

Use of Bacterial Source Tracking to Characterize Texas Watersheds

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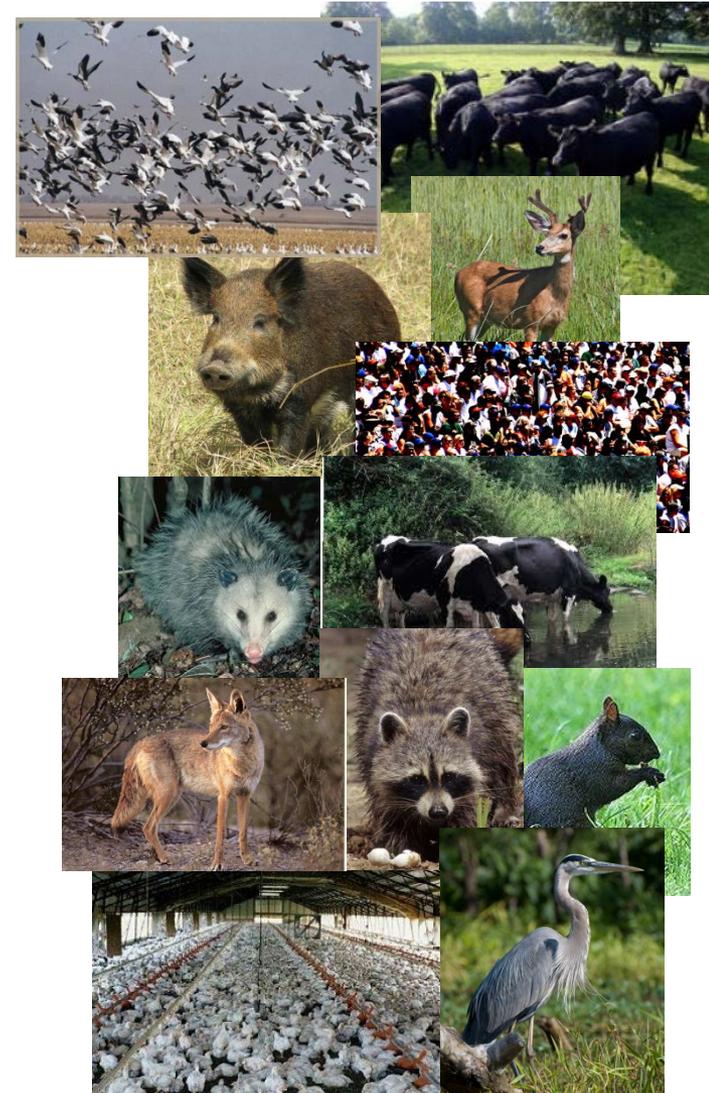
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Texas Water
Resources Institute
make every drop count

