

Texas Water Resources Institute

Support Analytical Infrastructure and Further Development of a Statewide Bacterial Source Tracking Library FY 10 State General Revenue Nonpoint Source Grant Program TSSWCB Project No. 10-50

Quarter no. 2 from 12/1/10 through 02/28/11

I. Abstract

- a. This quarter a meeting was held to discuss and agree on a protocol for isolate identification for TSSWCB projects. The need to expand the number of isolates in the state library was also discussed. Conference planning began in earnest and the committee received input from TDA and TCEQ on the material that they would like to see at the conference.

II. Overall Progress and Results by Task

TASK 1: Project Administration

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.*

The following actions have been completed during this reporting period:

- a. TWRI submitted the 1st Quarter, Year 1 report to TSSWCB on January 14, 2011.

2% Complete

Subtask 1.2: *TWRI will perform accounting functions for project funds and will submit appropriate Reimbursement Forms to TSSWCB at least quarterly.*

The following actions have been completed during this reporting period:

- a. Expenditures thus far have totaled \$54,053 or roughly 12% of total project funding.

12% Complete

Subtask 1.3: *TWRI will host coordination meetings, conference calls, or TTVN meetings with the TSSWCB, AgriLife 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.*

The following actions have been completed during this reporting period:

- a. A meeting was held December 17, 2010 to begin conference planning.
- b. A meeting was held on January 11, 2011 to discuss protocol for a 3, 4 or 6 way split on isolate identification. It has been decided that a six-way split of Human, non-avian livestock (including cattle), avian livestock, non-avian wildlife, avian wildlife, and pets would be useful and scientifically sound for identifying pollution sources for entire watersheds or large segments. Only a 3way split would be used when smaller groups of isolates need to be identified. The current library composition and the Lampasas stakeholder survey of concerns were discussed to determine what additional sources needed to be collected. Also discussed were formats for tables and graphs for future BST reports.

20% Complete

Subtask 1.4: TWRI will develop (Months 1-3), host and maintain (Months 4-24) a website (e.g., <http://bst.tamu.edu>) that will be used as a means 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.

The following actions have been completed during this reporting period:

- a. This quarter, the website <http://texasbst.tamu.edu/> was viewed by 9 unique visitors (2 in December 2010, 4 in January 2011, and 3 in February 2011).

55% Complete

Subtask 1.5: TWRI will work with AgriLife SCSC and AgriLife EP to prepare Technical Reports as required by project Tasks into published technical reports. These reports will be permanently housed in the TWRI online Reports Database.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

TASK 2: Support and maintain BST analytical infrastructure

Subtask 2.1: AgriLife EP and AgriLife SCSC will ensure needed operational and maintenance support for current BST analytical equipment (i.e., RiboPrinter) and general laboratory equipment is executed. This includes securing maintenance contracts, replacement parts and expendable supplies.

The following actions have been completed during this reporting period:

- a. A pricing agreement for RiboPrinter consumables is still undergoing negotiations with DuPont Qualicon.

10% Complete

Subtask 2.2: *AgriLife EP will hire a Postdoctoral Research Associate/Research Associate that will maintain laboratory operating capacities and technical expertise to conduct BST studies across the state. This FTE is intended to provide support on TSSWCB-funded projects in the Leon River, Lampasas River, and Buck Creek watersheds (projects 10-51, 06-11, 06-07).*

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

100% Complete

Subtask 2.3: *AgriLife SCSC will hire a Postdoctoral Research Associate that will maintain laboratory operating capacities and technical expertise to conduct BST studies across the state. This FTE is intended to provide support on TSSWCB-funded projects in the Little Brazos River tributaries, Big Cypress Creek, and Attoyac Bayou watersheds (projects 09-52, 09-55, 09-10).*

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

100% Complete

TASK 3: Quality Assurance

Subtask 3.1: *AgriLife SCSC and AgriLife EP will maintain and update (at least annually) statewide BST SOPs for ERIC-PCR, RP 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.*

The following actions have been completed during this reporting period:

- a. AgriLife SCSC and AgriLife EP have refined ERIC-PCR gel imaging protocols to ensure consistency between the different lab imaging systems.

