qPCR and Canine Microbial Source Tracking in Juanita and Thornton Creeks

Project Leads

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Jonathan Frodge – Seattle Public Utilities
Ryean-Marie Tuomisto – Kirkland Water Quality
Karen Reynolds – Environmental Canine Services
Testing Microbial Source Tracking Tools

CRITERIA
Fecal coliform
*E. coli*

HUMAN

- Human *Bacteroidetes* - EPA (HumM2)
- Human *Bacteroides* - EPA (Hu-2-Bac)

RUMINANT/COW

- Cow *Bacteroidetes* - EPA (Cow M2)
- Ruminant *Bacteroidales* (Rum-2-Bac)

The Usual Suspects
Human Source qPCR markers

$qPCR = \textit{quantitative Polymerase Chain Reaction}$

- not a culturing process – not relying on viable cells
- targets and quantifies DNA the most common human gut
  \textit{Bacteroides spp}

\textbf{Hu-2-Bac} - more specific than previous markers
(less false positives).

Sample ➔ membrane filtration
➔ frozen @ -80°C ***
➔ aliquot processed
➔ reported as copies/100ml
Canine source tracking

- Two canines trained to identify fecal material specifically originating from humans
- Both dogs lay down to indicate presence
- Successfully used in Santa Barbara, CA to identify human waste contamination (same company, different dogs)
  - *Van De Werfhorst et al.* (2014)
Urban Watersheds

Both Creeks

- Drain to swimming beaches with historically high fecal coliform
- Past intensive bacterial studies
- Great partners!
**Study Design**

Two samples collected at each site:

- Canine testing
- Lab testing
  - E. coli / FC
  - Hu-2-Bac

**Juanita Creek (73 samples)**

- **Day 1**: Bucket test in scent neutral field
- **Day 2**: site investigations
- **Day 3**: Bucket test in scent neutral field
- **Day 4**: site investigations

**Thornton Creek (77 samples)**

- **Day 1**: Bucket test in scent neutral field
- **Day 2**: site investigations
- **Day 3**: Bucket test in scent neutral field
- **Day 4**: site investigations
Controls in each basin (Day 1 & Day 3)

- **4 Positive Controls**: Influent diluted to 1%, 2%, 5%, and 10%
- **4 Negative Controls**: Distilled H₂O
- **4 Alternate Waste Samples**: 1% to 2% diluted cat/dog feces
- **2 Field Duplicates**
Canine Source Tracking

• Video:  http://vimeo.com/95309371
Thornton Creek Results

4,970,200
1,873,200
608,700
840,200
117,100

Hu-2-bac (copies/100 mL)
Juanita Creek Results

E. coli (cfu/100 mL)

*Hu-2-bac (copies/100 mL)*
Dogs performed well on controls
Mostly agreed but some variability
Canine hits did not agree with FC, EC, or Hu-2-Bac
Canine Scent Tracking Summary

Pros

- Rapid response for field work
- Control results were good – two exceptions.
- Great exposure

Cons

- Some variability between dogs
- Uncertain what exactly in waste stream dogs key on (may not be fecal material)
- Did not correlate well with laboratory results
- Follow up dye and smoke testing showed nothing
- Not local - cost $$$ for travel
Human *Bacteroides* qPCR Summary

**Pros**
- Specific to human gut bacteria
- Control results were good
- Quantitative results
- Can freeze samples for later analysis allowing for tiered processing

**Cons**
- Interference can inhibit reading
- DNA can last in environment depending on conditions
- Two week turnaround time
Acknowledgements

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- Doug Williams
- Logan Harris

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- Holli Brandt
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- Nathan Hart
- Ellen Stewart
- Adam Bailey

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- Ryean-Marie Tuomisto
- Wes Ayers
- Jenny Gaus
- Marie Jensen
- Most members of the Kirkland engineering and maintenance staff!!

