



Tracking the Source of Fecal Contamination to Western Galveston Bay

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Microbial Source Tracking

- Waterways receive fecal contamination from diverse sources
- Human waste contamination presents acute public health risk



Conceptual Model

- Metagenomic
- Culture/library-dependent



Water sample



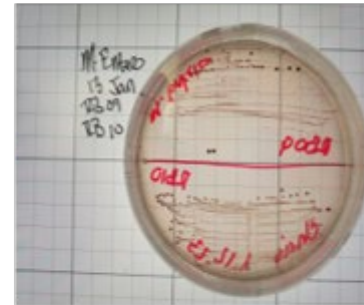
IDEXX Enterolert



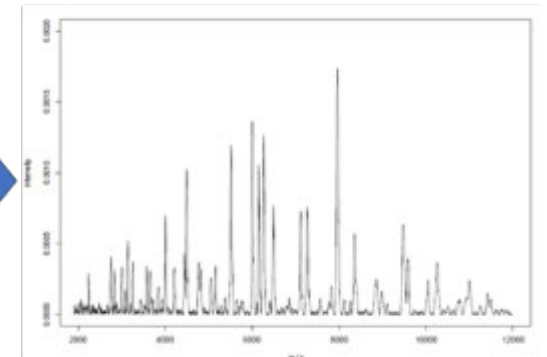
Concentrating
pipette