 **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**

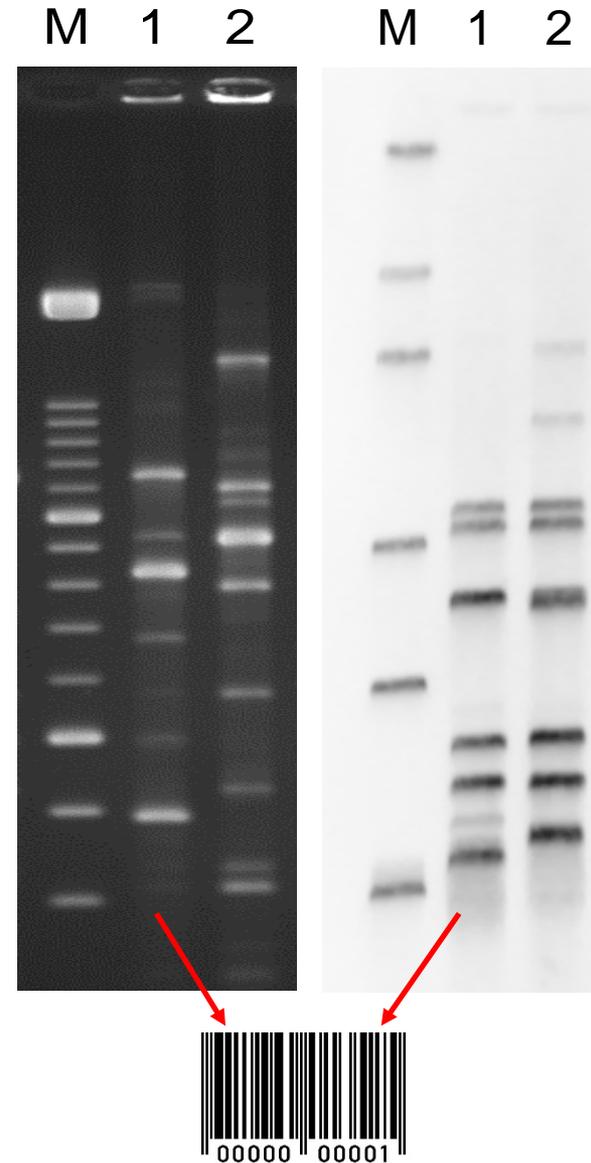
Library-Dependent BST Methods

Methods:

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

Advantages/Disadvantages:

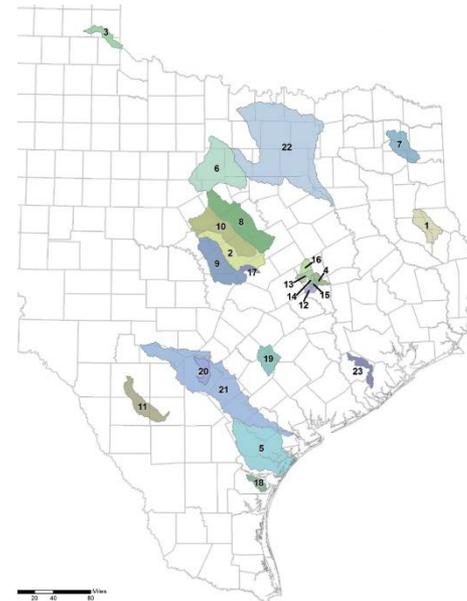
- More discriminating
- Allows ranking of sources
- Relatively expensive



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:

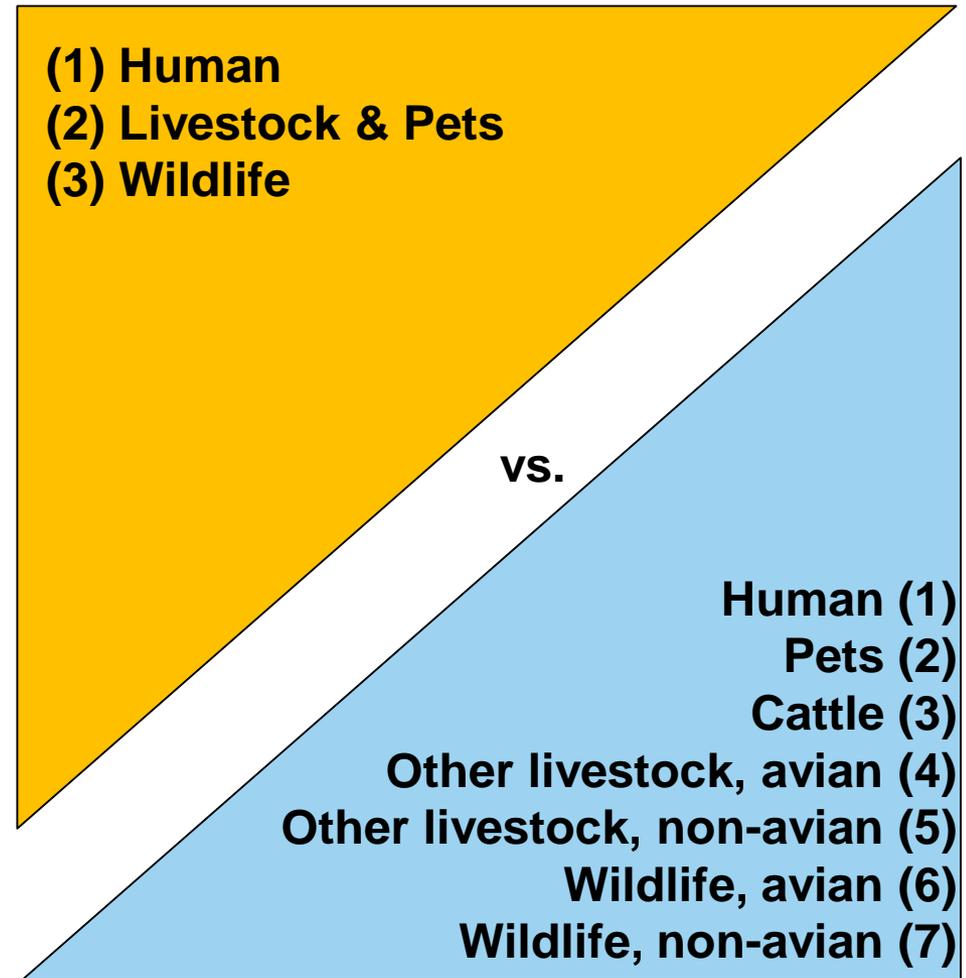
- Plum Creek
- San Antonio
- Lake Granbury
- Oyster Creek / Trinity River
- Waco / Belton Lake
- Little Brazos River Tributaries
- Attoyac Bayou



