

Bacterial Source Tracking (BST)

Citizens Advisory Group US EPA Gulf of Mexico Program

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Presentation Topics



- What is Bacterial Source Tracking?
- Scope of the Problem
- A National Collaboration
- Point vs Non Point Sources
- What is the State of the Science?
- One well developed method.
- How is the technology used to make a decision?
- Deliverables
- Promise of the technology
- Possible Role of the Citizens Advisory Group in Bacterial Source Tracking

Definition of Bacterial Source Tracking

- The determination of the animal origin of fecal bacteria in natural waters that result from point or non point pollution
- Directed analysis and remediation



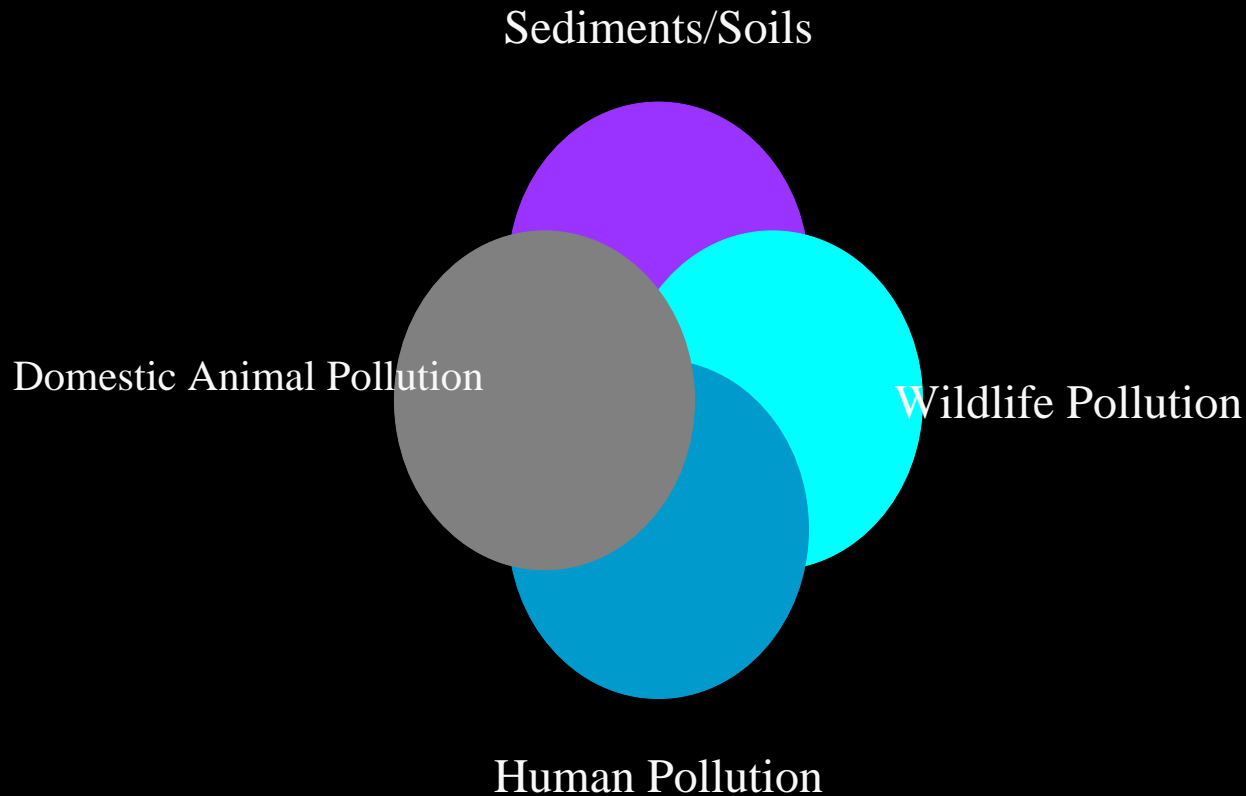
The world of water pollution

- Boil water notices
- Beach closings
- Closed shellfish waters
- Legal issues – i.e. human vs. bovine?
- Disease risk
- Regulation issues
- The problem of wildlife

