Use of Bacterial Source Tracking for Characterization of Watersheds

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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"**
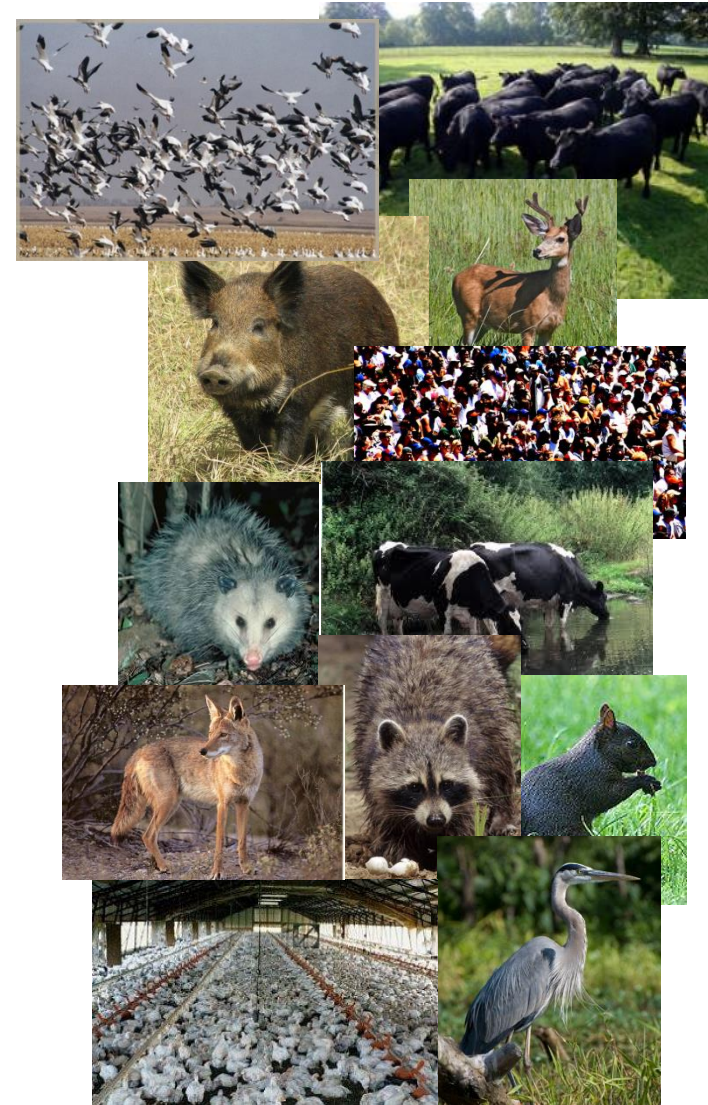
BST Target Organisms

- **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**

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

Isolate *E. coli* → DNA Fingerprint → Compare to Library → Source ID
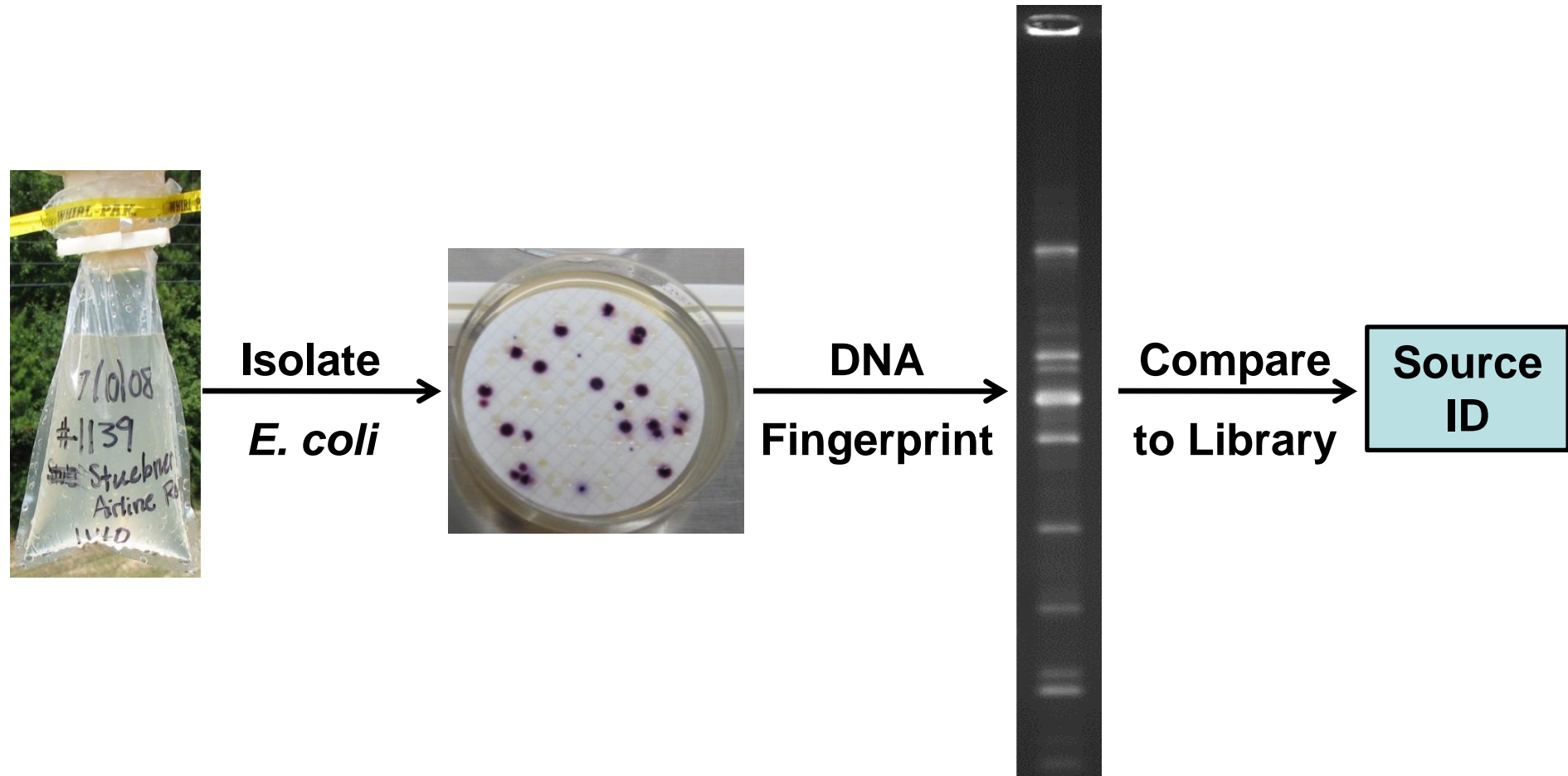
Library-Dependent BST Methods

