 25
Subtask 7.2	AgriLife SCSC and UTSPH EP will collaborate to determine the possibility of creating a separate "feral hog" source category in the Texas *E. coli* BST Library using existing data.			
	Start Date	Month 4	Completion Date	Month 25
Deliverables	• Highlights of work performed included in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 8	Education and Outreach			
Costs	$32,308			
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 8.1	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 25
Subtask 8.2	TWRI, UTSPH EP and AgriLife SCSC will promote the use of and provide resources on BST. TWRI, UTSPH EP 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. Finally, TWRI, UTSPH EP 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 25
Deliverables	• Summaries of outreach efforts included in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 9	Double Bayou BST Assessment			
Costs	$29,077			
Objective	To conduct BST analysis on water samples collected in the Double Bayou watershed to inform future implementation prioritization and support stakeholder education			
Subtask 9.1	HARC will coordinate and facilitate collection of 20 routine and stormwater samples in the Double Bayou watershed and arrange for sample delivery to AgriLife SCSC in College Station for BST analysis.			
	Start Date	Month 1	Completion Date	Month 25
Subtask 9.2	AgriLife SCSC will process samples received using traditional ERIC-RP BST methods and report results back to HARC for stakeholder engagement purposes.			
	Start Date	Month 1	Completion Date	Month 25
Deliverables	• Notation of data collection events in QPRs • Summary of BST findings in Final Report			

Tasks, Objectives and Schedules				
Task 10	Petronila and San Fernando Creek WPP Implementation			
Costs	$58,154			
Objective	To initiate WPP implementation activity in the Petronila and San Fernando Creeks watershed through continued stakeholder engagement, education and outreach activity and facilitated discussions regarding implementation activities			
Subtask 10.1	The watershed coordinator will continue stakeholder engagement efforts by participating in existing meetings with individuals, groups, and public forums and discuss WPP implementation plans, efforts, progress, and related needs.			
	Start Date	Month 1	Completion Date	Month 25
Subtask 10.2	The watershed coordinator will work to schedule and deliver WPP related education and outreach content as appropriate across the watershed for audiences as appropriate to support WPP implementation plans and activities.			
	Start Date	Month 1	Completion Date	Month 25
Deliverables	• Brief summary of meetings attended/hosted in QPRs and Final Report • Summary of engagement programs hosted in QPRs and Final Report			

Project Goals (Expand from Summary Page)

- Further evaluate, update and refine the Texas BST Library through continued personnel support; 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
- Initiate genetic sequencing to develop a source-specific bat molecular marker for library independent BST
- Assess potential for NGS techniques to expand BST assessment capacity in future watershed management
- Initiate WPP watershed coordination efforts to kickstart implementation activity through expanded and focused stakeholder engagement on priority issues in the Petronila and San Fernando Creek watersheds

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 needed level of training of AgriLife SCSC and UTSPH EP 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 Lavaca River watershed.
- Analysis of the presence and distribution of pathogens in water samples collected from sites in the Lavaca River watershed.
- Development of the NGS BST Library through the analysis of approximately 100 fecal samples collected by LNRA.
- BST analysis of 48 water samples.
- Evaluation of bioinformatics data from NGS methods to characterize fecal sources and pathogens in the Lavaca River watershed.
- Development/evaluation of a source-specific bat marker for library-independent BST and evaluation of dPCR for quantitative detection of markers.
- 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. 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.
- Completed BST assessment of water samples collected from the Double Bayou watershed
- Establishment of the watershed coordinator and engagement activities initiated in the Petronila and San Fernando Creek watersheds

2017 Texas NPS Management Program **Reference (Expand from Summary Page)**
Components, Goals, and Objectives
Component 1 – Explicit short- and long-term goals, objectives, and strategies that 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.
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.
Component 2 – Working partnerships and linkages to appropriate State, interstate, Tribal, regional, and local entities, private sector groups, and Federal agencies.
Component 3 – Balanced approach that emphasizes both statewide NPS programs and on-the-ground management of individual watersheds.
Component 5 – …Progressively address these identified waters by conducting more detailed watershed assessments…

