Texas State Soil and Water Conservation Board
State General Revenue Nonpoint Source Grant Program
FY2013 Project Workplan 13-50

PROJECT SUMMARY PAGE			
Title of Project	Statewide Bacterial Source Tracking Program for FYs 2013-2014		
Project Goals	Support BST analyses across the State through (1) maintenance of analytical infrastructure at public BST laboratories; (2) continued development and implementation of statewide BST template-SOPs; (3) delivery of informational materials on bacteria BMPs and the use and applicability of BST and the State-supported analytical labs; (4) further expansion and evaluation of the Texas *E. coli* BST Library; and (5) further development of suitable source-specific bacterial markers for library independent BST.		
Project Tasks	(1) Project Administration; (2) Quality Assurance; (3) Known Source Fecal Sample Collection; (4) Analytical Laboratory Capacity, Library Expansion, and Methods Development; (5) Outreach on Bacterial Source Tracking and BMPs; (6) Technical Assistance for Leon River WPP Update		
Measures of Success	• Updated BST template-SOPs for ERIC-RP, RP and *Bacteroidales* PCR • Data analyzed for approximately 100 known source *E. coli* isolates from TSSWCB project 11-50 for expansion of the Texas *E. coli* BST Library • Fingerprinting and analysis of 20 known-source *E. coli* isolates collected as part of TSSWCB project 11-51 • Expansion and evaluation of the Texas *E. coli* BST Library through analysis of approximately 100 targeted known source fecal samples • Evaluation of geographical and temporal stability of the Texas *E. coli* BST Library and diversity of source specific isolates • Development/evaluation of source-specific bacterial markers for library-independent BST • Outreach through website and delivery of BST and BMP informational materials to water resource professionals across the state		
Project Type	Implementation ( ); Education ( ); Planning ( ); Assessment (X); Groundwater ( )		
Status of Waterbody on *2010 Texas Integrated Report*	Segment ID Statewide	Parameter of Impairment or Concern bacteria	Category 4 and 5
Project Location (Statewide or Watershed and County)	Statewide		
Key Project Activities	Hire Staff (X); Surface Water Quality Monitoring ( ); Technical Assistance ( ); Education ( ); Implementation ( ); BMP Effectiveness Monitoring ( ); Demonstration ( ); Planning ( ); Modeling ( ); Bacterial Source Tracking (X); Other ( )		
2012 Texas NPS Management Program Reference	• Component 1 – LTG Objectives 1, 2, 3, 6 • Component 1 – STG 1C • Components 2, 3, 5		
Project Costs	$454,098		
Project Management	• Texas Water Resources Institute • The University of Texas Health Science Center at Houston School of Public Health, El Paso Regional Campus • Texas A&M AgriLife Research, Department of Soil and Crop Sciences • Texas A&M Institute of Renewable Natural Resources		
Project Period	October 1, 2012 – May 31, 2015		

Part I – Applicant Information

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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.
Texas Water Resources Institute (TWRI)	Project Coordination and Administration, Project Reporting, and Outreach (Tasks 1 and 5).
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, 3 and 4.
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, 3 and 4.
Texas A&M Institute of Renewable Natural Resources (IRNR)	Work in conjunction with UTSPH EP and AgriLife SCSC to perform work described in Task 3.

Part II – Project Information

Watershed Information				
Watershed or Aquifer Name(s)	Hydrologic Unit Code (12 Digit)	Segment ID	Category on 2010 IR	Size (Acres)
Statewide	N/A	N/A	4 and 5	N/A

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: *2010 Texas Integrated Report*, Clean Rivers Program Basin Summary/Highlights Reports, or other documented sources.
The 2010 303(d) List identified >300 contact recreation use impairments (waterbody-pollutant combinations) and 15 oyster water use impairments due to excessive bacteria (*E. coli*, *Enterococcus spp.*, or fecal coliform). These bacteria impairments account for more than half of all impairments on the 2010 303(d) List. This is more than 3 times as many impairments as the next largest number of a specific impairment type/pollutant. These indicator bacteria originate from human (WWTF, OSSF) and animal (wildlife, pets, livestock, feral hogs) sources and reach waterbodies through point source discharges, direct deposition, and NPS runoff.

Project Narrative

Problem/Need Statement

Protection of water resources is one of the most significant environmental challenges of the new millennium. Nonpoint sources (NPS) of pollution, including agricultural activities, can greatly impact water quality. One key component in effectively implementing a NPS pollution abatement program is the identification and assessment of sources of fecal pollution. Proper evaluation of these sources is needed to target best management practices (BMPs) and develop bacterial total maximum daily loads (TMDLs) or watershed protection plans (WPPs). This information may also be useful to properly assess risk in contact recreation, as many waterborne pathogens causing human illness do not colonize nonhuman hosts. According to the *2010 Texas Integrated Report*, there are over 300 impairments due to excessive bacteria.

