

# Leveraging DNA to Identify Bacteria Sources in Stormwater

SENY Stormwater Conference  
October 17<sup>th</sup> 2018  
Beacon, New York



# Providing Genetic & Analytic Solutions for Water

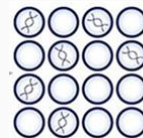


## Accredited\* Water DNA Lab

\*World's only [ISO 17025 Accredited MST Lab](#)



## Project & Site Analytics



Digital PCR



Pathogens (BSL2)



Nutrient Source Tracking



Host Fecal Score

# Increasing pressure to “get it right”

## How worried should New Yorkers be about sewage ending up in city waterways?

Combined sewer overflow is a real problem, dumping pollution into waterways—but how does it affect

By **Ashley Fettes** | Mar 30, 2018, 11:23am EDT

f t SHARE



Newtown Creek, where combined sewer overflow is one of the biggest sources of

Source of water contamination in Jones unclear, DEQ says



by **WILLIAM CRUM**

Published: Sat, August 18, 2018 1:56 PM | Updated: Sat, August 18, 2018 2:49 PM

The Oklahoma Department of Environmental Quality has been unable to identify a "definitive cause" of the contamination in the rural suburb's water supply.

E. coli was found in the Jones public water supply earlier this month.

## Sewage 'detectives' will search for Fairview Beach's waste problems

By CATHY DYSON THE FREE LANCE-STAR | Oct 7, 2018



monitoring for total  
was safe.

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Judge from Oklahoma

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# Lesson from other industries



Forensics

**God's signature: DNA profiling, the new gold standard in forensic science.**

Lynch M<sup>1</sup>.

 **Author information**

1 Department of Science & Technology Studies, Cornell University, 302 Rockefeller Hall, Ithaca, NY 14853-2401, USA.



Food Testing

## CDC Using DNA Testing to Find Source of Chipotle's E. Coli Outbreak

The exact source of the foodborne illness outbreak is still unknown.

by Whitney Filloon | @whitneyfilloon | Nov 6, 2015, 5:00pm EST

 TWEET  SHARE  PIN



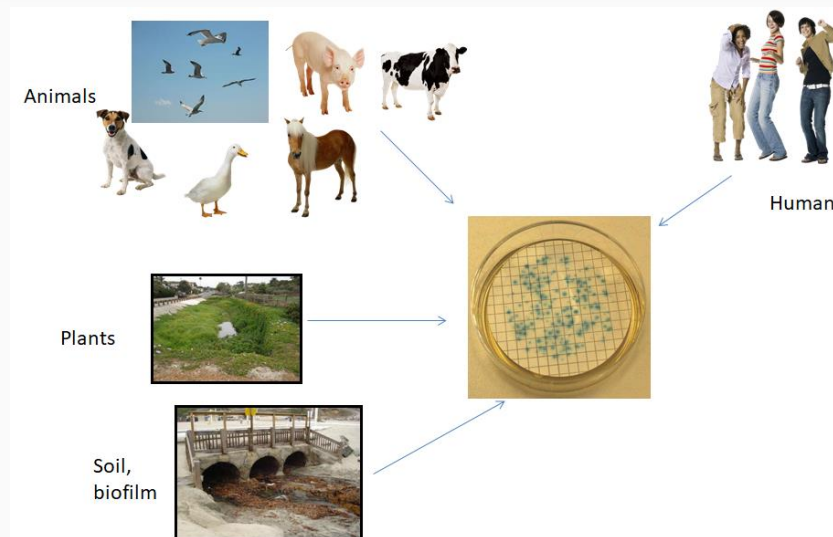
it's been  
messy yet  
#fightdir  
method

SHOP NOW

# How does genetic technology solve?

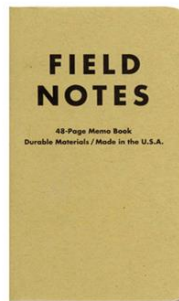
| Challenges handling fecal pollution in water | Microbial Source Tracking |
|--|---------------------------|
| <b>Where is the pollution coming from?</b>   | ✓                         |
| <b>Unclear who is responsible?</b>           | ✓                         |
| <b>How do I evaluate BMP effectiveness?</b>  | ✓                         |

# Available Tools - Legacy Testing (Culture FIB)



Concerns: Ineffective at discriminating between sources.

