NONPOINT SOURCE SUMMARY PAGE
FY02 319(h)
Project 02-10

1. Title of Project: Development of BST Library and Assessment of Bacterial Sources Impacting Lakes Waco and Belton

2. Project Goals/Objectives:

Laboratory Objectives:

To develop publicly available, comprehensively characterized genetic fingerprint and antibiotic resistance libraries of approximately 1,000 unique *E. coli* isolates from known animal and human sources in the Bosque and Leon River watersheds. Approximately 650 ambient water isolates from the above watersheds will be analyzed and compared to the libraries to determine the human or animal sources of bacteria, as well as 100 quality control isolates. To make progress towards the development of statewide libraries, approximately 100 *E. coli* isolates from known animal sources in South Texas will also be characterized. Standard methods will be used so that the libraries can be expanded through future Texas waterbody studies and data may be exchanged with other researchers.

Water Quality Assessment Objectives:

1. Review and evaluate existing data and information summarizing bacterial levels and potential sources to Lake Waco and Lake Belton.
2. Collect and analyze new data and information of known and specified quality to differentiate and quantify the relative contributions from each bacterial source into Lake Waco and Lake Belton via the Bosque and Leon rivers.
3. Coordinate and link this project with a parallel study that will be conducted by Texas A & M aimed at testing different molecular and non-molecular analytical methods of bacteria source tracking.
4. Provide sufficient documentation of the data and technical analyses conducted that will aid the TFB in communicating the assessment results to watershed stakeholders, TNRCC, and U.S. EPA, as necessary.
5. Obtain support for the technical sufficiency of the project from stakeholders, and the TNRCC, TSSWCB, and US EPA Region 6.

3. Project Tasks: (1) Coordination with project collaborators, (2) Literature review and summarize existing data and information, (3) Coordination meeting and bacterial source tracking workshop, (4) Sampling plan development, sanitary survey, and known-source sample collection for library development, (5) Quality management program, (6) Ambient water sampling and analysis, (7) Isolation, BOX-PCR Screening, and archiving of E. coli isolates, (8) Ribotyping of E. coli isolates, (9) Pulsed-Field Gel Electrophoresis (PFGE) analysis of E. coli isolates, (10) Antibiotic resistance analysis of E. coli isolates, (11) Data sharing, analysis of BOX-PCR, ribotype, PFGE, and antibiotic resistance profile data, and (12) reporting and presentation of findings.

4. Measures of Success: Development of publicly available, comprehensively characterized genetic fingerprint and antibiotic resistance libraries of unique *E. coli* isolates for determining the animal or human nonpoint source contamination of surface water. Apply these libraries to classify *E. coli* isolated from ambient water samples from Lake Waco and Lake Belton and their main tributaries, the Bosque and Leon Rivers, to identify the likely sources of bacterial contamination.

5. Project Type: Statewide

6. Waterbody Type: River

7. Project Location: Statewide

8. NPS Management Program Reference: State of Texas Agricultural/Silvicultural Nonpoint Source Management Program, Approved February 25, 2000.

9. NPS Assessment Report Status: Impaired(X) Impacted(X) Threatened( ) Other( )

10. Key Project Activities: Monitoring and Technical Assistance

11. NPS Management Program Elements: Implement milestones from the "1999 Texas Nonpoint Source Pollution Assessment Report and Management Program", which will include; (1) Coordinate with federal, state, and local programs, (2) TSSWCB is committed to technology transfer, technical assistance and support, administrative support and cooperation between agencies and program for the prevention of NPS pollution.

12. Project Costs: Texas Agricultural Experiment Station: $639,742 (Federal), $373,774 (Non-Federal), $1,013,516 (Total) **Attachment I**; Texas Farm Bureau Agricultural Service Company: $141,094 (Federal), $91,449 (Non-Federal), $232,543 (Total) **Attachment II;**

Total Project Cost: $780,836 (Federal), $465,223 (Non-Federal), $1,246,059 (Total)

13. Project Contractors:
Texas Farm Bureau Agricultural Service Company (TFB), Waco and
Texas Agricultural Experiment Station, Texas A&M University System Agricultural Research and Extension Center (TAMU), El Paso

14. Project Period: Three years

Development of BST Library and Assessment of Bacterial Sources Impacting Lakes Waco and Belton

Texas State Soil and Water Conservation Board
FY02 CWA Section 319(h)

Problem/Need Statement:

Protection of our water resources is one of the most significant environmental challenges of the new millennium. Nonpoint sources (NPS) of pollution, especially from agricultural activities, can greatly impact water quality. One key component in effectively implementing a NPS pollution management program, especially for impacted waterbodies such as Lake Waco and Lake Belton, is the identification and assessment of sources of bacterial contamination. Proper evaluation of these sources is needed to develop best management practices (BMPs) and microbial total maximum daily loads (TMDLs). 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.