Fecal coliform bacteria have extensively been used as an indicator of fecal pollution and the potential presence of other pathogenic microorganisms in water. It has been established that the fecal coliform bacterium *E. coli* is more closely associated with fecal pollution than other fecal coliform bacteria, which may normally reside and multiply in the environment. *E. coli* is a common inhabitant of animal and human intestines and recent studies have shown that isolates from humans and various host animals (e.g., cattle, chickens, and pigs) may differ genetically and phenotypically. Use of genetic and biochemical tests may allow the original host species to be identified and is referred to as bacterial source tracking (BST).

The premise behind BST is that genetic and phenotypic tests can identify bacterial strains that are host specific so that the original host species and source of the fecal contamination can be identified. Often *E. coli* or *Enterococcus* spp. are used as the bacteria targets in BST, as this provides a direct link with water quality standards which are usually based on one of these two indicators (Parveen, Portier et al. 1999; Dombek, Johnson et al. 2000; Graves, Hagedorn et al. 2002; Field, Chern et al. 2003; Hartel, Summer et al. 2003; Kuntz, Hartel et al. 2003; Stoeckel, Mathes et al. 2004; Harwood, Levine et al. 2005). While there has been some controversy concerning host specificity and survival of *E. coli* in the environment (Gordon, Bauer et al. 2002), this indicator organism has the advantage that it is known to correlate with the presence of fecal contamination and is used for human health risk assessments. BST of *E. coli*, therefore, has the advantages of direct regulatory significance and availability of standardized culturing techniques for water samples, such as EPA Method 1603 (EPA 2005).

BST is a valuable tool for identifying human and animal sources of fecal pollution. Comprehensive BST has been completed by UTSPH EP (formerly with Texas A&M AgriLife Research) for (1) the Lake Waco and Belton Lake watersheds, (2) several San Antonio area watersheds, (3) the Lake Granbury watershed, (4) Buck Creek, and (5) the Leon and Lampasas Rivers watersheds. The Waco/Belton and Buck Creek studies were funded by the TSSWCB through Clean Water Act §319(h) NPS grants from the U.S. Environmental Protection Agency (EPA) (TSSWCB projects 02-10 and 06-11, respectively) and the Leon and Lampasas project through state general revenue funds (TSSWCB project 10-51); while the San Antonio study and Lake Granbury studies were funded by the Texas Commission on Environmental Quality (TCEQ). In addition, AgriLife SCSC has completed BST projects for the Little Brazos River tributaries and Big Cypress Creek watersheds (TSSWCB projects 09-52 and 09-55, respectively). Additionally, with TSSWCB funding, BST projects are currently under way in the Leona River and Attoyac Bayou watersheds to assess water quality impairments (projects 11-50 and 09-10, respectively). A Texas *E. coli* BST Library has been developed based on known source isolates from the Waco/Belton, San Antonio, Granbury, Buck Creek, Big Cypress, Little Brazos River, Attoyac Bayou, Leon River, Lampasas River, Upper Trinity River and Upper Oyster Creek watersheds. The Texas *E. coli* BST Library (ver. 8-12) currently contains 1,669 *E. coli* isolates obtained from 1,455 different domestic sewage, wildlife, livestock and pet fecal samples. While this represents a significant step towards development of a statewide *E. coli* BST library, continued expansion of the library to include additional known source isolates from different Texas watersheds and different animal hosts is still needed. This will allow continued evaluation of the library for geographical stability and the diversity of source specific isolates to identify specific needs for future expansion and refinement of the library. The use of the Texas *E. coli* BST Library will provide for significant cost and time savings for the identification of NPS pollution in the development of TMDLs and WPPs.

A Task Force on Bacteria TMDLs was jointly established by the TSSWCB and the TCEQ in fall 2006. In the Task

Force's Report, a strategy to address current and future bacterial TMDLs and Implementation Plans (I-Plans) was outlined. The Task Force describes and makes recommendations for effective use of BST methods that have been used in Texas. These include enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR), RiboPrinting (RP), Kirby-Bauer antibiotic resistance analysis (KB-ARA), carbon source utilization (CSU), and *Bacteroidales* PCR. The Task Force recommended using library-independent methods such as *Bacteroidales* PCR for preliminary qualitative analyses and library-dependent methods (e.g., ERIC-PCR and RP) if more quantitative data are required. Further characterization of known source *E. coli* for expansion of the Texas *E. coli* BST Library and continued support of established BST analytical infrastructure will help achieve the recommendations of the Task Force.

