Bacterial Source Tracking Using DNA Profiles Of E. coli Bacteria
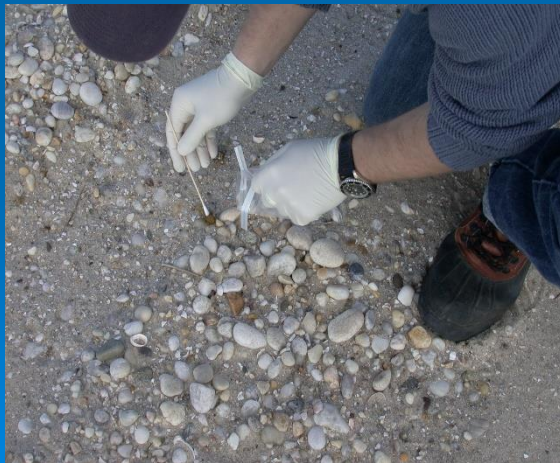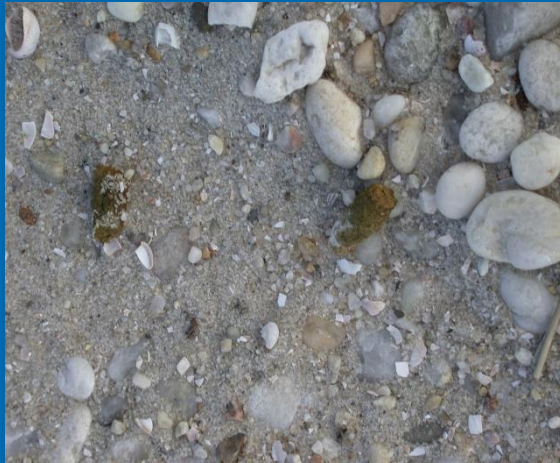
Sources Of Contamination

- NPS is the primary cause of reduced water quality on L.I.
- PEP CCMP identifies NPS, specifically stormwater runoff, as the major source of pathogens in the Estuary
- Identification of exact sources of bacteria will determine best remediation strategy - Bacterial Source Tracking

Collection of Samples

- ➢ Set up known source library.

- ➢ Source samples (scat) are collected from numerous known animals.

- ➢ Unknowns are water samples collected from impacted areas.

Source samples are taken from individual animal species that are common around watershed areas.

Muskrat

Red Fox

Raccoon

Black Duck

Mute Swan

Cormorant

Deer

Mallard Duck

Horse

Herring Gull

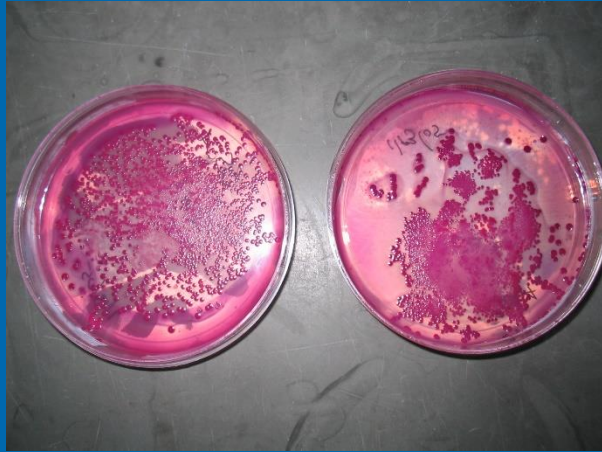Greater Black-Backed Gull

Canada Goose

Dog

RESTROOM

Human

Presumptive E. coli

➢ Samples are taken back to the lab and prepared for analysis.

➢ Sample is added to MUG media with an inverted durham tube, and incubated over night.

➢ Presumptive E. coli - turbidity, gas in the inverted durham tube, and MUG fluoresces under UV light.

Cultivation of <u>E. coli</u>

> ➢ Sample is serial diluted and streaked onto Violet Red Bile Agar (VRBA) to selectively grow individual colonies of <u>E. coli</u>. Plates are incubated overnight.

> ➢ Individual colonies are selected and streaked to grow bacterial "lawns" on Nutrient Agar (NA) plates. Plates are incubated overnight.

