qPCR and Canine Microbial Source Tracking in Juanita and Thornton Creeks

Project Leads

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Ryean-Marie Tuomisto – Kirkland Water Quality
Karen Reynolds – Environmental Canine Services

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Water and Land Resources Division

Seattle Public Utilities

City of Kirkland Washington

Environmental Canine Services LLC
Protecting Our Precious Resources with Nature's Gift
www.ecsk9s.com

Testing Microbial Source Tracking Tools

CRITERIA

Fecal coliform

E. coli

RUMINANT/COW

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

HUMAN

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

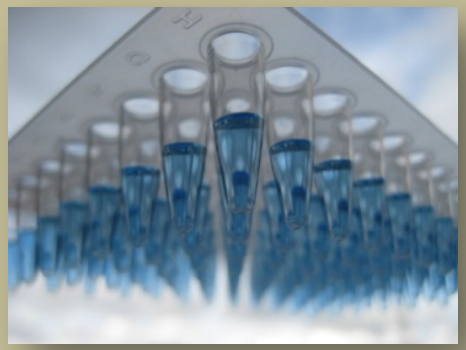
Human Source qPCR markers

qPCR = quantitative Polymerase Chain Reaction

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

Hu-2-Bac - more specific than previous markers (less false positives).

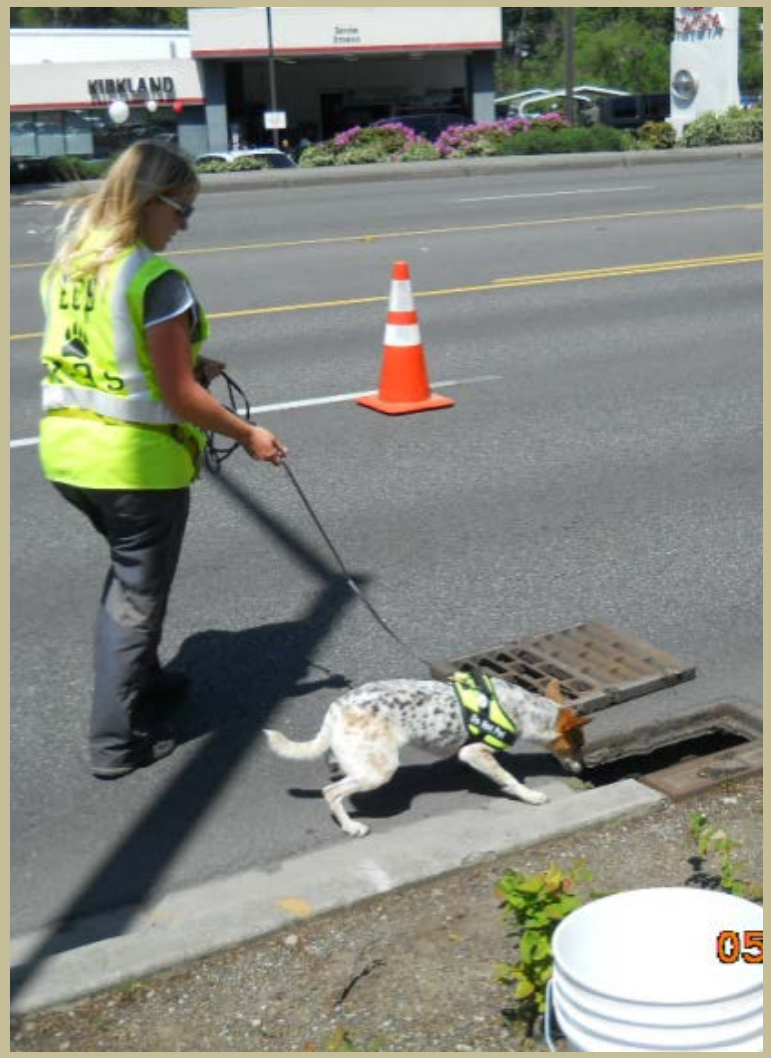
Sample → membrane filtration
→ frozen @ -80°C ***
→ aliquot processed
→ reported as copies/100ml



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)

Molly



Crush

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)

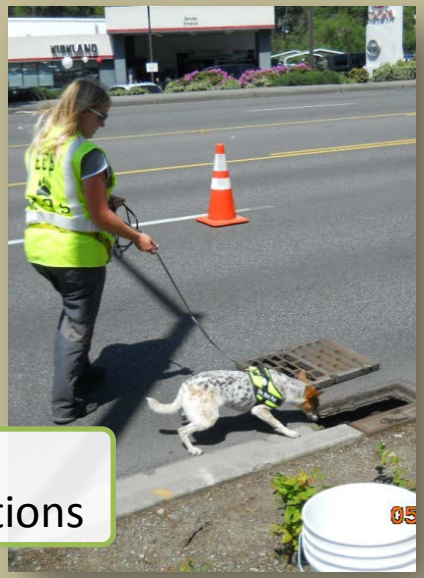
Day 1
- Bucket test in scent neutral field
- site investigations

Day 2
- site investigations

Thornton Creek (77 samples)