20% Complete

Subtask 3.2: *AgriLife SCSC and AgriLife EP will coordinate to ensure that needed personnel training is kept on par between the groups to ensure congruity statewide.*

The following actions have been completed during this reporting period:

- a. a. AgriLife SCSC and AgriLife EP are working together to explore an atypical *E. coli* mTEC phenotype that we have found in watersheds across the state.

20% Complete

Subtask 3.3: *AgriLife SCSC and AgriLife EP will work with AgriLife BAEN, USDA-ARS, USDA-NRCS WRAT, and others to develop strategies for 1) reconciling BST and modeling results (SELECT, SWAT, etc.) and 2) using BST in model development, calibration, and validation.*

The following actions have been completed during this reporting period:

- a. AgriLife SCSC, AgriLife EP and a modeler (Karthikeyan) are collaborating on a paper comparing BST and modeling results.

20% Complete

Subtask 3.4: AgriLife EP and AgriLife SCSC will work with public and private laboratories across the state which are exploring the use of BST. AgriLife 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 AgriLife EP and AgriLife SCSC (subtask 3.1).

The following actions have been completed during this reporting period:

- a. AgriLife EP gave comment on Leon WPP draft report to correct reporting of BST results (via Mel Vargas)

10% Complete

Subtask 3.5: AgriLife EP and AgriLife SCSC will work with AgriLife BAEN, USDA-ARS, USDA-NRCS WRAT, USGS, TIAER, and selected river authorities to define appropriate ambient water sampling protocols to provide desired statistical confidence with BST findings. The level of sampling adequate for statistical characterization of sources and determination of environmental conditions influencing source contributions (often wet versus dry weather conditions) will be described.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

TASK 4: Promotion of BST

Subtask 4.1: AgriLife SCSC with assistance from TWRI will develop a publication that describes the extent of BST work conducted to date in Texas. This report should compare and contrast methodologies and results. This publication should build on the discussion in the Task Force Report.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Subtask 4.2: TWRI will develop flyers, one-pagers, tri-folds or other appropriate printed media that can be used to 1) promote 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) promote the analytical lab capability of public BST labs which the state has invested. Printed media should be appropriately developed for each target audience including, but not limited to, 1) state and federal agencies with water quality responsibilities and jurisdictions (TCEQ, TPWD, DSHS, GLO, USEPA (Region 6 and GOMP), USGS, NOAA), 2) political subdivisions of state government (municipal, county), including TML and TAC, that may be involved in watershed planning processes focused on abatement of bacterial contamination, 3) livestock producer organizations such as TFB, TSCRA, ICA, TAD, TCFA, TSGRA, TPPA, TPF, TWA, 4) private and public water quality labs, and 5) the general public. As appropriate, TWRI will include information about BST in general, and this project specifically, in the tx H2O, New Waves e-letter, AgriLife News.

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Subtask 4.3: *TWRI, AgriLife EP, and AgriLife SCSC will promote BST by making presentations at conferences, workshops, seminars and other appropriate venues such as WEF/WEAT, TSCRA/TFB/TWA Annual Conventions, ASABE, TCEQ Environmental Trade Fair.*

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Subtask 4.4: *TWRI, AgriLife EP and AgriLife SCSC will work to inform other researchers/academia who are engaged in BST in Texas (Edrington, Brinkmeyer, Alam) about the methods and approaches recommended by the Task Force and being implemented by the State.*

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

TASK 5: Texas *E. coli* BST Library expansion, PCR marker development/refinement and *E. coli* isolate selection method evaluation

Subtask 5.1: *AgriLife EP and AgriLife SCSC will isolate E. coli from known source fecal samples. Known source fecal samples should primarily fill gaps identified in other TSSWCB-funded BST projects, as well as, archived known sources identified through subtask 5.5, and gaps identified through subtask 6.3. Approximately three isolates from each fecal sample 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 will be further analyzed using RP for inclusion in the Texas E. coli BST Library. AgriLife EP and AgriLife SCSC will equitably split workload.*