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

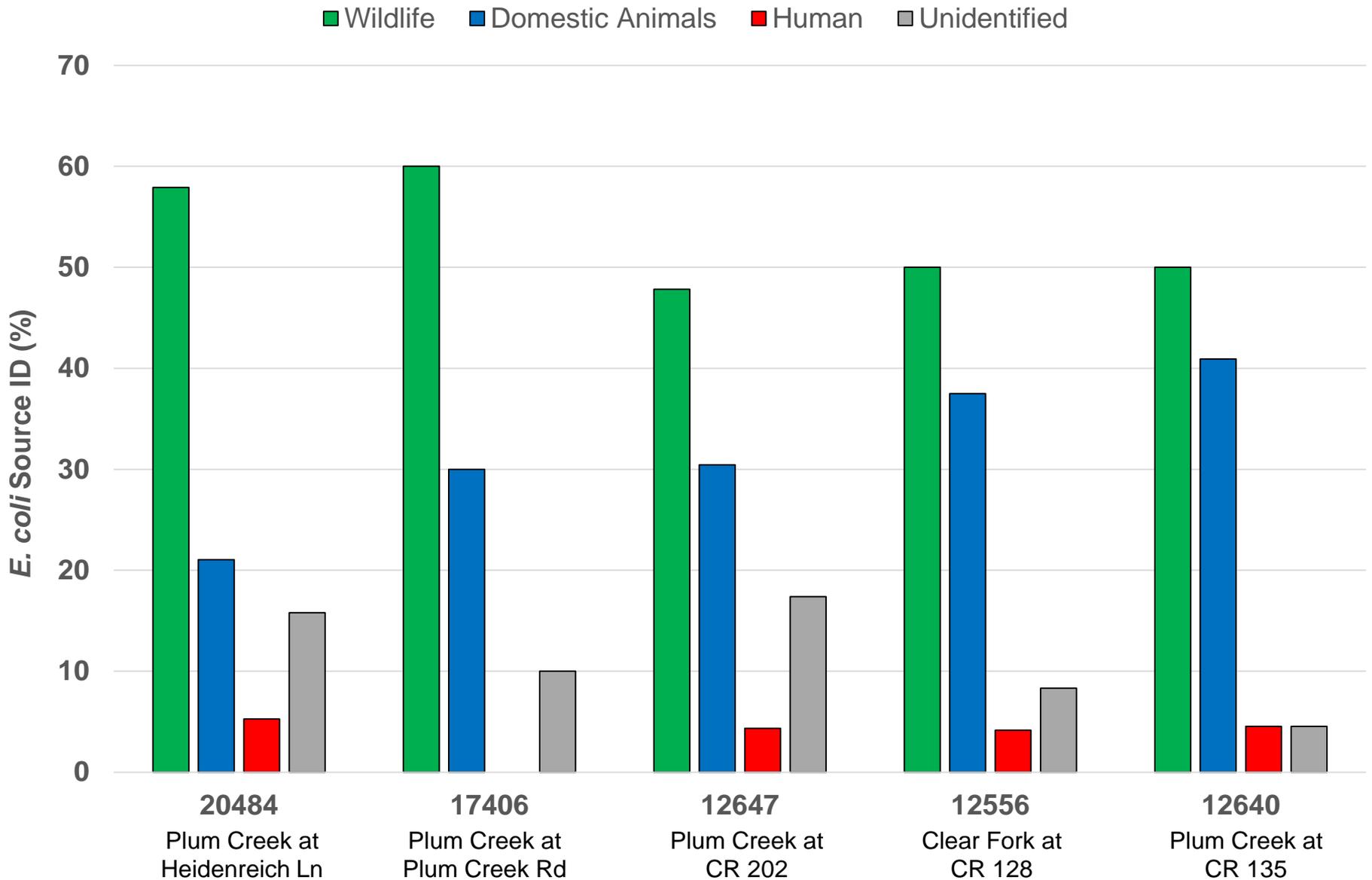
Three-way v. Seven-way Split of Results

- **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)