The Task Force Report identified certain Research and Development (R&D) needs to advance understanding of bacteria. Specifically, 30 types of studies or research needs in 6 categories (including Characterization of Sources and Bacterial Source Tracking) were identified. This list was not exhaustive and no attempt was made to prioritize these activities. As such, there is a need to update, expand and prioritize these BST-related R&D activities.

Lastly, the state of BST science, methodologies, application and confidence has evolved greatly in the past few years. A host of new information is currently available, yet not readily distributed or known to state and federal agency personnel. To address this, the *2012 BST – State of the Science Conference* was held. To build on the success of this conference, continued outreach and technology transfer is needed to foster dialogue and collaboration and bring water resource managers up to speed on advances in BST technologies, methodologies, applications and results.

Project Narrative

General Project Description (Include Project Location Map)

The Texas *E. coli* BST Library is dynamic, with new isolates being added with each successive BST project. The current library (ver. 8-12) contains known source isolates from over a dozen watersheds, as well as wildlife isolates from South Texas. Under this project, ERIC-RP data for approximately 100 known source *E. coli* isolates from the Leona River watershed (TSSWCB Project 11-50) will be provided by AgriLife SCSC to UTSPH EP for analysis and expansion of the state library. In addition, approximately 100 known source fecal samples from targeted animal sources will be collected and analyzed for *E. coli* to further expand the state library and provide additional datasets for library evaluation. In particular, the state library has very few *E. coli* from wildlife species such as mice, rabbits, nutria and squirrels. By collecting some of these known source samples from a previously studied watershed (e.g., Leon River watershed), the temporal stability of the library will also be assessed. The geographic stability of the library will be evaluated by performing watershed exclusive and inclusive statistical analyses. In addition, the fingerprint diversity of source-specific *E. coli* isolates will be investigated to help evaluate the strain representativeness of the library. This will allow the project team to identify specific needs for the future expansion and refinement of the library.

There have been significant developments in library-independent BST methods, including bacterial genetic markers specific to different animal sources and humans (i.e. Bernhard and Field 2000; Dick, Bernhard et al. 2005; Scott, Jenkins et al. 2005; Hamilton, Yan et al. 2006). Library-independent methods are cost-effective, rapid, and potentially more specific than library-dependent methods. Concerns with many of the recently developed library-independent approaches include uncertainties regarding geographical stability of markers and the difficulty of interpreting results in relation to regulatory water quality standards and microbial risk, since some target microorganisms are not regulated. More importantly, these library-independent methods can only detect a limited range of pollution sources and are currently only semi-quantitative. For example, the *Bacteroidales* PCR (Bernhard and Field 2000; Dick, Bernhard et al. 2005) can detect fecal pollution from ruminants, humans, dogs, horses and pigs; but currently no further discrimination is possible. Despite these limitations, this method may be very useful for the rapid and inexpensive assessment of the possible sources of fecal pollution impacting a waterbody. UTSPH EP (under TSSWCB project 10-50) has generated promising preliminary results for a *Bacteroidales* PCR method to detect feral hog fecal pollution, as well as identified possible genetic targets for discriminating human and animal *E. coli*. A simple library-independent method for distinguishing human from animal *E. coli* would be quite useful for BST studies. Current research in this area at UTSPH EP is based on sequence analysis of ERIC-PCR products from isolates identified through data mining of the Texas *E. coli* BST Library. Library-independent source-specific methods have recently been described for poultry (Weidhaas et al. 2010) and cattle (Shanks et al. 2010). Importantly, UTSPH EP has observed some cross-reactivity of animal fecal DNA with *Bacteroidales* PCR markers, especially for the human HF183 marker. This occurred for some known source wildlife samples in the Buck Creek project (TSSWCB project 06-11) which were collected from a remote site which had very limited human access. This may explain the unexpected and frequent occurrence of water samples positive for the human marker at this site. To help explore the issue of cross-reactivity, all 100 known source fecal samples collected under this project will be analyzed for the human HF183 marker. Further development and evaluation of these library-independent methods will be conducted for possible inclusion into Texas' BST toolbox.

Due to the current and anticipated need for BST studies in Texas, statewide BST analytical infrastructure needs to be maintained appropriately. This not only includes the needed maintenance and repairs of analytical equipment; but also the continued support, training, and retention of skilled personnel. 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 hire and train graduate students or a postdoctoral student at UTSPH EP and retain (or hire) graduate students or a postdoctoral associate at AgriLife SCSC. Training needs for each individual laboratory's personnel will be coordinated to ensure appropriate technology transfer and comparability of BST data.