The following actions have been completed during this reporting period:

- a. 40 Known-source (wildlife) *E.coli* isolates from ‘*Fate and Transport of E.coli in rural Texas Landscapes and Streams*,’ conducted by Dr. Karthikeyan in the Cedar Creek watershed were archived for BST downstream applications.
- b. Current state library composition sorted by source and watershed was used to determine gaps in the library. (questions about possum, aquatic animals—nutria and beaver—sewage vs septage and dairy vs beef cattle were raised. Need more avian livestock!!)
- c. As part of this project the Leon and Lampasas studies will increase the number of sources samples collected from 100 total to 200 total. Currently a total of 21 *E. coli* positive samples from the Leon and Lampasas studies have been collected; 82 isolates have been archived, 52 isolates have been screened by ERIC-PCR and 30 isolates have been selected for the local libraries which will then be screened for inclusion in the Texas *E. coli* BST library.

15% Complete

Subtask 5.2: AgriLife EP and AgriLife SCSC will quantify species-specific bacteria production (E. coli and Enterococcus) in feces and measure the variability of this production. While bacteria content of feces has been reported in literature for some species and has been summarized in some reports used in Texas bacteria projects, often this information has been limited to fecal coliform. Known source fecal samples from subtask 5.1 should be used.

The following actions have been completed during this reporting period:

- b. No activity to report this quarter.

0% Complete

Subtask 5.3: Utilizing known source fecal material, AgriLife SCSC and AgriLife EP will utilize the best available bacterial indicators to further develop and refine species-specific bacteria markers for Bacteroidales PCR. Specifically, efforts will be made on markers to 1) differentiate between ruminants (primarily cattle and deer), 2) identify poultry, and 3) differentiate between domestic swine and feral hogs.

The following actions have been completed during this reporting period:

- b. No activity to report this quarter.

0% Complete

Subtask 5.4: AgriLife SCSC and AgriLife EP will coordinate to conduct comparison studies to evaluate differences in E. coli isolate selection using isolation methods EPA 1603, EPA 1604 and the IDEXX methods. Six water samples will be processed using the EPA 1603 and 1604 methods and the IDEXX method. Ten E. coli isolates per sample per enumeration/isolation (total of 180 isolates) will be analyzed using the ERIC-RP. The suitability of utilizing E. coli isolates processed using methods other than EPA 1603 in BST will be determined.

The following actions have been completed during this reporting period:

- b. No activity to report this quarter.

0% Complete

Subtask 5.5: AgriLife EP and AgriLife SCSC will work to quantify the extent (quantity) and nature (method) of known source library samples that have been collected by out-of-state labs (Harwood @ University of South Florida, Source Molecular Corp., IEH Laboratories & Consulting Group, Ellender @ University of Southern Mississippi) and other in-state labs (Brinkmeyer @ TAMU-Galveston) for Texas BST projects. To the extent practical and appropriate, AgriLife EP and AgriLife SCSC will work to incorporate known source library samples from these 3rd party labs into the Texas E. coli BST Library. AgriLife EP and AgriLife SCSC and will work with AgriLife BAEN to incorporate known source fecal samples collected through TSSWCB project 07-06 into the Texas E. coli BST Library.

The following actions have been completed during this reporting period:

- a. Dr. Mott prepared a list of isolates and pertinent source information. AgriLife EP selected the isolates of interest from the list and is awaiting isolates.

10% Complete

Subtask 5.6: *TWRI, AgriLife EP and AgriLife SCSC will collaborate to 1) expand and update on the list of BST-related R&D activities identified by the Task Force, and 2) prioritize the updated list of BST-related R&D activities.*

The following actions have been completed during this reporting period:

- b. No activity to report this quarter.

0% Complete

TASK 6: BST workshop and state of the science conference delivery and development

Subtask 6.1: *AgriLife SCSC and AgriLife 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 meetings, seminars and workshops, as appropriate, to learn of new and improved BST methods being employed elsewhere.*

The following actions have been completed during this reporting period:

- a. No activity to report this quarter.