Methods:

- **DNA fingerprinting**

  - **Enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction (ERIC-PCR)**

  - **RiboPrinting® (RP)**

Advantages/Disadvantages:

- **More discriminating**

- **Allows ranking of sources**
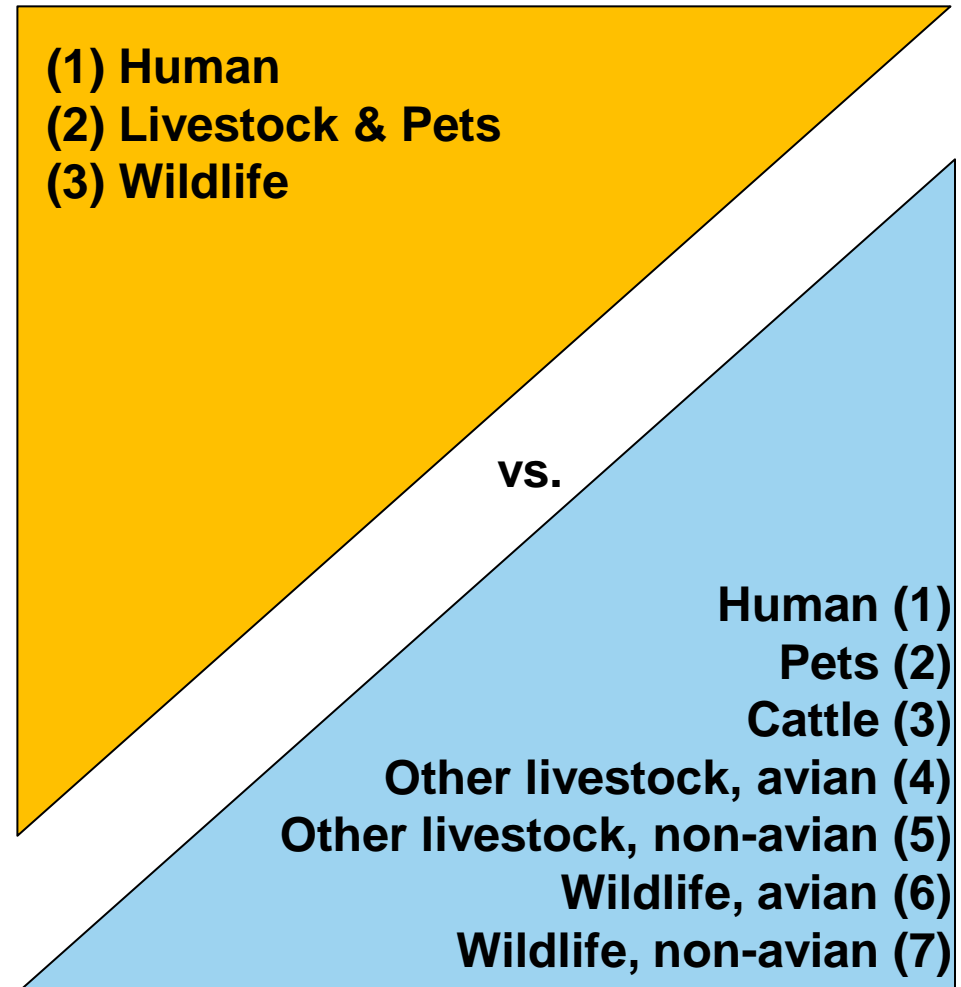
- **Relatively expensive**
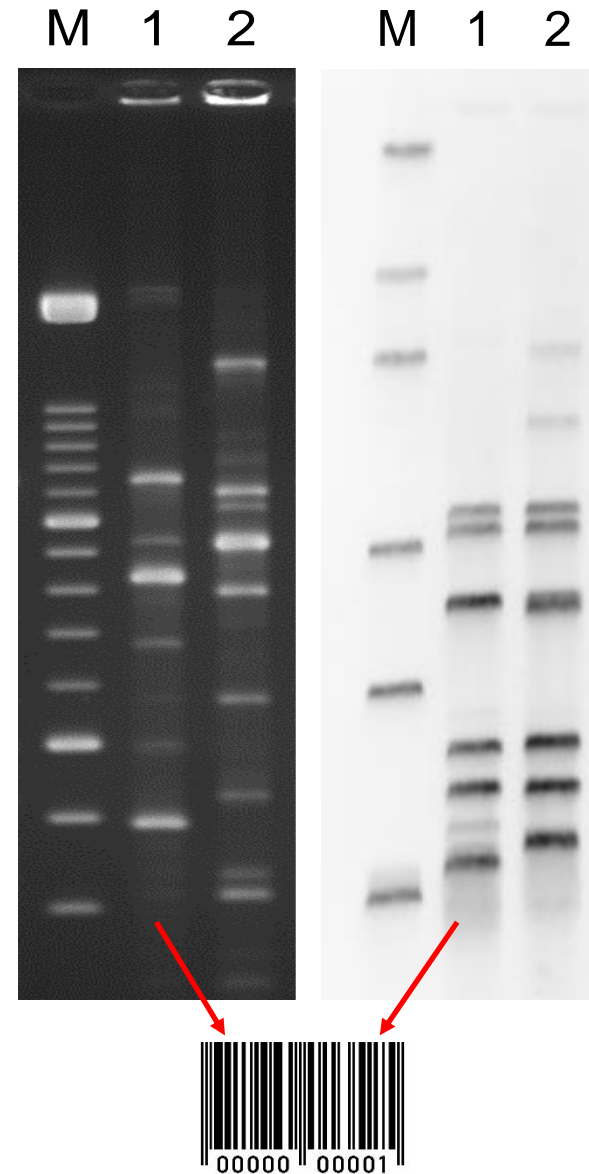
Texas *E. coli* BST Library (v. 03-20)