Day 3
- Bucket test in scent neutral field
- site investigations

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 H_2O

4 Alternate Waste Samples

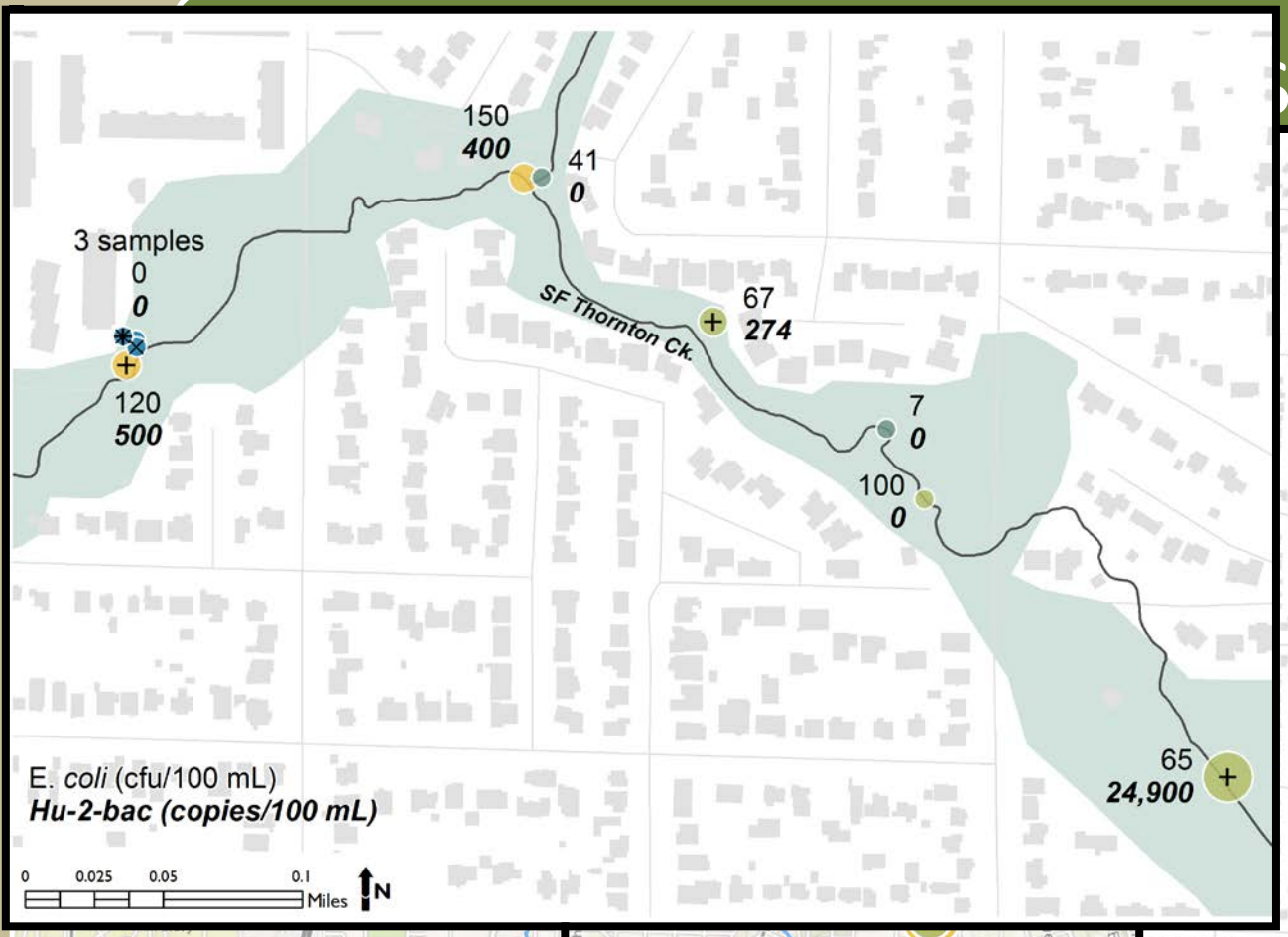
- 1% to 2 % diluted cat/dog feces

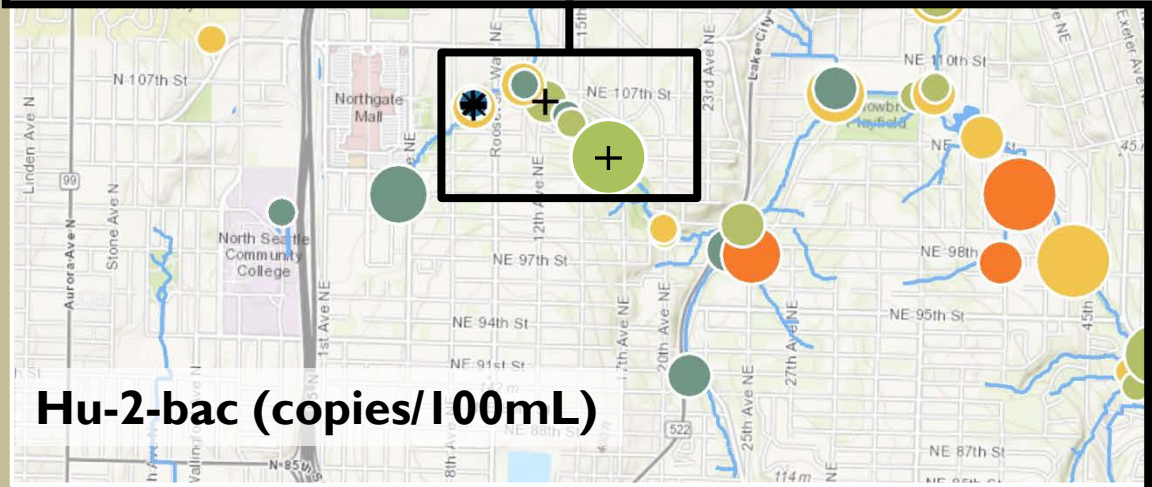
2 Field Duplicates