# Available Tools - Field Observations

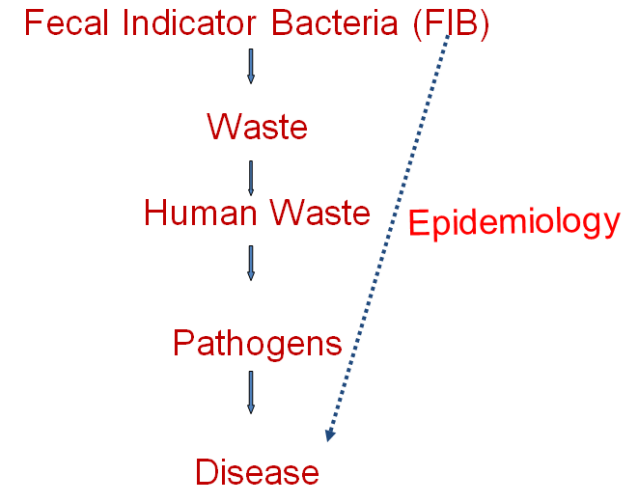


Concerns: Circumstantial and subjective evidence. Difficult to defend.

# Consequence

- Hinders source abatement
  - Source identification must precede mitigation
- Weakens the chain of inference
  - Not all sources present the same level of human health risk
    - Non-fecal < fecal
    - Non-human < human

## Basis for Monitoring: The Chain of Inference





# DNA-based Microbial Source Tracking

- There are special microbes that are only associated with a given source
  - Host and gut microbes co-evolve
    - Physiological difference of the gut
    - Dietary difference between hosts
- MST provides a set of methods to identify sources of contamination

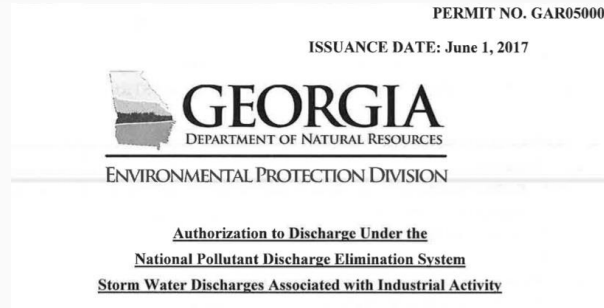


# Microbial Source Tracking - Maturity

|                                 |   |  |
|---------------------------------|---|--|
| <b>Precedent</b>                | ✓ | Projects in >40 States                       |
| <b>Credible Tests</b>           | ✓ | National Validation ( <a href="#">SIPP</a> ) |
| <b>Access to Technology</b>     | ✓ | Laboratories ( <a href="#">Accredited</a> )  |
| <b>Objective Interpretation</b> | ✓ | <a href="#">Host Fecal Score</a>             |

# MST's in stormwater permitting

## GA NPDES Industrial Storm Water General Permits



**C.2.4.2** Scientific testing, such as DNA analysis, may be used to document that bacteriological constituents found in stormwater discharges from the facility are not present as a result of industrial activity at the site or are below the impaired waters benchmark for fecal coliform.

Permittees must submit the testing program to EPD and obtain approval prior to conducting the testing. The results of the testing must demonstrate that bacterial contamination from industrial activity does not contribute to a violation of water quality standards.

# Sampling and Testing Plan

- Fecal Bacteria Hotspots
- Collecting Near Physical Sources
- Represent Watershed's Spatial Variability

## Sampling Sites



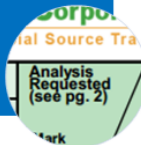
- Wet/Dry Weather Sampling
- Seasonal Changes
- Significant Number of Events to Represent Temporal Variability

## Sampling Events



- Focus on Anthropogenic Sources (Human, Dog, Agriculture)
- Most Likely Wildlife Source (Birds, Deer, ect)

## Tests Per Sample



~\$250-\$800/sample

# Tools

Source Tracking Trend Heat Map (in development)