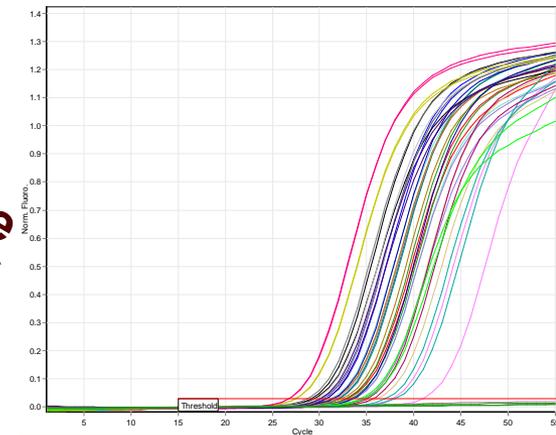
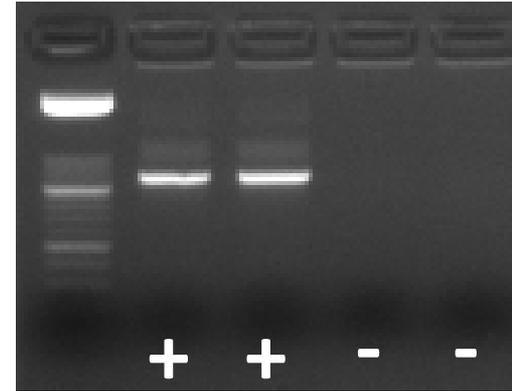
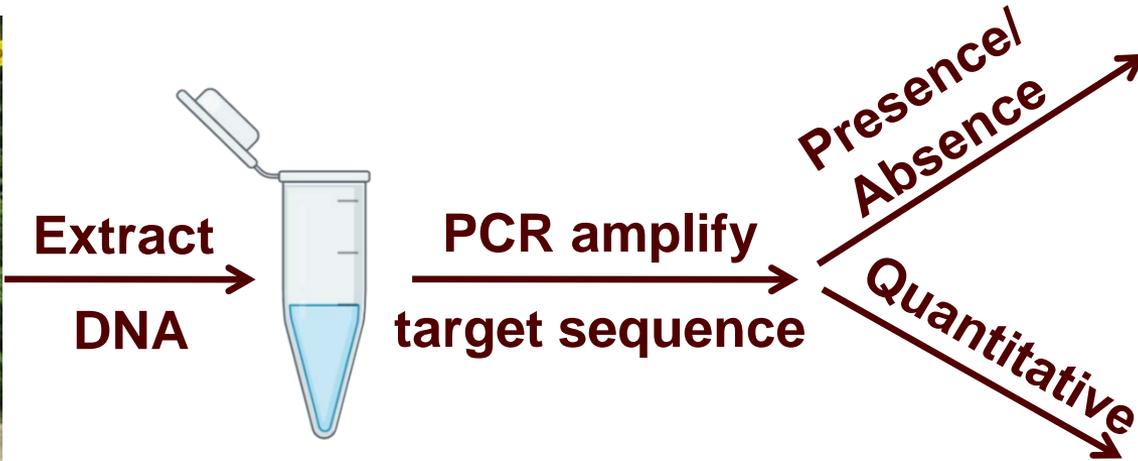
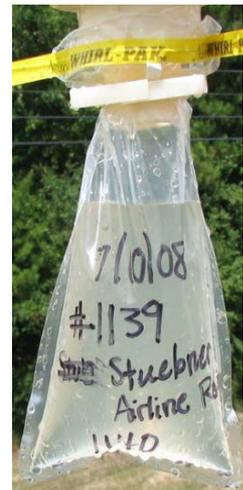
Library Independent BST