dPCR



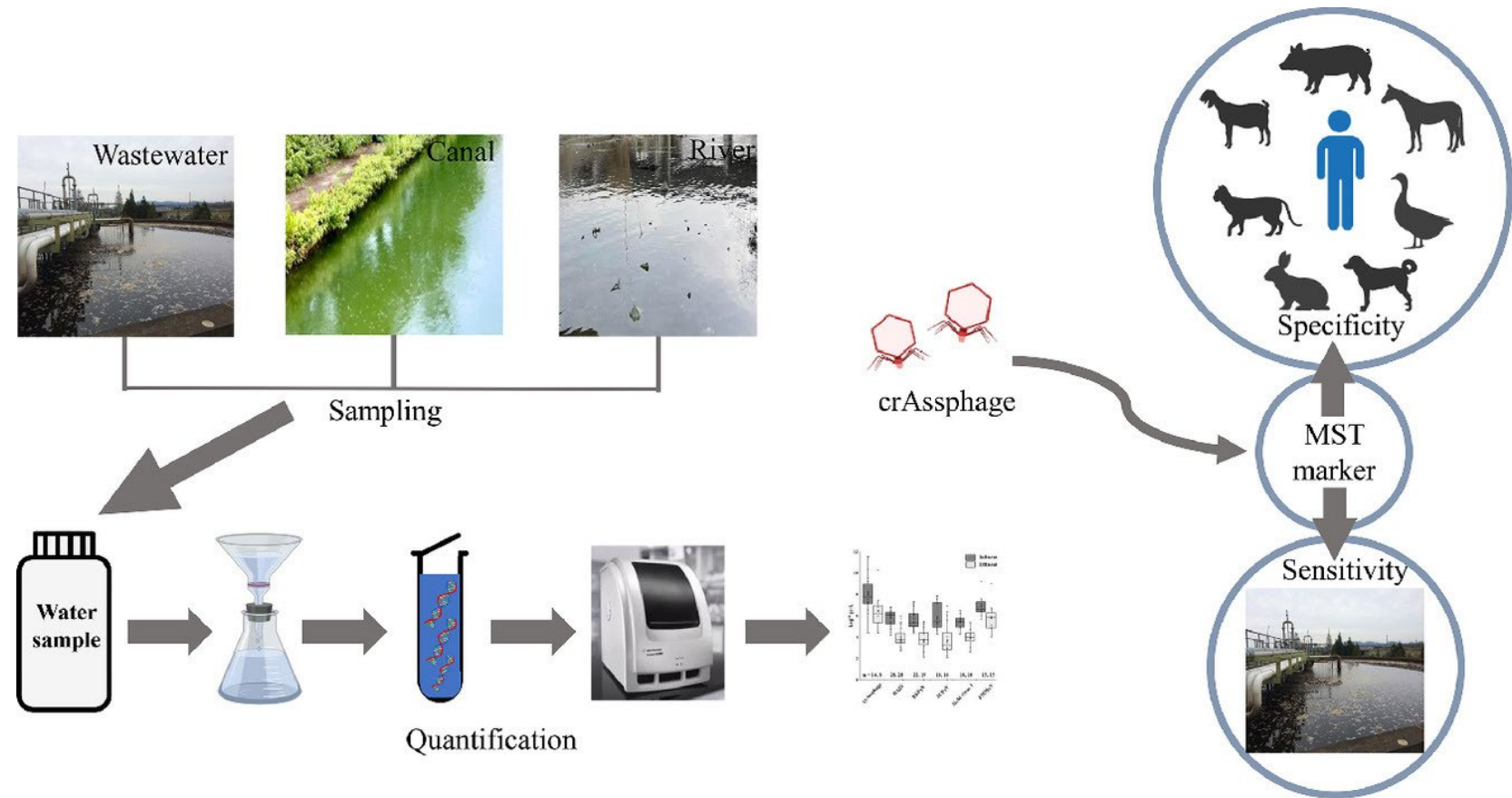
Differential and
selective media



Mass spectra

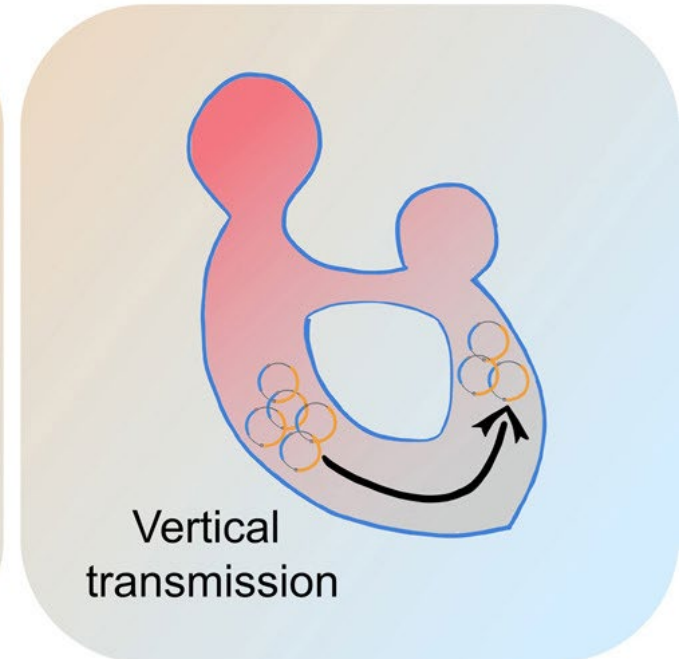
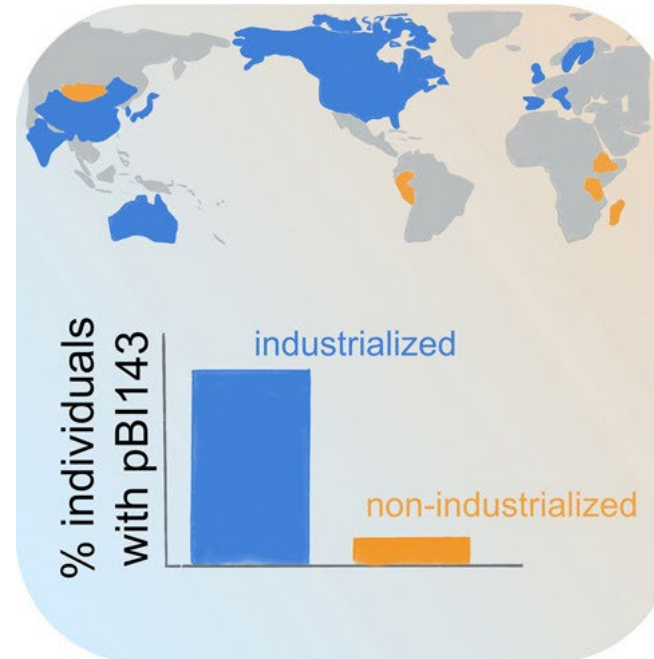
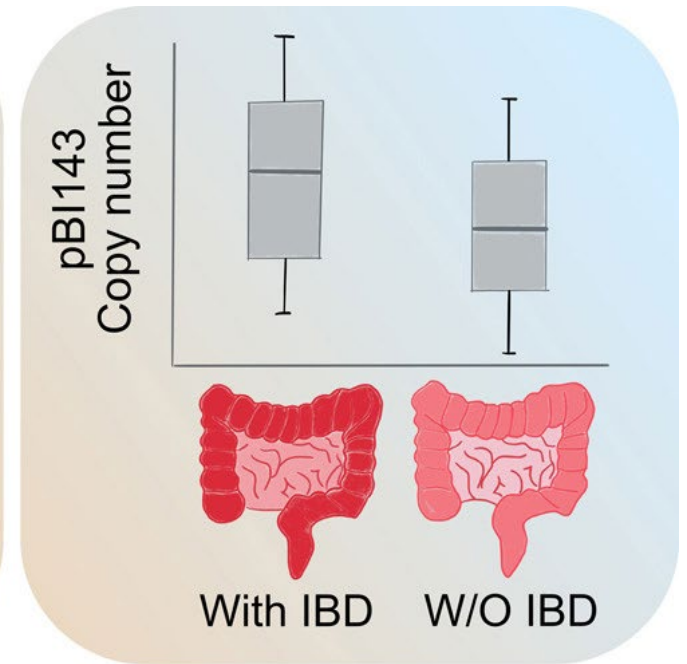
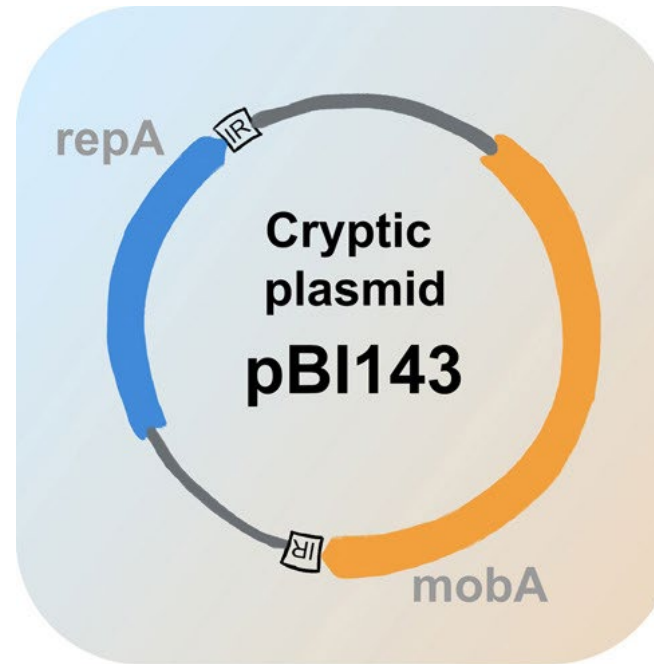
crAssphage

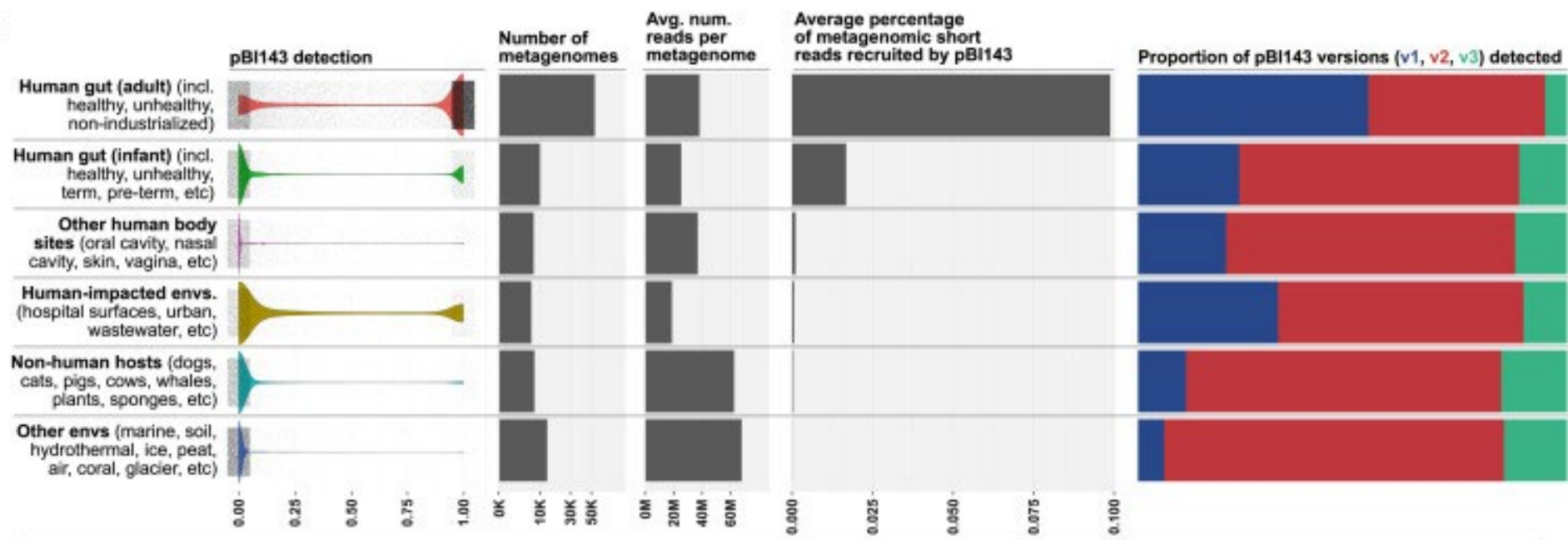
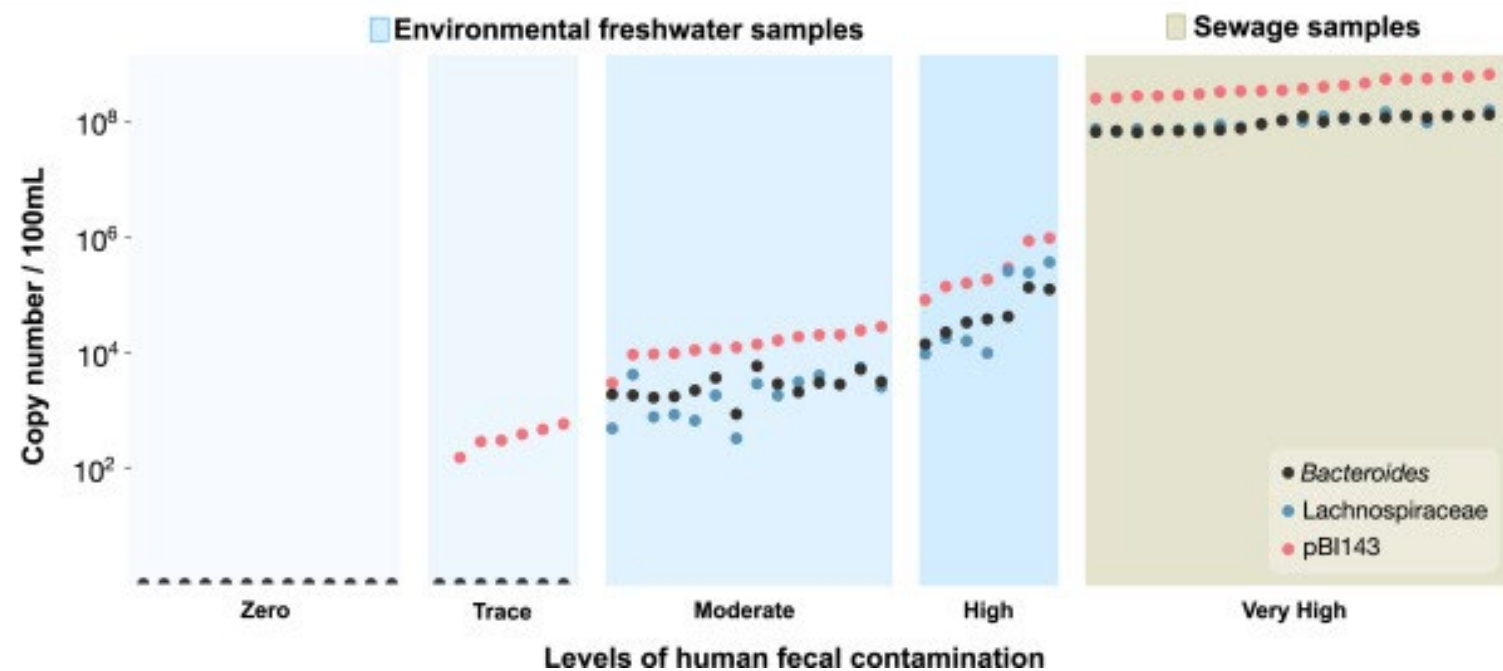
- bacteriophage found in high concentrations in human waste
- Emerging tool for tracking human waste in receiving waters