0% Complete

Subtask 6.2: *TWRI, AgriLife SCSC and AgriLife EP will cooperate to establish a Conference Planning Team that serves as the advisory committee for planning a statewide BST workshop (subtask 6.4) and agency specific meetings. Planning activities will include setting meeting agendas, providing information for the development of meeting materials and identifying invited speakers for the statewide BST workshop.*

The following actions have been completed during this reporting period:

- a. A BST Conference Planning meeting was on December 17, 2010 to discuss members of the Conference Planning Committee:
 - i. Dr. George Di Giovanni, Texas AgriLife Research Center at El Paso (Chair)
 - ii. Dr. Terry Gentry, Texas A&M University
 - iii. Dr. Joanna Mott, Texas A&M University – Corpus Christi
 - iv. Dr. R. Karthikeyan, Texas A&M University
 - v. Dr. R. Srinivasan, Texas A&M University
 - vi. Dr. Jorge Santo Domino, U.S. Environmental Protection Agency
 - vii. Dr. Valerie (Jody) Harwood, University of South Florida
- b. Formal invitation letters were sent to the Planning Committee on December 20. The program coordinator also worked with Planning Committee to establish the date for the Conference – February 28-29, 2012.
- c. The Conference was added to the project website (<http://texasbst.tamu.edu/>) and will be updated with information as it becomes available.
- d. The program coordinator continued in the conference venue search and worked with the Planning Chair, Dr. Di Giovanni to narrow venue options:
 - i. Embassy Suites San Marcos
 - ii. T Bar M Resort/Conference Center, New Braunfels
- e. Discussions were underway during this quarter with the venues, as well as other venues that could possibly accommodate the needs of the Conference.
- f. The project manager submitted a request to state and federal agencies on February 8 to give them the opportunity to provide input and gain perspective in regards to the

- 2012 Conference and what they would like to see at the conference.
- i. Input from the Texas Department of Agriculture and the Texas Commission on Environmental Quality's Water Quality Planning Division was received (by March 1) and emailed to the Conference Planning Committee on March 3 for further review.
 - ii. Additional comments from the Planning Committee were sent to Chair, Dr. Di Giovanni, to take the lead on preparing a conference outline.
 - iii. A conference call to discuss further will be scheduled for next quarter.
- g. Planning Committee will develop an outline for the conference and then discuss with the rest of the project partners and TSSWCB.

30% Complete

Subtask 6.3: TWRI will work to coordinate BST meetings with targeted agencies (TCEQ, TDA, TPWD, GLO, DSHS, USEPA, and selected river authorities). The intent of these meetings is further the understanding of agency staff on BST and to develop action strategies to address issues raised by agency staff regarding the validity of BST in general, and methods and the Texas E. coli BST Library particularly.

The following actions have been completed during this reporting period:

- c. No activity to report this quarter.

0% Complete

Subtask 6.4: TWRI will coordinate the planning of a statewide BST workshop to be held in Austin in summer/winter 2011/2012. The purpose of this workshop will be to 1) highlight the extent of BST work that has been and is being conducted in the state, 2) discuss the scientific advances and improvements in the application of BST, and 3) identify research needs to further the science of BST. TWRI will handle all meeting logistics, speaker invitations, meeting materials preparation and advertising/promotion of the meeting.

The following actions have been completed during this reporting period:

- a. See subtask 6.2

15% Complete

III. Related Issues/Current Problems and Favorable or Unusual Developments

- a. Current state library composition sorted by source and watershed was used to determine gaps in the library. (questions about possum, aquatic animals—nutria and beaver—sewage vs septage and dairy vs beef cattle were raised.)
- b. More avian livestock samples are needed.

c. Projected Work for Next Quarter

- a. Conference venue will be decided.
- b. Planning Committee will develop an outline for the conference and then discuss with the rest of the project partners and TSSWCB.
- c. Collection of samples will continue and be expanded.