Delivering educational and informational programming regarding BST is also a critical need. Although the Task Force recommended the usage of BST, the TSSWCB and TCEQ adopted the general process laid out by the Task Force on the use of BST, and BST has been successfully employed in many watersheds across the state, BST is still not being used to its full potential in Texas. To provide greater outreach to water resource managers in Texas, the project team

will participate in conferences including the 2013 and 2014 TCEQ Environmental Trade Fair and Conference and other events in Texas. Flyers, one-pagers, tri-folds or other appropriate printed media developed through previous projects will be used to 1) describe the general use of BST consistent with the Task Force Report, 2) discuss the appropriate application of BST in identifying fecal contamination sources, and 3) review the analytical lab capability of public BST labs which the state has invested.

TWRI will continue to host and maintain the BST website (http://texasbst.tamu.edu/) to disseminate educational materials, project updates, science updates, and other outreach efforts to advance the science and application of BST in Texas and nationally.

This project will advance the recommendations of the Task Force by updating, expanding, and prioritizing BST-related R&D needs. Additionally, this project will work towards accomplishing R&D needs identified in the Report:

- Investigation and refinement of library-independent BST methods, and determine which library-independent BST methods are best suited for Texas. Specifically, this will include work on feral hog, poultry, and human markers.
- Continue expansion and refinement of the Texas *E. coli* BST Library.
- Continued investigations into the geographic stability of the Texas *E. coli* BST Library and refinement of library isolate selection.

While previous studies have utilized appropriate quality assurance and quality control mechanisms as identified in project-specific QAPPs, the volume of current and anticipated BST studies across the State favors the development and implementation of BST template-SOPs. BST template-SOPs developed under TSSWCB projects 08-50 and 08-51 have provided for the continued development and use of the Texas *E. coli* BST Library by multiple laboratories and will also support and improve inter-laboratory comparison of BST results. In this project, ERIC-PCR, RP and *Bacteroidales* PCR template-SOPs will be reviewed and updated accordingly to ensure that they are current and up to date with applicable methods, technologies and markers.

In order to reduce bacteria and other pollutant contributions to streams, TWRI will also coordinate a Southwestern United States Stream Conference Workshop titled: Riparian Vegetation Workshop – Putting the 'green' into streambank stabilization in San Antonio in 2013. Establishment of riparian vegetation is one of the most important components of streambank stabilization and stream restoration, but it can also be one of the most challenging. This informative half day workshop will focus on the role of riparian vegetation, overcoming challenges of riparian management and restoration, and methods of establishment. It will also discuss budgeting for and monitoring of riparian vegetation restoration efforts and the techniques for managing invasive species. Instructors for the workshop will be from multiple states including Arkansas, Oklahoma, New Mexico and Texas. Further, with assistance from the USDA-NASS Texas Field Office, a stratified random sampling scheme will be implemented to support assessment of barriers to bacteria BMP adoption in conjunction with TSSWCB Project #12-08.

Project Goals (Expand from Summary Page)

Support BST analyses across the State through (1) continued personnel support and operation and maintenance of analytical infrastructure at public BST laboratories; (2) 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; (3) delivery of information on BMPs and materials that give an overview of BST activities in Texas to date and describe the use, capabilities and applicability of BST and the services provided by the State-supported analytical labs to local, state and national stakeholder audiences; (4) continued development of the Texas *E. coli* BST Library by incorporating additional known source fecal sample isolates; and, (5) further development of suitable source-specific bacteria markers for library independent BST.

Measures of Success (Expand from Summary Page)
• 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 utilization of BST applications statewide. • Data analysis for approximately 100 known source *E. coli* isolates from the Leona River (TSSWCB project 11-50) for expansion of the Texas *E. coli* BST Library • Fingerprinting and analysis of 20 known-source *E. coli* isolates collected as part of TSSWCB project 11-51 *Instream Bacteria Influences from Bird and Bat Habitation of Bridges* • Expansion of the Texas *E. coli* BST Library through the analysis of approximately 100 known source fecal samples collected by IRNR • Evaluation of geographical and temporal stability of the Texas *E. coli* BST Library and diversity of source specific isolates • Development/evaluation of new source-specific bacterial markers (e.g., poultry, feral hog from domestic swine,deer from other ruminants) for library-independent BST • 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 • Continued outreach through delivery of BST and BMP 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

