Use of Bacterial Source Tracking for Characterization of Watersheds

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TEXAS A&M AGRILIFE RESEARCH

Texas Water Resources Institute
make every drop count

UTHealth | School of Public Health
The University of Texas Health Science Center at Houston

What is Bacterial Source Tracking?

- Used to determine the sources of fecal contamination
- Based on uniqueness of bacteria from individual sources
- A variety of different methods are used
- Often works best as part of a “toolbox approach”

BST Target Organisms

- **Bacterial v. Microbial Source Tracking**
- **Different targets:**
  - ***E. coli***
  - ***Bacteroidales***
  - **Bacteriophage**
  - **Human viruses**
  - **Animal cells**
  - **Chemicals**

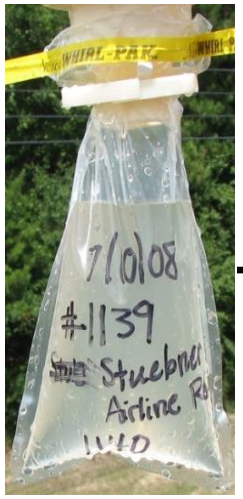
BST Approaches

- **Culture-based (library-dependent)**
  - Isolate bacteria
  - Phenotypic/genotypic characterization
  - Compare to isolates from known-source samples
- **Marker-based (library-independent)**
  - Extract DNA from samples
  - Use PCR-based methods to detect/quantify source-specific markers
- **Sequencing-based**
  - 16S rRNA gene, metagenomic

History of BST Use in Texas

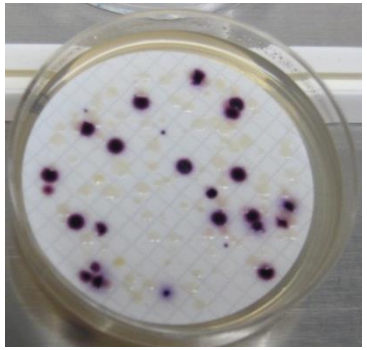
- **Lake Waco/Belton Project Findings**
  - **Initiated Sep. 2002 with funding from TSSWCB**
  - **4-method composite performed better than individual methods**
  - **2-method composites appeared promising**
    - **ERIC-ARA = lower cost but more sample & data processing**
    - **ERIC-RP = higher cost but automated**
- **TMDL Task Force Report – 2007**
  - **Confirmed ERIC-RP as recommended method**

Use of Texas *E. coli* BST Library for Identifying Water Isolates



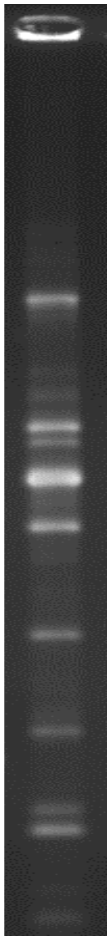
Isolate

E. coli



DNA

Fingerprint



Compare

to Library

Source ID

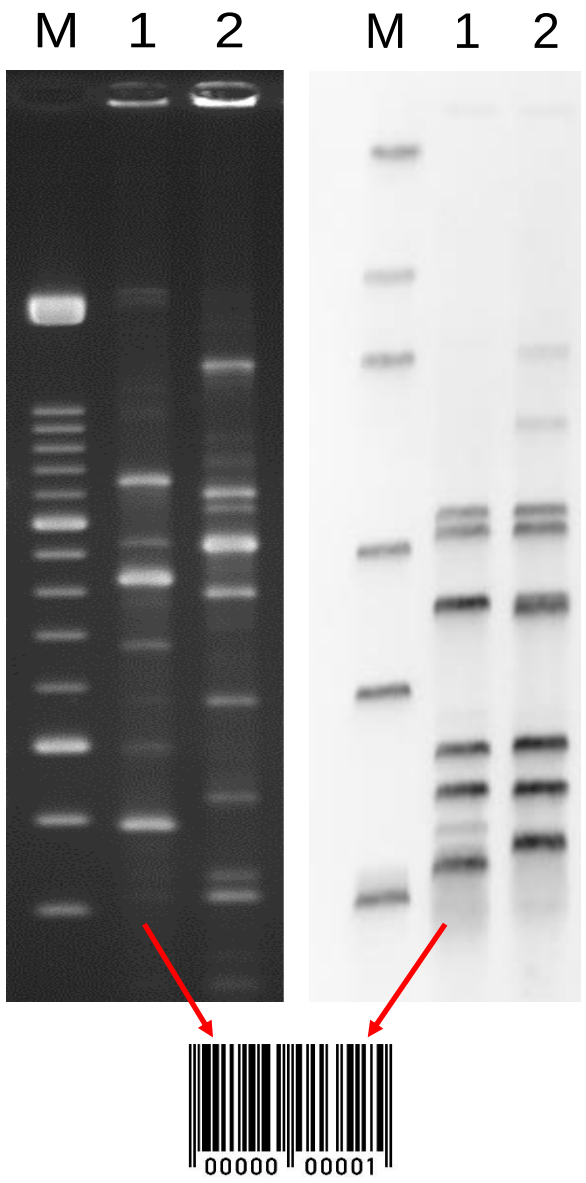
Library-Dependent BST Methods

Methods:

- DNA fingerprinting
  - Enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction (ERIC-PCR)
  - RiboPrinting® (RP)

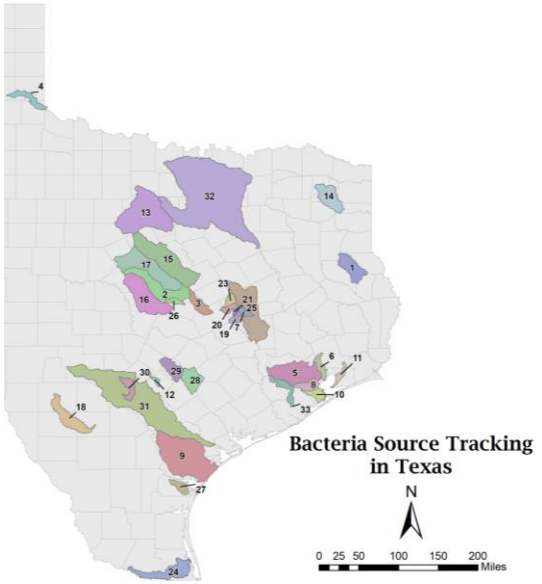
Advantages/Disadvantages:

- More discriminating
- Allows ranking of sources
- Relatively expensive



Texas *E. coli* BST Library (v. 03-20)

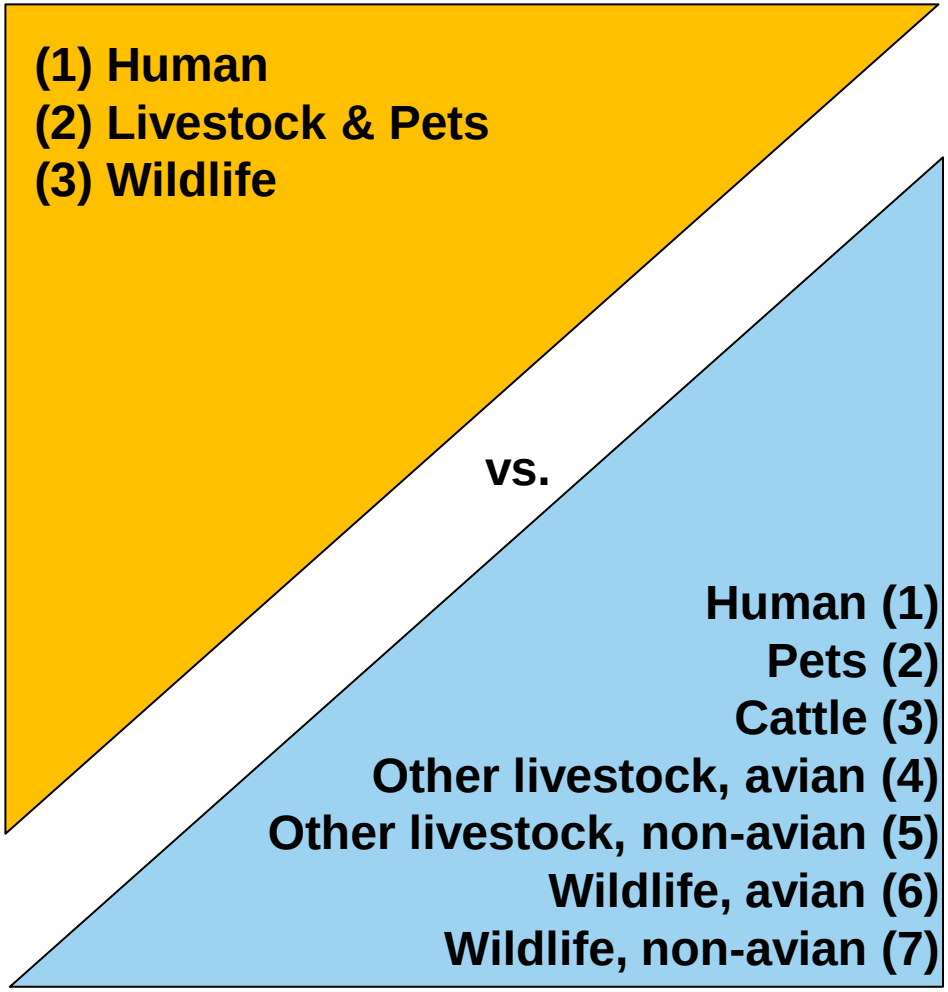
- **Contains 1,912 *E. coli* isolates from 1,653 different human and animal samples**
- **Developed by collecting over 4,000 domestic sewage, wildlife, livestock, and pet fecal samples and screening over 7,000 isolates for clones and host specificity**
- **Samples from >20 watersheds across Texas for BST including:**
  - **Plum Creek**
  - **San Antonio**
  - **Lake Granbury**
  - **Oyster Creek / Trinity River**
  - **Waco / Belton Lake**
  - **Little Brazos River Tributaries**
  - **Attoyac Bayou**