E. coli Verification

- API 20E verification system used

- Isolates that are positively identified as E. coli are processed through PFGE

- 6 Isolates for each source sample, 15 isolates for each water sample

DNA Preparation

- Isolates are grown in Luria broth.
- Each isolate is washed with several buffers and enzymes to release DNA.
- DNA is restricted using Not I restriction enzyme.
- Plugs are then prepared for Pulse Field Gel Electrophoresis.

Pulse Field Gel Electrophoresis

➢ DNA plugs are cut and embedded in a platform of agarose to create the gel. Standards are also run on the gel to aid in molecular weight determination and as quality control.

➢ The gel is subjected to electrical pulses over a 20 hour period.
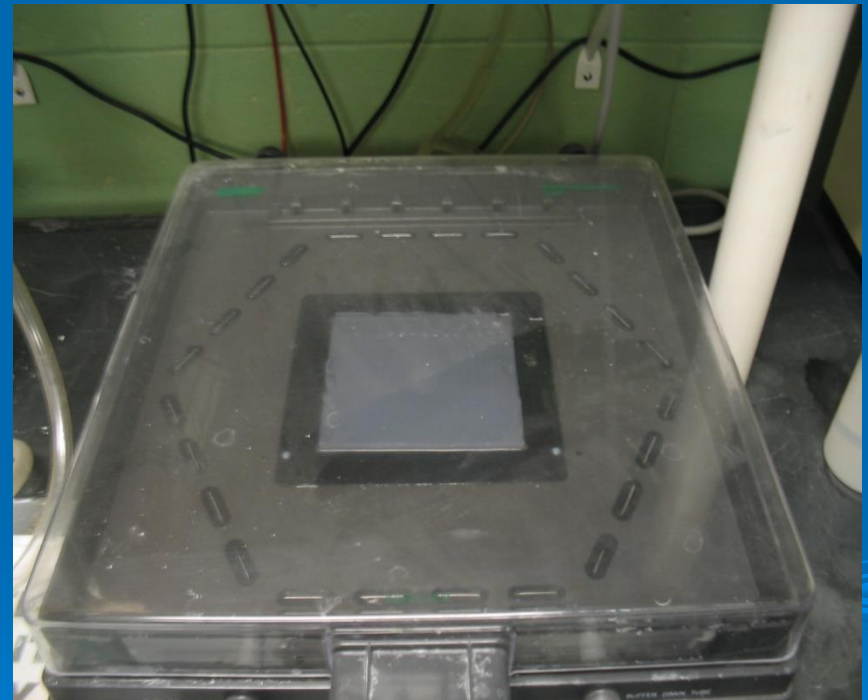
Gel Image

➢ After electrophoresis, the gel is stained using Ethidium Bromide (EtBr).

➢ The gel is then photographed using a light box.The image is then digitized and stored on the computer.

Quantifying Gels
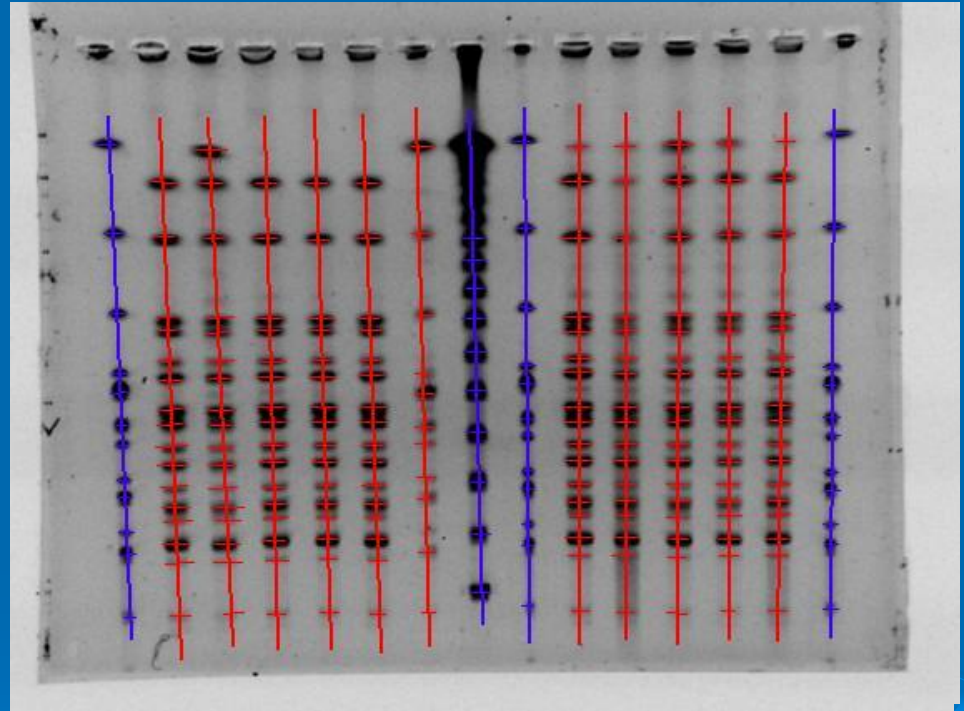
➢ Gels are viewed using Quantity One software.