***2012 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…

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Tasks, Objectives and Schedules				
Task 1	Project Administration			
Costs	$23,750			
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 15th of March, June, September, and December. QPRs shall be distributed to all Project Partners and posted on the project website.			
	Start Date	Month 1	Completion Date	Month 32
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 32
Subtask 1.3	TWRI will host coordination meetings or conference calls with the TSSWCB, UTSPH EP, and AgriLife SCSC at least quarterly 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 32
Subtask 1.4	TWRI will work with AgriLife SCSC and UTSPH EP to develop a Final Report that summarizes activities completed, conclusions reached during the project, and the extent to which project goals and measures of success have been achieved.			
	Start Date	Month 1	Completion Date	Month 32
Deliverables	• QPRs in electronic format • Reimbursement Forms, and necessary supporting documentation, in hard copy format • Final Report in electronic and hard copy formats			

Tasks, Objectives and Schedules				
Task 2	Quality Assurance			
Costs	$5,000			
Objective	Develop and implement data quality objectives (DQOs) and quality assurance/control (QA/QC) activities to ensure data of known and acceptable quality are generated through this project. Update and implement statewide BST template-SOPs.			
Subtask 2.1	TWRI will work with UTSPH EP, AgriLife SCSC, and IRNR to develop a QAPP for activities in Tasks 3-4 consistent with *EPA Requirements for Quality Assurance Project Plans (QA/R-5)* (May 2006) and the *TSSWCB Environmental Data Quality Management Plan* (August 2007).			
	Start Date	Month 1	Completion Date	Month 3
Subtask 2.2	TWRI will submit revisions and necessary amendments to the QAPP as needed.			
	Start Date	Month 4	Completion Date	Month 32
Subtask 2.3	AgriLife SCSC and UTSPH EP will maintain and update, at least annually, the 7 statewide BST template-SOPs for collection of fecal samples for BST, isolation of *E. coli*, archival of *E. coli* isolates, ERIC-PCR, RP, pre-processing of water samples for *Bacteroidales* PCR, and *Bacteroidales* PCR consistent with *EPA Guidance for Preparing Standard Operating Procedures (SOPs) (QA/G-6)* and the *TSSWCB Environmental Data Quality Management Plan* so that they include the most recent advances in BST science, methodologies, markers and technologies.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 2.4	AgriLife SCSC and UTSPH EP will coordinate to ensure that needed personnel training is kept on par between the groups to ensure congruity statewide.			
	Start Date	Month 1	Completion Date	Month 32

Subtask 2.5	UTSPH EP and AgriLife SCSC will work with public and private laboratories across the state which are exploring the use of BST. UTSPH EP and AgriLife SCSC will work to ensure that methodologies and QA/QC mechanisms adopted by these other laboratories are as congruent as possible with SOPs utilized by UTSPH EP and AgriLife SCSC (subtask 2.1).			
	Start Date	Month 1	Completion Date	Month 32
Deliverables	• QAPP for Tasks 3-4 approved by TSSWCB in both electronic & hard copy formats • Approved revisions and amendments to QAPP • Updated statewide BST template-SOPs			

Tasks, Objectives and Schedules				
Task 3	Known Source Fecal Sample Collection			
Costs	$30,000			
Objective	To expand the Texas *E. coli* BST Library through the collection of approximately 100 known source fecal samples.			
Subtask 3.1	TWRI will work with IRNR to collect known source fecal samples.			
	Start Date	Month 1	Completion Date	Month 2
Subtask 3.2	TWRI and IRNR will work with UTSPH EP and AgriLife SCSC to develop a targeted list of needed species/watersheds for fecal sample collection and plan for their collection and delivery. This list should primarily fill gaps in the Texas *E. coli* BST Library identified in other TSSWCB-funded BST projects. Targeted species will include small mammals such as mice, squirrels, nutria and rabbits. In addition, samples will be collected from at least one previously studied watershed (e.g., Leon River) in order to determine the temporal stability of the Texas *E. coli* BST Library. Approximately 50 known source fecal samples from each of 2 watersheds (Leon and San Antonio Rivers) are budgeted for collection (total of 100 samples). TWRI, UTSPH EP, and AgriLife SCSC will review the draft QAPP with IRNR and discuss and resolve issues as necessary.			
	Start Date	Month 2	Completion Date	Month 4
Subtask 3.3	IRNR will collect fecal samples in accordance with the plan developed in Subtask 3.2 and work closely with UTSPH EP and AgriLife SCSC to coordinate delivery of the samples to the appropriate lab. IRNR will communicate with a select group of organizations, agencies and businesses in each of the 2 targeted watersheds to arrange and resolve any access concerns and gather input to improve geographic targeting of sample collection. Travel plans, scheduling, and routing maps will be prepared prior to deploying the field crew. IRNR will deploy the field crew to collect known source samples from each targeted watershed. IRNR will coordinate closely with 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 submitted to TWRI.			
	Start Date	Month 4	Completion Date	Month 15
Deliverables	• Map of watersheds targeted for known source sample collection • Proposed list of needed species recommended for fecal sample collection • MS Excel summary data sheets cataloguing known source samples collected			