Estimated Load Reductions Expected (Only applicable to Implementation Project Type)
N/A

Part III – Financial Information

Budget Summary	
Category	Total
Personnel	$ 179,425
Fringe Benefits	$ 56,667
Travel	$ 7,431
Equipment	$ 0
Supplies	$ 45,010
Contractual	$ 231,828
Construction	$ 0
Other	$ 41,512
Total Direct Costs	$ 561,873
Indirect Costs (≤ 15%)	$ 84,281
Total Project Costs	$ 646,154

Budget Justification		
Category	Total Amount	Justification
Personnel	$ 179,425	• SCSC Co-PI: $144,732 @ 1.95 months– $24,577 • TWRI Program Manager (TBD): $64,970 @ 1.92 months – $10,552 • TWRI QA Officer (TBD): $75,000 @ 0.96 months – $6,090 • SCSC Research Associate TBD: $40,296 @ 12 months – $41,200 • SCSC TBD Post-Doc: $53,041 @ 11.32 months - $51,461 • SCSC Hourly Laborer TBD: $35/hr @ 160 hrs - $5,600 • SCSC Student Labor (TBD 3 students): $17/hr @520 $26,520 • Sea Grant Principal Investigator: $106,154 @ 0.24 months - $2,219 • Sea Grant Program Coordinator: $53,592 @ 2.4 months - $11,206 *named positions are budgeted with a 3% annual pay increase in all 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	$ 56,667	Fringe for faculty and staff is calculated at 18.8% salary plus $825 per month. Fringe benefits for eligible students is calculated at 11% salary plus $560 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.) *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.

Travel	$ 7,431	SCSC Travel to state meetings & El Paso airfare, car rental, lodging and per diem and mileage @ State Rate: $1,000 SCSC Travel to retrieve samples from The Woodlands car rental, lodging and per diem and mileage @ State Rate: $810 SCSC Travel to national meetings airfare, car rental, lodging and per diem and mileage @ State Rate: $2,000 TWRI Travel to federal and state meetings mileage, lodging and per diem @ State Rate: $921 TWRI Travel for bat fecal collection mileage @ State Rate: $500 Sea Grant Travel: 4,000 miles @ the state rate = $2,200
Equipment	$ 0	N/A
Supplies	$ 45,010	• SCSC Lab filtration and DNA extraction (up to 100 known-source samples; 48 water samples; 50 bat samples): $4,200 • SCSC Miscellaneous project supplies: $1,000 • DNA sequencing supplies (up to 48 samples): $24,000 • MinIon Enhanced Package: $4,500 • Routine water samples *E. coli* Filtration supplies: $560 • Stormwater samples *E. coli* Filtration supplies: $200 • Double Bayou ERIC RP supplies: $9,750 • Sea Grant supplies for meetings and demonstrations: $800
Contractual*	$ 231,828	UTSPH EP: $174,086 Lavaca-Navidad River Authority: $9,398 HARC - $13,000 Texas A&M University Corpus Christi - $35,344
Construction	$ 0	N/A
Other	$ 41,512	TWRI Communication Services: $900 SCSC DNA sequencing (up to 198 samples (water, known source, bat fecal)): $19,800 SCSC Conference Registration: $800 SCSC Bioinformatics services (up to 100 hours): 6,000 SCSC general maintenance on equipment: $1,000 SCSC Riboprinter maintenance: $8,800 SCSC Publication Fees: $2,912 Hazardous waste disposal fees: $100 TAMU Genomics Core facility use fees: $1,200
Indirect	$ 84,281	Per the RFP requirements, indirect costs are limited at 15% of total direct costs. $561,873 Total Direct Costs * 15% = $84,281