BACTERIAL SOURCE TRACKING

Take home messages: expect complex mixtures of organisms,
do not expect instant solutions, expect interest to remain high

Best Current Results: **Local**, State, Regional, National, International



Overall Goals

- To develop appropriate BST methods which are selective, sensitive and cost effective...which
- Determine the source(s) of pollution of water...and
- Give federal and state regulatory agencies the ability to control and remediate pollution



Scope of the Problem



National Concerns:

The Problem

Origin of fecal pollution of water

The Need

Identify methods & indicators

Define local, State, Regional and national protocols

The Solution

Effective Bacterial Source Tracking Database(s) & Methods

Federal/State Concerns:

Effect on Agricultural Activities

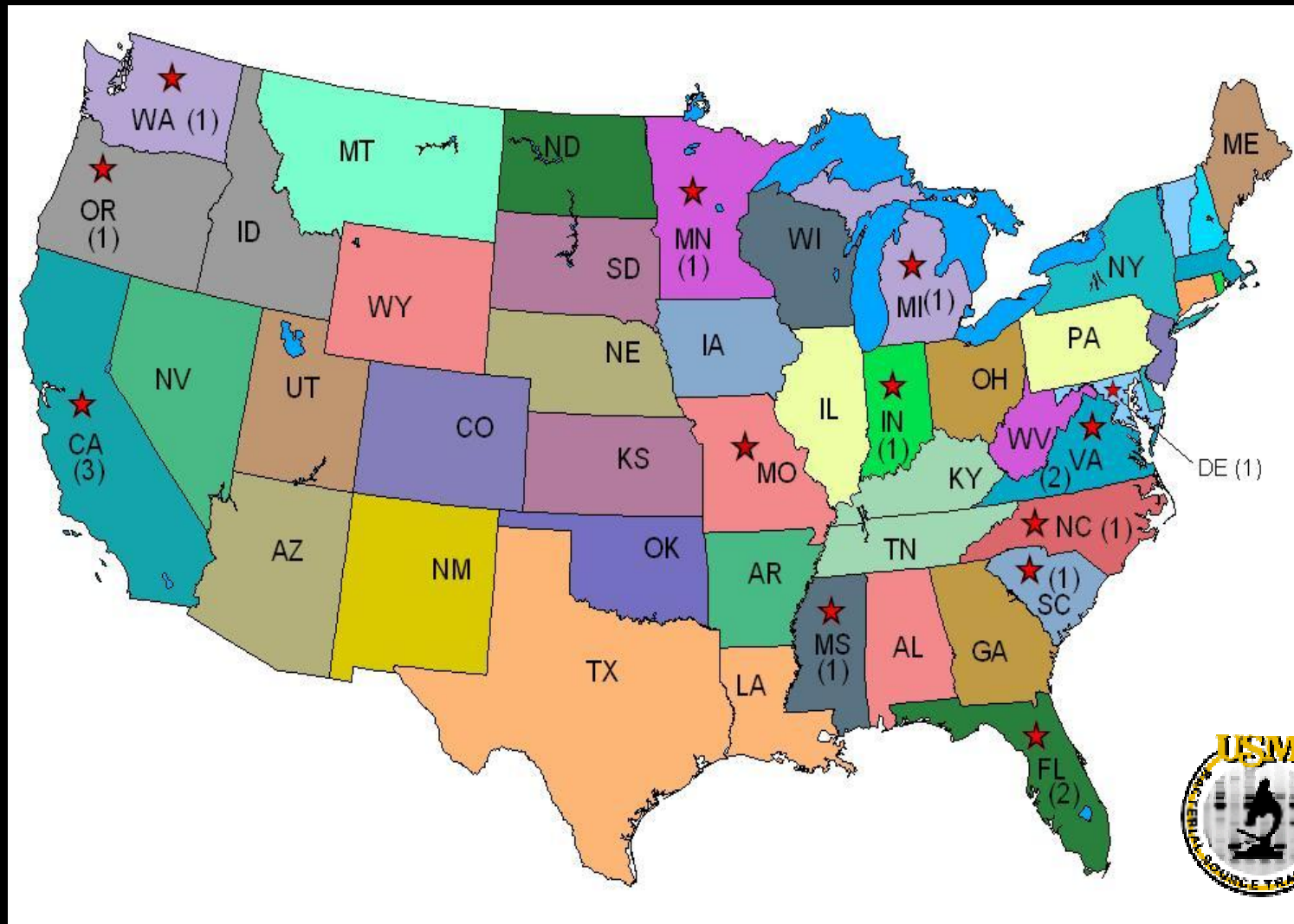
Effect on Economic Development

TMDL surveys

Beach Closing

Health Risk to Population

BACTERIAL SOURCE TRACKING A NATIONAL EFFORT



Bacterial Source Tracking

- *Federal-State Collaborators/Interested Partners*
 - US EPA Gulf of Mexico Program
 - NOAA Coastal Assistance Impact Program
 - US Department of Agriculture
 - Sea Grant Program
 - US Geological Survey
 - Southern California Coastal Water Resources Authority

 - Departments of Agriculture
 - Departments of Environmental Quality