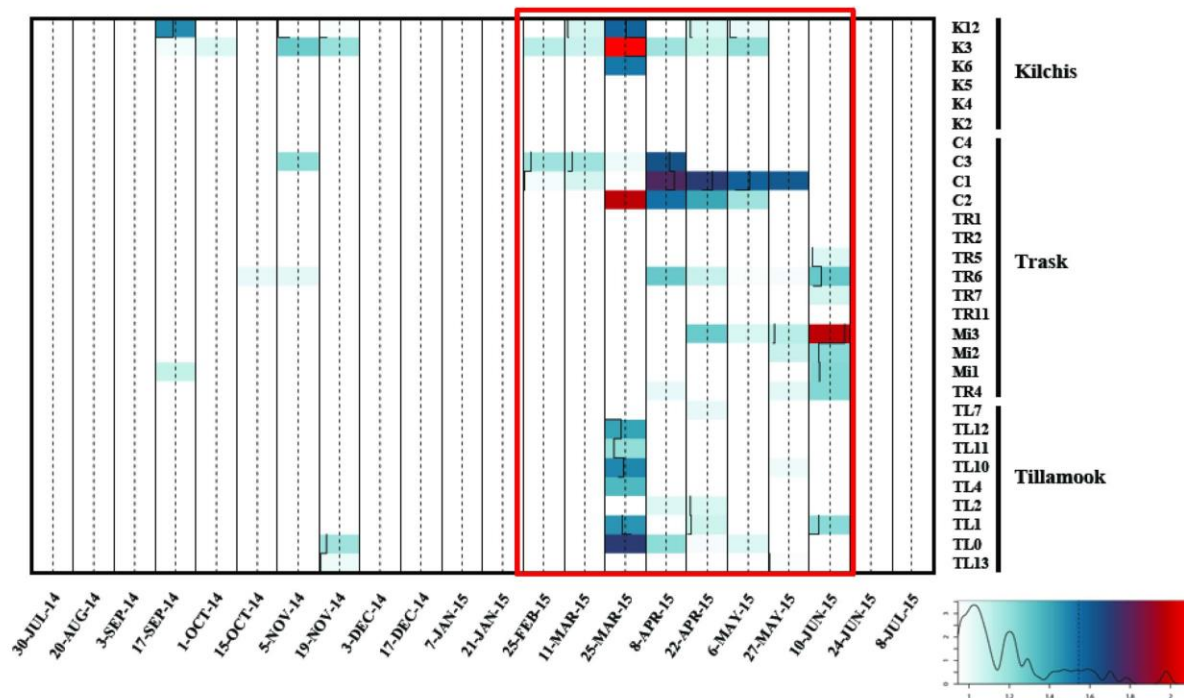
**Goal:** Identification of fecal pollution source spatial and temporal trends contributing to water impairment

- 29 sites in Tillamook Basin, OR
- Chronic water quality impairment (*E. coli* MPN)
- Bimonthly sampling for 12-months (n = 696)
- Urban, residential, agricultural and wildlife pollution sources
- Land use high resolution mapping
- 8 MST qPCR assays
- Partners
  - EPA Region 10 Laboratory
  - Oregon Department of Agriculture
  - Oregon Department of Environmental Quality
  - Tillamook Estuaries Partnership