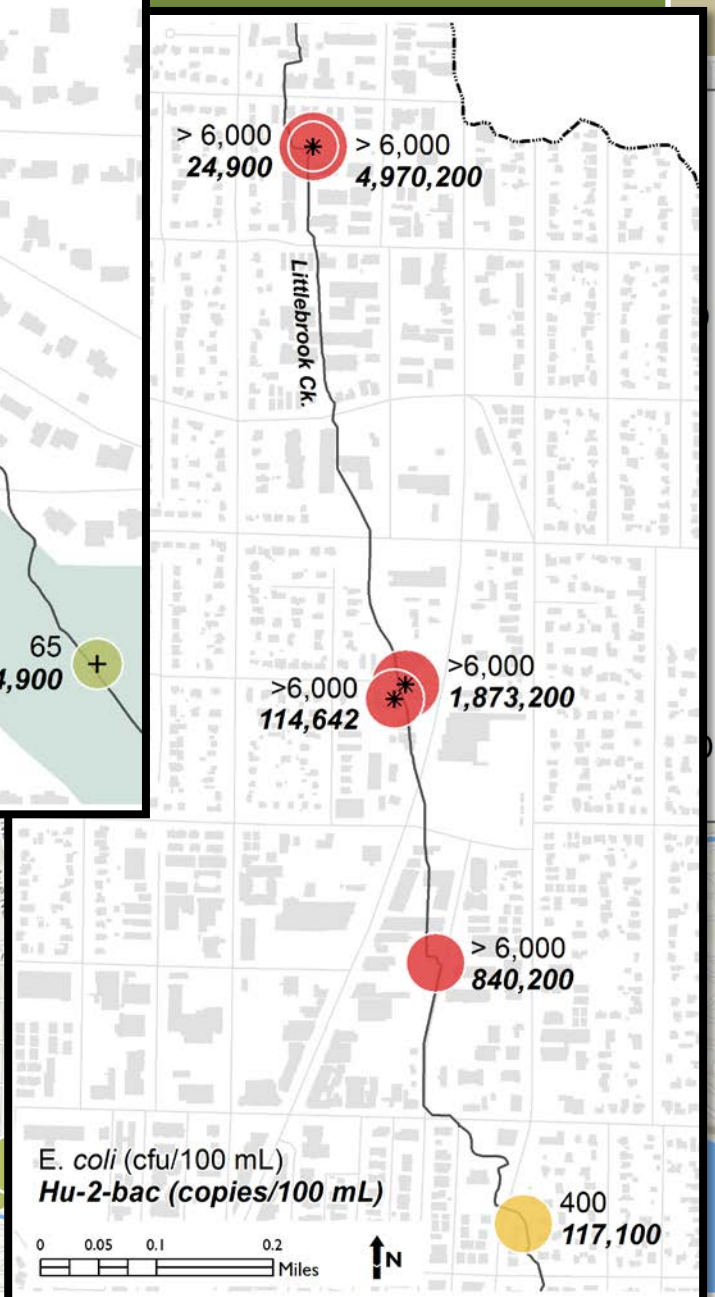
Canine Source Tracking

- Video: http://vimeo.com/95309371

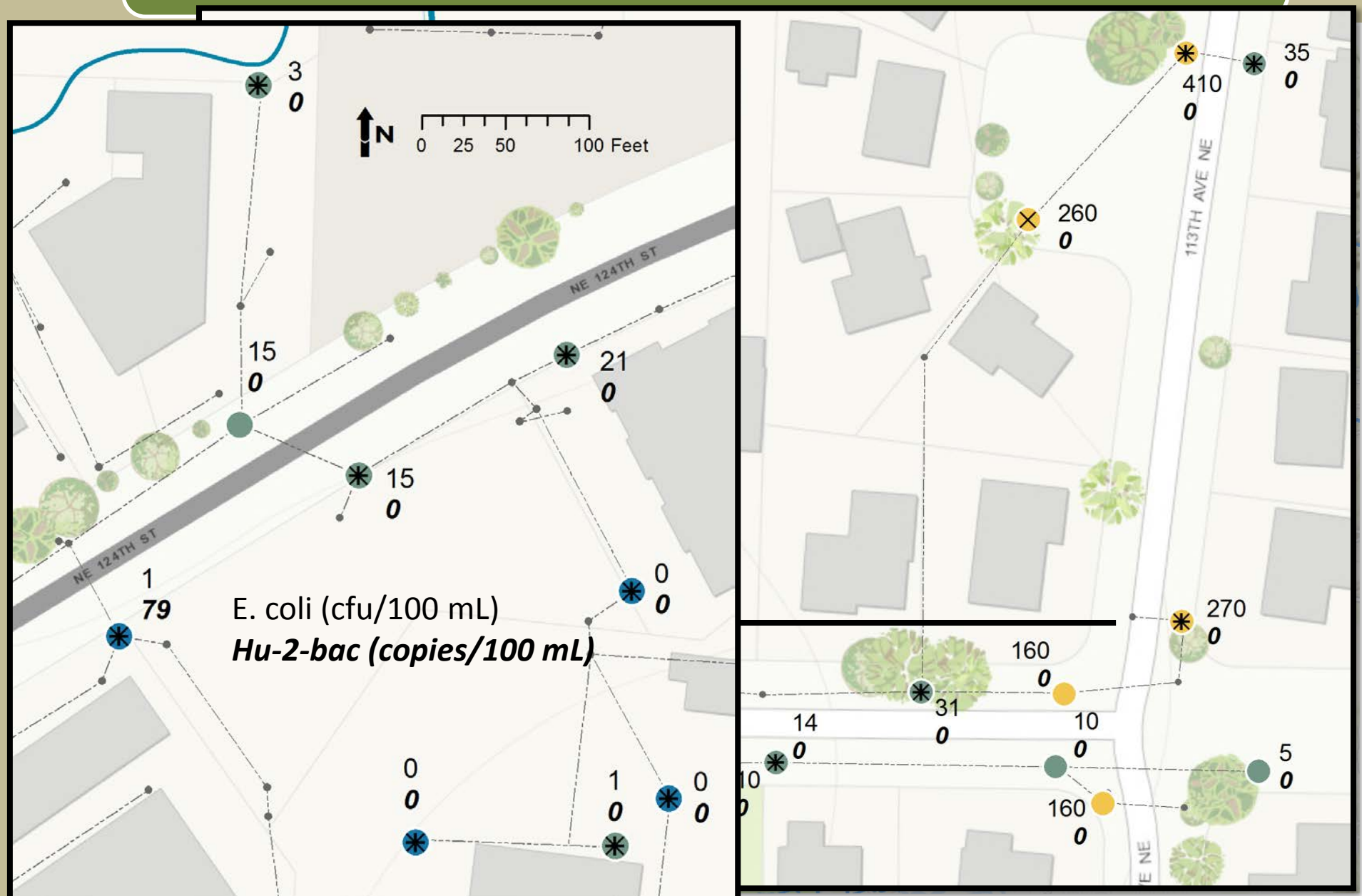
sults


150
400
41
0
3 samples
0
0
SF Thornton Ck.
67
274
120
500
7
0
100
0
65
24,900
E. coli (cfu/100 mL)
Hu-2-bac (copies/100 mL)
0 0.025 0.05 0.1 Miles
N