**Environmental Canine Services:**
- Karen Reynolds
- Gwen – Molly’s handler
- Aryn – Crush’s handler
Questions?
Hu-1-Bac vs Hu-2-Bac

Graph showing the correlation between Hu-1-Bac [cells 100mL⁻¹] and Hu-2-bac [copies 100mL⁻¹].
Hu-1-Bac vs Hu-2-Bac - ranked
<table>
<thead>
<tr>
<th>Organism or Gene Marker Name</th>
<th>Method and Result Units</th>
<th>Type of fecal Pollution Indicated</th>
<th>Performance Characteristics</th>
<th>Notes</th>
<th>Cost for Complete Analysis</th>
<th>Cost for Prep and Hold</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bacteroides spp.</strong></td>
<td>Membrane filtration &amp; Real Time qPCR</td>
<td>Indicates Possible Human Source</td>
<td>Highly Sensitive(^1) (detects target at low concentrations)</td>
<td>Presence indicates recent human contamination</td>
<td><strong>$125</strong></td>
<td><strong>$50.00</strong></td>
</tr>
<tr>
<td>Hu1Bac</td>
<td>Cells/100 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Bacteroides spp.</strong></td>
<td>Membrane filtration &amp; qPCR</td>
<td>Indicates Possible Human Source</td>
<td>Top performing human marker in 2013 study(^2)</td>
<td>Presence indicates recent human contamination</td>
<td><strong>$125</strong></td>
<td><strong>$50.00</strong></td>
</tr>
<tr>
<td>Hu2Bac</td>
<td>Copies/ml</td>
<td></td>
<td></td>
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<tr>
<td><strong>Bifidobacteria spp.</strong></td>
<td>Culture Based</td>
<td>Indicates Possible Human Source</td>
<td>High Confidence. (Any level of detection is significant)</td>
<td>Presence indicates recent human contamination</td>
<td><strong>$175</strong></td>
<td>N\A</td>
</tr>
<tr>
<td></td>
<td>Cells/100 ml</td>
<td></td>
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<tr>
<td><strong>Ruminant</strong></td>
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<tr>
<td><strong>Rum2Bac</strong></td>
<td>Membrane filtration &amp; qPCR</td>
<td>Indicates Possible Ruminant Source</td>
<td>High Confidence. Top performing ruminant marker in 2013 study(^3)</td>
<td></td>
<td><strong>$125</strong></td>
<td><strong>$50.00</strong></td>
</tr>
<tr>
<td></td>
<td>Copies/ml</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><strong>Rhodococcus spp.</strong></td>
<td>Culture Based</td>
<td>Indicates Possible Ruminant Source</td>
<td>High Confidence</td>
<td></td>
<td><strong>$300</strong></td>
<td>N\A</td>
</tr>
<tr>
<td></td>
<td>Cells/100 ml</td>
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</tbody>
</table>

**Costs here for single samples and may be discounted in larger batches.**
Thornton Creek Results

LEGEND

- ★ Follow-up sampling

Hu-2-bac (size) [copies/100mL]
- 0
- 1 - 1,000
- 1,001 - 10,000
- 10,001 - 100,000
- 100,001 - 1,000,000
- > 1,000,000

E. Coli (color) [CFU/100mL]
- 0
- 1 - 50
- 51 - 100
- 101 - 410
- 411 - 1,000
- 1,001 - 10,000

Lake Washington
Thornton follow up *E. coli*

Pre-storm *E. Coli* (sampled 10/8/14)

10/13-14 Precip = 0.60”

Post-storm *E. Coli* (sampled 10/14/14)
Thornton follow up Hu-2-Bac

Pre-storm Hu-2-bac (sampled 10/8/14)
10/13-14 Precip = 0.60”
Post-storm Hu-2-bac (sampled 10/14/14)
Juanita follow up *E. coli*

**Pre-storm E. Coli** (sampled 10/8/14)

10/13-14 Precip = 0.60”

**Post-storm E. Coli** (sampled 10/14/14)
Juanita follow up Hu2Bac

Pre-storm Hu-2-bac (sampled 10/8/14)
10/13-14 Precip = 0.60”

Post-storm Hu-2-bac (sampled 10/14/14)