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 *Escherichia coli* (*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 inhabit 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 animal to be identified, referred to as bacterial source tracking (BST). Molecular tools appear to hold the greatest promise for BST, providing the most conclusive characterization and level of discrimination for isolates. Of the molecular tools available, ribosomal ribonucleic acid genetic fingerprinting (ribotyping) and pulsed-field gel electrophoresis (PFGE) are emerging as versatile and feasible BST techniques. A phenotypic characterization method, antibiotic resistance profiling, also has the potential to identify the human or animal origin of isolates. However, reference “libraries” of bacterial genetic fingerprints and antibiotic resistance profiles are needed to correctly identify the source of bacteria isolated from environmental water samples.

General Project Description:

This project has two general objectives (1) to assess the water quality in Lake Waco and Lake Belton with regard to the relative contributions of bovine, human, and other animal contributions to the water bodies and (2) to develop libraries, genetic and biochemical, that can be used in determining the animal or human nonpoint source contamination of surface water.

Water Quality Assessment

The proposed project will include the review and evaluation of existing data and information pertaining to bacterial contributions and sources to Lake Waco and Lake Belton. New data, of known and specified quality, will be collected and analyzed to differentiate and quantify the relative contributions of bovine livestock and other human and animal bacteria sources into Lake Waco and Lake Belton. This assessment and differentiation between bacteria sources will utilize, and be coordinated with, the development of bacteria source tracking libraries generated by Texas A&M. This project will provide sufficient documentation of the data and technical analyses conducted that will aid the TFB in communicating the assessment results to watershed stakeholders, TSSWCB, TNRCC, and U.S. EPA, as necessary. It is also a goal of this project to obtain support for the technical sufficiency of the project from stakeholders, and the TSSWCB, TNRCC, and US EPA Region 6.

Library Development

The proposed project will include the development of publicly available BOX-PCR, RiboPrinter ribotype, and PFGE genetic fingerprint and antibiotic resistance profile libraries generated from approximately 1,000 unique *E. coli* isolates from known animal, human and wastewater sources from the Bosque River/Lake Waco and Leon River/Lake Belton watersheds. Approximately 750 water isolates from the above watersheds will be analyzed and compared to the libraries to determine the human or animal sources of bacterial contamination. To make progress towards the development of statewide libraries, approximately 100 *E. coli* isolates from known animal sources in South Texas will also be characterized. A multiphasic approach using standardized methods will be used so that the libraries can be expanded through future Texas 303(d) impaired waterbody studies and data may be exchanged with other researchers.

A critical element of this proposal is the use of the Qualicon RiboPrinter Microbial Characterization System for automated ribotyping of *E. coli* isolates. The RiboPrinter was originally developed for use in identification and BST of microbial isolates for the food industry. There are several advantages of using the RiboPrinter:

- The RiboPrinter is the only automated system for high-throughput genetic typing of bacteria. All bacterial isolate sample processing is automated using standardized reagents and a robotic workstation, providing an exceptional level of reproducibility. Up to 32 samples may be analyzed per day, whereas manual ribotyping methods may require up to several days to complete.
- The system has built-in data analysis of ribotypes, including automated searching of the Qualicon ribotype library and custom identification libraries. This reduces the awkward and subjective interpretation of ribotype fingerprints.
- The electronic ribotype libraries can be shared with other users via a modem, allowing rapid dissemination of data. Furthermore, since the system employs standardized methods and reagents, results obtained from other laboratories using the system are directly comparable.

- Researchers with the United States Geological Survey, Purdue University Calumet, New York Department of Environmental Protection, University of Hawaii and University of Georgia have recently begun using the RiboPrinter system for BST, creating the possibility of data sharing and development of an expansive ribotype library.

E. coli isolates will be selected using an approach to maximize the diversity of strains represented in the library. Livestock and wildlife fecal samples, municipal wastewater treatment plant effluent, septic system and dairy waste lagoon samples will be obtained from a variety of sources in the Bosque and Leon River watersheds. Sources will be identified through a sanitary survey to be conducted by Parsons (Austin, TX) as part of a Texas Farm Bureau sponsored project. Parsons will be responsible for collecting samples and shipping to the El Paso Agricultural Research and Extension Center Environmental Microbiology Laboratory (Di Giovanni) for isolation of *E. coli*. *E. coli* will be isolated from the samples using standard microbiological methods. Briefly, fecal specimens will be used to inoculate lauryl tryptose broth to enrich coliform bacteria. Enriched samples will be inoculated onto EC medium, selective for coliforms and *E. coli*. Colonies from EC medium will be streaked onto McConkey medium to isolate lactose-fermenting coliforms. Colonies resembling *E. coli* will be cultured in LB broth/MUG and UV fluorescence will provide confirmation of *E. coli*.

Confirmed *E. coli* bacterial colonies will be screened using a repetitive sequence polymerase chain reaction (BOX-PCR) method. BOX-PCR is another genetic fingerprinting method used for BST and will be used to identify unique *E. coli* isolates from each sample and eliminate further analysis of identical isolates (clones). At least one *E. coli* isolate from each fecal, wastewater, etc. sample will be included in the library, even if it is identical to a previously isolated *E. coli*. Therefore, abundant/common strains will be sufficiently represented in the libraries. It is anticipated that over 3,000 *E. coli* colonies will be screened by BOX-PCR, therefore a large library of BOX-PCR fingerprints will be generated. Cultures of selected isolates will be archived and subcultures will be shipped to the other investigators for further analysis.