Contractual Budget Justification – LNRA		
Category	Total Amount	Justification
Personnel	$ 4,198	TBD Field Specialist: $40.37 per hour @ 2 hours per week @ 52 weeks
Fringe Benefits	$ 0	N/A
Travel	$ 4,100	Travel to College Station: 300 miles @ state rate * 12 trips = $1,980 Travel throughout watershed for fecal sample collection: $1,118 Travel to Seguin for non-CRP months (8 events) of sampling: $1,002
Equipment	$ 0	N/A
Supplies	$ 1,100	Field and lab supplies (coolers, waders, bottles, Whirlpaks, sample collection materials, etc.) = $1,100
Contractual*	$ 0	N/A
Construction	$ 0	N/A
Other	$ 0	N/A
Indirect	$ 0	N/A

Contractual Budget Justification – UTSPH-El Paso		
Category	Total Amount	Justification
Personnel	$ 103,033	El Paso (former) PI (Mena): $150,380 at 0.51 months ($6,406) El Paso Lab Manager (Monserrat): $40,170 at 18.0 months ($60,255) El Paso (former) Postdoc (Gitter): $56,500 at 1.69 months ($7,978) El Paso PI (Gitter): $104,000 at 2.51 months ($21,727) El Paso Graduate Student (TBD): $40,000 at 2 months ($6,667)
Fringe Benefits	$ 34,457	El Paso (former) PI (Mena): 22% of personnel ($1,409) El Paso Lab Manager (Monserrat): 36% of personnel ($21,692) El Paso (former) Postdoc (Gitter): 36% of personnel ($2,872) El Paso PI (Gitter): 28% of personnel ($6,084) El Paso Graduate Student (TBD): 36% of personnel ($2,400)
Travel	$ 2,453	Round-trip travel to Texas A&M University in College Station, Texas for cross-training for lab analyses (2 trips)
Equipment	$ 0	N/A
Supplies	$ 10,500	Laboratory supplies to ensure that lab is operational and able to conduct BST analyses • Media: $3,500 • Gloves: $750 • Petri dishes: $300 • Misc. lab materials (biohazard bags, labeling tape, etc.): $3,360 • Agar, TBE buffer, molecular biology grade water, etc.: $1,040 • Pipette tips: $1,550
Contractual*	$ 0	N/A
Construction	$ 0	N/A
Other	$ 936	Crystal Ball Software and license support for two years = $501 GraphPad Prism Software (data visualization) = $380 Minitab software (data analysis) = $55
Indirect	$ 22,707	15% Total Direct Costs

Contractual Budget Justification – Houston Advanced Research Center (HARC)		
Category	Total Amount	Justification
Personnel	$ 6,952	Research Scientist, $95,600 @ average of 2% FTE Senior Research Assistant, $71,500 @ average of 2% FTE Program Coordinator $73,043@ 0.18% FTE
Fringe Benefits	$ 3,337	Based on actual fringe benefit costs at 48% of salaries.
Travel	$ 0	N/A
Equipment	$ 0	N/A
Supplies	$ 0	N/A
Contractual	$ 0	N/A
Construction	$ 0	N/A
Other	$ 1,015	IT & Facilities Fee - includes actual costs of providing computer support (network, licenses, etc.) and offices (depreciation, housekeeping, etc.) in support of this project. These costs are not part of HARC's indirect costs and are estimated based on recent historical data.
Indirect	$ 1,696	HARC's approved IDC rate is 53% of modified total direct costs, but for this project HARC is voluntarily limiting the indirect cost reimbursement to 15% MTDC.

Subaward Budget Justification – Texas A&M Corpus Christi (TAMUCC)		
Category	Total Amount	Justification
Personnel	$ 19,279	TAMUCC Professor ($160,000 @ 0.472 months): $6,287 Graduate Student ($38,400 @ 4 months): $12,992 *named positions are budgeted with a 3% annual pay increase in all years (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 the aggregate, will not exceed total effort estimates for the entire project.)
Fringe Benefits	$ 3,611	Fringe for faculty and staff is calculated at 18.8% salary plus $825 per month Graduate Student Fringe = Salary *11% + $560/month *Fringe benefits estimates are based on salary 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	$ 0	N/A
Other	$ 7,844	Graduate student tuition
Indirect Costs	$ 4,610	15% of Modified Total Direct Costs