## Field Studies:

### MST in Action - Spatial and Temporal Trends in Avian Pollution



## Field Studies:

### MST in Action - Spatial and Temporal Trends Other Sources

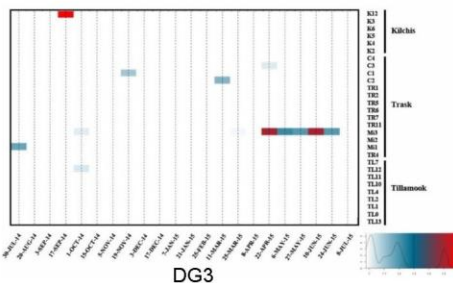
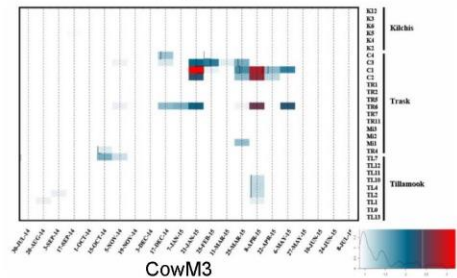
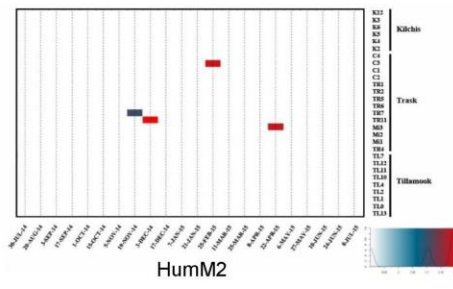
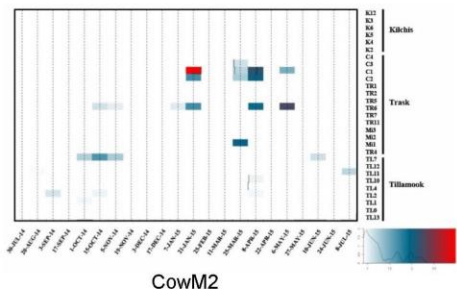
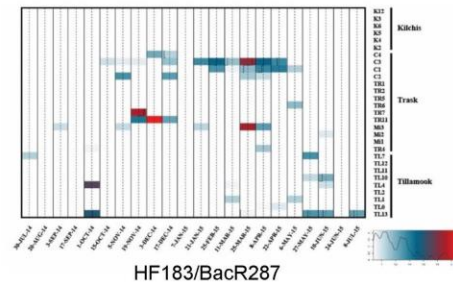
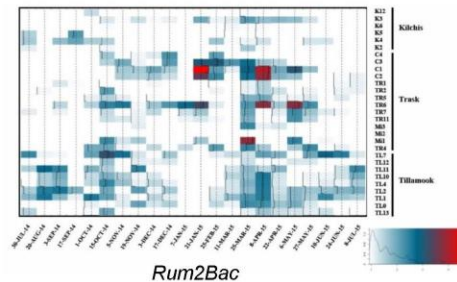
- Spatial trends