- **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

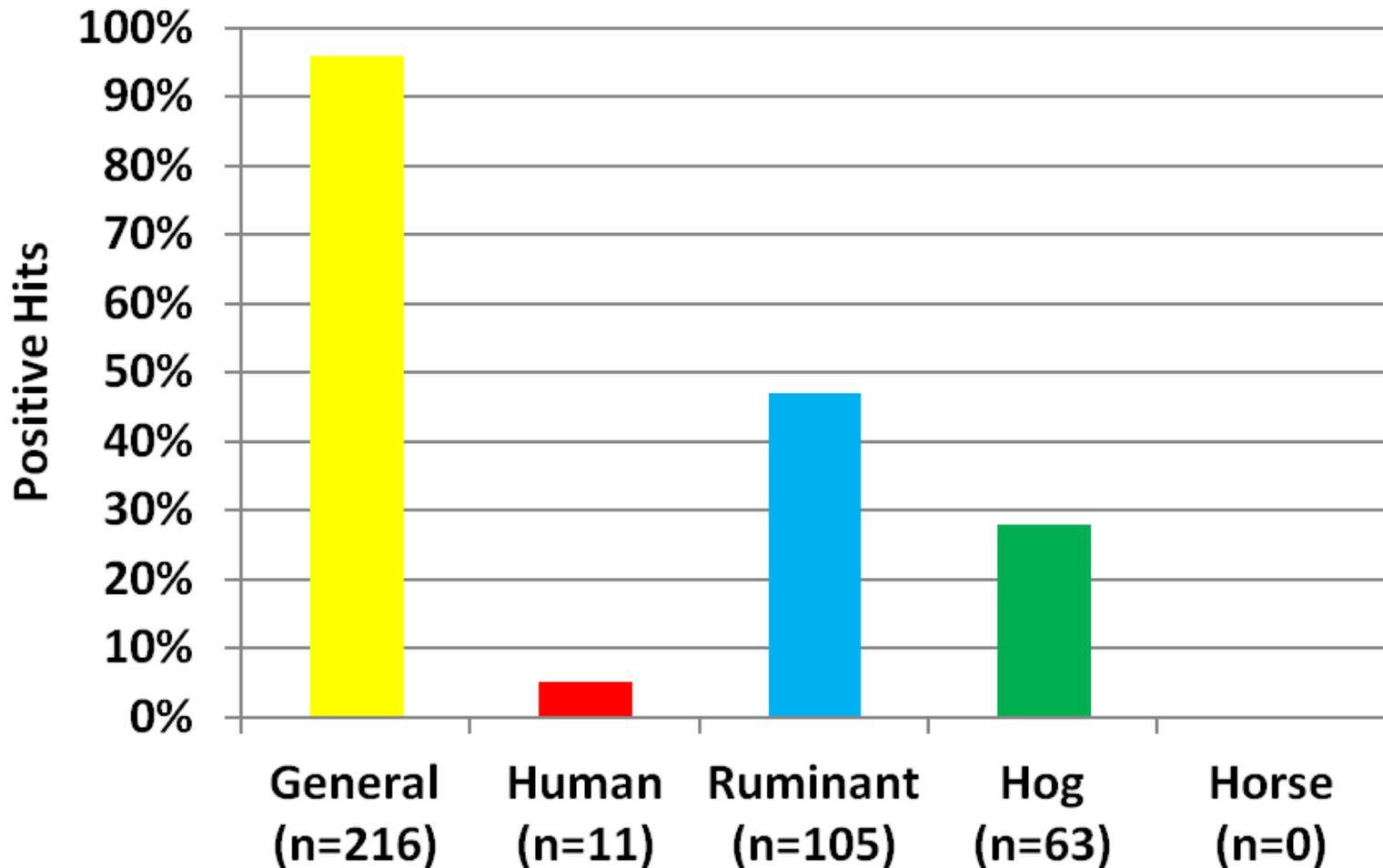


Advantages:

- Cost
- Time

Bacteroidales BST Results

Base Flow Samples (n=225)