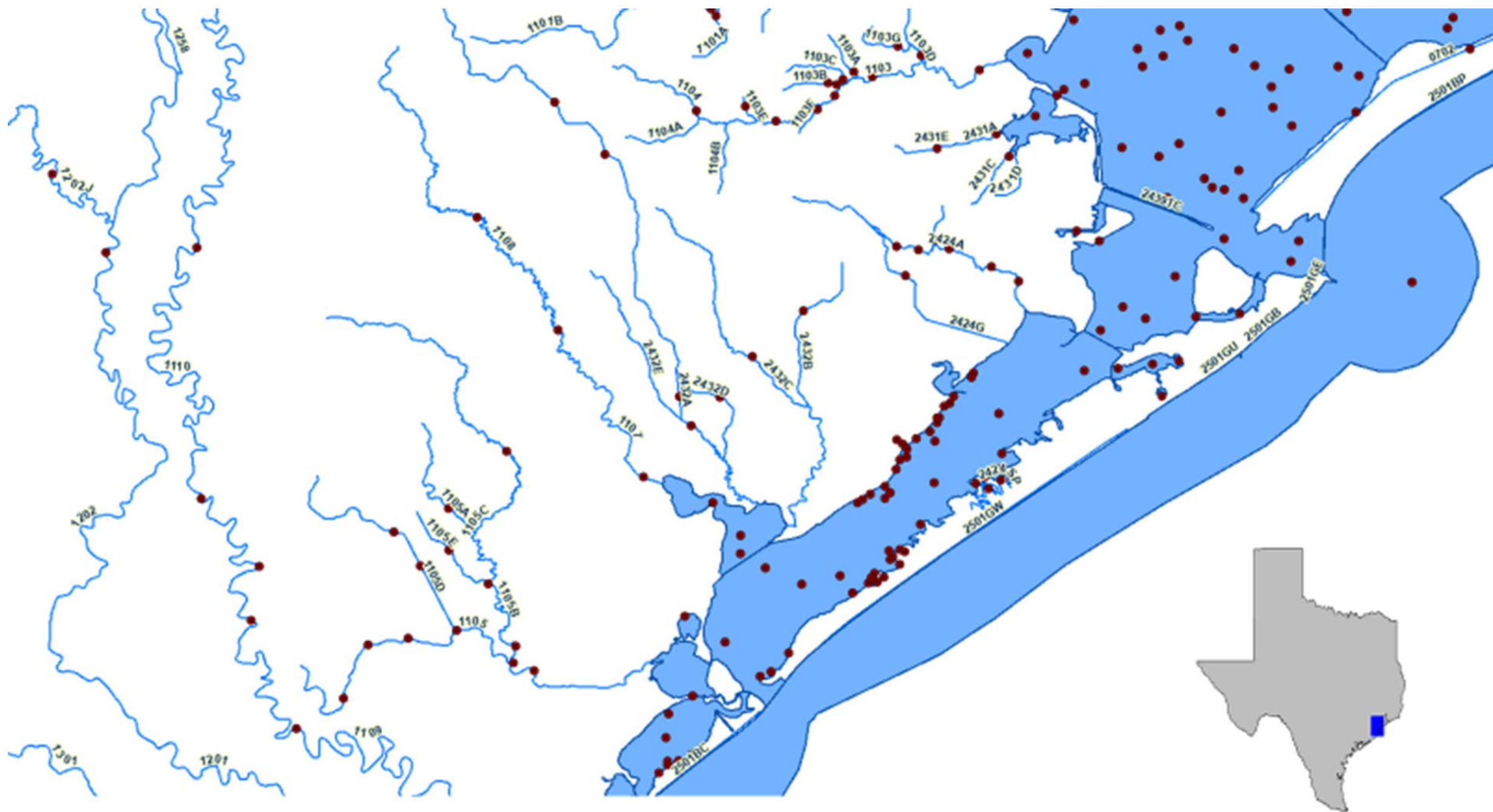


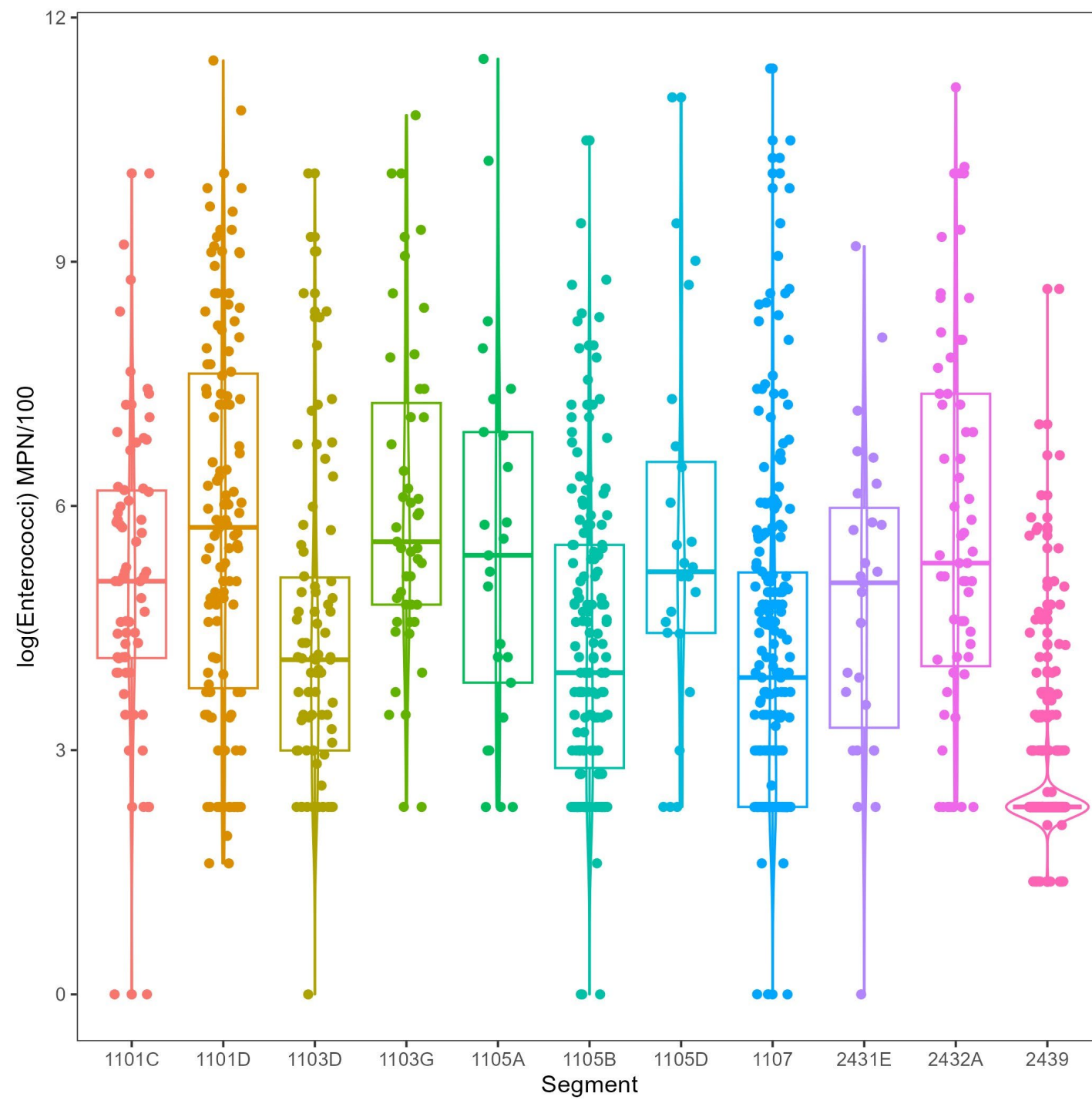
pBI143

- ubiquitous across industrialized gut microbiomes
- 14 x as numerous as crAssphage



