

- Leveraging Fecal DNA to
- enhance water quality

•Evidence-based guidelines for Microbial Source Tracking

•March 1, 2018  
•SC Association of Stormwater Managers



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- Increasing pressure on government/dischargers to “get it right”



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- Available Tools - Legacy Testing (Culture FIB)



•Concerns: Ineffective at discriminating between sources.



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### •Available Tools - Field Observations



•Concerns: Circumstantial and subjective evidence. Difficult to defend.

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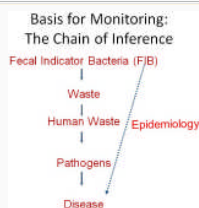
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### •Consequence

- 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



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### Human MST Method Standardization: Detection Technologies

- Microarray
- Deep sequencing
- End-point PCR
- Quantitative real-time PCR
- Digital PCR
- Immuno-magnetic separation
- Terminal restriction fragment length polymorphism
- Selective bacterial culturing
- Antibiotic resistance profiling
- Chemical detection
- Canine scent detection



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### •Microbial Source Tracking

Source: [1] Park, Biological and Ecological Sources of Foodborne Pathogens (2014), p. 174

Cellcore Microbial Source Identification Manual

PATHOGENS

Support sites of the microbial source tracking research and technology development project. The project is supported by the National Science Foundation.

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### •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

Source: [1] Park, Biological and Ecological Sources of Foodborne Pathogens (2014), p. 174

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### •DNA-based Microbial Source Tracking

#### How do qPCR quantify?

Grow cells vs. "grow" DNA

Source: [1] Park, Biological and Ecological Sources of Foodborne Pathogens (2014), p. 174

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•Microbial Source Tracking - Maturity

<b>Precedent</b>	Clean Beach Initiative Projects Boston Projects in >40 States
<b>Credible Tests</b>	Validated Methods (SIPP)
<b>Access to Technology</b>	Laboratories (Accredited)
<b>Objective Interpretation</b>	Host Fecal Score

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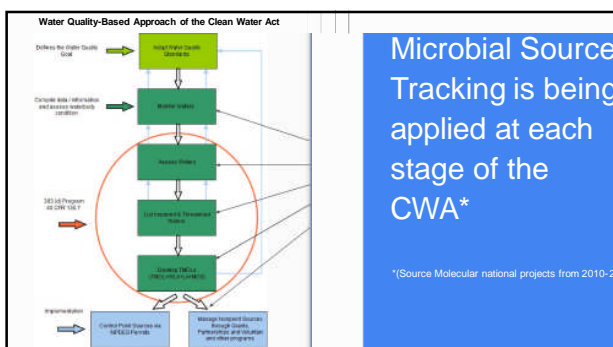
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•How does genetic technology solve?

Water Pollution Challenges	Microbial Source Tracking
Where is the pollution coming from?	
Which site(s) do I prioritize?	
How do I evaluate permittees' BMP effectiveness?	

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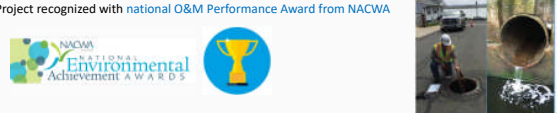
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•Where is the pollution coming from - Boston Water and Sewer

- 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




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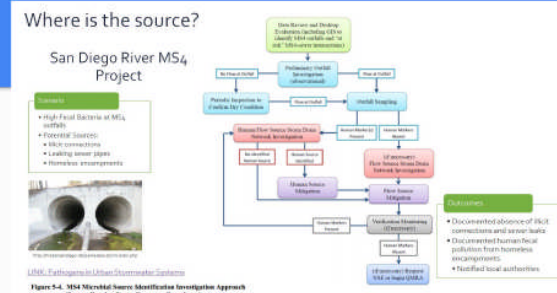
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Where is the source?  
San Diego River MS4 Project



**Outcomes:**

- Documented absence of illicit connections and sewer leaks
- Documented nonpoint pollution from residential encroachment
- Notified local authorities

UNW, Pathways to Urban Stormwater Systems  
Figure 5-4. MS4 Nonpoint Source Identification Investigation Approach  
(Source: Brandon Roth, Guyton Consulting)

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•Site Prioritization - Florida DEP/Martin County

Summary of Costs / Ranking (Total)

Site ID	Name	Priority	Score	Cost (\$)	Rank
1	...	...	...	...	...
2	...	...	...	...	...
...	...	...	...	...	...
10	...	...	...	...	...
Total Cost				\$1,020,000	...

Ranking Summary

Marker	Score	Weight	Weighted Score
Human	12	12	144
Biological	12	12	144
...	...	...	...
<b>Total Score</b>	<b>99.55</b>	<b>90.85</b>	<b>85.51</b>

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•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


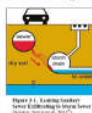



Figure 1.1. Sewer exfiltration from a storm drain into the ocean. Source: National SCSW.

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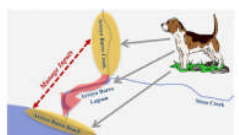
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•Demonstrating BMP effectiveness - Santa Barbara

**Arroyo Burro Beach Source Tracking Project**  
Multiple Source Markers to test hypotheses

**Microbial Source Tracking in a Coastal California Watershed Reveals Canines as Controllable Sources of Fecal Contamination**  
Jarrod S. Krizan et al. 2014



**Observations**

- High use of Beaches
- Refractory Sources
- Homeless
- Marine
- Gulf
- Dog

**Conclusions**

- Marine not detected
- Gulf confirmed at lagoon and beach
- Dog markers reduced after targeted public outreach
- Human markers associated with homeless encampments

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•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 on top of rich DNA test data enables analytics

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•Summary

**Define Project Objectives**

- IDDE
- Compliance Demonstration
- Natural Source Exclusion
- Site Specific Objectives
- Infrastructure Asset Management

Initial Hypothesis For Source of Bacteria

High Levels of Bacteria in Stormwater

Land Use within Watershed

Fecal Bacteria

Fecal Bacteria Hotspots

Wet/Dry Weather Sampling

Seasonal Changes

Represent Temporal Variability

Focus on Anthropogenic Sources

Most Likely Wildlife Source

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•Summary

**Sample and Test Plan**

- Sampling Sites**
  - Fecal Bacteria Hotspots
  - Collecting Near Physical Sources
  - Represent Watershed's Spatial Variability
- Sampling Events**
  - Wet/Dry Weather Sampling
  - Seasonal Changes
  - Represent Temporal Variability
- Tests Per Sample**
  - Focus on Anthropogenic Sources (Human, Dog, Agriculture)
  - Most Likely Wildlife Source (Birds, Deer, ect)

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•Summary

**HFS Application: Prioritizing Remediation**

**HUMAN FECAL SCORE FOR SITE RANKING**

**HFS Application: Real Site Scores**

Human Fecal Score

Site

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•Simple/Clear, Complete, Objective, Standardized

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 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**

\*22 Source Molecular

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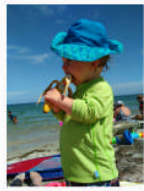
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**•Thank You**

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