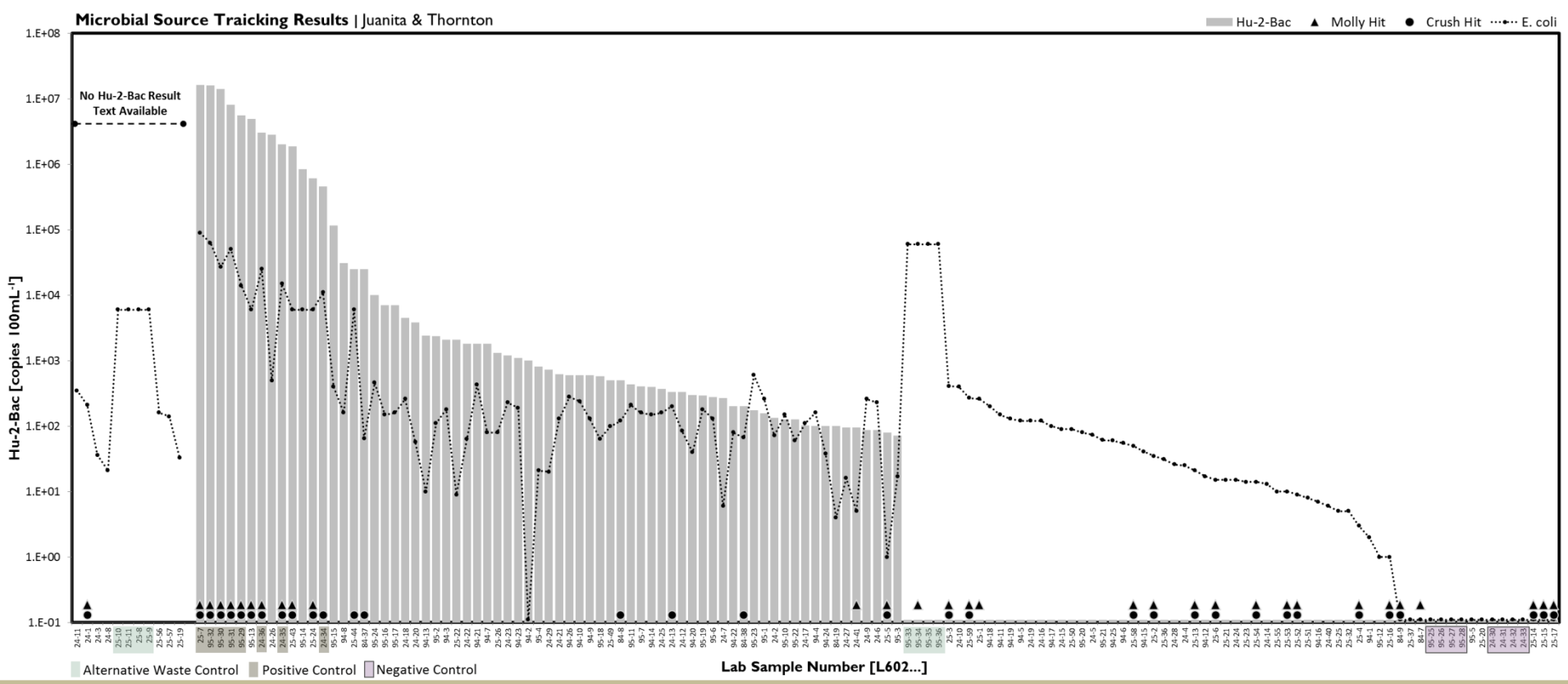

N 107th St
Northgate Mall
NE 107th St
NE 110th St
North Seattle Community College
NE 97th St
NE 98th
NE 94th St
NE 95th St
NE 91st St
NE 87th St
Hu-2-bac (copies/100mL)


> 6,000
24,900
> 6,000
4,970,200
Littlebrook Ck.
>6,000
114,642
>6,000
1,873,200
> 6,000
840,200
400
117,100
E. coli (cfu/100 mL)
Hu-2-bac (copies/100 mL)
0 0.05 0.1 0.2 Miles
N

Juanita Creek Results



Canine and Lab Results



- 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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- Aryn – Crush's handler

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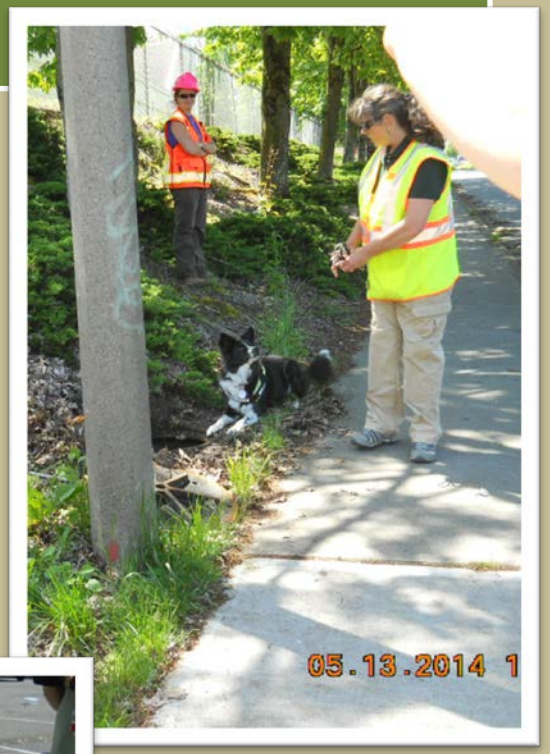
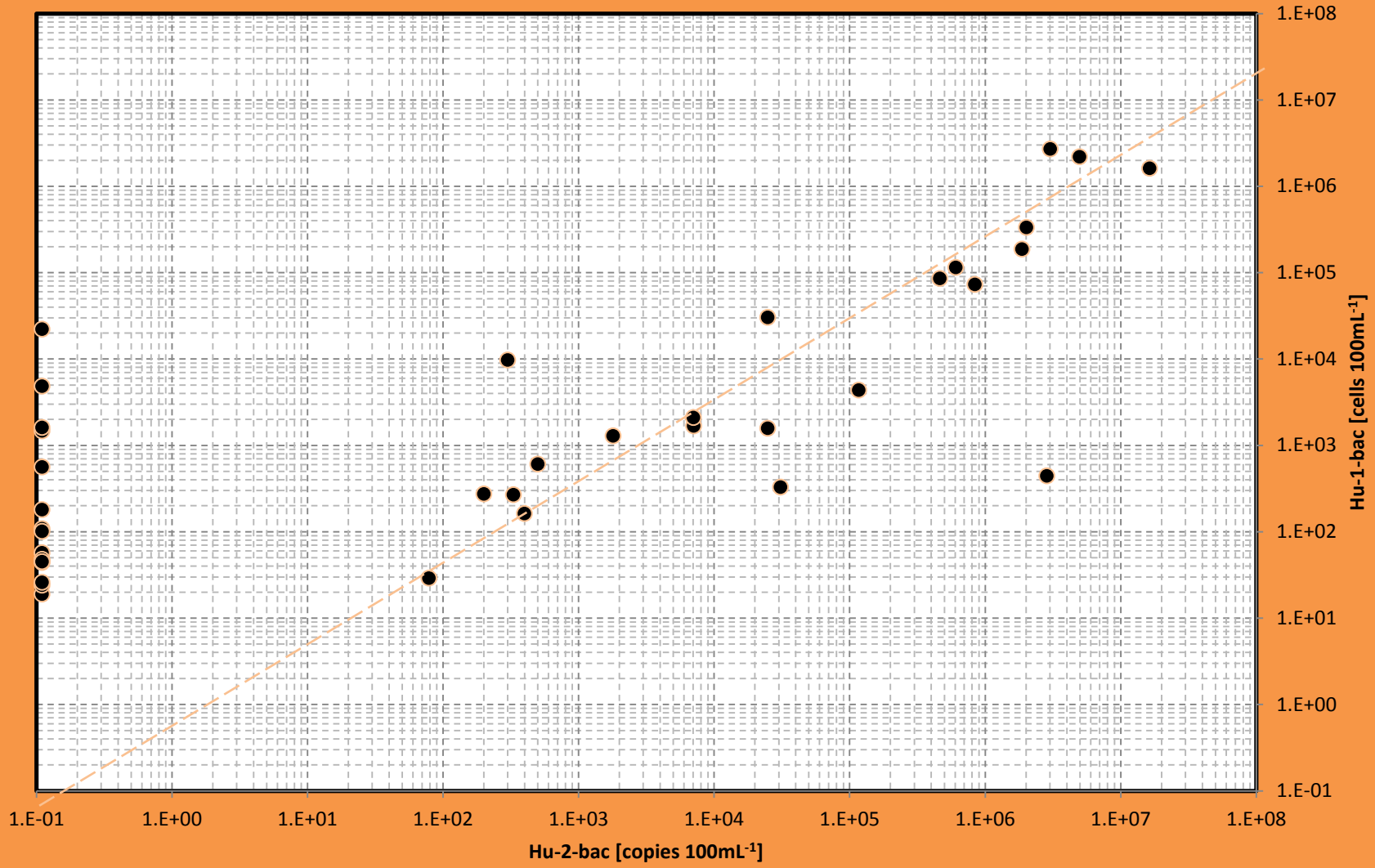
- Ryean-Marie Tuomisto
- Wes Ayers
- Jenny Gaus
- Marie Jensen
- Most members of the Kirkland engineering and maintenance staff!!