- **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**

Bacteria Source Tracking in Texas

- **Additional isolates being added from ongoing and future BST projects in other areas of Texas**
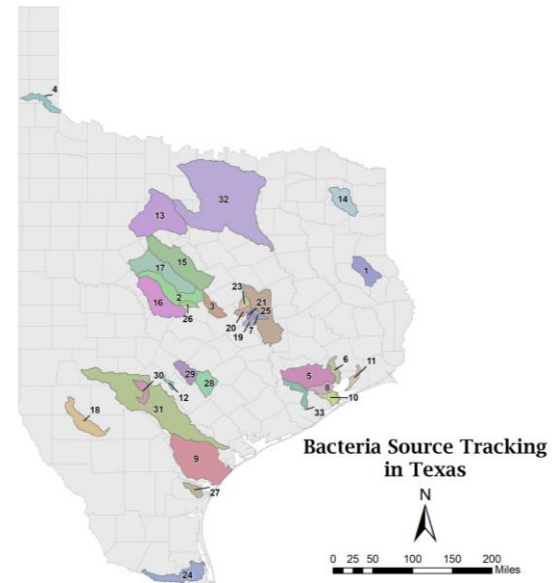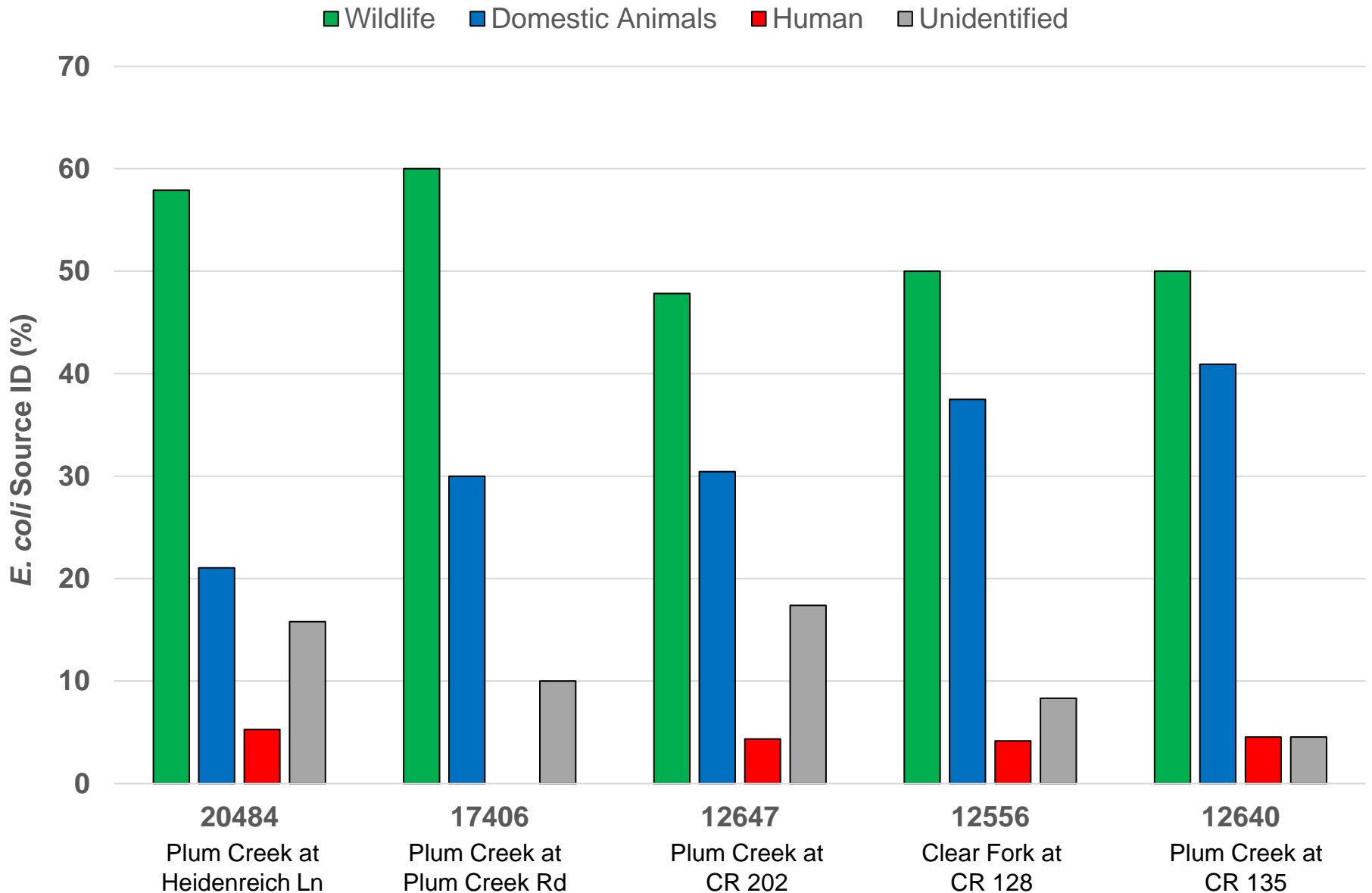
Three-way v. Seven-way Split of Results