A**B**

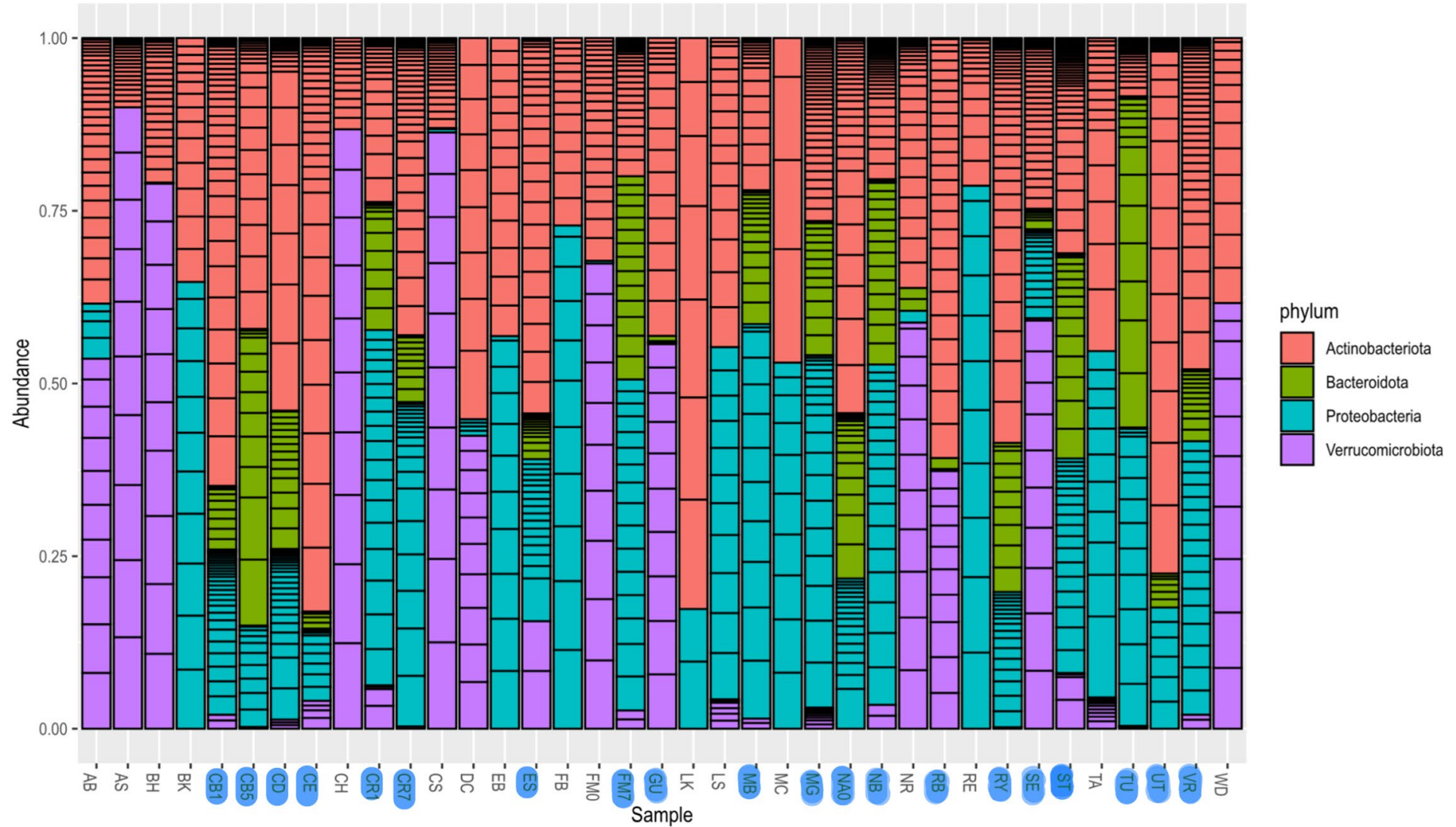




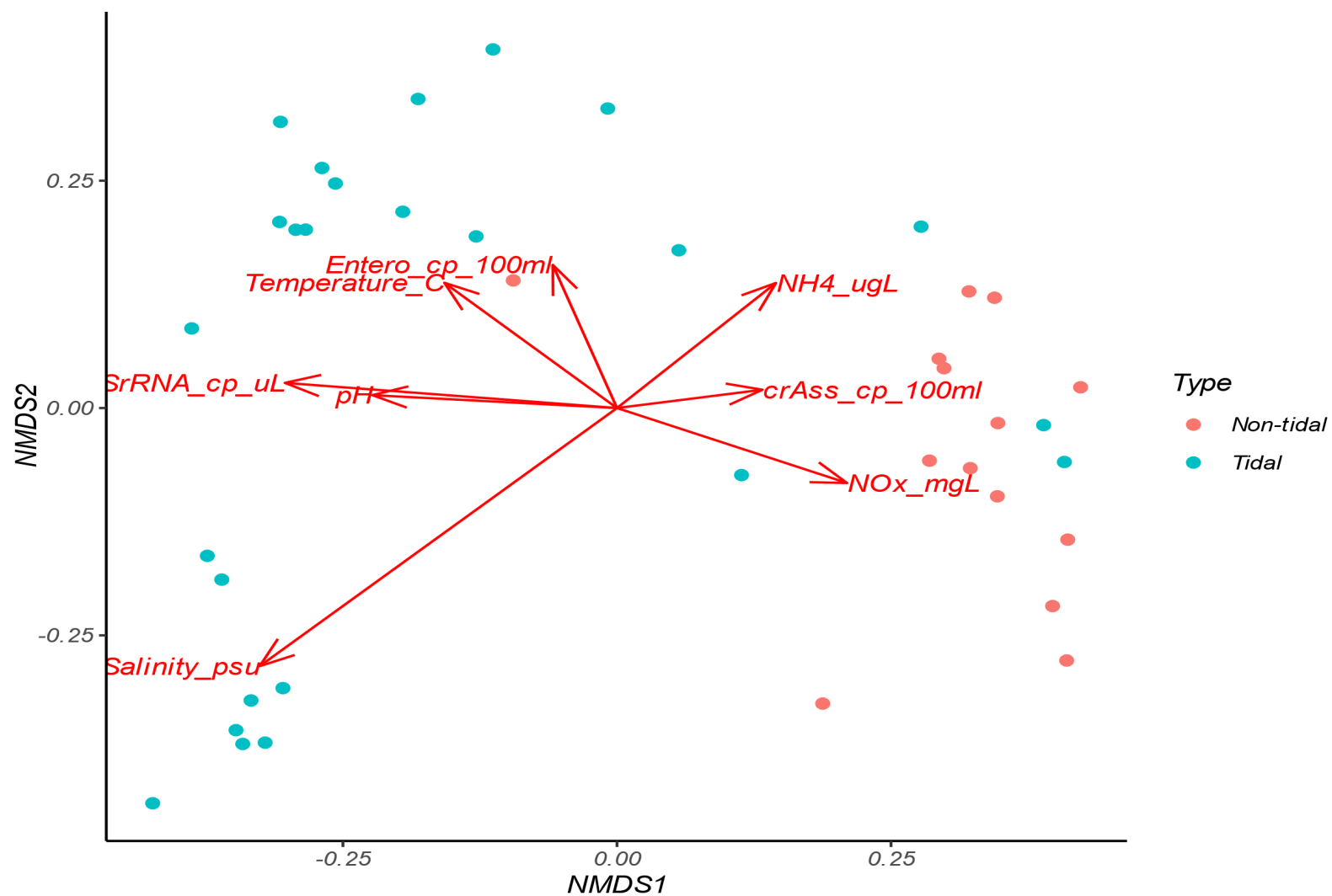
Metagenomic Methods

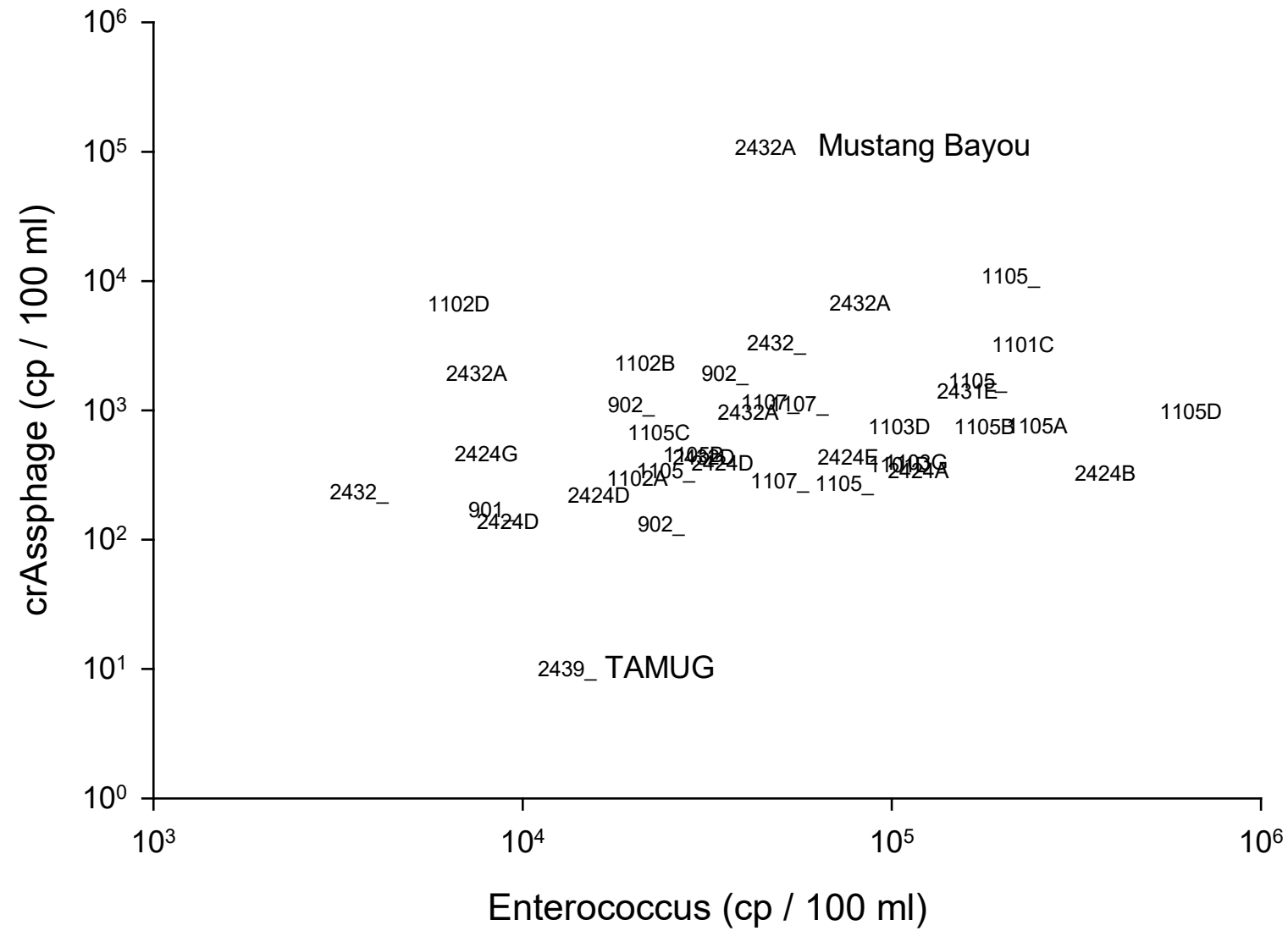
- Stations in tributaries to Western Galveston Bay were sampled in July 2023 in conjunction with the Environmental Institute of Houston (EIH).
- Water samples were acidified to capture phages and cells and concentrated via membrane filtration using duplicate 0.45-micron filters.