 - Departments of Wildlife
 - Departments of Marine Resources
 - Departments of Health



Bacterial Source Tracking

- **Point vs. Non point sources**

- **Point sources: readily identifiable input where waste is discharged to a receiving water**

- **Examples:**

- Accidental spills, Waste Lagoons, Confined Animal Feed Lots, Garbage Transfer Stations, Land Application Sites, Lift Stations, Marinas, Sewer Pipelines crossing over streams, Water Treatment Plants, Wastewater Treatment Facilities, Septic tanks

- **Non point sources represent an ever increasingly important component of pollution as point sources are identified and controlled.**



BACTERIAL SOURCE TRACKING

POINT SOURCES



INDUSTRIAL WASTE



DAIRY FARMING



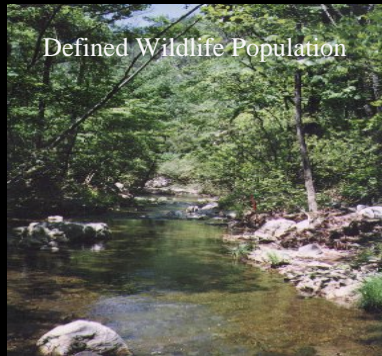
AQUACULTURE



MUNICIPAL WASTE



Defined Wildlife Population



POULTRY OPERATIONS



Bacterial Source Tracking

- **Nonpoint source pollution: Pollution from diffuse sources associated with rainfall, snowmelt, tidal action, water moving over or through the ground**
- **Examples:**
 - crops, livestock manure lagoons
 - construction, dredging, sludges, logging, urban runoff, sediments, pet wastes
 - wildlife
 - faulty septic systems
- **Non-point sources represent the most formidable challenge for research and regulation**