Following BOX-PCR analysis, isolates will be ribotyped at the EP AREC using the Qualicon automated RiboPrinter using the restriction enzyme *Hin*d III. This enzyme has been compared to other restriction enzymes by other investigators and found to be useful for BST of *E. coli*. At least two other investigators (Dr. Rose, University of South Florida; Dr. Tseng, Purdue-Calumet) are currently using the RiboPrinter and *Hin*d III for BST of *E. coli*. Both of these investigators have stated they are willing to freely share data, creating the possibility of rapidly expanding the ribotype library.

The isolates will be further characterized using pulsed-field gel electrophoresis (PFGE), another leading method for BST. PFGE is currently being used by the Centers for Disease Control and Prevention (CDC) to track foodborne *E. coli* O157:H7 and *Salmonella* isolates. The isolates will be shipped to TAMU Food and Environmental Microbiology Laboratory (Pillai) for PFGE analysis. The standardized CDC protocol for PFGE analysis of *E. coli* will be followed. CDC currently uses this standardized protocol

as the basis of their "PulseNet" outbreak surveillance network, which allows public health laboratories nationwide to quickly compare their PFGE fingerprints to the CDC central reference library.

An additional BST technique, antibiotic resistance profiling, will be performed by the TAMU Corpus Christi Environmental Microbiology Laboratory (Mott). This technique follows methods used in the clinical laboratory for evaluating the antibiotic resistance of bacterial isolates. Commonly, the disk diffusion method is used which involves measuring the diameter of zone of inhibition of bacterial growth around a filter disk impregnated with a specific antibiotic. By comparison to resistant and susceptible control strains, the response of the *E. coli* isolates can be determined. To further standardize and automate the assay, an image analysis system will be used to measure the zones of inhibition and provide electronic archival of data.

By using the combination of BOX-PCR, RiboPrinter ribotyping, PFGE, and antibiotic resistance profiling, one of the most comprehensive and accessible libraries of *E. coli* isolates for environmental BST will be developed. This multiphasic approach will allow the state of Texas to keep pace with developments in BST technology. The validity of the study and conclusions will be strengthened through the use of multiple techniques to identify the sources of contamination. It is also anticipated that approximately 100 blind QC *E. coli* isolates will be analyzed throughout the course of the project to evaluate the robustness of the methodologies and laboratory performance. By using standardized methods, the libraries can be expanded through future projects and the data shared with other BST investigators and regulatory agencies. Peer-reviewed publication of project results is also a goal of the proposed work.

The expertise in molecular environmental microbiology and water quality required for this project exists within the Texas A&M University System. The Texas A&M El Paso Agricultural Research and Extension Center (EP AREC) will coordinate sample collection with Parsons personnel. Dr. George D. Di Giovanni's laboratory at the EP AREC will isolate *E. coli* from samples, perform BOX-PCR screening, RiboPrinter ribotyping, and archiving of bacterial cultures. Dr. Suresh D. Pillai's laboratory at Texas A&M University - College Station (TAMU) will perform PFGE analysis of isolates. Dr. Joanna Mott's laboratory at Texas A&M University – Corpus Christi (TAMU-CC) will perform the antibiotic resistance profiling. The respective laboratories will also be responsible for coordinating data sharing efforts with other researchers using the respective methods. All libraries will be made publicly available for other agencies and testing laboratories.

Tasks, Objectives, Schedules, and Estimated Costs:

Task I: Coordination between Project Collaborators (TFB & TAMU)
Costs:
$25,878 (Federal) 13,956 (Non-Federal) $39,834 (Total) Task is 3% of Total
Objective: The project has two parallel objectives and timeframes that must be linked. The Lake Waco/Lake Belton water quality assessment aims to provide data and information that can be used to make recommendations of different water quality management strategies for reducing bacteria loadings; the library development seeks to test different analytical methods for creating a genetic fingerprint library the state might use in the future. The TFB, TAMU and TSSWCB must coordinate efforts to ensure that the objectives of the bacterial source tracking library development and the water quality assessment are compatible and complimentary. Coordination is also required to ensure that the data quality objectives of this project are maintained throughout the implementation and execution of the project. Technical assistance and collaboration between the TFB, TSSWCB, TAMU, and TNRCC are necessary to ensure that project deliverables are well coordinated and complimentary of other efforts and projects in the state that are related to addressing bacteria impairments.

Deliverables:
Meetings and conference calls are the primary deliverable under this task.