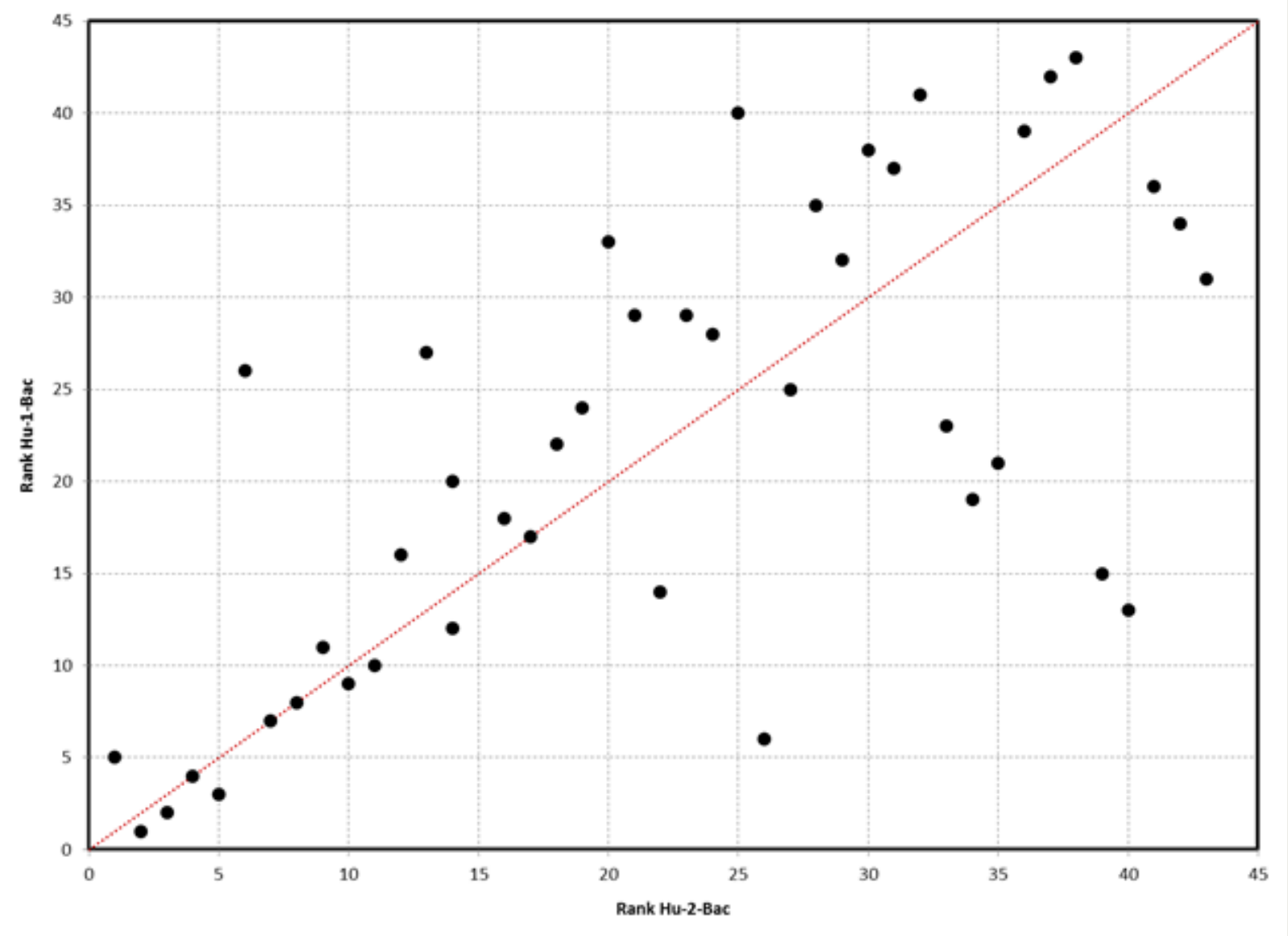
Questions?