BACTERIAL SOURCE TRACKING NON-POINT SOURCES



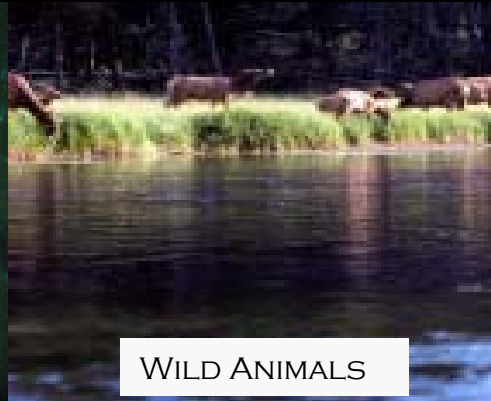
MARINE FISH



PLANT MATTER



WILD ANIMALS



SHORE BIRDS



© Don Getty

MIGRATING BIRDS



Sooty Shearwaters, Colac Bay, New Zealand, copyright Duncan Poyser

SEDIMENTS



State of the Science



- Decision Tree (many current ways to proceed)
 - Microbe Selection
 - Genotypic methods vs. Phenotypic methods
 - Fingerprint methods - PCR, TRLP, Ribotyping, ARA, CSU,
 - Library vs. Non library methods
 - Type of statistical analysis

State of the Science – personal observations



- Enterococci preferable to E. coli
- Genotypic methods preferable to phenotypic methods
 - PCR preferable to ribotyping
- Library vs. non library methods
 - Library methods require considerable effort (no. isolates, manpower) but are being used by many researchers
 - Non library methods save considerable time and expense but are poorly developed at present

BASIC STEPS IN RESEARCH PROCESS

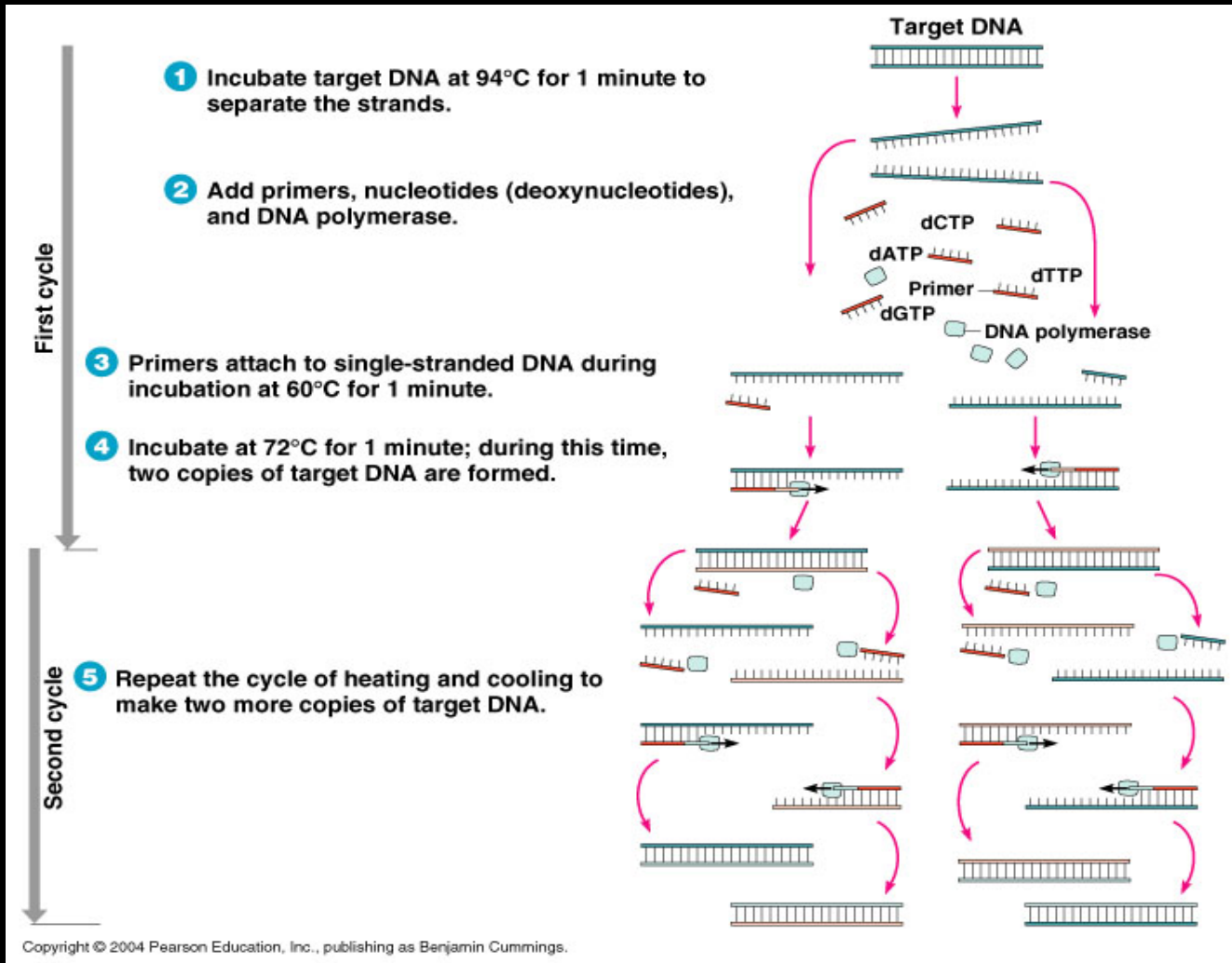
- Build a library of animal samples and isolates
- Focus on animals most likely to affect environment
- Utilize the most important indicator species
- Fingerprint isolates using the best methods
 - Reliability; Statistically significant measures
- Apply the best analysis software
- Test the protocols with blind known isolates
- Refine the protocols as needed
- Apply the methods to unknown samples from environmental samples
- Transfer knowledge to Federal/State agencies