- Land use
- Waste management practices

- Temporal trends

- Weather conditions
- Agricultural practices
- Wildlife activities

- Varies by assay

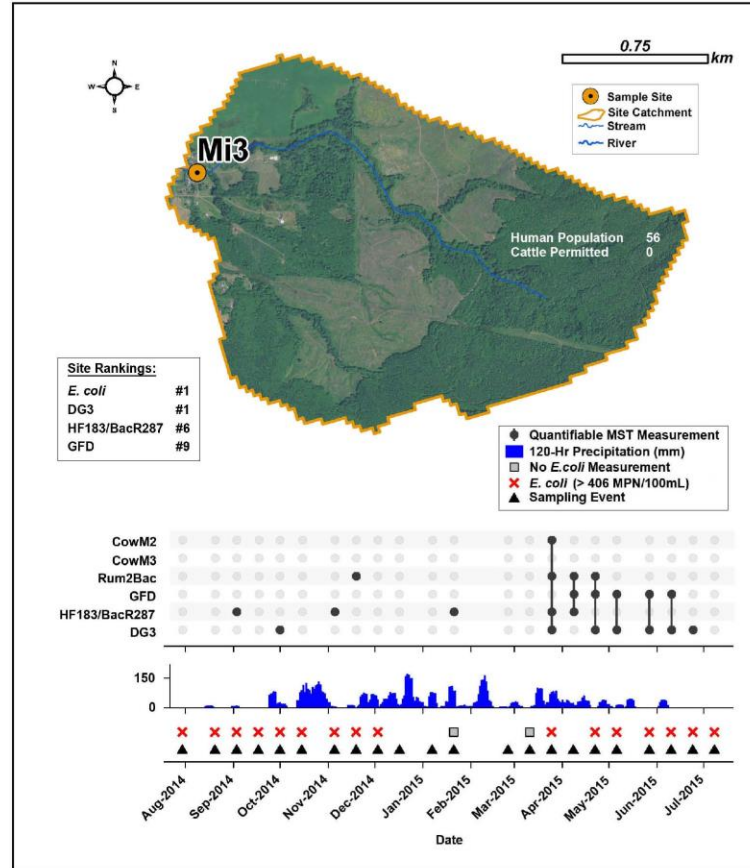




## Field Studies:

### MST in Action - Mi3 Site Profile

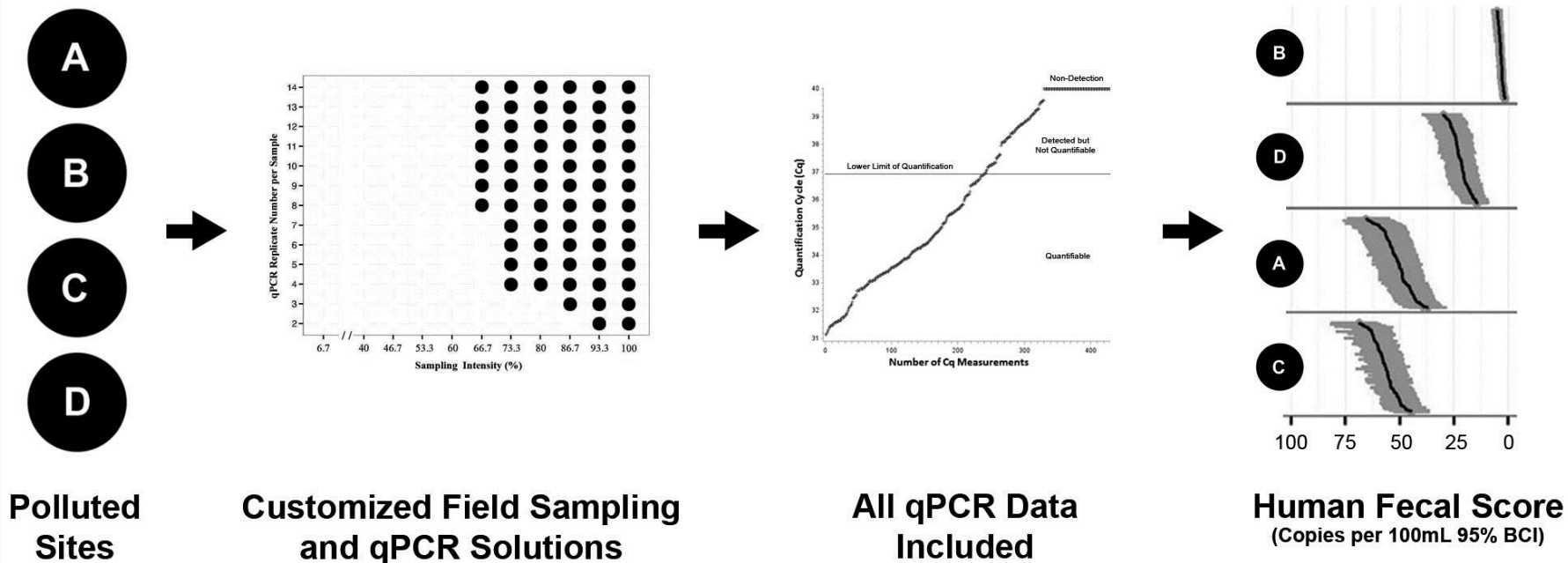
- Trask River
- *E. coli* exceedance (80%)
- Seasonal dog pollution, target local breeding facility
- Possible bird migration impact
- Non-point human impact during wet season
- Ruminant in spring, likely AFO
- Management recommendation
  - Sanitary survey in Spring
  - Target AFO, septic system, and dog facility