- DNA from each filter was extracted using commercial kit
- Next-gen sequencing was done on amplicons generated with primers for the V4 region.
- Absolute quantification of crAssphages and *Enterococcus* species was performed using an Applied Biosystems QuantStudio™ in single 2-plex reaction

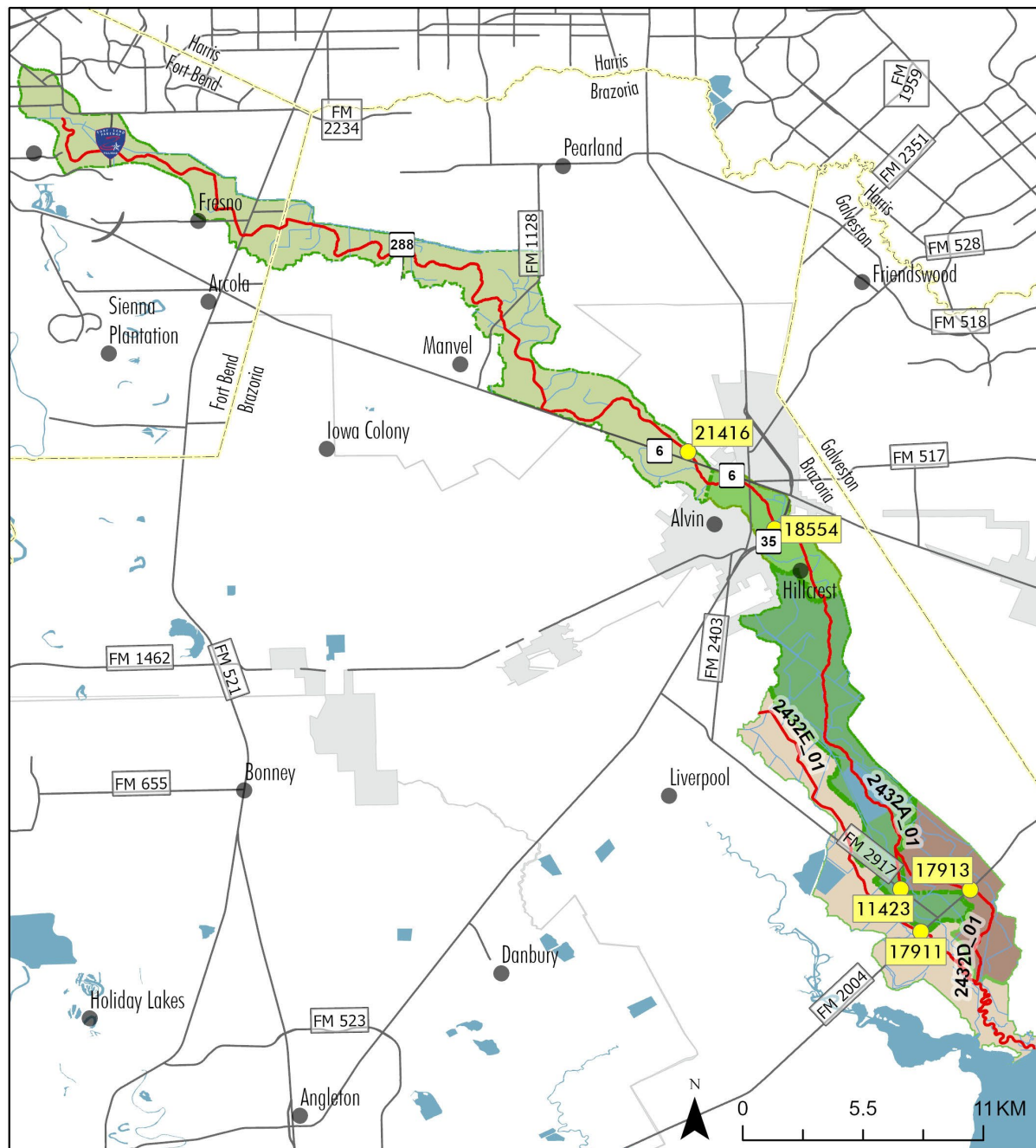
16s rRNA amplicon sequence variants



Non-metric multidimensional Scaling plot of bacterial community structure in tidal and non-tidal sites