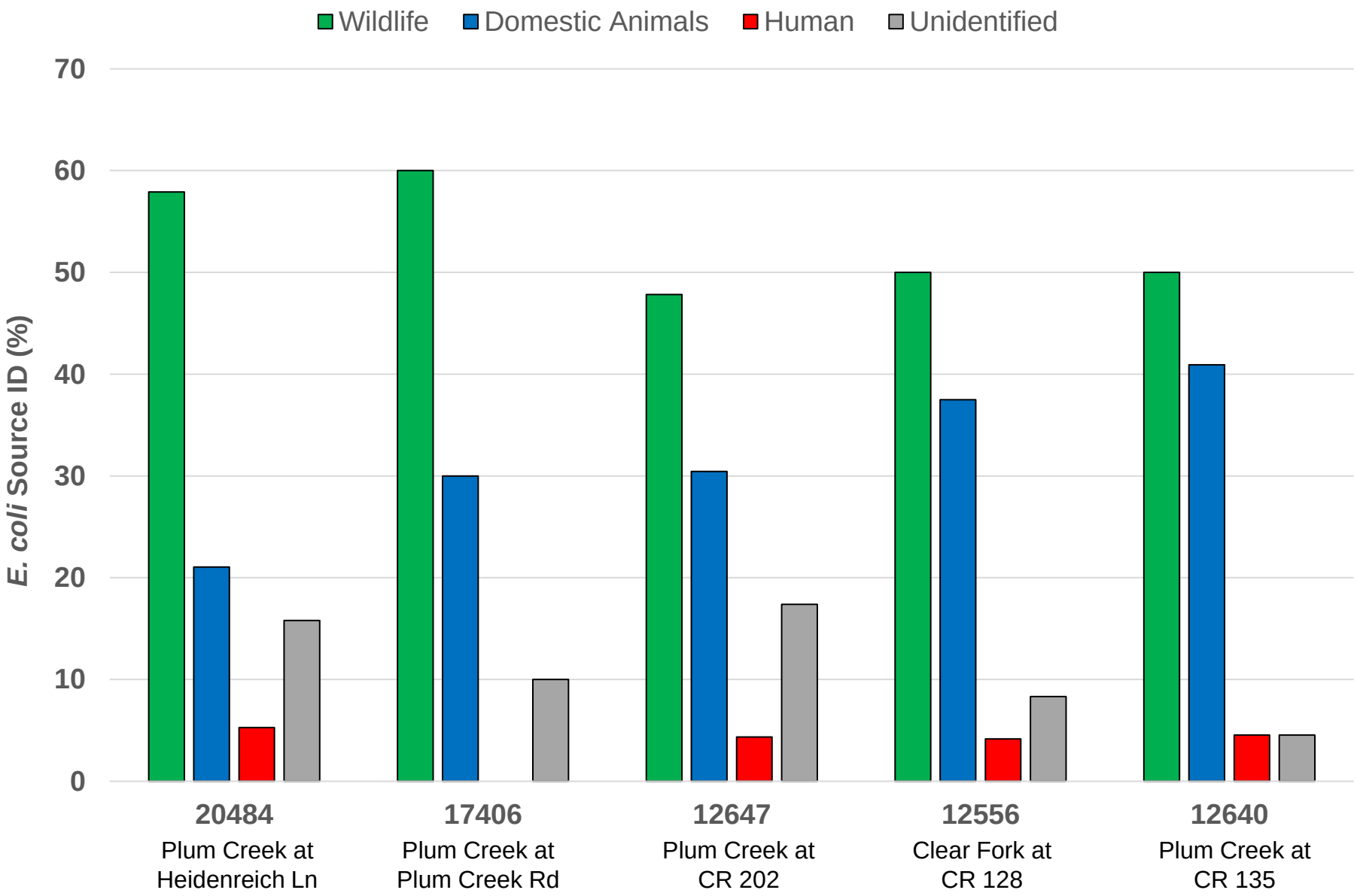
- **Additional isolates being added from ongoing and future BST projects in other areas of Texas**

Three-way v. Seven-way Split of Results

- **Using the results**
  - Is it from human sources?
  - Is it from livestock?
  - Is it from wildlife?
- **Biology**
  - Large variety of wildlife
  - Geographical and temporal differences
  - Cosmopolitan strains
- **Statistics**
  - Number of isolates collected
  - May only use three-way split for limited studies