Task II: Literature Review and Summarize Existing Data and Information (TFB)
Costs:
$13,578 (Federal) $9,304 (Non-Federal) $22,882 (Total) This Task is 2% of Total
Objective: Available literature, data, and information germane to describing the contributions, both spatially and temporally, and sources of bacterial loading in Lake Waco and Lake Belton will be acquired. The data analyses will include discussion of temporal (inter-annual, seasonal) and spatial trends in water quality, an evaluation of potential sources, and an identification of data gaps. The relevant data and information will include:

- reported wastewater permit information, including permit limits, self-reported effluent quality data, violations, and inspection reports;
- hydrologic data;
- land use, population density, and the extent of use of on-site sewerage systems (septic tanks) in the watershed;
- livestock density and agricultural practices in the watershed from the most recent county-level agricultural census, as well as the abundance and type of confined animal feeding operations;
- wildlife surveys associated with the watersheds of Lake Waco and Lake Belton;
- topography, soil, and vegetation information; and
- special studies and published reports for Lake Waco and Lake Belton.

This task will include an explanation of how BST methods provide useful data to assist in water quality management efforts where bacterial contamination is a concern.

Deliverables:

- Technical memorandum summarizing the results that will be modified and incorporated into the final report. Final report will be in electronic format

Task III: Coordination Meeting and BST Workshop (TFB & TAMU)

Costs:

$14,278 (Federal) $9,304 (Non-Federal) $23,582 (Total) Task is 2% of Total

Objective: The TFB will hold a project initiation meeting with the key project participants, TSSWCB, US EPA and TNRCC to discuss the objectives, scope and schedule of the project. In addition, TAMU will work with TFB to design and conduct a full day workshop that will:

1. Describe the technical methods and benefits of using BST;
2. Develop draft data quality objectives and a preliminary sampling plan; and
3. Conduct training for collecting library samples from known fecal sources.

Task IV: Sampling Plan Development, Sanitary Survey, and Known-Source Sample Collection for Library Development (TFB & TAMU)

Costs:

$53,028 (Federal) $32,569 (Non-Federal) $85,597 (Total) This Task is 7% of Total

Objective: A sampling plan and data quality objectives (DQO) for both Lake Waco and Lake Belton watersheds will be developed based on the requirements of the TFB, review of the existing data and information, and the recommendations developed from the BST Workshop. An additional step that has proven very useful in developing a sampling plan is a sanitary survey of the watershed. The primary goal of the sanitary survey is to identify the potential sources of fecal matter to Lake Waco and Lake Belton. Wildlife and domestic animal sources of observed scat can be identified. Concentrated waterfowl areas and bird rookeries or bat colonies may be identified. The utilization of waterways by wildlife, as well as dogs, cats, and other domestic animals are also assessed. Human influences are also typically identified, including malfunctioning septic systems and sewer overflows. Septage and fecal samples from various wildlife and domestic animals are collected for culturing and ribotyping to develop a local DNA library of known sources.

Following the sanitary survey, samples of fecal matter will be collected from the major potential sources of fecal matter in the watersheds. These sources will include domestic animals, wildlife, and human sources. In all, over 1,000 known source samples from the Lake Belton and Lake Waco watersheds will be collected and sent to the EP AREC laboratory for development of the libraries. All sample collection procedures and documentation will be specified in a quality assurance project plan.

Deliverables:

- Technical memorandum summarizing the results of the sanitary survey and known source sample collection that will be modified and incorporated into the final report.
- Sampling plan will be prepared as part of the quality assurance project plan.

Task V: Quality Management Program (TFB & TAMU)

Costs:

$22,378 (Federal) $13,956 (Non-Federal) $36,334 (Total) This task is 3% of Total

Objective: Quality assurance is an important part of the project. It ensures that data and information collected under the project are of appropriate type and quality for their intended uses. Quality assurance will involve thorough planning, coordination, and implementation. Thorough planning, with documentation in a quality assurance project plan (QAPP), can greatly assist the project manager in controlling costs and meeting project objectives on schedule. The quality assurance process will ensure defensible products and decisions. The QAPP will specify project objectives; include the project management structure and key roles and responsibilities; criteria for assessing the validity of existing data and information; the data quality objectives for the project; the sampling and analysis plan; the staff training and communication plan; the data management plan; standard operating procedures; technical assessments, including field and laboratory audits; data verification and validation; and a final data quality report and validity assessment.

Other key water quality monitoring entities within the watershed will be considered as the QAPP is developed. Preparation of the QAPP will need to respond to findings of the Literature Review and Summary of Existing Data and Information task, above, in addition to input from the City of Waco, BRA, and TSSWCB. No data collection will begin until the QAPP has been presented to the TFB, TSSWCB and USEPA, as appropriate. Every member of the project team will be knowledgeable of the quality assurance project plan.

Accuracy is one of the primary measures of data quality. It measures how close the result of a measurement is to the true value. In MST, the primary measure of accuracy is the average rate of correct classification (ARCC). ARCC is the percent of time that a sample known to be from a given source (e.g., human) is correctly classified as such by the BST method. Approximately 100 E. coli isolates from known sources will be sent to the EP AREC to obtain a true determination of the accuracy and reproducibility of each method. A “blind” procedure will be used with these samples: only persons collecting the sample will know and record the true source of these isolates. This will ensure an accurate measure of the quality of data is obtained.