Hu-1-Bac vs Hu-2-Bac



Hu-1-Bac vs Hu-2-Bac - ranked

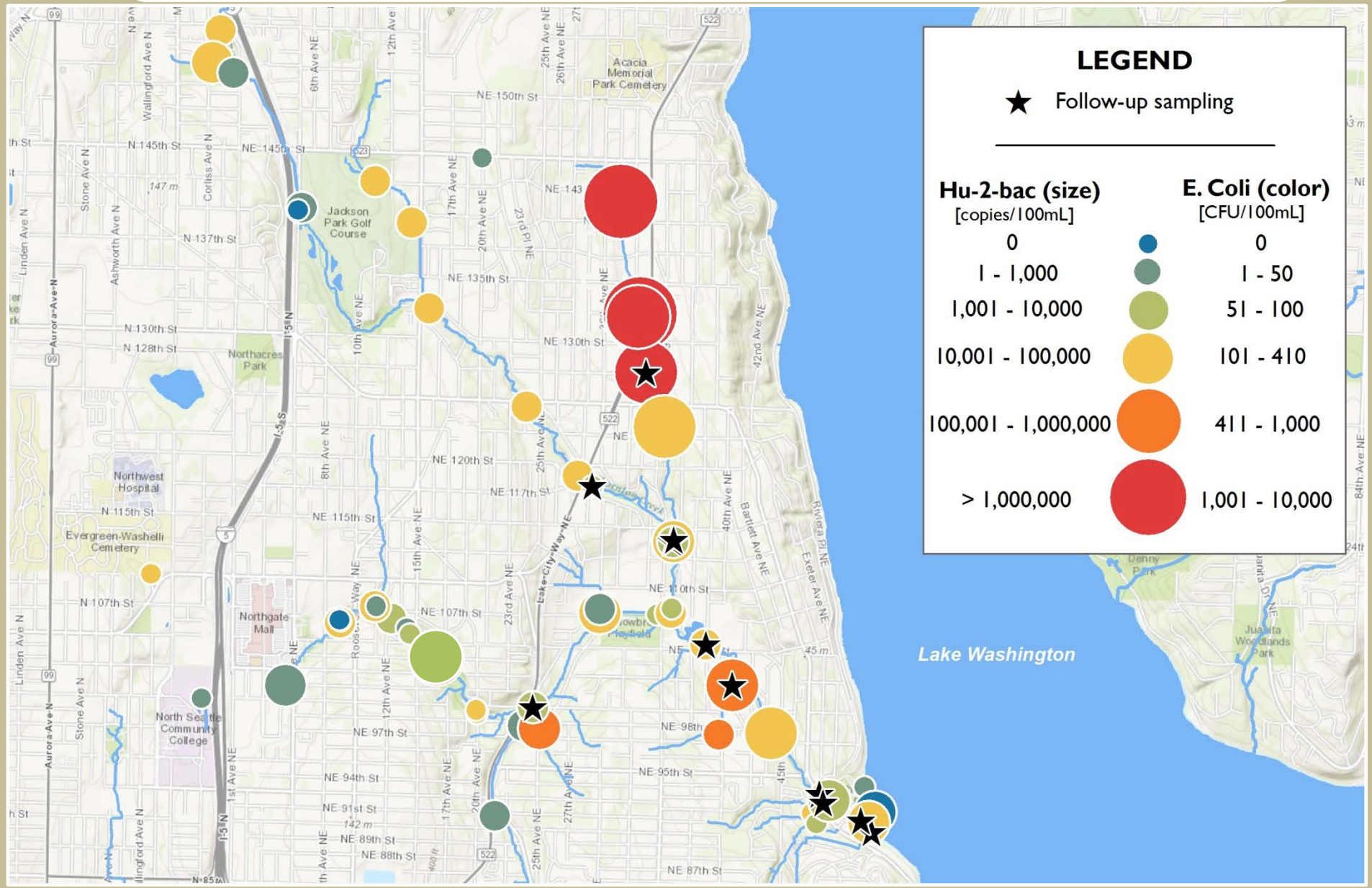


King County Environmental Lab Services

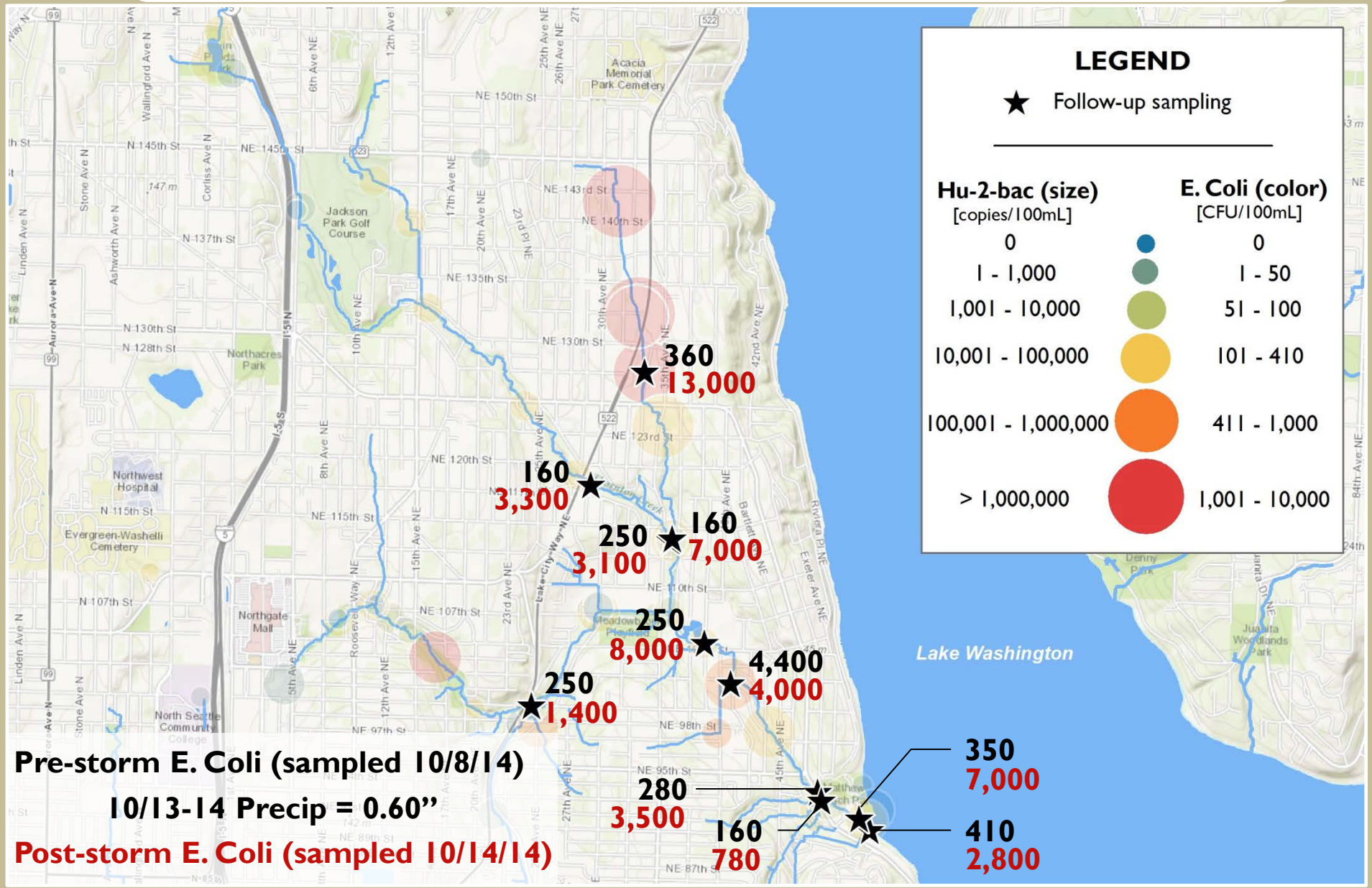
	Organism or Gene Marker Name	Method and Result Units	Type of fecal Pollution Indicated	Performance Characteristics	Notes	Cost for Complete Analysis	Cost for Prep and Hold
Human							
	Bacteroides spp. Hu1Bac	Membrane filtration & Real Time qPCR Cells/100 ml	Indicates Possible Human Source	Highly Sensitive[1] (detects target at low concentrations)	Presence indicates recent human contaminatio n	$125	$50.00
	Bacteroides spp. Hu2Bac	Membrane filtration & qPCR Copies/ml	Indicates Possible Human Source	Top performing human marker in 2013 study[2]	Presence indicates recent human contaminatio n	$125	$50.00
	Bifidobacteria spp.	Culture Based Cells/100 ml	Indicates Possible Human Source	High Confidence. (Any level of detection is significant)	Presence indicates recent human contaminatio n	$175	N\A
Ruminant							
	Rum2Bac	Membrane filtration & qPCR Copies/ml	Indicates Possible Ruminant Source	High Confidence. Top performing ruminant marker in 2013 study [3]		$125	$50.00
	Rhodococcus spp.	Culture Based Cells/100 ml	Indicates Possible Ruminant Source	High Confidence		$300	N\A