Legend

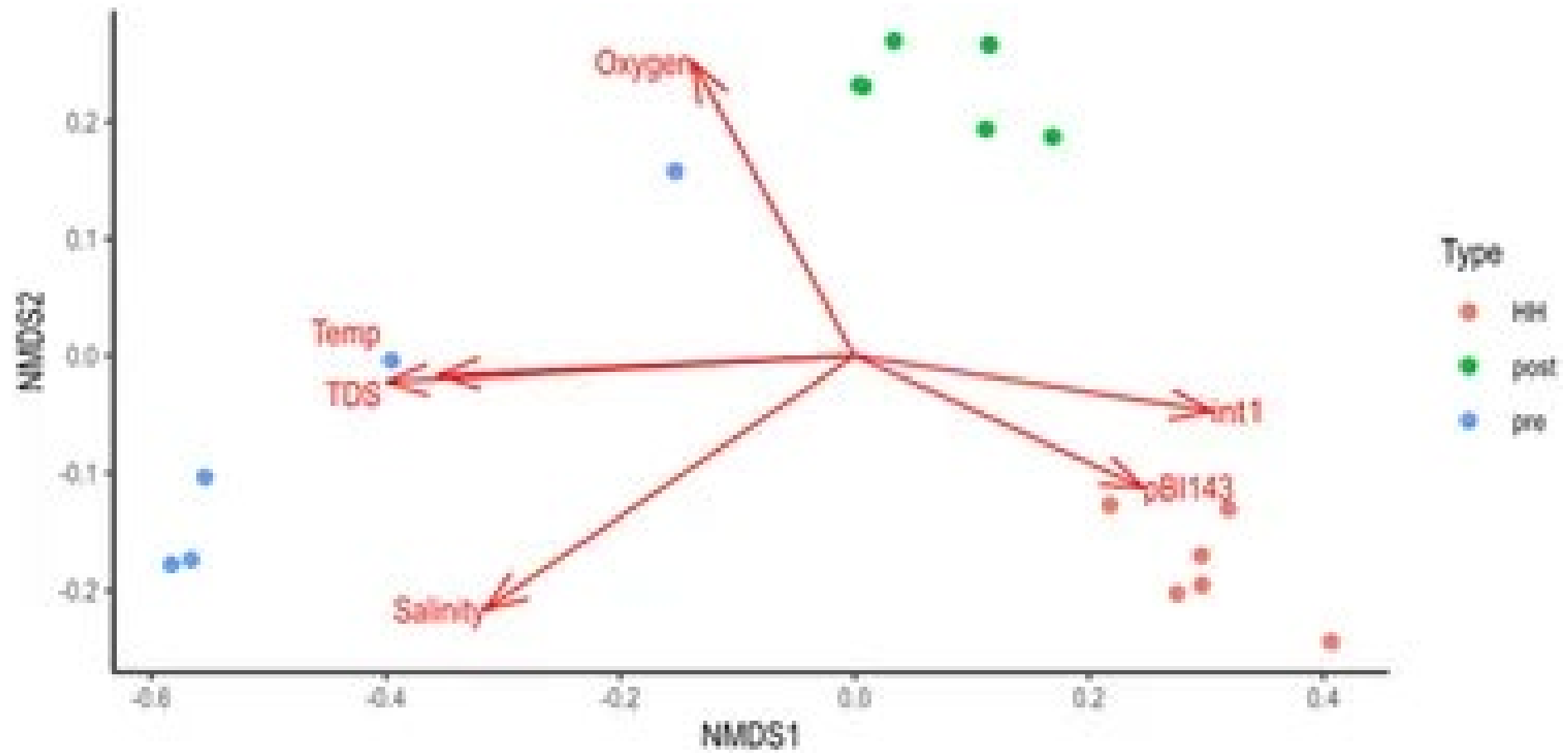
- SWQM Station
- City
- Streams
- Impaired AU
- Major Road
- ▭ City Boundary
- ▭ County Boundary

Subwatershed

- ▭ Mustang Bayou (2432A_01)
- ▭ Mustang Bayou (2432A_02)
- ▭ Mustang Bayou (2432A_03)
- ▭ Persimmon Bayou (2432D_01)
- ▭ New Bayou (2432E_01)