➢ Lanes are defined (up to 15 per gel).

➢ Bands are recognized and adjusted.

➢ Standards are set using known molecular weight markers.

Raw Data

➢ Standards determine the molecular weights of bands in source and water sample isolates.

➢ Molecular weights are exported into spreadsheets.

Lane Number	Band Number	Band Type #	Mol. Wt. KDa	Sample Name
10	1		651.994	#10 of dec1
10	2		558.658	#10 of dec1
10	3		375.252	#10 of dec1
10	4		354.472	#10 of dec1
10	5		325.56	#10 of dec1
10	6		272.536	#10 of dec1
10	7		232.496	#10 of dec1
10	8		206.021	#10 of dec1
10	9		198.548	#10 of dec1
10	10		181.238	#10 of dec1
10	11		153.109	#10 of dec1
10	12		122.888	#10 of dec1
10	13		62.536	#10 of dec1
10	14		32.737	#10 of dec1

JMP MANOVA Analysis

➢ Molecular weight data is imported into a JMP table for MANOVA comparison analysis

Source	<100-1	<100-2	<100-3	<100-4	<100-5	100-1	100-2	100-3
Iron Pier	71.7	88.3	0	0	0	155	181.8	190.8
Iron Pier	74.2	87.3	99.3	0	0	141	165.8	0
Iron Pier	73.3	89.7	0	0	0	100.5	114.2	142.1
Iron Pier	73.6	89.6	0	0	0	101.3	114.3	144
JM 107	32.6	77.3	93.9	0	0	114.7	140.1	184.5
Lambda	47.5	94	0	0	0	142.6	192.9	0
Iron Pier	32.7	62.5	0	0	0	122.9	153.1	181.2
Iron Pier	32.1	76.2	93	0	0	102.9	117.9	145.8
Iron Pier	77.9	95.5	0	0	0	104.2	119.4	149.2
Iron Pier	78.5	90.7	0	0	0	102.8	119	146.2
Iron Pier	66.5	0	0	0	0	117.7	149.8	178.4
Iron Pier	75.3	89.4	99.8	0	0	112.6	139.8	156.5
JM 107	78.6	91.1	0	0	0	116.3	136.9	180.1
Lambda	90.9	0	0	0	0	136.3	190.7	0
Iron Pier	75.6	89.7	99.2	0	0	112.9	137.8	155.4
Iron Pier	75.7	88.7	99.2	0	0	113	139.9	170
Iron Pier	68.3	81	0	0	0	131.2	159.2	180.6
Iron Pier	70.7	0	0	0	0	114	121.9	162

JMP Database Comparisons

Currently we have 9 databases that we run water samples through:

- Human vs. Not-Human
- Bird-Wildlife-Domestic
- Bird vs. Not-Bird
- Wildlife vs. Not-Wildlife
- Dog vs. Not-Dog

- Horse vs. Not-Horse
- Birds by species
- Wildlife by species
- Entire Library (14 individual species)

JMP Databases

➤ All samples are run through each of the 9 databases, but there is usually a logic chain that can be followed to find the best match.

➤ A sample is first run through HUMAN vs. Not-Human. If it comes out strongly Not-Human, we will run it through BIRD-WILDLIFE-DOMESTIC.