# Tools

## Project Level Probabilistic Modeling

## HUMAN FECAL SCORE FOR SITE RANKING



**STANDARDIZED PROCEDURE**

# Tools

qPCR MST Automated Platform

## Next Steps - qPCR MST Automated Platform



<https://youtu.be/1IJYHeglt4M>

# Site Prioritization - Florida DEP/Martin County

## Summary of Costs / Ranking (Total)

| Ranking   | Communities  | # of Parcels | Totals Cost               |                       |                       | Cost Per Parcel           |                       |                       |
|-----------|--|--------------|---------------------------|-----------------------|-----------------------|---------------------------|-----------------------|-----------------------|
|           |  |              | Vacuum Collection System* | Gravity Sewer System* | Grinder Pump System** | Vacuum Collection System* | Gravity Sewer System* | Grinder Pump System** |
| 1         | Martin Downs / Sunset Gardens (Old Palm City) Area | 1078         | \$13,532,368              | \$20,852,239          | \$16,412,724          | \$12,553                  | \$19,343              | \$15,225              |
| 2         | Golden Gate Subdivision                            | 775          | \$9,580,163               | \$15,656,572          | \$11,923,663          | \$12,373                  | \$20,202              | \$15,385              |
| 3         | Deau Rivage Subdivision                            | 256          | \$3,974,236               | \$5,909,089           | \$4,029,218           | \$15,524                  | \$23,082              | \$15,739              |
| 4         | Gaines Ave Area                                    | 277          | \$4,445,731               | \$6,817,429           | \$4,447,102           | \$16,050                  | \$24,612              | \$16,055              |
| 5         | Hibiscus Park Area                                 | 1349         | \$14,165,566              | \$18,617,161          | \$18,924,589          | \$10,501                  | \$13,801              | \$14,029              |
| 6         | Port Salerno / New Monrovia Area                   | 878          | \$10,536,133              | \$14,665,435          | \$12,878,941          | \$12,000                  | \$16,703              | \$14,668              |
| 7         | Salerno / Manatee Pocket Area                      | 478          | \$5,434,932               | \$7,838,844           | \$6,905,908           | \$11,370                  | \$16,441              | \$14,448              |
| 8         | Not  |              |                           |                       |                       |                           |                       | \$16,802              |
| 9         | Bay  |              |                           |                       |                       |                           |                       | \$15,452              |
| 10        | Sea  |              |                           |                       |                       |                           |                       | \$23,178              |
| 11        | Sea  |              |                           |                       |                       |                           |                       | \$15,308              |
| 12        | Town of Seawall's Point                            | 931          | \$11,559,281              | \$16,658,298          | \$14,024,705          | \$12,416                  | \$17,893              | \$15,064              |
| 13        | Rio / St. Lucie (East)                             | 331          | \$4,223,304               | \$6,408,478           | \$4,894,485           | \$12,759                  | \$19,361              | \$14,787              |
| 14        | Rosewalk / Gallop Day                              | 25           | --                        | \$1,173,085           | \$437,729             | --                        | \$46,950              | \$17,509              |
| 15        | Shurt Yacht & Country Club                         | 504          | \$7,065,980               | \$9,910,480           | \$7,596,348           | \$14,020                  | \$19,664              | \$15,072              |
| 16        | Four Rivers Subdivision                            | 106          | --                        | \$3,171,303           | \$1,985,847           | --                        | \$29,919              | \$18,449              |
| 17        | Cause Creek Country Club                           | 381          | \$6,462,014               | \$10,091,624          | \$6,385,546           | \$16,903                  | \$25,849              | \$16,500              |
| 18        | North Rivers Shore - Phase 2                       | 292          | \$4,186,403               | \$6,398,525           | \$4,484,893           | \$14,337                  | \$21,125              | \$15,359              |
| 19        | Tropical Farms Area                                | 652          | \$9,846,995               | \$14,796,607          | \$10,299,471          | \$15,102                  | \$22,094              | \$15,797              |
| 20        | River's End Subdivision                            | 113          | --                        | \$3,050,607           | \$2,011,467           | --                        | \$26,997              | \$17,801              |
| 21        | Vista Salerno / US 1 Area                          | 234          | \$3,404,083               | \$4,781,277           | \$3,558,041           | \$14,547                  | \$20,433              | \$15,205              |
| 22        | Rio / St. Lucie (West)                             | 97           | --                        | \$2,197,773           | \$1,582,174           | --                        | \$22,657              | \$16,311              |
| 23        | Captain's Creek Subdivision                        | 167          | --                        | \$4,591,743           | \$2,769,291           | --                        | \$27,495              | \$16,583              |
| 24        | Lake Grove Subdivision                             | 76           | --                        | \$2,027,803           | \$1,955,236           | --                        | \$26,083              | \$18,358              |
| Totals*** |  | 10,358       | \$118,082,117             | --                    | \$20,150,037          |                           |                       |                       |
|           |  |              | Total Cost****            | \$138,232,154         |                       |                           |                       |                       |