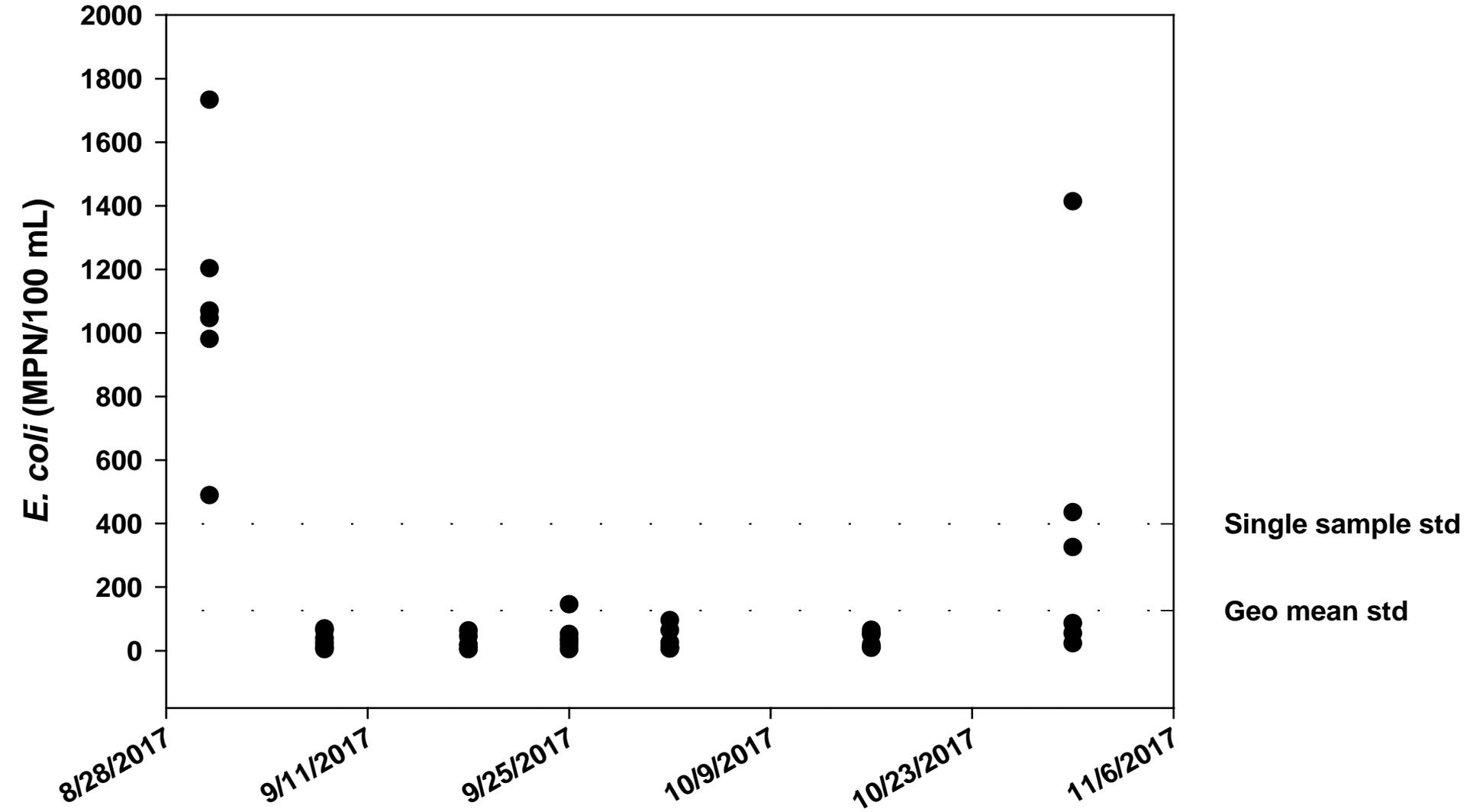


Hurricane Harvey Flooding

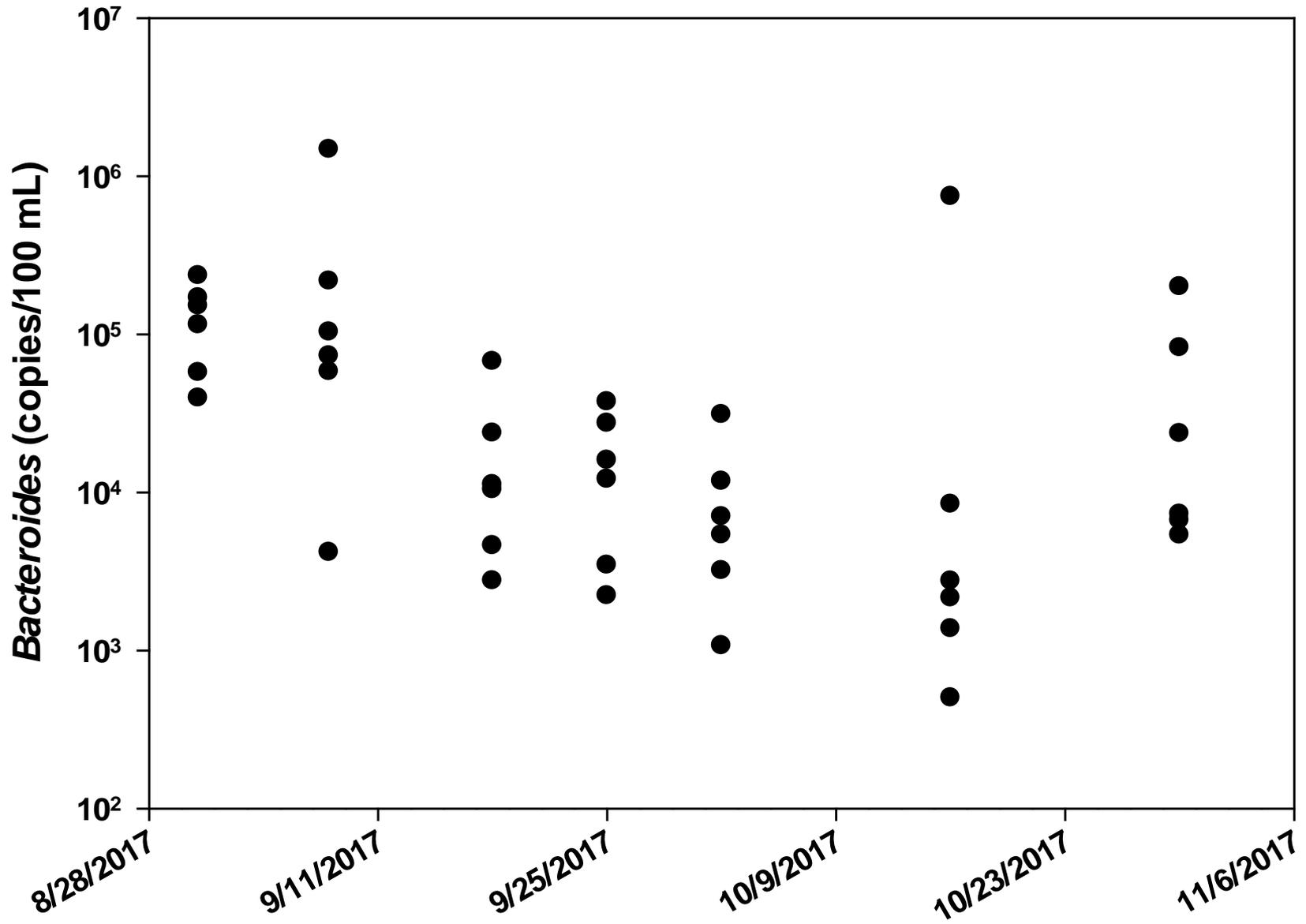
- **Six locations in the southeastern Houston area around Clear Lake**
- **Surface water samples collected as soon as sites accessible following the hurricane and then every 1-2 weeks for ~2 months**
- **Measured *E. coli* and used qPCR for general and human markers**