Plum Creek BST Results
5 Sampling Sites (3-Way Split)



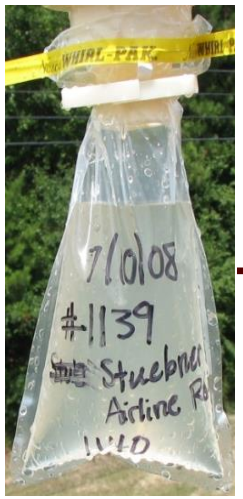
Library Independent BST

- Most common approach targets *Bacteroidales*
- *Bacteroidales* – human and animal fecal bacteria, more abundant than *E. coli*
- Markers available for
  - Ruminants (cattle, deer, elk, sheep, horses, llama)
  - Humans
  - Horses
  - Birds
  - Hogs
- Limited markers for wildlife
- Relationship to *E. coli* and pathogens uncertain
- Some highly specific, but tradeoff between specificity and sensitivity

Brevibacterium LA35 Poultry Marker

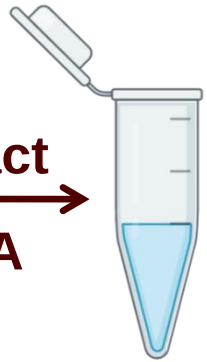
- Developed by Harwood lab at University of South Florida (Weidhaas et al., 2013)
- Tested samples from eastern, central, and southern Texas
  - 58 poultry fecal and litter samples
  - 119 livestock and wildlife fecal samples
- Results
  - Poultry litter (48/58 positive = 83% sensitivity)
  - Non-target (1/119 positive = >99% specificity)

Library Independent BST



Extract

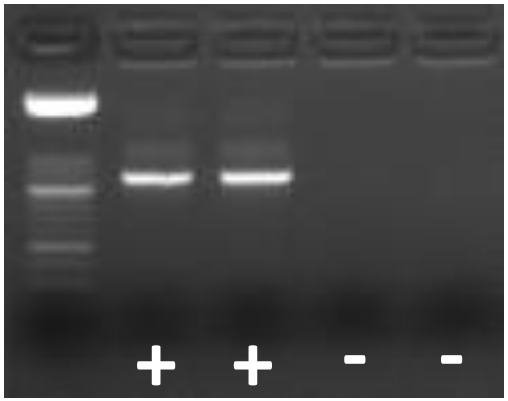
DNA



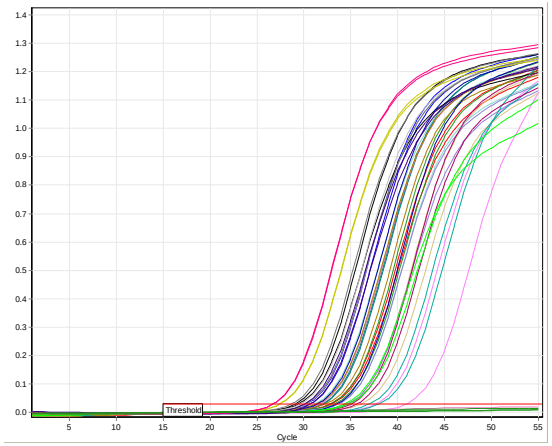
PCR amplify

target sequence

Presence/ Absence



Quantitative



<u>Advantages</u>:

- **Cost**
- **Time**

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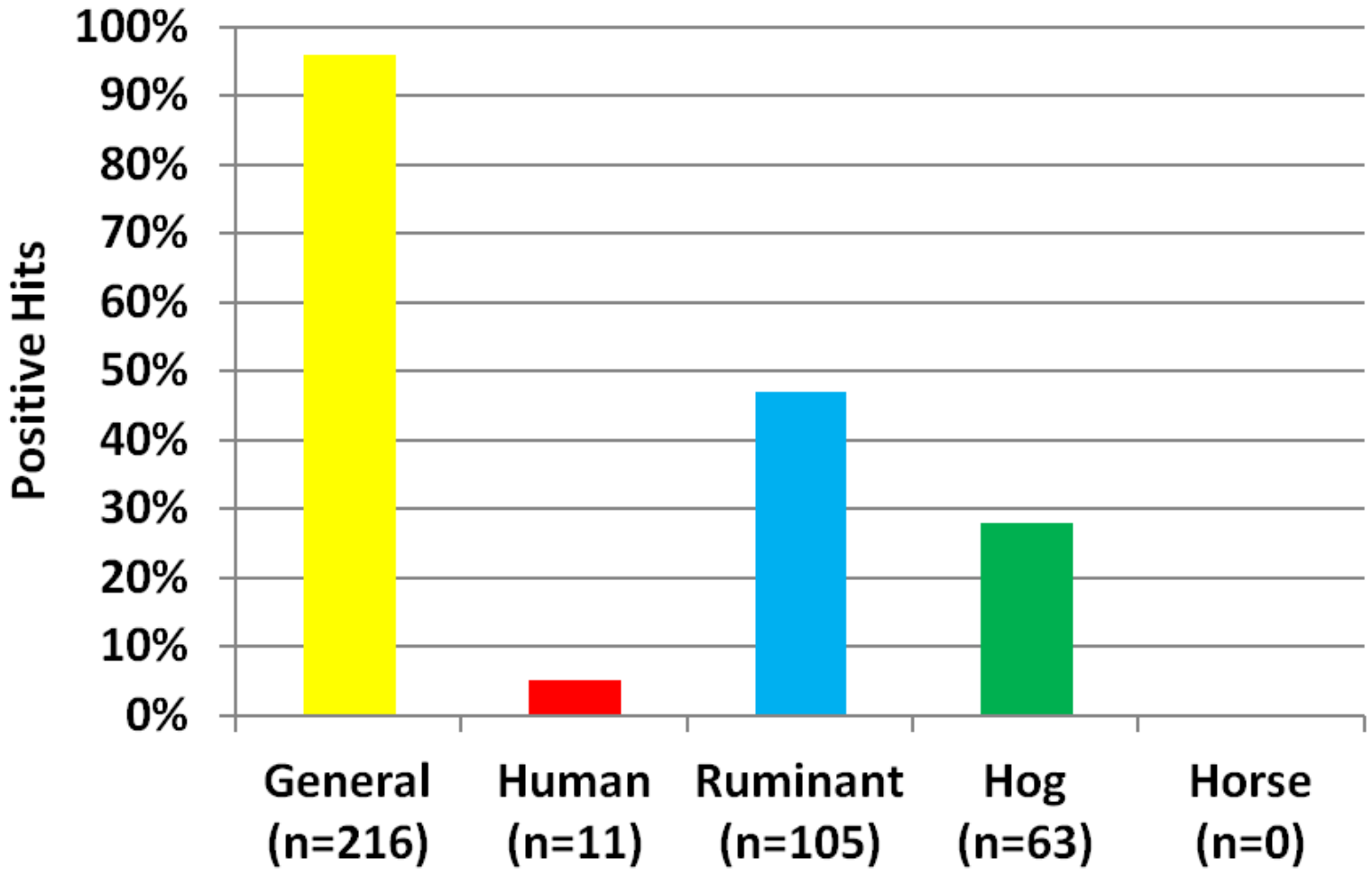
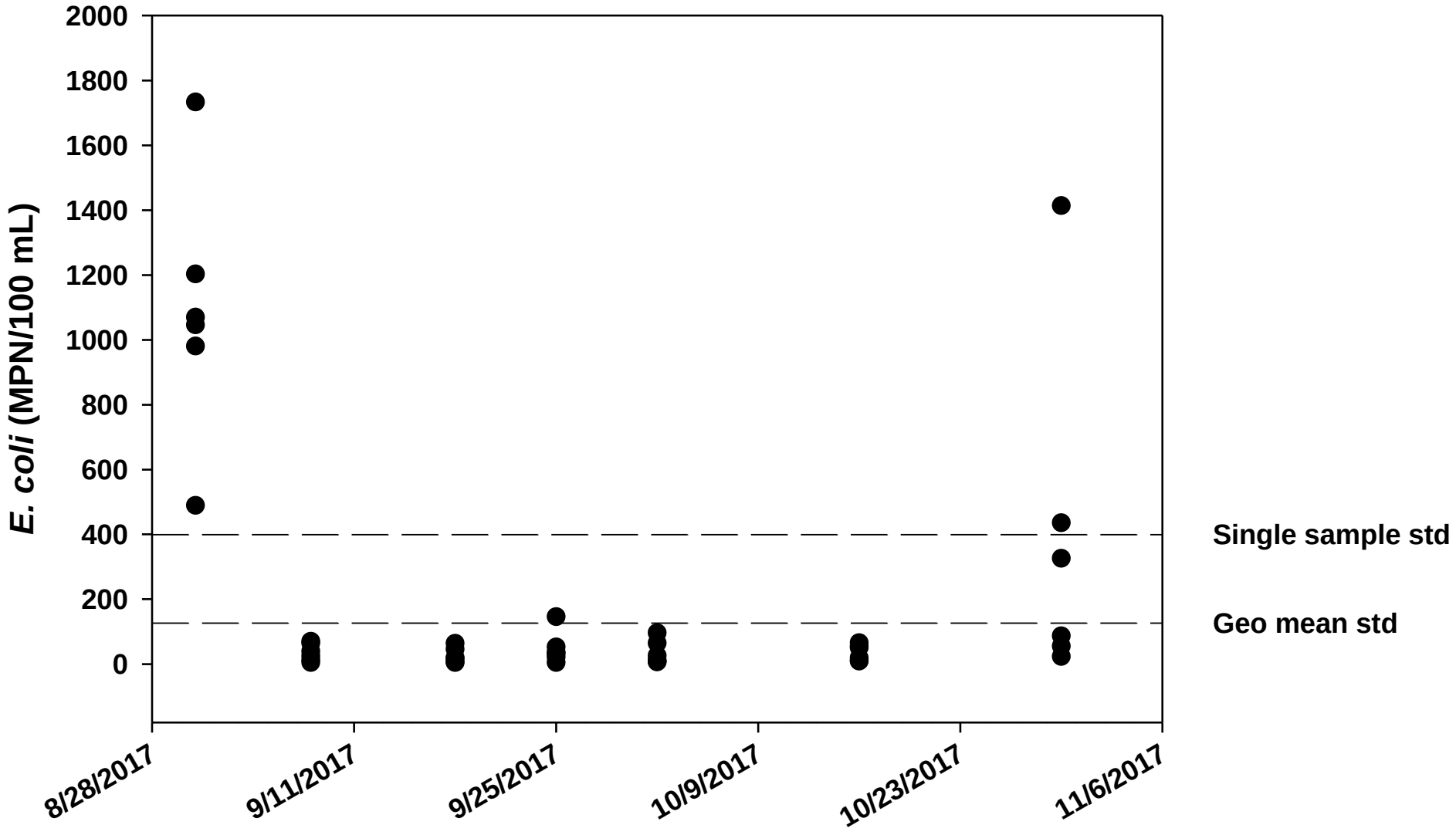
Bacteroidales BST Results
Base Flow Samples (n=225)