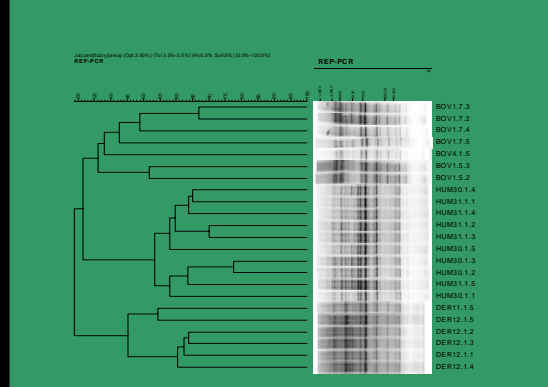
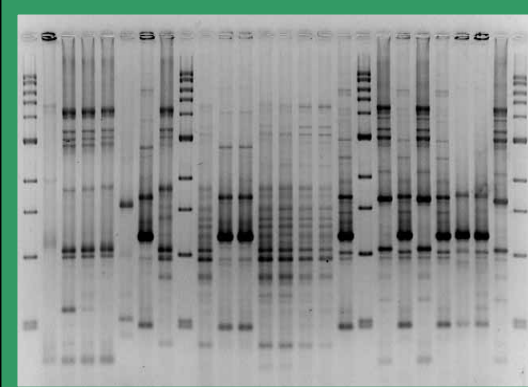
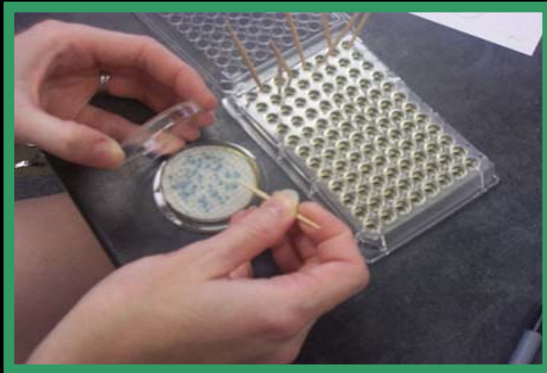
One method in detail

- PCR: How is it done?