One or more meetings with TFB and TSSWCB staff will be convened to discuss and review data quality objectives for the project, in accordance with USEPA guidance.[1]

- The types of data and information required to support these decisions will be discussed.
- The boundaries and limitations of the study will be identified.
- Lab analysis methods will be defined.
- The costs of various levels of uncertainty (and decision errors) for each decision to be made will be discussed and weighed.
- Data quality objectives that meet acceptable decision error rates and do not exceed project constraints will be finalized.

A plan for data collection that meets the agreed quality objectives for data will be developed and optimized considering site-specific factors. The complete data collection plan, with specific frequencies and locations for monitoring, will be provided to TSSWCB and USEPA for their review in an effort to obtain support for the project design as part of the quality assurance project plan.

In addition to the problem definition and project objectives, data quality objectives and monitoring and analysis plan, key elements of the QAPP include project management structure, roles and responsibilities, approvals, staff qualifications and training requirements, analytical procedures, quality control procedures, equipment required, a data management plan, field data and chain-of-custody forms, documentation, audits and other quality assessments and responses, reports to management, data review, data validation, and data verification, and reconciliation with user requirements.

Deliverables:

- QAPP
- Data validation and verification report, including an evaluation of the accuracy and precision of each BST method, in the final report

Task VI: Ambient Water Sampling and Analysis (TFB)

Costs:
$94,405 (Federal) 55,827 (Non-Federal) $150,232 (Total) This task is 12% of Total

Objective: To collect ambient water samples from 7 sites in Lake Waco and 6 sites in Lake Belton on ten dates over a 1-year period. These dates would likely include at least five dry weather events and at least three post-rainfall events. The sampling areas would include a deep-water area near the drinking water intake, as well as the major tributaries (Bosque and Leon Rivers). Approximately five water samples would be collected on each date from each site, and ten samples from the vicinity of the drinking water intake, for a total of 650 ambient water samples during the study. *E. coli* in water samples will be enumerated via standard membrane filter culture methods by the City of Waco

[1] U.S. EPA. 2000. EPA QA/G4.

laboratory. Following enumeration, the *E. coli* on membrane filters will be shipped overnight to EP AREC for source identification.

***Task VII: Isolation, BOX-PCR Screening, and Archiving of* E. coli *Isolates* (EP AREC)**

Costs:

$106,453 (Federal) $65,131 (Non-Federal) $171,584 (Total) This task is 14% of Total.

Objective: To isolate *E. coli* from known animal and human source fecal samples, septic systems and wastewater effluent samples from the Bosque and Leon River watersheds for the development of a reference *E. coli* culture collection. Approximately 3,000 confirmed *E. coli* isolates will be screened using BOX-PCR to identify unique strains, with approximately 1,000 isolates selected for additional analyses (ribotyping, PFGE, antibiotic resistance profiling) and archival of bacterial cultures. Approximately 650 unknown isolates from Bosque and Leon River watershed water samples will be analyzed for BST. In addition, 100 isolates obtained from South Texas (Mott) known wildlife and animal sources, and 100 blind QC samples will be analyzed. A standardized BOX-PCR protocol for *E. coli* BST developed by Sadowsky et al., University of Minnesota will be used.

7.1 The laboratory technician will coordinate sample shipment or collection (when necessary) with Parsons following the sanitary survey of the watersheds. (Start Date: Month 1; Completion Date: Month 16)

7.2 Approximately 3,000 *E. coli* bacterial colonies will be screened using BOX-PCR and fingerprints analyzed using Applied Maths BioNumerics software to identify unique strains. This screening process will ensure that the greatest diversity of *E. coli* isolates will be included in the reference culture collection. The BOX-PCR fingerprints will also be considered for interpretation of BST analyses. Bacterial cultures of all unique *E. coli* isolates will be archived for future analyses and dissemination to other laboratories. (Start Date: Month 1; Completion Date: Month 16)

Deliverables:

- Quarterly reports
- Inventory of archived *E. coli* isolates
- Publicly available BOX-PCR reference library of *E. coli* isolates from known human and animal sources

***Task VIII: Ribotyping of* E. coli *Isolates* (EP AREC)**

Costs:

$219,593 (Federal) $130,262 (Non-Federal) $349,855 (Total) This task is 28% of Total.

Objective: To develop a reference RiboPrinter ribotype library of approximately 1,000 unique *E. coli* isolates obtained in Task I. Approximately 650 unknown isolates from Bosque and Leon River watershed water samples will be analyzed for BST. In addition, 100 isolates obtained from South Texas (Mott) known wildlife and animal sources, and

100 blind QC samples will be analyzed. (Start Date: Month 4; Completion Date: Month 18)

Deliverables:

- Quarterly reports
- Publicly available electronic ribotype fingerprint library of approximately 1,100 unique *E. coli* isolates from known human and animal sources

***Task IX: Pulsed-Field Gel Electrophoresis (PFGE) Analysis of* E. coli *Isolates* (TAMU)**

Costs:

$116,017 (Federal) $69,784 (Non-Federal) $185,801 (Total) This task is 15% of Total.