Tasks, Objectives and Schedules				
Task 4	Analytical Laboratory Capacity, Library Expansion, and Methods Development			
Costs	$295,348			
Objective	Support BST analyses across Texas, through continued operation and maintenance of BST laboratory analytical infrastructure, including equipment and personnel. Evaluate and expand the statewide *E. coli* BST library through the analysis of ERIC-RP data provided by AgriLife SCSC for approximately 100 *E. coli* known source isolates obtained from the Leona River watershed (TSSWCB Project 11-50) and the addition of known source fecal samples collected through Task 3 and TSSWCB project 11-51. Develop and refine library-independent markers.			
Subtask 4.1	UTSPH EP and AgriLife SCSC will maintain BST analytical equipment (e.g., RiboPrinter) and general laboratory equipment. This includes securing maintenance contracts, replacement parts, and expendable supplies and purchase of a new computer for the UTSPH EP RiboPrinter system.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 4.2	UTSPH EP will retain (or hire) a Graduate Student or Postdoctoral Research Associate that will 1) maintain laboratory operating capacities and technical expertise to conduct BST studies across the state, 2) aid in the evaluation, expansion and maintenance of the Texas *E. coli* BST Library, 3) evaluate library-independent methods and markers, and 4) provide support on TSSWCB project 12-10 *BST to Support Adaptive Management of the Arroyo Colorado WPP*.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 4.3	AgriLife SCSC will retain (or hire) Graduate Students and/or a Postdoctoral Research Associate that will 1) maintain laboratory operating capacities and technical expertise to conduct BST studies across the state, 2) continue BST efforts in support of TSSWCB projects 09-10 *Development of a WPP for Attoyac Bayou*, 11-50 *Assessment of Water Quality and Watershed Planning for the Leona River*, and 11-51 *Instream Bacteria Influences from Bird and Bat Habitation of Bridges*, and 3) evaluate new poultry marker(s) for library-independent BST.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 4.4	UTSPH EP and AgriLife SCSC will expand the statewide *E. coli* BST library through the analysis of ERIC-RP data provided by AgriLife SCSC for approximately 100 *E. coli* known source isolates obtained from the Leona River watershed (TSSWCB Project 11-50). Additionally, UTSPH EP and AgriLife SCSC will isolate *E. coli* from approximately 100 known source fecal samples collected through Task 3, which should primarily fill gaps in the library identified in other TSSWCB-funded BST projects. Approximately three isolates from each fecal sample (for a total of approx. 300 isolates) will be analyzed using ERIC-PCR for inclusion in the Texas *E. coli* BST Library; based on the ERIC-PCR fingerprint patterns, approximately half of the isolates (150) will be further analyzed using RP for inclusion in the Texas *E. coli* BST Library. UTSPH EP and AgriLife SCSC will equitably split workload. AgriLife SCSC will also fingerprint (ERIC-RP) and analyze 20 known-source *E. coli* isolates collected as part of TSSWCB Project 11-51.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 4.5	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 1	Completion Date	Month 32
Subtask 4.6	Using known source fecal material, AgriLife SCSC and UTSPH EP will utilize the best available bacterial indicators to evaluate and further develop/refine source-specific bacterial PCR markers. Specifically, efforts will be made on markers to 1) identify poultry litter/manure pollution, 2) evaluate the use of genetic targets based on ERIC-PCR products to differentiate human and animal derived *E. coli,* 3) differentiate between domestic swine and feral hogs, 4) differentiate deer from other ruminants by continued analysis of existing data on deer fecal microbial communities, and 5) evaluate the occurrence of human HF183 marker cross reactivity for all 100 known source animal samples collected under Task 3.			
	Start Date	Month 1	Completion Date	Month 32