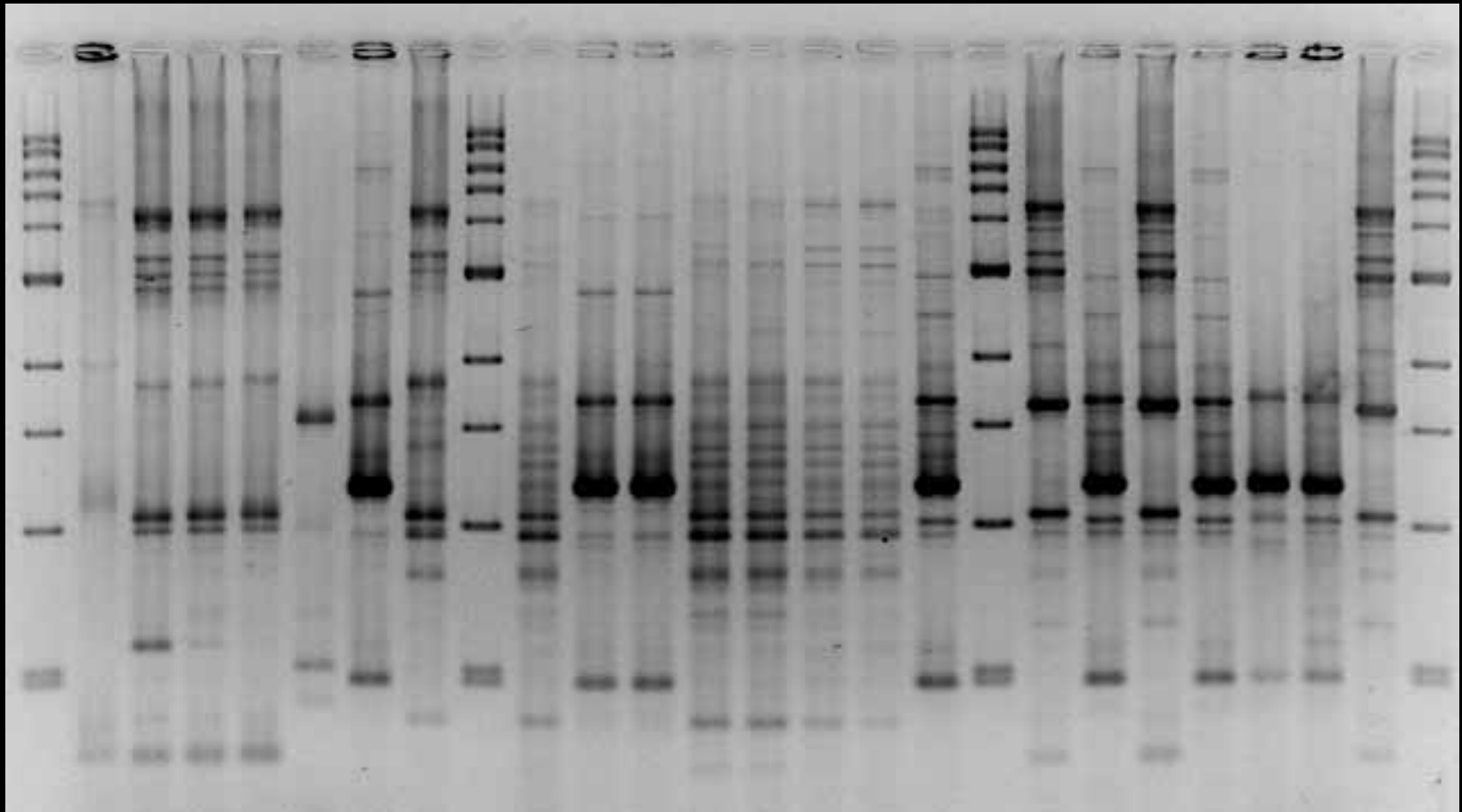
Polymerase Chain Reaction



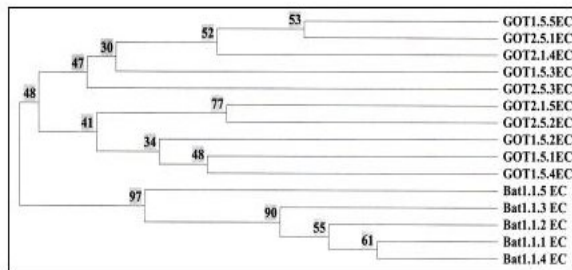
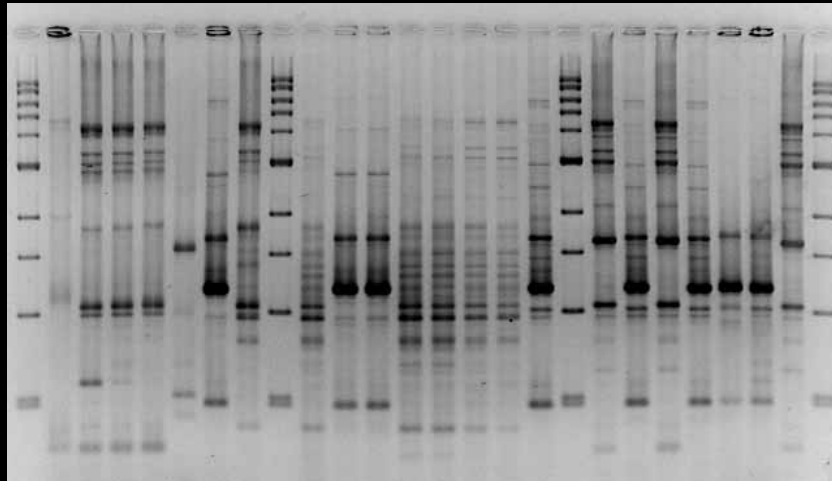
From Isolation to Cladogram



BOX Fingerprint



BACTERIAL SOURCE TRACKING



Dendrogram showing the relatedness of the E coli isolates

→ GEL FINGERPRINT



DIGITIZE ISOLATE BAND PATTERN



IDENTIFY/CONFIRM BANDS



PLACE BAND DATA INTO
BIONUMERICS