Objective: To develop a reference PFGE library of approximately 1,000 unique *E. coli* isolates obtained in Task I. Approximately 650 unknown isolates from Bosque and Leon River watershed water samples will be analyzed for BST. In addition, 100 isolates obtained from South Texas (Mott) known wildlife and animal sources, and 100 blind QC samples will be analyzed. The standardized method currently used by the Centers for Disease Control (CDC) PulseNet surveillance network will be used. (Start Date: Month 4; Completion Date: Month 18)

Deliverables:

- Quarterly reports
- Publicly available electronic ribotype fingerprint library of approximately 1,100 unique *E. coli* isolates from known human and animal sources

***Task X: Antibiotic Resistance Profiling of* E. coli *Isolates* (TAMU-CC)**

Costs:

$83,228 (Federal) $51,174 (Non-Federal) $134,402 (Total) This task is 11% of Total.

Objective: To develop a reference antibiotic resistance profile library of approximately 1,000 unique *E. coli* isolates obtained in Task I. Approximately 650 unknown isolates from Bosque and Leon River watershed water samples will be analyzed for BST. In addition, 100 isolates obtained from South Texas (Mott) known wildlife and animal sources, and 100 blind QC samples will be analyzed. The standardized Kirby Bauer Disk Diffusion method with a panel of 10 antibiotics will be used. Zones of inhibition will be measured using an automated image analyzer to ensure uniformity for future comparisons with *E. coli* isolates from unknown sources. (Start Date: Month 4; Completion Date: Month 18)

Deliverables:

- Quarterly reports
- Publicly available electronic antibiotic resistance profile library of approximately 1,100 unique *E. coli* isolates from known human and animal sources

Task XI: Data Sharing, Analysis of BOX-PCR, Ribotype, PFGE, and Antibiotic Resistance Profile Data, and Final Report

Objective: To obtain additional BOX-PCR, RiboPrinter ribotype, PFGE fingerpints of *E. coli* isolates from other researchers to expand and test our library. Compare all BST data to determine utility of single or multiple analyses for source classification of isolates and determine average rates of correct classification (ARCC). Determine the source classification of Bosque and Leon River watershed water isolates and identify the likely nonpoint sources of bacterial contamination in the watersheds.

11.1 Final Report summarizing library of archived *E. coli* isolates from this project and characteristics of additional isolates provided by other researchers through data sharing; levels of source discrimination based upon BOX-PCR, RiboPrinter ribotype and PFGE fingerprints, and antibiotic resistance profiles. (Start Date: Month 20; Draft due Month 21; Final Draft due: Month 24)

Deliverables:

- Final Report that will be useful to the regulatory and scientific communities. Final report will be in electronic format

Task XII: Reporting and Presentation of Findings (TFB& TAMU)

Costs:

$32,000 (Federal) $13,956 (Non-Federal) $45,956 (Total) This task is 3% of Total.

Objective: To prepare a written report that summarizes the monitoring strategy, analytical methods used and the results of the BST data by source category. During the final stages of the project a maximum of two public presentations and two internal presentations to summarize the results of the study will be made, if appropriate forums occur.

Coordination, Roles and Responsibilities:

Participating organizations and agencies and their roles in this project:

- **Texas A&M El Paso Agricultural Research and Extension Center – Project Lead (EP AREC)** Responsible for coordination of sample collection, isolation of *E. coli*, BOX-PCR screening, culture archiving and shipping to TAMU and TAMU-CC, RiboPrinter ribotyping and data analysis of fingerprints. Responsible for Final Report. See Attachment I for TAM-ARC/EC Budget
- **Texas A&M University-College Station (TAMU)** Responsible for PFGE analysis of isolates. Assist in completion of Final Report.
- **Texas A&M University-Corpus Christi (TAMU-CC)** Responsible for antibiotic resistance profile analysis of isolates. Also responsible for providing approximately 100 *E. coli* cultures isolated from known animal sources in South Texas. Assist in completion of Final Report.
- **Texas Farm Bureau Agricultural Service Company** Responsible for coordination of the Microbial Source Tracking Group (including Brazos River

Authority and City of Waco), review of existing data and information on potential bacterial sources in the watersheds of Lake Waco and Lake Belton. Texas Farm Bureau will contract Parsons Engineering Science, Inc. to complete a BST workshop, and development of a sampling plan and QAPP. Parson Engineering Science, Inc. will also perform a sanitary survey of the Bosque River/Lake Waco and Leon River/Lake Belton watersheds, and collect known source samples for library development. Collect ambient water samples from Lake Waco, Lake Belton, Bosque River, and Leon River. Culture and enumerate *E. coli* in ambient water samples, and ship to EP AREC. Prepare final report assessing sources of fecal contamination. See Attachment II for TFB Budget

- **Texas State Soil and Water Conservation Board** Responsible for overall project coordination and oversight as well as being a liaison between TX A & M and the Texas Farm Bureau Agricultural Service Company.

Measures of Success:

- Development of publicly available, comprehensively characterized molecular fingerprint and antibiotic resistance profile libraries of approximately 1,100 unique *E. coli* isolates for determining the animal or human nonpoint source contamination of surface water. Use the reference libraries to determine the sources of approximately 650 Bosque and Leon River watershed water *E. coli* isolates and identify the likely nonpoint sources of bacterial contamination in the watersheds.