## Ranking Summary

| Ranking                  | 1   | 2           | 3           | 4        | 5                             | 6                        | 7                             | 8                             | 9                             | 10                            | 11                            | 12                            | 13                            | 14                            | 15                            | 16                            | 17                            | 18                            | 19                            | 20                            | 21                            | 22                            | 23                            | 24                            |
|--------------------------|---|-------------|-------------|----------|-------------------------------|--------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Communities              | Martin Downs / Sunset Gardens (Old Palm City) | Golden Gate | Deau Rivage | Bay Area | Port Salerno / Manatee Pocket | Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket | Port Salerno / Manatee Pocket |
| Population Density       |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Potable Systems          |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Classified Surface       |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Proximity Surface        |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| FEMA / Plain             |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Ground Table             |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Soil Type                |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Surface Water Management |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Nitrogen Contribution    |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Human Biological Markers |   |             |             |          |                               |                          |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |                               |
| Total Score              | 99.35   | 90.85       | 85.51       | 84.37    | 82.93                         | 82.30                    | 81.90                         | 80.66                         | 79.64                         | 79.36                         | 76.33                         | 74.57                         | 73.98                         | 73.15                         | 72.69                         | 72.10                         | 71.65                         | 71.35                         | 71.17                         | 70.80                         | 68.62                         | 68.52                         | 65.74                         | 53.53                         |

# Where is the pollution coming from - Boston Water and Sewer

- **First ever** effectiveness assessment of MS4 IDDE program using DNA markers
- Human markers measured at outfalls regardless of degree of IDDE completion, and conventional tools (test kits) found to be insufficiently sensitive or specific for detecting illicit discharges
- New IDDE procedures now recommended, including DNA markers to improve program effectiveness
- Outcome will be greater bacteria and phosphorus reduction (at **lower unit cost and greater health benefit** than Green Infrastructure), **moving City closer to TMDL compliance**
- Project recognized with **national O&M Performance Award from NACWA**

Geosyntec  
consultants



# Demonstrating BMP effectiveness - Santa Barbara

## **Santa Barbara Beaches** (with UCSB), for SWRCB under Clean Beaches Initiative grant

- Infrastructure sources investigated and largely ruled out
- Homeless and bather sources continue to be evaluated
- DNA markers have been an essential complement to conventional tools (dye, CCTV, GIS, etc.)
- Management actions recommended based on study results, **improving public health protection at high use beaches**
- Prior work was **first ever** to document and publish on sewer exfiltration into stormdrains, shedding new light on this important source for agencies nationwide

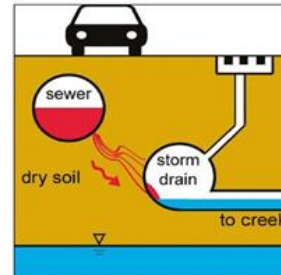
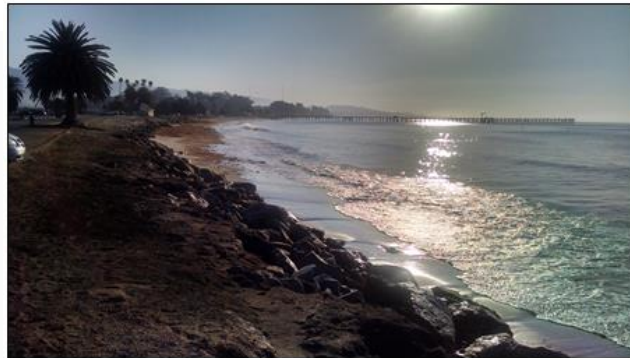


Figure 3-1. Leaking Sanitary Sewer Exfiltrating to Storm Sewer  
(Source: Sercu et al. 2011<sup>4</sup>)



# Summary

Genetics is a superior tool for water quality monitoring

It can be used for effective investigations of bacteria impairment

Building a data driven approach based on rich DNA analytics

A powerful tool for regulatory prioritization (QMRA/Natural Source Exclusions)

# Thank You



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