- **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

(1) Human
(2) Livestock & Pets
(3) Wildlife

vs.

Human (1)
Pets (2)
Cattle (3)
Other livestock, avian (4)
Other livestock, non-avian (5)
Wildlife, avian (6)
Wildlife, non-avian (7)

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

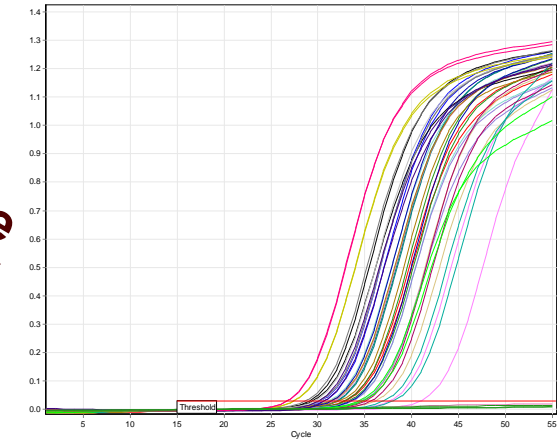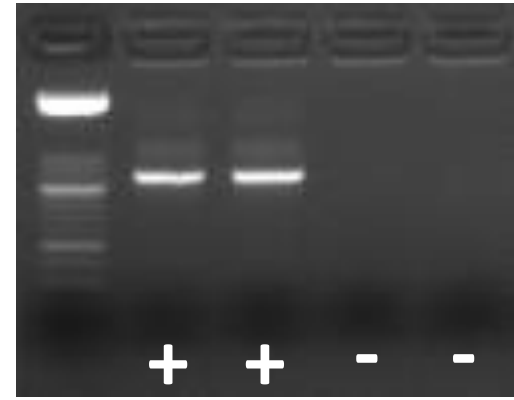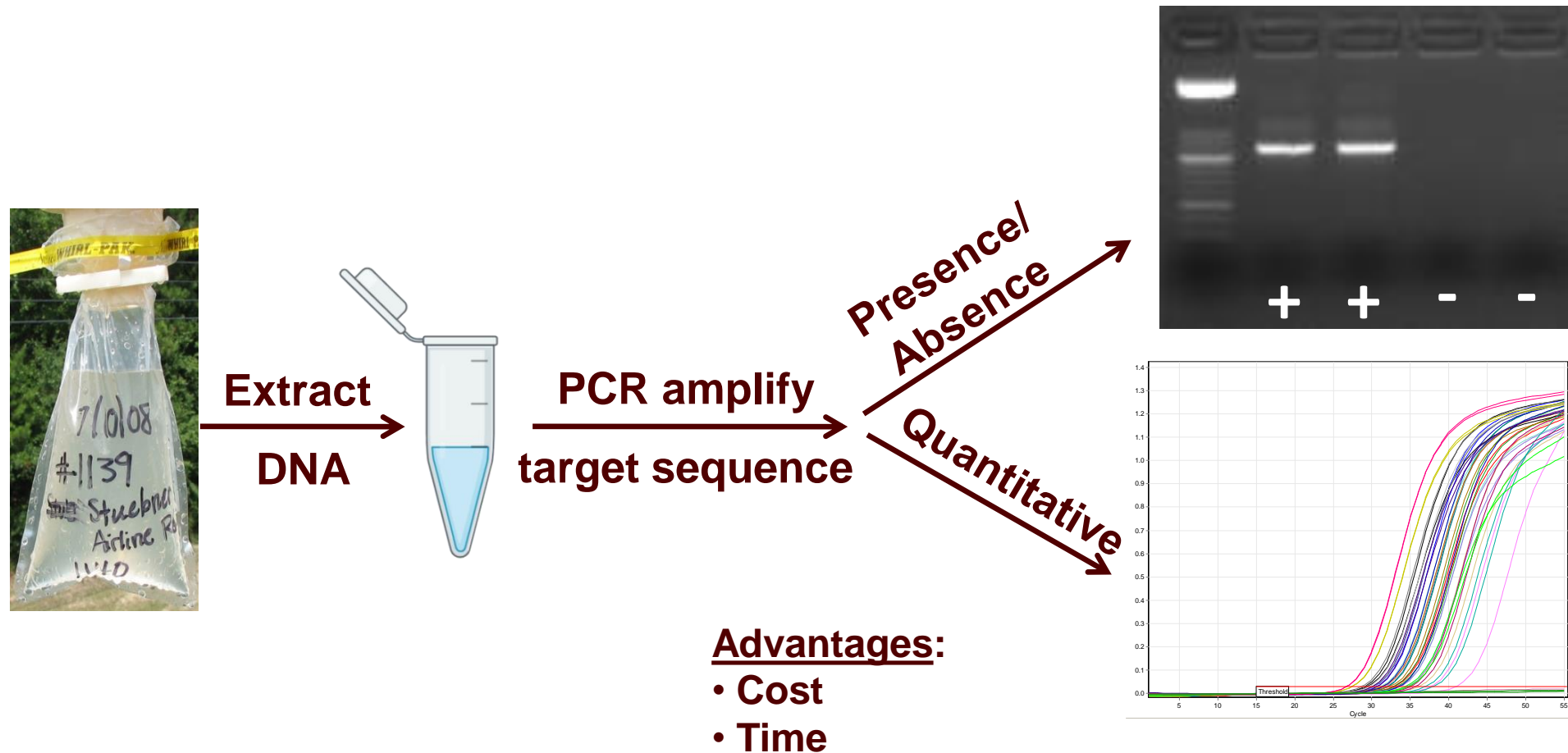# Library Independent BST

- 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
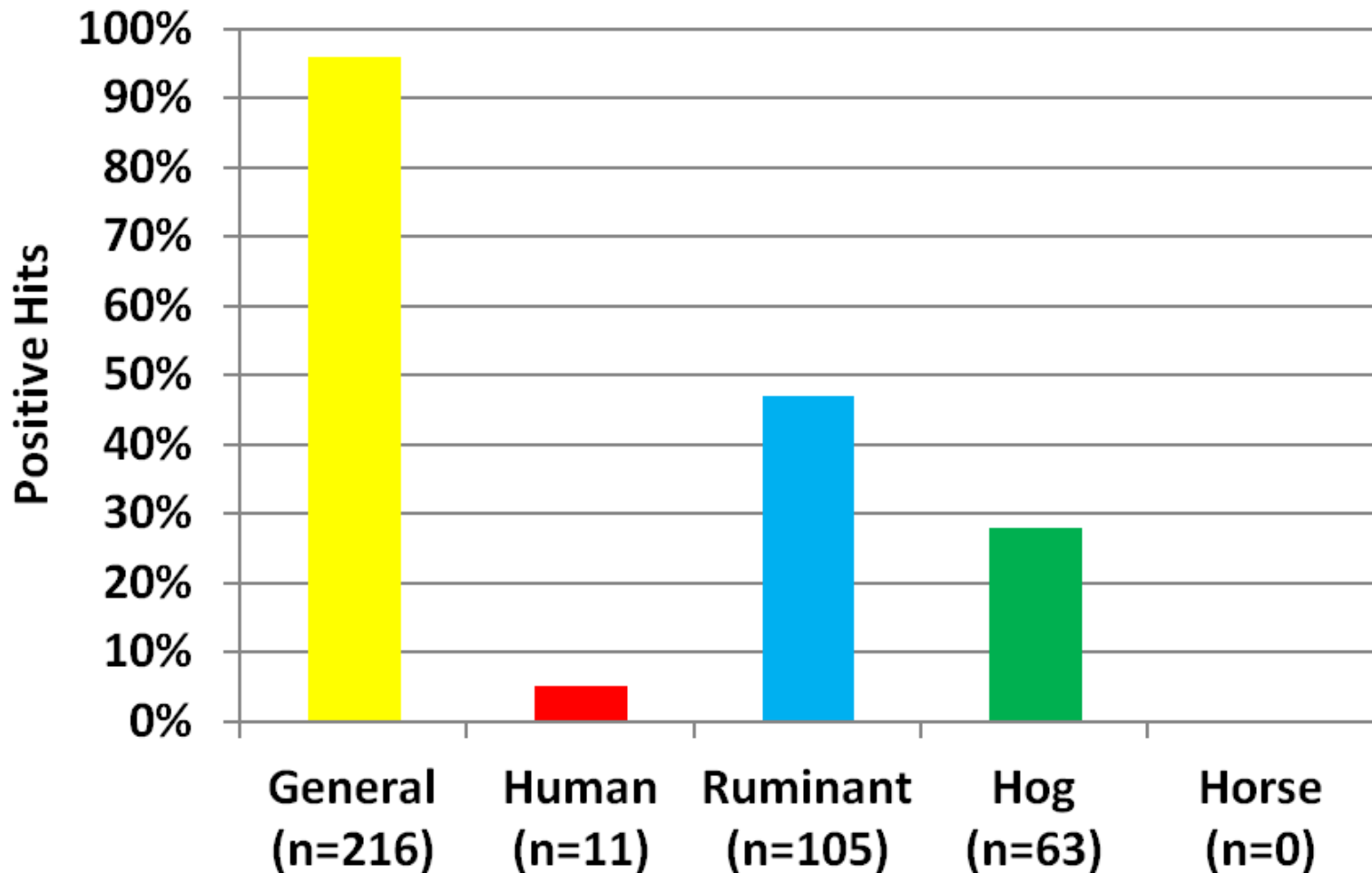
Brevibacterium LA35 Poultry Marker