Subtask 4.7	AgriLife SCSC and UTSPH EP will cooperate with other entities nationwide to ensure that the most up-to-date and accurate BST approaches are implemented in Texas by attending and participating in BST-related meetings, seminars and workshops, as appropriate, to learn of new and improved BST methods being employed elsewhere.			
	Start Date	Month 1	Completion Date	Month 32
Deliverables	• Highlights of work performed included in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 5	Outreach on Bacterial Source Tracking and BMPs			
Costs	$65,000			
Objective	To further outreach regarding bacteria BMPs as well as the science of BST and its application through improving the statewide knowledge base regarding current BST practices, BMPs, scientific advances, improvements in the application of BST and incorporating information from other areas of the nation into the BST approaches utilized in Texas.			
Subtask 5.1	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 32
Subtask 5.2	TWRI, UTSPH EP, and AgriLife SCSC will periodically meet with natural resource agencies, including but not limited to USEPA-R6, TCEQ, TPWD, TDA, GLO, DSHS, and selected river authorities, to advance the general knowledge and understanding of agency staff on BST and to develop action strategies to address issues raised by agency staff regarding the use of BST in Texas.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 5.3	TWRI, UTSPH EP, and AgriLife SCSC will distribute the educational brochures developed through TSSWCB Project 10-50 (subtask 4.2). TWRI, UTSPH EP, and AgriLife SCSC will develop additional flyers, one-pagers, tri-folds or other appropriate printed media, as needed, 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 which the State has invested. As appropriate, TWRI will include information about BST in general, and this project specifically, in the txH2O magazine, the Conservation Matters e-mail newsletter, and AgriLife Today news.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 5.4	TWRI, UTSPH EP, and AgriLife SCSC will promote the use of and provide resources on BST by participating in conferences, workshops, seminars, and other appropriate venues, including but not limited to the 2013 and 2014 TCEQ Environmental Trade Fair, WEF/WEAT events in Texas, TSCRA/TFB/TWA annual conventions, and ASABE events in Texas.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 5.5	TWRI, UTSPH EP and AgriLife SCSC will work to inform other researchers/academia who are engaged in BST in Texas (e.g., Edrington, Brinkmeyer, Alam, Ward) about the methods and approaches recommended by the Task Force and being implemented by the State.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 5.6	To build on the success of the *2012 BST – State of the Science Conference*, TWRI, UTSPH EP, and AgriLife SCSC will evaluate the need for and timing of a follow-up conference. If the need is substantiated, TWRI, UTSPH EP, and AgriLife SCSC will initiate planning and logistics for a follow-up conference.			
	Start Date	Month 1	Completion Date	Month 32

Subtask 5.7	With assistance from the USDA-NASS Texas Field Office, a stratified random sampling scheme will be implemented using a target population of beef cattle producers who completed 2012 Census of Agriculture forms. The sample will be stratified according to NASS district and beef cattle herd size. USDA-NASS will provide Texas A&M Department of Soil & Crop Sciences with a list of unique identifying numbers that will be placed on all survey materials so that response/non-response can be tracked. The USDA-NASS Texas Field Office will also assist with logistics related to compiling, stuffing, and mailing survey materials that will include an introductory postcard, the first survey packet with cover letter and survey instrument, a reminder postcard, and a second survey packet with cover letter and survey instrument. This information will support assessment of barriers to BMP adoption in conjunction with TSSWCB Project #12-08.			
	Start Date	Month 1	Completion Date	Month 32
Subtask 5.8	In order to reduce pollutant contributions to streams, including bacteria, TWRI will coordinate a Southwestern United States Stream Restoration Conference Workshop titled: Riparian Vegetation Workshop – Putting the 'green' into streambank stabilization in San Antonio in 2013.			
	Start Date	Month 1	Completion Date	Month 12
Deliverables	• Summaries of outreach efforts included in QPRs and Final Report			

Tasks, Objectives and Schedules				
Task 6	Technical Assistance for Leon River WPP Update			
Costs	$35,000			
Objective	Work with the Leon River WPP stakeholders to review and address comments from EPA's nine element consistency review of the Leon River WPP.			
Subtask 6.1	Schedule and attend a meeting to gain input and support from the stakeholders on the strategies, proposed answers and rebuttals of the comments submitted by EPA. Also participate on planning conference calls as needed.			
	Start Date	Month 11	Completion Date	Month 17
Subtask 6.2	PowerPoint presentation to stakeholders to summarize key issues of response to comments for discussion at stakeholder meeting.			
	Start Date	Month 11	Completion Date	Month 17
Deliverables	• Response to EPA comments on the Leon WPP (Draft and Final) • PowerPoint presentation			

Part III – Financial Information

Budget Summary		
Category	Costs	
Personnel	$	125,983
Fringe Benefits	$	31,165
Travel	$	12,065
Equipment	$	9,000
Supplies	$	14,149
Contractual	$	188,371
Construction	$	0
Other	$	33,357
Total Direct Costs	$	414,090
Indirect Costs (≤ 15%)	$	40,008
Total Project Costs	$	454,098