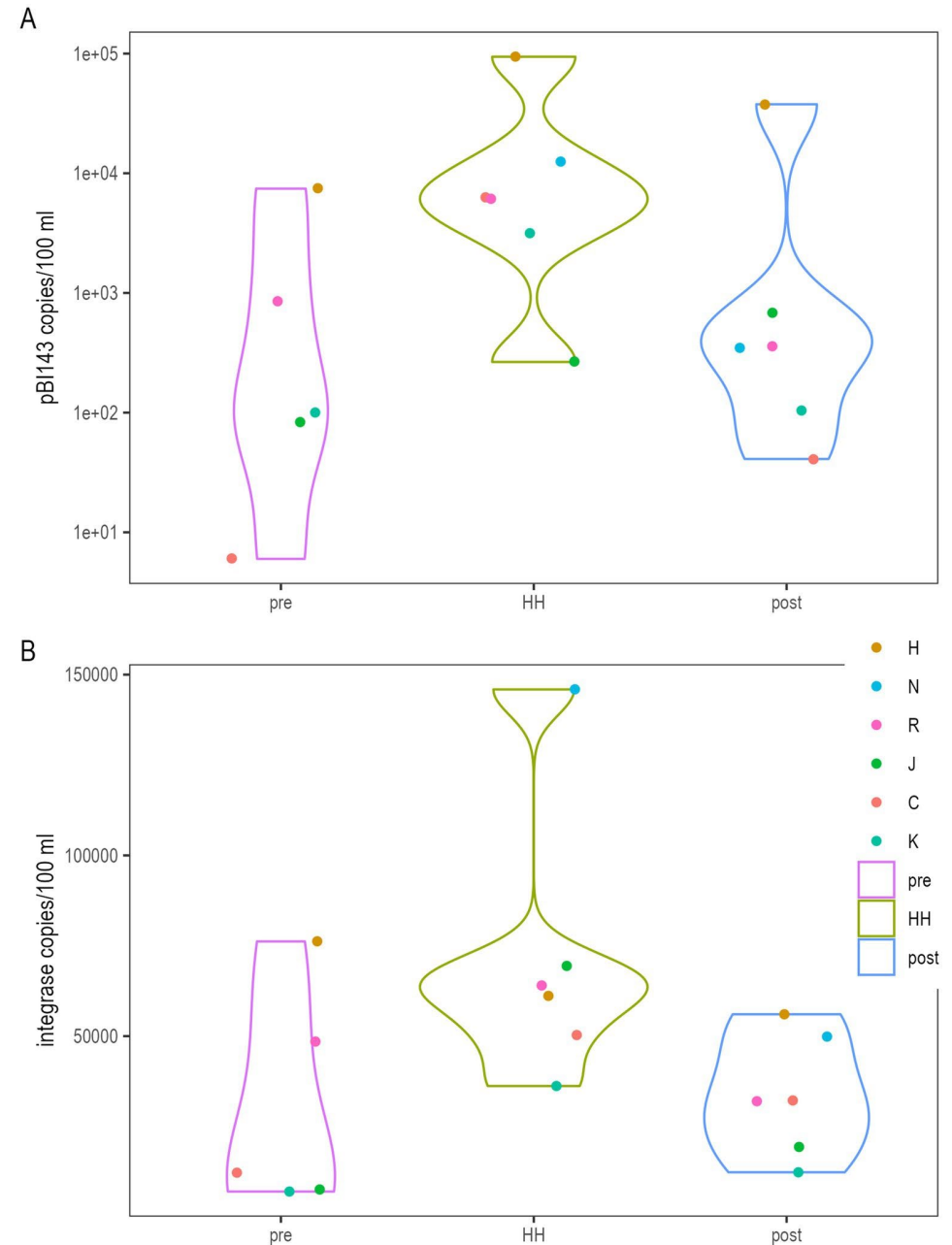


Hurricane Harvey

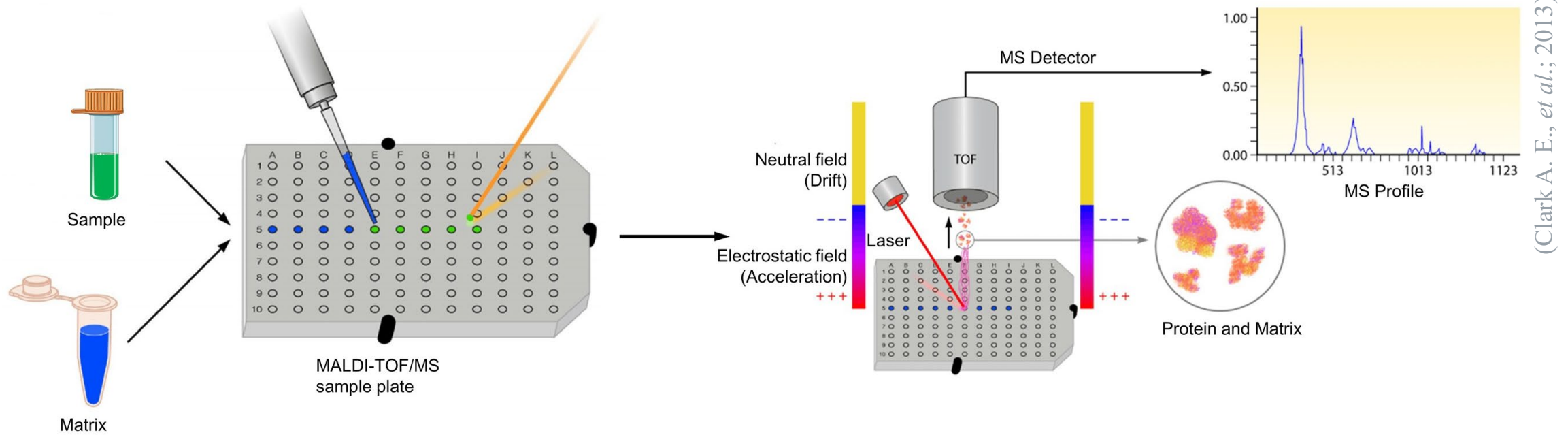


HH - dPCR

- Samples pulled from freezer
- Multiplexed assay developed for pBI143 and antibiotic resistance genes
- Floodwater samples had higher level of pBI143 and integrase