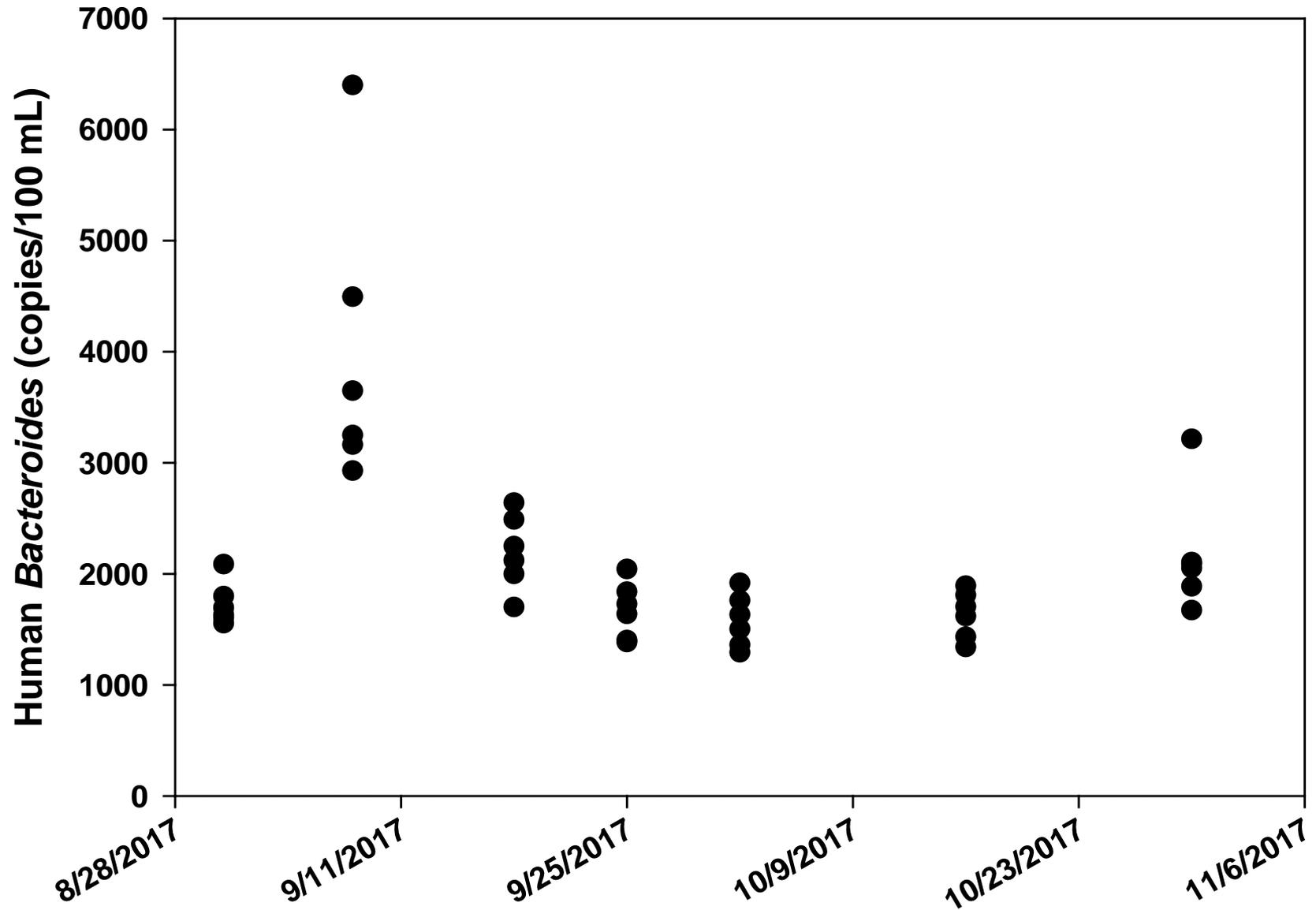
E. coli Levels



Total *Bacteroides* Levels (GenBac)

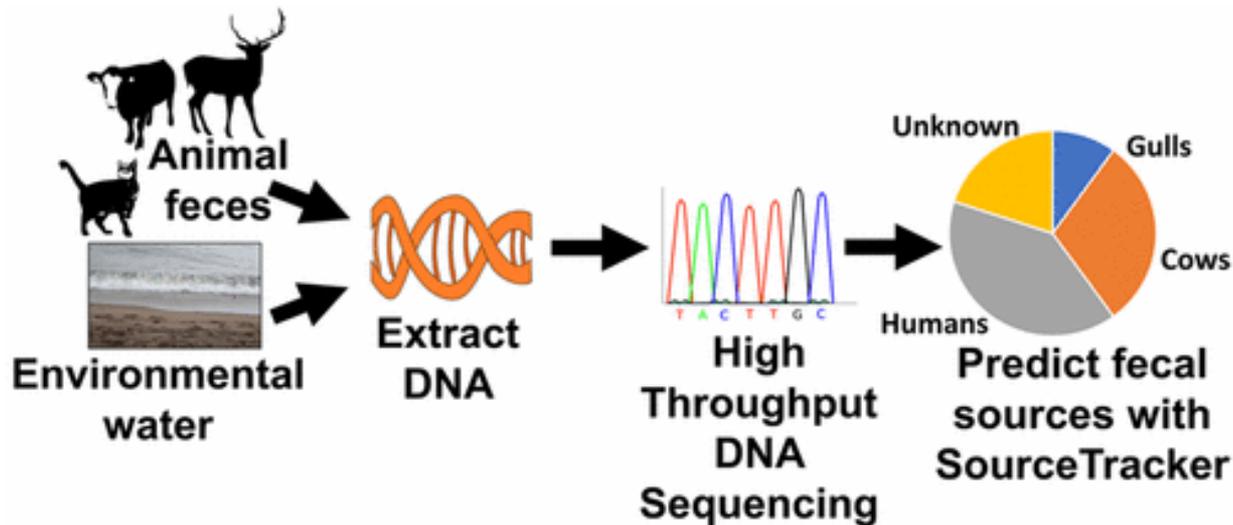


Human *Bacteroides* Levels (HumM2)



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)



- **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 \pm Standard Deviation) Using the FL Blinded Source Samples Alone^a

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

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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