Costs here for single samples and may be discounted in larger batches.

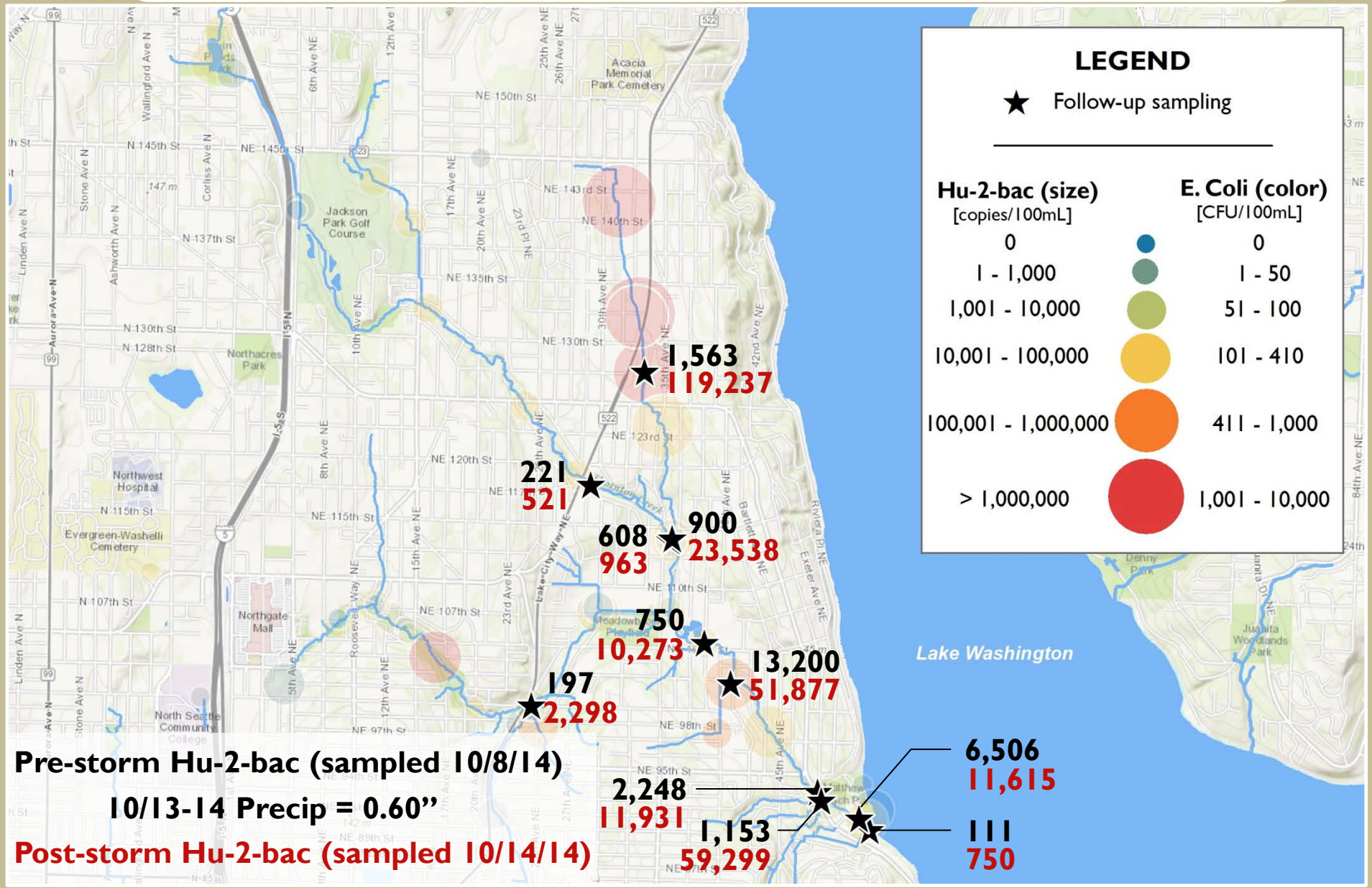
Thornton Creek Results



Thornton follow up *E. coli*



Thornton follow up Hu-2-Bac

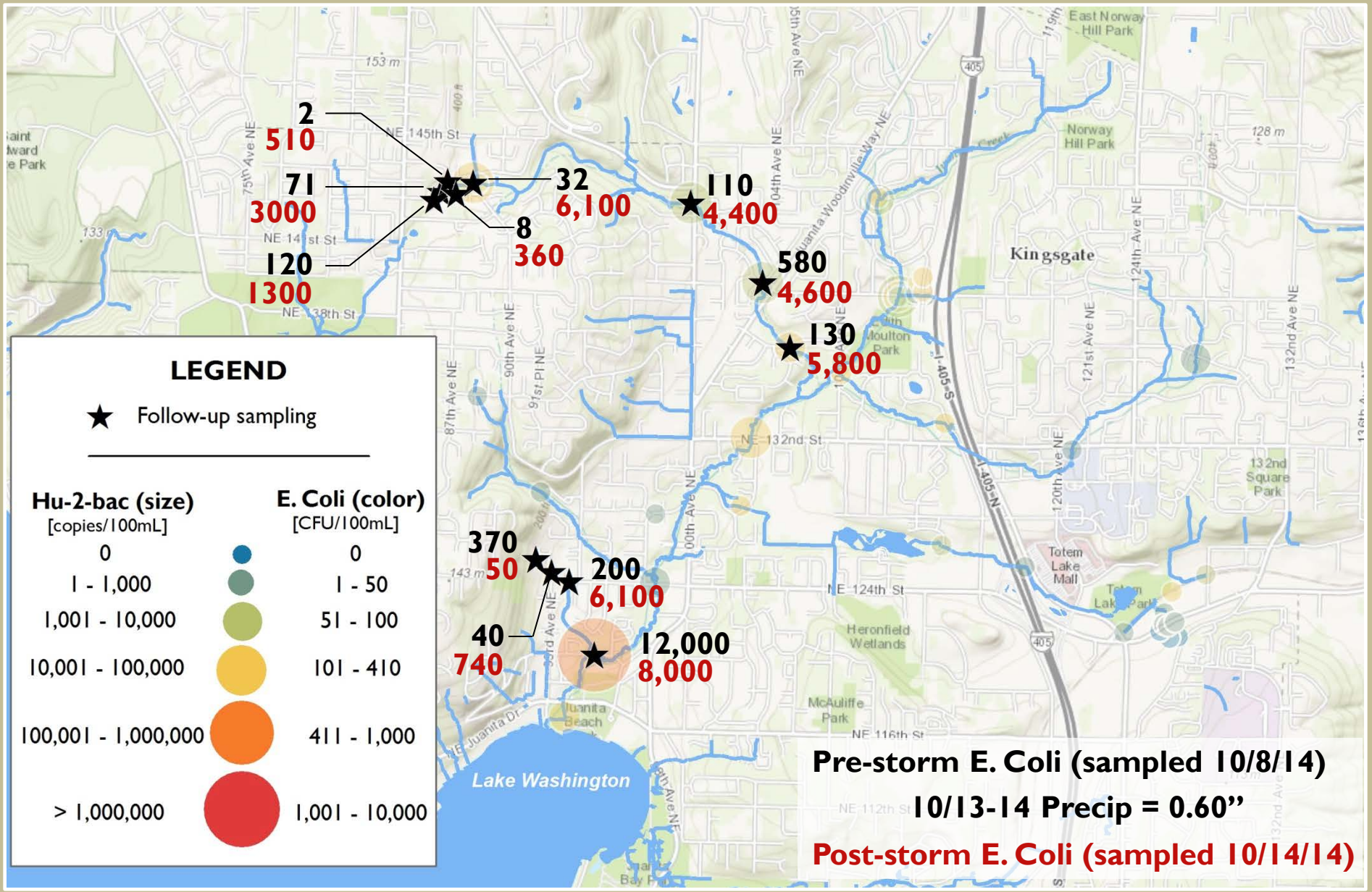