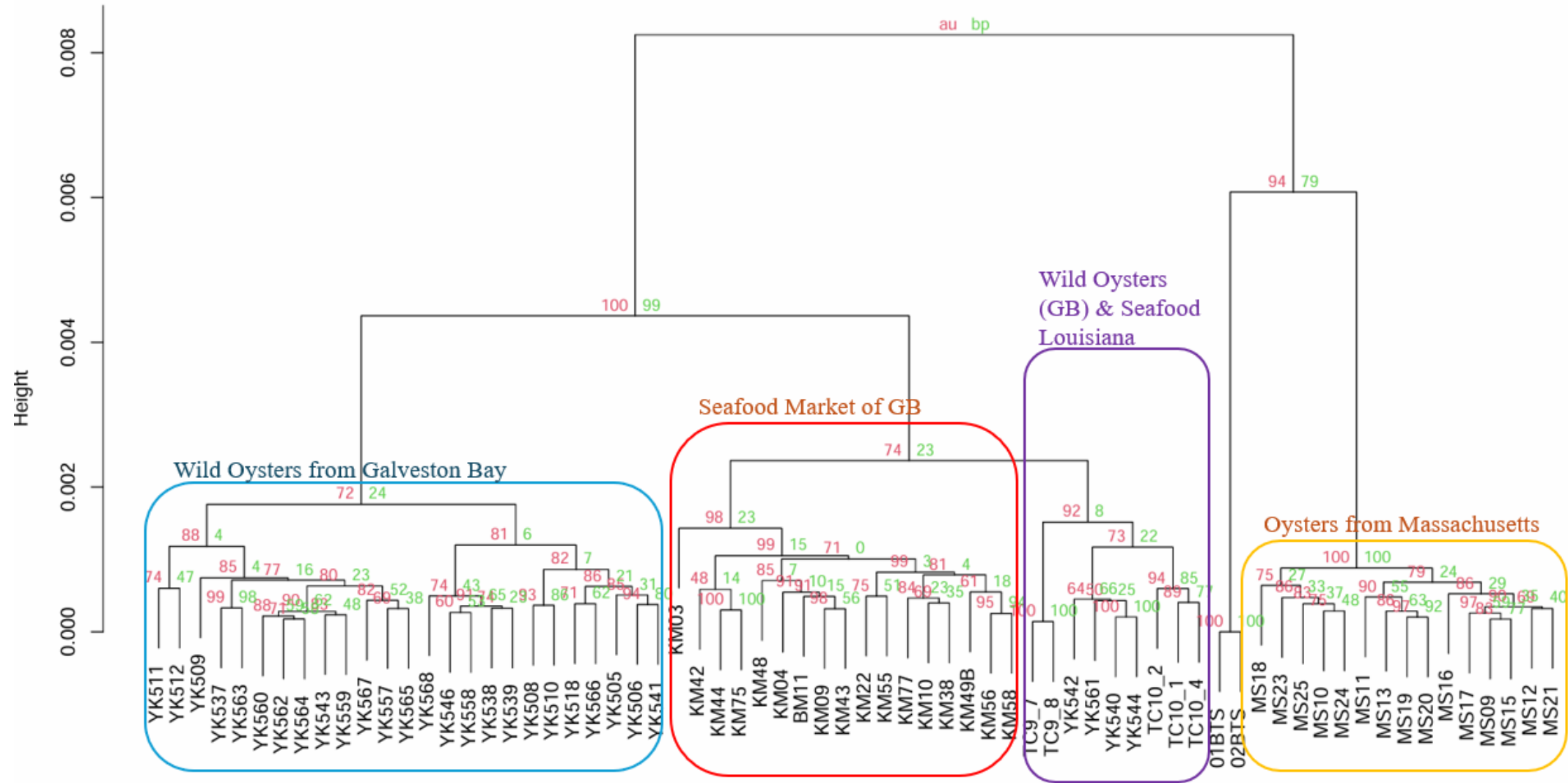


MALDI-TOF Mass Spectrometry

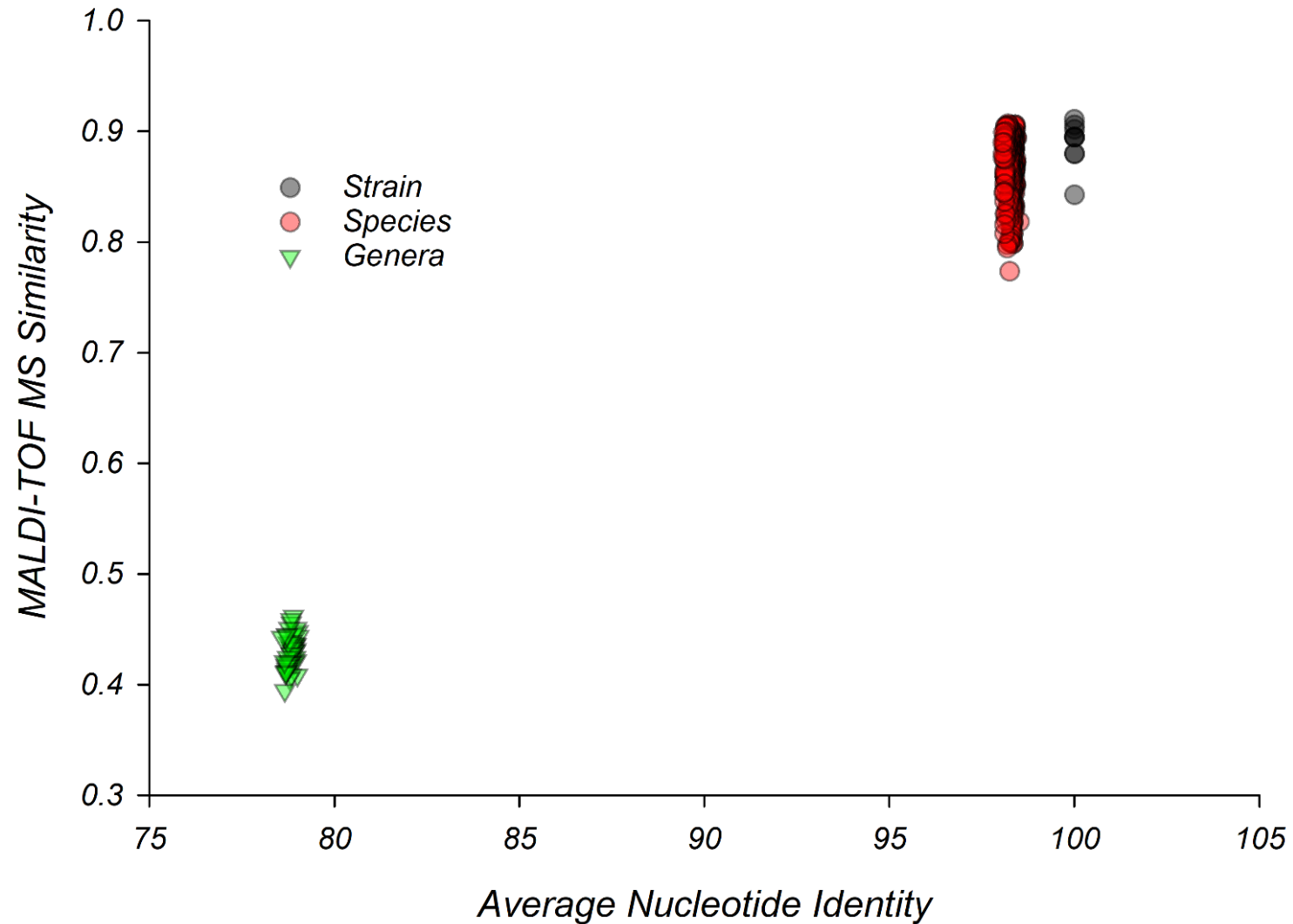


- MALDI-TOF MS identifies isolates through protein characterization through molecular weight and peptide “fingerprinting”.
- Each isolates gets Bruker identification and score.
- Bruker scores above 2 are acceptable for species level identification; between 1.7-2 are acceptable for genus level identification; under 1.7 have no reliable ID.

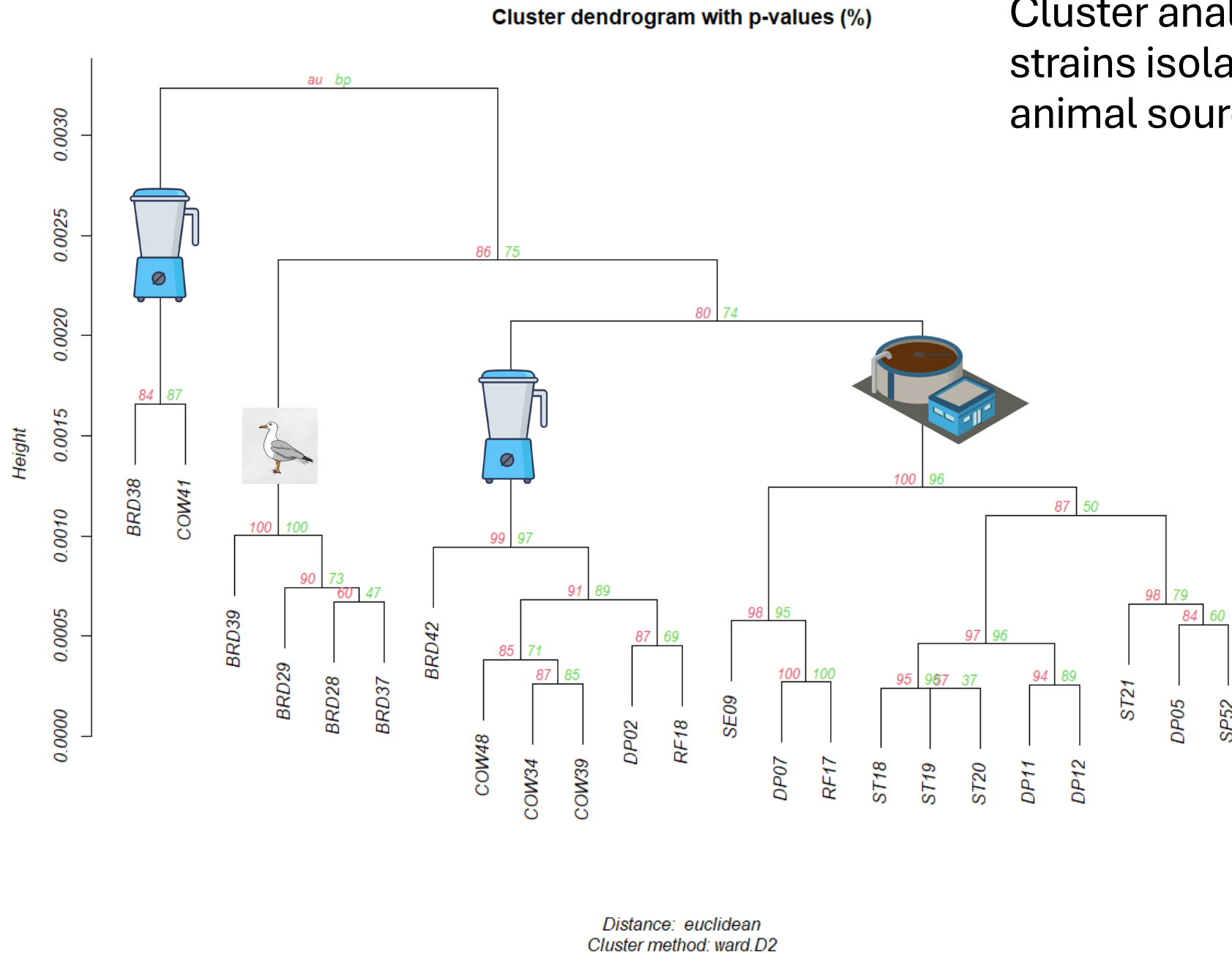
Cluster Dendrogram of *V.parahaemolyticus*



Comparison of Resolution of WGS to MALDI-TOF MS



Cluster analysis of *E. faecium* strains isolated from human and animal sources



- *Enterococcus* species isolated from various sources were identified with a MALDI Biotyper® system.
- Mass spectra generated from isolates that were identified as *E. faecium* selected for cluster analysis.
- Source-specific clusters associated with human waste or seagulls are indicated by icons.
- Clusters corresponding to a mixture of source types are indicated by the icon of a blender.

Conclusions

- dPCR and metagenomic analysis suggests
 - fecal contamination from human sources is high in Mustang Bayou
 - pBI143 high in floodwaters generated by Hurricane Harvey
- MALDI-TOF shows promise for MST

Acknowledgements



- Natasha Zarnstorff and citizens scientists at the GBF
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- Michael Allen (co-PI) and Yan Zhang (UNTHSC)
 - dPCR
 - Targeted metagenomics
- Haley Stevens (sewage and septic sample analysis)
- Tetyana King (*Vibrio*) and DSHS (WGS)
- Terry Gentry (TX A&M) / Hurricane Harvey project