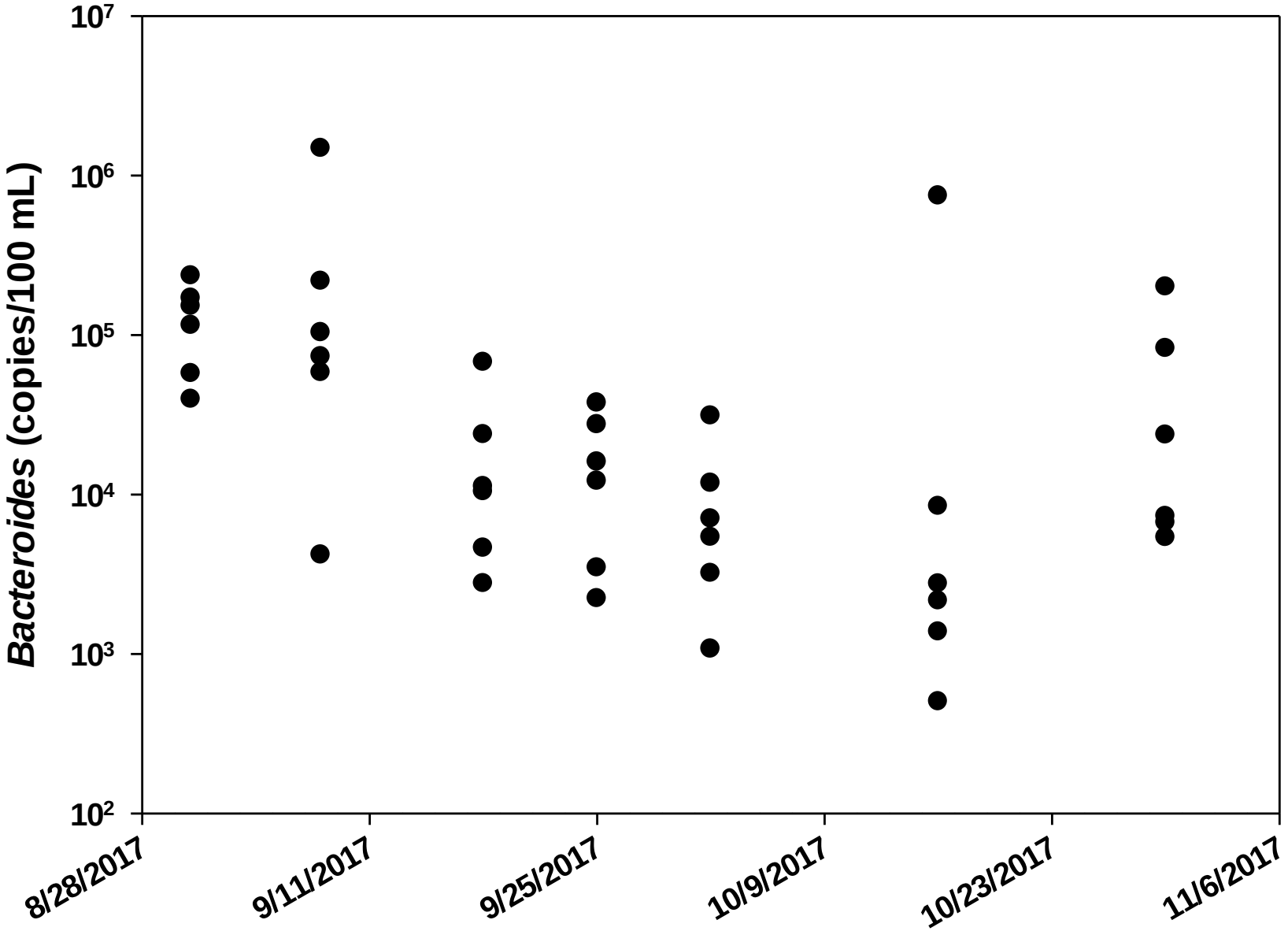
Hurricane Harvey Flooding

- **Six locations in the southeastern Houston area around Clear Lake**
- **Surface water samples collected as soon as sites accessible following the