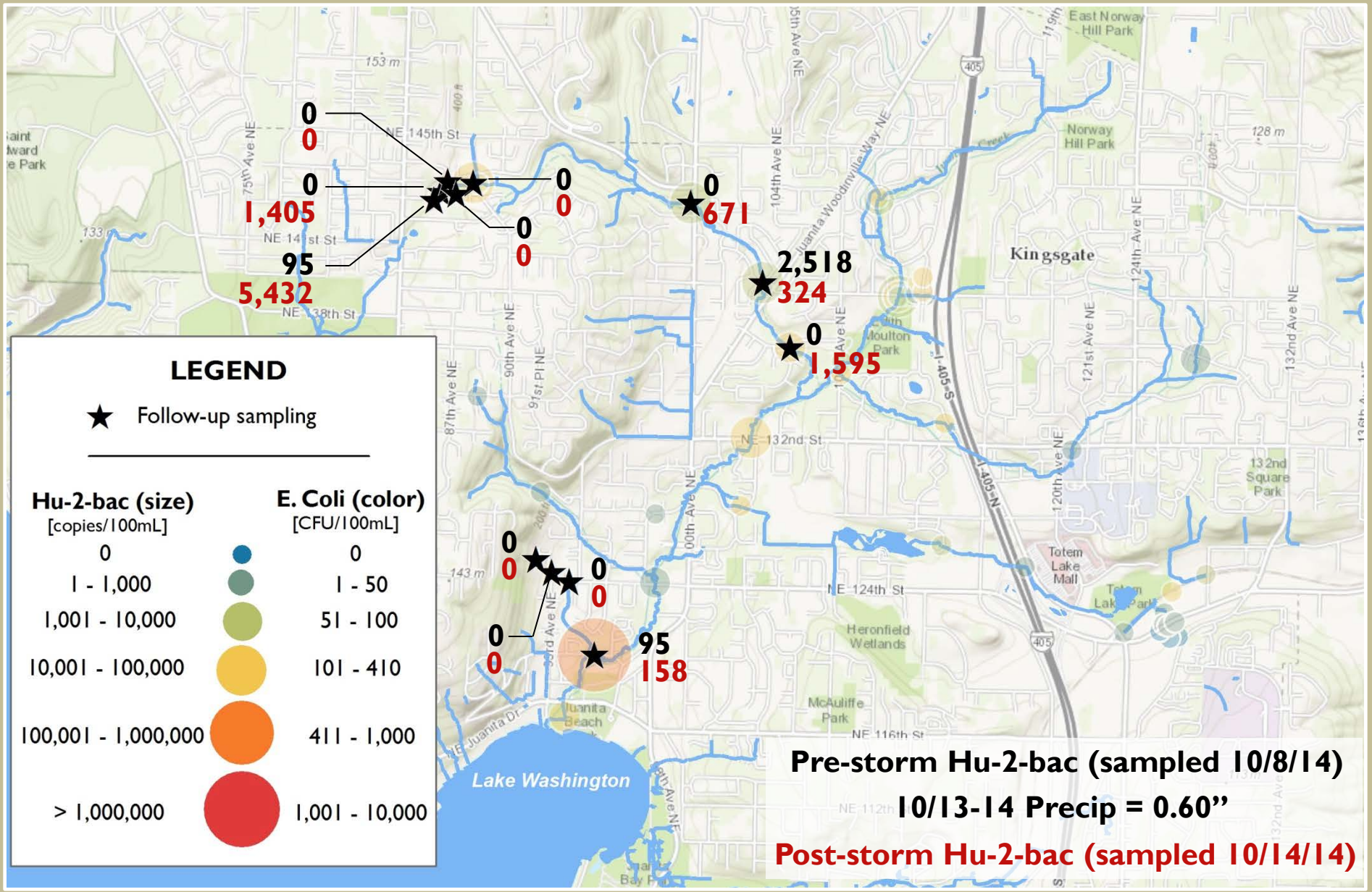


Pre-storm Hu-2-bac (sampled 10/8/14)

10/13-14 Precip = 0.60"

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

Juanita follow up *E. coli*



Juanita follow up Hu2Bac