➤ From here we may be able to narrow it down further, if, say a sample may show a good match to BIRD.

➤ We can now try to pinpoint a specific bird, by running the sample through the Birds by species database.

➤ Finally, the sample is run through the entire library.

MANOVA Analysis Showing Predicted Sources:

Area	Prob[HUMAN]	Prob[LAMBDA]	Prob[Non-Human]	Pred Source	Match %
Iron Pier	0.00083	0	0.99917	Non-Human	Non-Human 100%
Iron Pier	0.90109	0	0.09891	HUMAN	HUMAN 90%
Iron Pier	0.99992	0	0.00008	HUMAN	HUMAN 100%
Iron Pier	0.99994	0	0.00006	HUMAN	HUMAN 100%
JM 107	0.04252	0.00003	0.95745	Non-Human	Non-Human 96%
Lambda	0	1	0	LAMBDA	LAMBDA 100%
Iron Pier	0.48581	0	0.51419	Non-Human	Non-Human 51% Human 48%
Iron Pier	0.83273	0.00006	0.16721	HUMAN	HUMAN 83%
Iron Pier	0.92448	0.05057	0.02494	HUMAN	HUMAN 92%
Iron Pier	0.99875	0	0.00125	HUMAN	HUMAN 100%
Iron Pier	1	0	0	HUMAN	HUMAN 100%
Iron Pier	0.93366	0	0.06634	HUMAN	HUMAN 93%
JM 107	0.37463	0.01003	0.61535	Non-Human	Non-Human 62%
Lambda	0.00001	0.99999	0	LAMBDA	LAMBDA 100%
Iron Pier	0.4095	0	0.5905	Non-Human	Non-Human 59% Human 41%
Iron Pier	0.95854	0	0.04146	HUMAN	HUMAN 96%
Iron Pier	0.01977	0.00033	0.9799	Non-Human	Non-Human 98%
Iron Pier	0.99999	0	0.00001	HUMAN	HUMAN 100%

	HUMAN/NOT HUM.	BIRD/WILD/DOM	BIRD/NOTBIRD	BIRD BY SPECIES	TOTAL LIBRARY	SOURCE
#3 of dec1	NON 100%	BIRD 100%	BIRD 100%	CORM 100%	CG 92%	BIRD
#4 of dec1	HUMAN 90%					HUMAN
#5 of dec1	HUMAN 100%					HUMAN
#6 of dec1	HUMAN 100%					HUMAN
#7 of dec1	XX					XX
#8 of dec1	XX					XX
#10 of dec1	NON 51%					UNKNOWN
#11 of dec1	HUMAN 83%					HUMAN
#12 of dec1	HUMAN92%					HUMAN
#13 of dec1	HUMAN 100%					HUMAN
#4 of dec2	HUMAN 100%					HUMAN
#5 of dec2	HUMAN 93%					HUMAN
#7 of dec2	XX					XX
#8 of dec2	XX					XX
#10 of dec2	NON 59%					UNKNOWN
#11 of dec2	HUMAN 96%					HUMAN
#12 of dec2	NON 98%	BIRD 98%	BIRD 99%	GBBG 96%	MD 77%	BIRD
#13 of dec2	HUMAN 100%					HUMAN

Results Of Water Samples Run Through The 9 Databases:

ISOLATE LABEL	SAMPLE #	HUMAN-NONHUMAN	ALL BUT HUMAN	BIRD-NOT BIRD	WILD - NOT WILD
#9 of 1.37wfiltercce	1-W3006	NON 90%	WILD 89%	NOT 60%	WILD 94%
#10 of 1.38wcce	1-W2194	NON 100%	WILD 75%	NOT 78%	WILD 83 %
#11 of 1.38wcce	1-W2194	NON 100%	WILD 76%	NOT 77%	WILD 83%
#12 of 1.38wcce	1-W2194	NON 100%	WILD 77%	NOT 77%	WILD 84%
#13 of 1.38wcce	1-W2194	NON 100%	WILD 79%	NOT 78%	WILD 86%
#3 of 1.38wcce	1-W2194	NON 100%	DOM 72%	NOT 87%	NOT 57%
#5 of 1.38wcce	1-W2194	NON 100%	WILD 78%	NOT 77%	WILD 84%
#6 of 1.38wcce	1-W2194	NON 100%	WILD 75%	NOT 77%	WILD 83%
#7 of 1.38wcce	1-W2194	NON 100%	WILD 77%	NOT 78%	WILD 84%
#2 of 1.35wcce	1-W3005	NON 100%	DOM. 57%	NOT 78%	NOT 56%
#3 of 1.35wcce	1-W3005	NON 100%	DOM. 56%	NOT 78%	NOT 54%
#5 of 1.35wcce	1-W3005	NON 100%	DOM. 55%	NOT 78%	NOT 55%
#6 of 1.35wcce	1-W3005	NON 100%	DOM. 59%	NOT 77%	NOT 59%
#7 of 1.35wcce	1-W3005	NON 100%	DOM. 65%	NOT 76%	NOT 67%