hurricane and then every 1-2 weeks for ~2 months**
- **Measured *E. coli* and used qPCR for general and human markers**

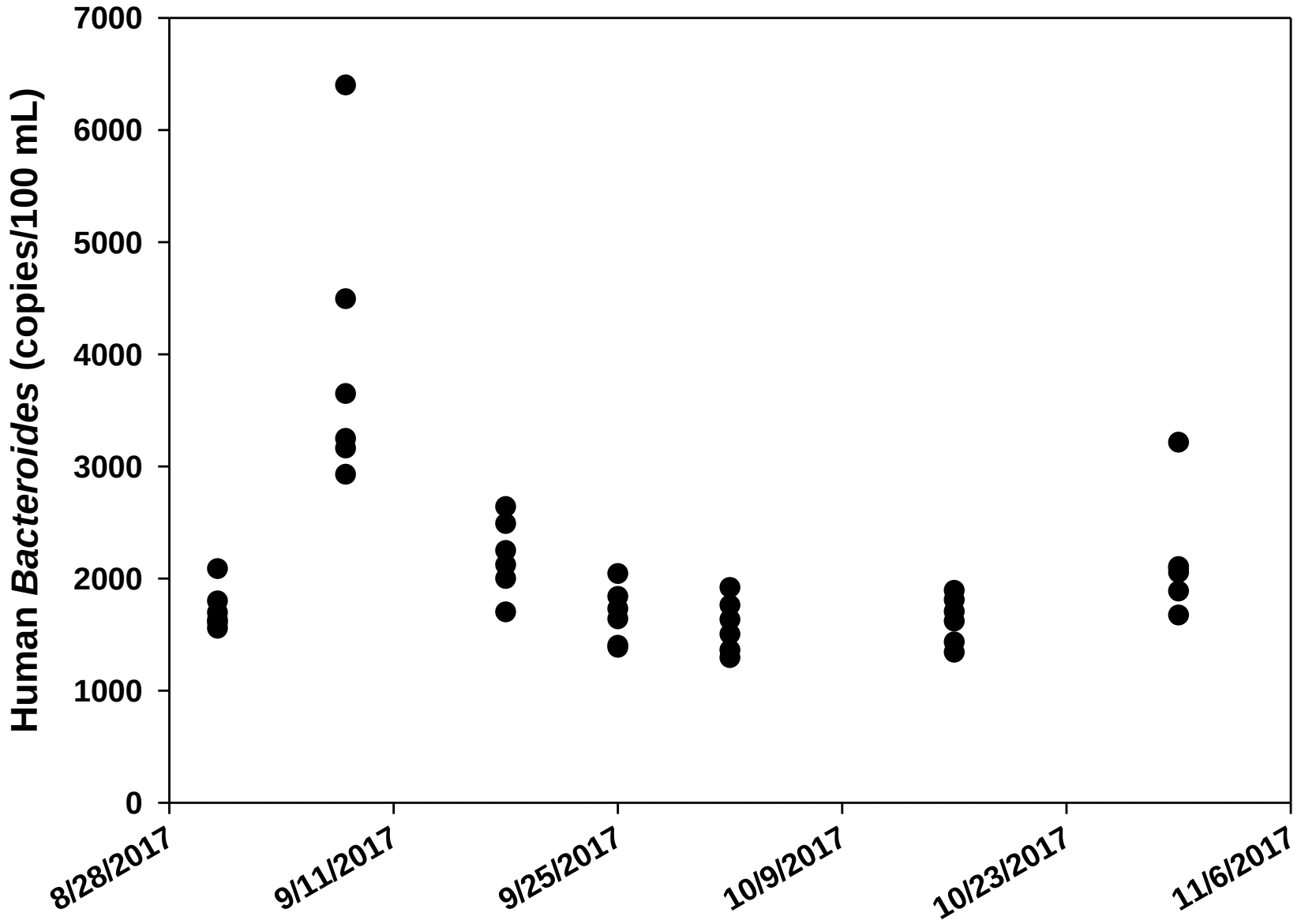
E. coli Levels



Total *Bacteroides* Levels (GenBac)