Reference to Project in the NPS Management Program:
Category: Agriculture

Project Leads:

Name:	Ned Meister
Address:	P.O. Box 2689 Waco, TX 76702-2689
Phone#	254-751-2457
Affiliation:	**Texas Farm Bureau**

Name:	George D. Di Giovanni, PhD
Address:	1380 A&M Circle El Paso, TX 79927
Phone#	915-859-9111
Affiliation:	**Texas A&M El Paso Agricultural Research and Extension Center**

ATTACHMENT I

TAES – El Paso

		Federal	Non-Federal	Total
A.	Senior Personnel			
	Co-Principal Investigators			
	George Di Giovanni			
	50% Time, 12 Calendar Months/Yr	$0	$ 71,050	$71,050
	Suresh Pillai			
	5% Time, 1 Calendar Months/Yr 1	360	0	$360
	5% Time, 11 Calendar Months/Yr 1	0	3,958	$3,958
	15% Time, 11 Calendar Months/Yr 2	0	12,229	$12,229
B.	Other Personnel			
	Technicians			
	To Be Named (Dr. Pillai's lab)			
	5% Time, 12 Calendar Months/Yr	0	2,132	$2,132
	50% Time, 12 Calendar Months/Yr	24,360		$24,360
	To Be Named (Dr. Di Giovanni's lab)			
	75% Time, 12 Calendar Months/Yr	0	36,540	$36,540
	100% Time, 12 Calendar Months/Yr	64,720	0	$64,720
	Graduate Research Assistant			
	To Be Named (Dr. Pillai)			
	12.5% Time, 12 Calendar Months/Yr 1	0	4,000	$4,000
	50% Time, 12 Calendar Months/Yr 2	0	16,480	16,480
	Total Salaries and Wages	89,440	146,388	$235,828
C.	Fringe Benefits			
	@ 15.5% & 8.25% of salaries and wages	13,863	21,205	$35,068
	Group Health Insurance	13,519	17,205	30,724
	Total Fringe Benefits	27,382	38,410	$65,792
	Total Salaries, Wages, and Fringe Benefits	116,822	184,797	$301,620
D.	Equipment	168,000	0	$168,000
	RiboPrinter $99,000			
	Bio Safety Cabinet $9,000			
	2 - Minus 80 C freezer $16,000			
	PFGE Equipment - $30,000			

		Genetic Analysis Software $10,000			
E.	Travel	For state and/or national meeting Di Giovanni and Pillai @ $2,000 ea	4,000	0	$4,000
F.	Participant Support Costs		0	0	$0
G.	Other Direct Costs				
	1. Materials and Supplies		162,940	0	$162,940
		Refrigerator $4,000 Isolation and archival of 1950 *E. coli* $15,600 BOX-PCR Equipment - $2,000 BOX-PCR 3450 isolates $17,250 RiboPrinting 2200 isolates $78,320 PFGE 1950 isolates $68,250			
	2. Publication Costs		500	0	$500
	3. Consultant Costs		0	0	$0
	4. Computer Costs		3,000	0	$3,000
	5. Subawards - TAMUCC		77,035	30,328	$107,363
	6. Other		24,000	0	24,000
		RiboPrinter Service Contract Yr 2, $15,000 PFGE Service Contract, Yr 2 - $3,000 Sample collection supplies and shipping $6,000			
	Total Other Direct Costs		267,475	30,328	$297,803
H.	Total Direct Costs		556,297	215,125	$771,422
I	Indirect Costs				
	@ 15% of total direct costs		83,445	0	$83,445
	@ 45.5% of modified total direct		0	84,083	$84,083
	Difference of indirect cost rates		0	74,566	74,566
	Total Indirect Costs		83,445	158,649	$242,094
J.	Total Costs		$ 639,742	$ 373,774	$1,013,516

Itemized Budget Justification

1. Texas A&M El Paso Agricultural Research and Extension Center

Funding is requested for a Laboratory Technician (50% time) to process samples at a rate of approximately $18,612 per year (including fringe and indirect costs) for 2 years at EP AREC for total of $37,617. A Natural Resource Specialist one month salary with benefits is requested for laboratory analysis and quality control and undergraduate students (TAMU-CC) are requested to perform supervised laboratory procedures (total $8,993).

Due to the volume of samples to be processed and potential presence of human pathogens, several pieces of equipment are needed for the isolation, archiving and analysis of *E. coli* isolates. These include a Biological Safety Cabinet, Class II, Type A at $9,000; a refrigerator for sample storage at $4,000; two -80°C freezers for archiving bacterial cultures (EP AREC) and PFGE samples (TAMU) at $8,000 each;; a desktop computer system dedicated to genetic fingerprinting analysis with printer and specialized software to handle complex statistical and graphical analysis of genetic fingerprints at $13,000.

Funding for a RiboPrinter to perform automated ribotyping at $99,000 is requested. This is the only automated instrument available for ribotyping. This instrument provides unprecedented reproducibility, data comparability and sample throughput compared to manual ribotyping.