- **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 → (gel image with + + − −)

Quantitative → (qPCR amplification curves)

Advantages:
- Cost
- Time

Created with BioRender.com

Bacteroidales BST Results
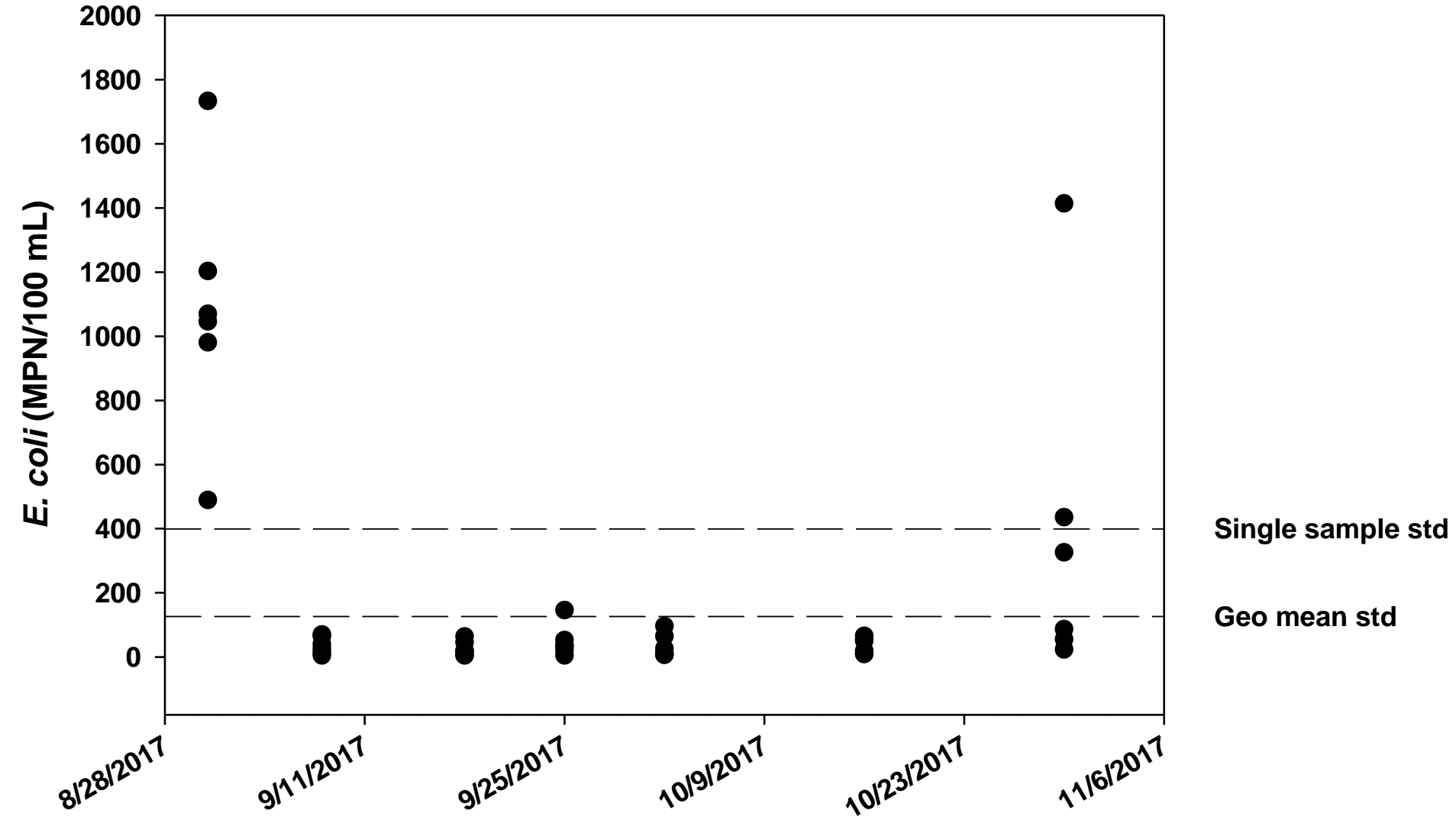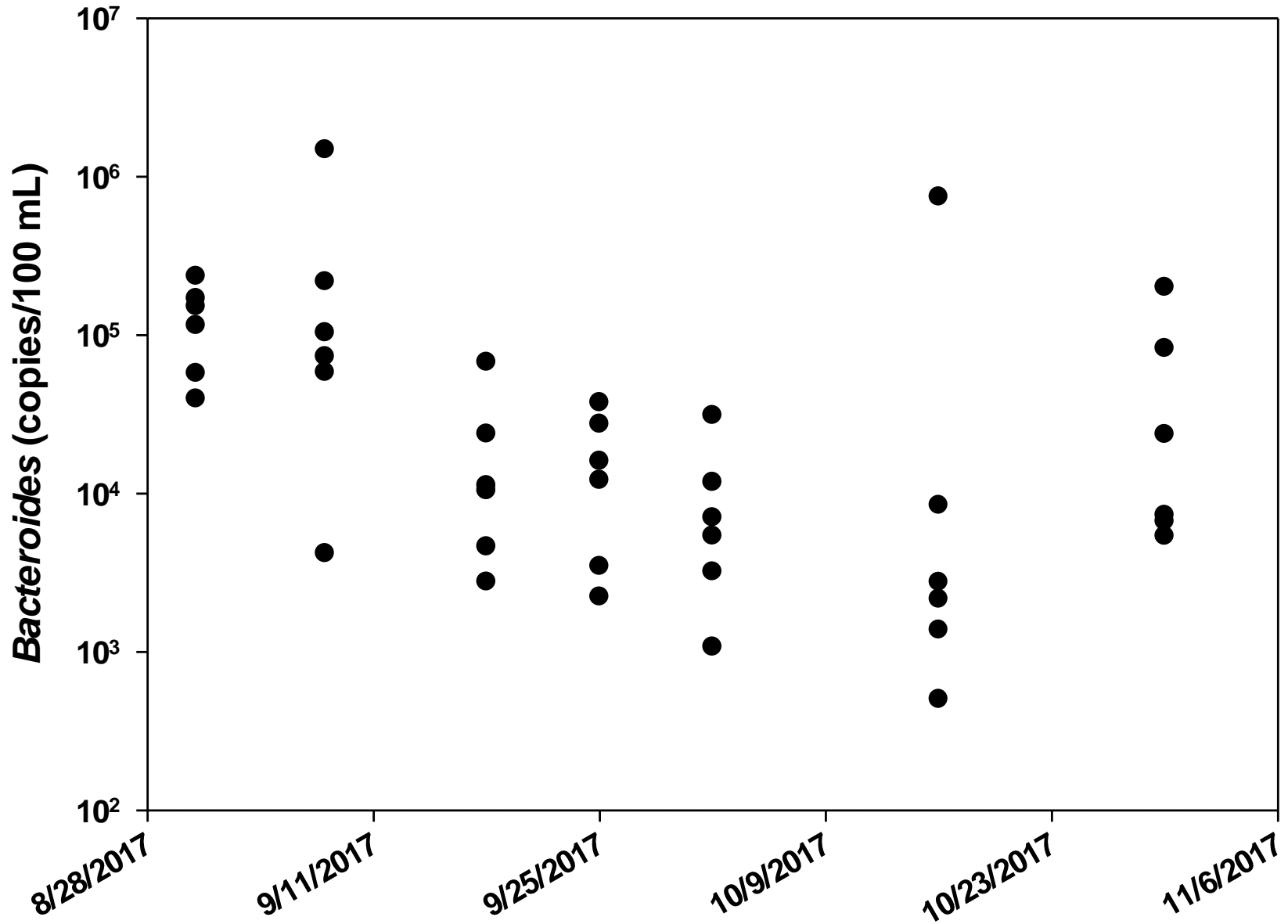## Base Flow Samples (n=225)