Budget Justification		
Category	Total Amount	Justification
Personnel	$ 125,983	• TWRI Associate Director (0.1 FTE) = $17,454 • SCSC Assistant Professor (0.08 FTE) = $17,499 • IRNR Website Administrator (0.04 FTE) = $3,341 • IRNR Extension Assistant (0.16 FTE) = $6,839 • IRNR Research Scientist (0.16 FTE) = $9,558 • Postdoctoral Associate (0.8 FTE) = $64,960 • Undergraduate Student Labor (0.15 FTE) = $6,332
Fringe Benefits	$ 31,165	Calculated at 17.2% of Personnel (9.9% for Graduate Students) to cover FICA, UCI, WCI, and retirement. Additional $474/mo. ($376/mo. For Graduate Students) prorated per %FTE is calculated for group health insurance.

Travel	$ 12,065	• TWRI Associate Director & Extension Program Specialist ($4,592)
		  o Per diem ($1,157)
		  o Lodging ($1,900)
		  o Airfare ($550)
		  o Transportation and parking fees ($125)
		  o Concur Fees ($160)
		  o TWRI State Vehicle Mileage (0.50/mi) = $700
		• IRNR Extension Assistant & Research Scientist ($2,631)
		• SCSC Assist. Prof. & Grad Students ($4,842)
		  o National Meetings ($3,542)
		  o State Meetings ($1,300)

Equipment	$ 9,000	• Windows 7 computer with removable storage

Supplies	$ 14,149	• IRNR fecal collection supplies = $224
		• SCSC supplies = $13,925
		  o Computer for postdoc = $1,000
		  o ERIC-RP Supplies for project 11-51 ($53 x 20 isolates) = $1,060
		  o E. coli isolation/archival from fecal samples ($25 x 50) = $1,250
		  o ERIC-RP Supplies for new projects ($8 x 150 ERIC; $45 x 75 RP) = $4,575
		  o Eval/Development Supplies = $4,000
		  o Lab supplies = $2,040

Contractual	$ 188,371	• UTSPH EP = $153,371 • Task 6 = $35,000
Construction	$ 0	N/A

Other	$ 33,357	• TWRI Booths at Environmental Trade Fair ($825) • TWRI various conference registration fees for presenting on BST project ($1,254) • TWRI Shipping ($111) • Banner displays for booths and conferences ($600) • RiboPrinter Preventative Maintenance and Service (for RiboPrinters at both UTSPH EP and AgriLife SCSC) (TWRI) = ($3,600) • Instructor Travel & Registration – Southwest Stream Restoration Conference ($5,000) • SCSC DNA Sequencing for library independent markers, conference registration, general maintenance on equipment, NELAP Lab accreditation fees ($10,325) • IRNR fecal collection and shipping ($1,455) • IRNR Game Cameras needed for collecting ($1,958) • BST Brochures ($1,000) • USDA-NASS ($7,229)
Indirect	$ 40,008	15% of Modified Total Direct Costs (Total minus Contractual >$25,000 per contract and Equipment)

Contractual Budget Justification – UTSPH EP		
Category	Total Amount	Justification
Personnel	$ 69,665	• El Paso: Di Giovanni, PI (0.1 FTE) = $34,960 • El Paso: Classified Staff (Truesdale 4 months @ 1.0 FTE, Casarez 4 months @ 0.6 FTE) = $22,319 • El Paso: Grad Student (0.25 FTE) = 12,386
Fringe Benefits	$ 21,243	• El Paso: Di Giovanni @ 24% of personnel = $8,390 • El Paso: Classified Staff @ 30% of personnel + longevity pay and insurance premium = $10,128 • El Paso: Grad Student @ 22% of personnel = $2,725
Travel	$ 116	• Di Giovanni State or Regional Meeting = $116
Equipment	$ 0	N/A
Supplies	$ 42,342	• E. coli isolation and archival from known source fecal samples ($25 x 50) = $1,250 • ERIC-RP supplies ($8 x 150 ERIC, $45 x 75 RP) = $4,575 • Bacteroidales human HF183 PCR analysis of fecal samples ($75 x 100) = $7,500 • Supplies for library independent method eval/development, sequencing = $16,417 • Computer ($500) & software ($8,500) for DuPont Qualicon RiboPrinter = $9,000 • Refrigerator and general maintenance (Biological Safety Cabinets, freezers and refrigerators) = $3,600
Contractual	$ 0	N/A
Construction	$ 0	N/A
Other	$ 0	N/A
Indirect	$ 20,005	15% of Modified Total Direct Costs