Funding is requested for the purchase of PFGE equipment at $30,000. For commercial PFGE analysis of the number of isolates in this study it would cost approximately $330,000. In addition to cost savings, purchasing the PFGE equipment for this project allows for a) total control on QA/QC of the equipment and protocols, b) unlimited access to the equipment for this project, c) availability of this equipment for future State of Texas projects.

Funding for an antibiotic plate zone reader bioassay system with software and computer ($32,000) is requested. This will allow rapid, efficient and uniform analysis of bacterial growth in the presence of antibiotics and can be used for future work as the *E. coli* library is expanded and for comparison with watershed isolates.

Travel funds are also requested for Drs. Di Giovanni ($2,000), Pillai ($2,000) and Mott ($1,000) to attend state and/or national meetings to present project results ($5,000 total).

Indirect costs are requested at 15% of total direct costs with the difference of the indirect cost rate provided by the Texas Agricultural Experiment Station as matching funds.

ATTACHMENT II

Object Class Category	Federal Funds	Non-Federal	Total Cost
1.Personnel			
Director, Commodity and Regulatory Activities @ 120 hours for 2 years	$0	$6,269	$6,269
Associate Director, Commodity and Regulatory Activities @ 80 hours for 2 years	$0	$2,870	$2,870
Associate Director, Commodity and Regulatory Activities - Livestock @ 80 hours for 2 years	$0	$2,574	$2,574
Subtotal Personnel	$0	$11,713	$11,713
2.Fringe Benefits			
Fringe	$0	$0	$0
Subtotal Salary and Fringe	$0	$11,713	$11,713
3.Travel	$0	$500	$500
4.Equipment	$0	$0	$0
5.Supplies	$0	$0	$0
6.Contractual			
Parsons Engineering Science, Inc.	$123,744	$0	$123,744
Texas Farm Bureau Agricultural Service Company	$0	$25,000	$25,000
Brazos River Authority	$0	$20,302	$20,302
City of Waco	$15,350	$33,934	$49,284
Audit- Certified Public Accountant	$2,000	$0	$2,000
Subtotal Contractual	$141,094	$79,236	$220,330
7.Construction	$0	$0	$0
8.Other	$0	$0	$0
9.Total Direct Costs	$141,094	$91,449	$232,543
10.Indirect Costs	$0	$0	$0
11.Total Project Costs	**$141,094**	**$91,449**	**$232,543**

Itemized Budget Justification:

Personnel costs include salary expenses for Texas Farm Bureau Staff to coordinate the collection of scat samples, from private property locations, that will be used to develop the DNA library. Itemized as follows:

Name	Labor Classification	Hourly Labor Rate	Expected Number of Hours
Ned Meister	Director, Commodity and Regulatory Activities – Water Quality	$52.24	120
Don Petty	Associate Director, Commodity and Regulatory Activities – Wildlife	$35.87	80
Jon Johnson	Associate Director, Commodity and Regulatory Activities – Livestock	$32.17	80

Travel costs include expenses for the above-described sampling/scat collection at a mileage rate of 36.5 cents per mile and a per diem (meals only) of $25 per day.

Supplies include sterile sample bottles, petri dishes, sterile media, filters, and other reagents for the culture and enumeration of *E. coli* by membrane filtration.

Contractual costs include a contract with Parsons, Inc., whose budget is described separately. Also included is $2000.00 for additional auditing requirements for the Texas Farm Bureau Agricultural Service Company as well as funding for sample collection by the City of Waco.

Miscellaneous costs include boat use fees, room rental for a workshop, copies, and faxes.

Itemized Budget Justification: Contractual to Parson Engineering.

Personnel: 1832 total staff hours at Parsons reduced rate schedule (attached). These rates include fringe benefits.

Name	Labor Classification	Hourly Labor Rate	Expected Number of Hours
Mel Vargas	Project Manager	$111.20	310
Kirk Dean	Principal Scientist & QA Manager	$86.00	310
Randy Palachek	Supervising Scientist, senior biologist for sanitary survey	$108.10	60
Curt Burdorf	Senior Engineer, sanitary survey and sample collection	$81.60	200
Chris Ryon	Engineer, sample collection	$68.40	200
Kareem Al-Khafaji	Associate Engineer, sanitary survey and sample collection	$57.00	251
Garner Peterson	Associate Scientist, GIS analyst and data manager	$51.60	100
To be assigned	Senior Specialist II, administrative	$55.70	200
To be assigned	Specialist II, administrative	$35.60	201

Travel includes 9,234 total miles at 36.5 cents per mile, 28 total days at a per diem (lodging plus meals) of $85, and 2 roundtrip flights to El Paso from Austin at $250 each for project coordination and to perform a laboratory audit.

Supplies include field sampling supplies, such as live traps (for known source sample collection), sterile sample bottles, ice and coolers, sterile collection swabs and sterile rinse water.

Miscellaneous direct costs include boat rental (11 days at $250 /day), multi-parameter water quality probe rental (11 days at $50/day), overnight shipping of samples to the El Paso laboratory (40 coolers at $50 each), other postage, photocopies, and GIS workstation use fees.