COMPARE ISOLATES USING
STATISTICAL

STRATEGIES (DENDOGRAMS;
CLUSTER

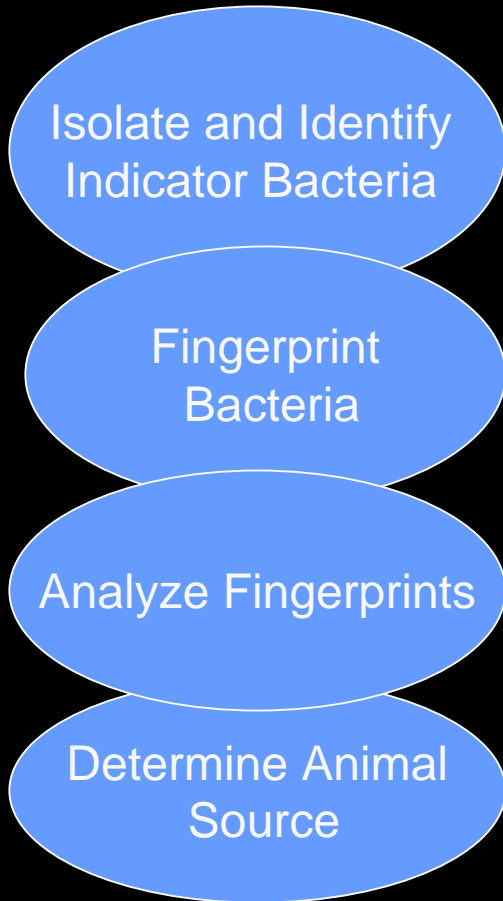
ANALYSIS)



Bacterial Source Tracking

- Using the data to make decisions

IDENTIFY SOURCE OF POLLUTION IN A WATER QUALITY QUESTION



- Perception of water quality problem
- Source tracking identifies the origin
- Proactive, remedial actions taken
- Management occurs
- Situation improves