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**
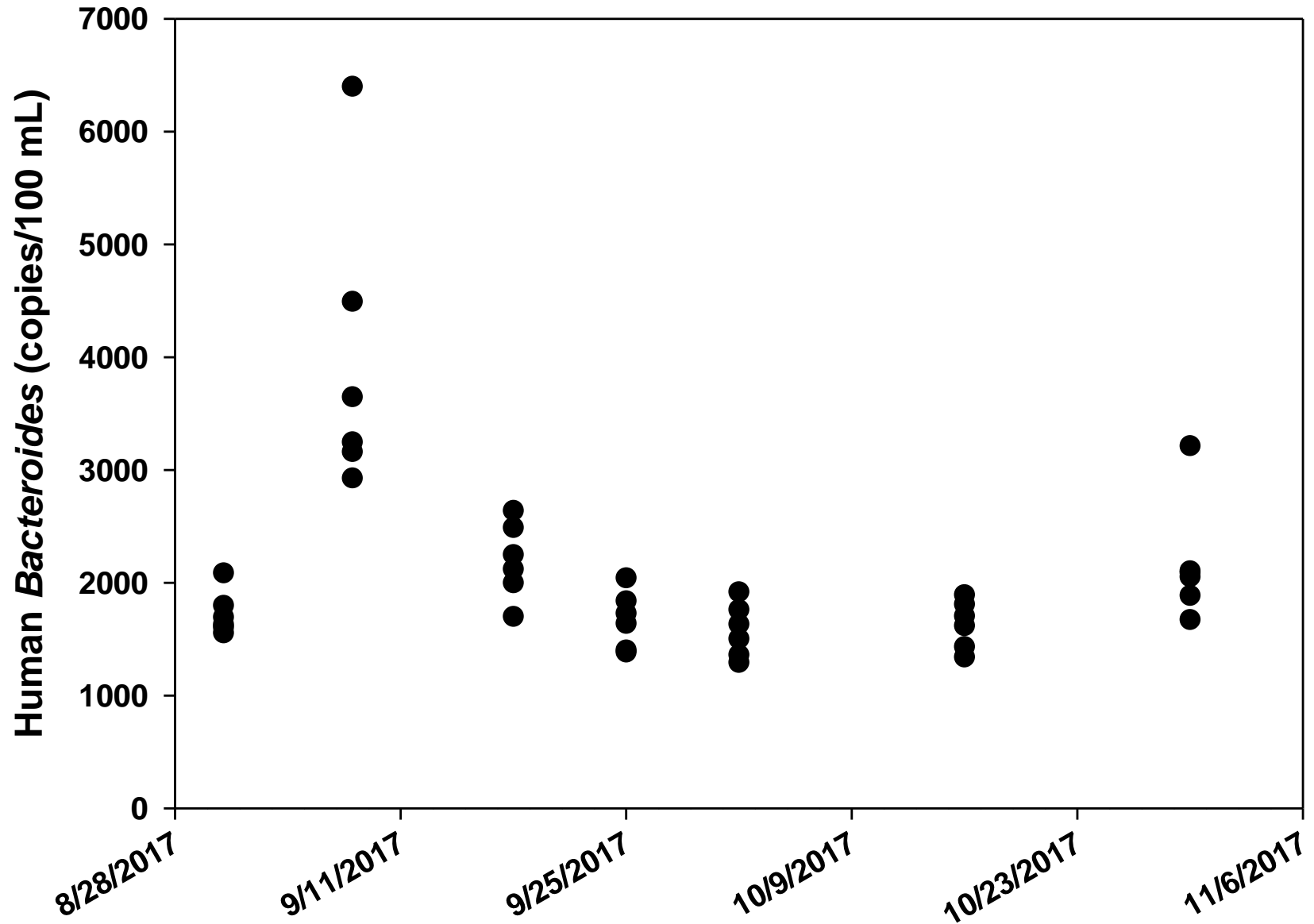
E. coli Levels

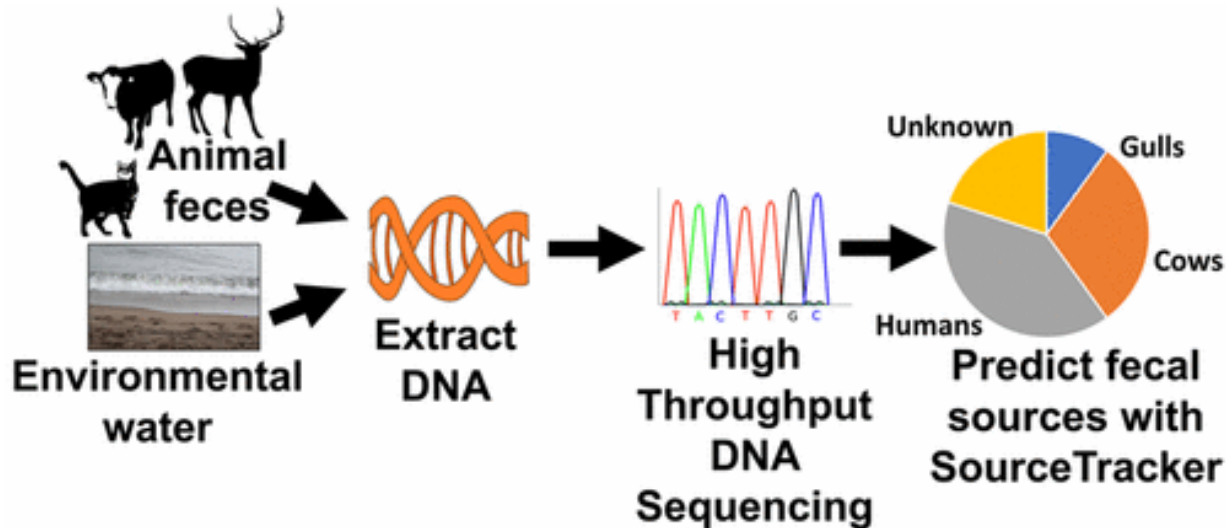# Total *Bacteroides* Levels (GenBac)

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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