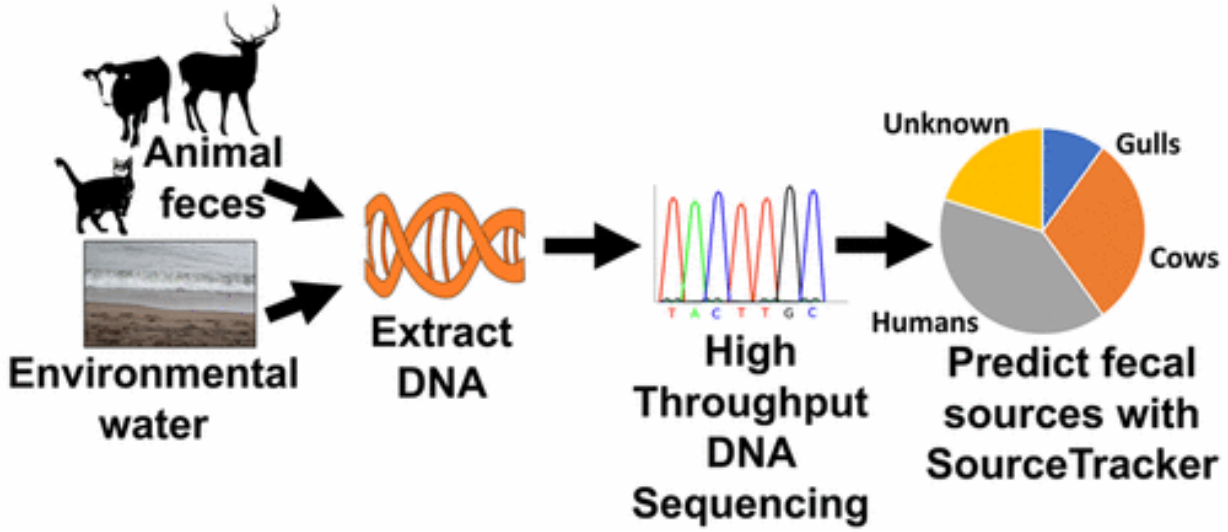


Human *Bacteroides* Levels (HumM2)



Sequencing-Based BST

- **High-throughput DNA sequencing (HTS) to identify microbiome in water samples**
- **Compare to microbiomes in known-source samples using programs such as SourceTracker (Knights et al., 2011)**



Brown et al. (2017)

- **Staley et al. (2018) spiked samples with various fecal mixtures**
- **HTS approach 91% accurate in identifying sources with no false negatives**
- **Overall, strong correlation between source contributions and volume spiked**

Table 1. Proportions of Spiked Source Material (% vol/vol) in Blinded Sink Samples and SourceTracker Sink Predictions (% Mean ± Standard Deviation) Using the FL Blinded Source Samples Alone[a]

sample ID	sample composition	cow	horse	cat	dog	WWTP
	all sources					
SW01	expected	2.0	2.0	2.0	2.0	2.0
	SourceTracker	3.3 ± 0.2	27.4 ± 0.5	6.5 ± 0.8	44.3 ± 0.8	11.8 ± 0.8
	dog					
SW25	expected	0.0	0.0	0.0	10.0	0.0
	SourceTracker	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	93.7 ± 0.5	1.2 ± 0.3

Staley et al. (2018)

Use of BST Results

- **Reconcile with:**
  - ***E. coli* enumeration data**
  - **Land use**
  - **Watershed source survey**
  - **Modeling**
  - **Stakeholder input**
  - **Common sense**

How to Start a BST Project?

- Government and commercial BST labs
- What is the goal of BST?
  - Characterize watershed or monitor specific sources?
  - How many potential sources?
    - All, most numerous…
    - One or a few (e.g., human)
  - What level of resolution is needed?
    - Individual species
    - Groups (e.g., humans, domesticated animals, and wildlife)
    - Presence/absence, relative ranking, or absolute number for various sources

Costs of a BST Project?

- **Current BST costs:**
  - **ERIC-RP = $250/isolate**
  - ***Bacteroidales* PCR**
    - **General + one specific marker = $250/sample**
    - **General + four specific markers = $325/sample**
  - **Sequencing-based = ?**
- **Example watershed:**
  - **Three sites**
  - **Samples collected monthly for one year**
  - **ERIC-RP five isolates per sample**
  - **3 sites x 12 sampling events x 5 isolates/sample [180 total isolates] x $250/isolate = $45,000**
  - **Does not include sample collection, initial sample processing, and transport to lab**

Questions?

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