IDENTIFY SOURCE OF POLLUTION IN A WATER QUALITY QUESTION



- Proactive, remedial actions
 - Fence installed to limit animal access to water body
 - Faulty septic system repaired
- Management of the problem
 - Agency analyzes problem and implements change
- Situation improves
 - Decrease in bacterial levels in water; drop in the cost of sample analysis

Application to State Problems - Current



- Hines County Farm study
 - Quality of lake water in isolated system
- Gulfport Street Study
 - Beach closures along MS Gulf Coast
- Leaf River analysis ⇒
 - Testing value of database
- Extension service work
 - Possible watershed contamination



Application to Water Quality



- Lake water contamination summary
 - Database of 87 known isolates from cattle and geese
 - 40 unknown water samples
 - >90% compatibility with highest degree of confidence
 - 3 isolates traced with high degree of confidence to cattle and geese
 - Proof that the process works

Application to Beach Closures



- Gulfport street (old sewerage conduits)
 - Known watershed boundaries
 - Sample taken from lift stations, manholes, dogs, seagulls, beach isolates, closure isolates
 - Limit = $>90\%$ compatibility with highest degree of confidence
 - 22 beach closure isolates from watershed yielded indicated that 15 showed the highest degree of confidence and were classified as seagull and sewage origin

Possible Application to State Needs



Wildlife, Fisheries and Parks

- Tourism, State parks (Flint creek)

• Health

- Tracking pathogens

- bioterrorism

• Marine Resources

- Source of fecal coliforms in oyster growing waters.

• Public Service

- Work force development (Crime Lab)

Deliverables

- Immediate assistance to state and local personnel on BST issues
- Statewide database
- Guidelines for regulators
- Technical expertise
- Training in DNA analysis
- Assistance to state military infrastructure on bioweapons fingerprinting
- Applications of BST to other state issues such as bioremediation, and pathogen tracking
- Positive impact on Economic Development



BACTERIAL SOURCE TRACKING – SUMMARY

WHAT IS THE PROMISE OF THE TECHNOLOGY?

- **Excellent chance of success = Automation**
 - Integrate Typhoon DNA analysis
 - Automate the PCR reaction
 - Develop BST Robotics as a means of reducing manual labor, allowing faster sample processing and data analysis, producing better coverage of the State's water resources.
 - Additional studies in which technology is taken “to the field” to demonstrate the value of the technology.



BACTERIAL SOURCE TRACKING

- WHAT MIGHT BE THE ROLE OF THE CITIZEN ADVISORY GROUP IN THE FUTURE OF BST?
 - √ SUPPORT AN EXPANDED RESEARCH FOCUS
 - √ ASSIST FORMATION OF A CENTER FOR BST RESEARCH TO EXIST FOR ALL GULF STATES TO SERVE AS A PROCESSING CENTER, DATA REPOSITORY AND ANALYSIS FOR ANY STATE WISHING TO SUBMIT A SAMPLE — NOMINAL FEE — CENTER TO ALSO SETUP BEST METHODS OF DOING BST FOR EVERYONE
 - √ POLITICAL — ASSISTANCE IN THE FORMATION OF A GULF STATES BST RESEARCH NETWORK



Web Based – BST Information



www.quickbase.com Information concerning samples and isolates used in this investigation can be found at **<http://www.quickbase.com>**. Go to this site, register, and send an e-mail to Dr. Shiao Wang (Shiao.Wang@usm.edu) to let him know the name you used to register under. He will then instruct Quickbase to give you access to the database. You will receive an e-mail notification that you have access. Click on the link and you'll see the list of samples and isolates. The list is updated regularly.

www.usm.edu/bst Our BST website containing methods and results, comments and other critical information about this effort in Mississippi. On line teaching through this site is being developed.

BACTERIAL SOURCE TRACKING



Thank you for this opportunity

R.D. Ellender