ISOLATE LABEL	DOG - NO DOG	HORSE/NO HORSE	BIRDS	WILDLIFE	TOTAL LIBRARY	LIKELY SOURCE
#9 of 1.37wfiltercce	NOT 96%	NOT 95%	CG 94%	MUSK 100%	DEER 82%	WILD-LIKELY DEER OR MUSK
#10 of 1.38wcce	NOT 67%	NOT 87%	GBBG 55%	MUSK 90%	MUSK 78%	MUSKRAT
#11 of 1.38wcce	NOT 87%	NOT 88%	GBBG 60%	MUSK 93%	MUSK 80%	MUSKRAT
#12 of 1.38wcce	NOT 68%	NOT 89%	GBBG 68%	MUSK 93%	MUSK 80%	MUSKRAT
#13 of 1.38wcce	NOT 68%	NOT 89%	GBBG 73%	MUSK 95%	MUSK 83%	MUSKRAT
#3 of 1.38wcce	DOG 79%	HORSE 51%	MD 59%	RF 90%	DOG43DEER30	DOMESTIC, LIKELY DOG
#5 of 1.38wcce	NOT 68%	NOT 89%	GBBG 71%	MUSK 94%	MUSK 82%	MUSKRAT
#6 of 1.38wcce	NOT 67%	NOT 87%	GBBG 58%	MUSK 89%	MUSK 78%	MUSKRAT
#7 of 1.38wcce	NOT 67%	NOT 88%	GBBG 61%	MUSK 91%	MUSK 80%	MUSKRAT
#2 of 1.35wcce	DOG 67$	NOT 64%	MD 95%	RF 95%	DOG 31%	NOT HUMAN, NOT BIRD
#3 of 1.35wcce	DOG 65%	NOT 63%	MD 95%	RF 94%	DOG 30%	NOT HUMAN, NOT BIRD
#5 of 1.35wcce	DOG 64%	NOT 62%	MD 95%	RF 94%	DOG 29%	NOT HUMAN, NOT BIRD
#6 of 1.35wcce	DOG 65%	NOT 59%	MD 95%	RF 94%	DOG 29%	NOT HUMAN, NOT BIRD
#7 of 1.35wcce	DOG 67%	NOT 54%	MD 95%	RF 95%	DOG 29%	NOT HUMAN, NOT BIRD

Results For Hashamomuck Pond: D.E.C. Station 1.1

Isolate Label	Sample Number	Sample Location	Date	Predicted Source
#12 of 1.20filterwcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	DOMESTIC ANIMAL
#13 of 1.20filterwcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	DOMESTIC ANIMAL
#14 of 1.20filterwcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	HUMAN
#10 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	DOMESTIC ANIMAL
#11 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	HORSE
#13 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	DEER
#14 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	DEER
#2 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN
#3 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN,NOT WILDLIFE
#4 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	DOMESTIC ANIMAL
#6 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN,NOT WILDLIFE
#7 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN,NOT WILDLIFE
#9 of 1.21wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN,NOT WILDLIFE
#2 of 1.22.1wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	WILDLIFE
#2 of 1.23wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN
#3 of 1.23wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN
#4 of 1.23wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN
#6 of 1.23wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN
#7 of 1.23wcce	1-W2189	HASHAM. STA. 1.1 WET	Sep-03	NOT HUMAN
#5 of 1.30wfiltercce	1-W3001	HASHAM STA 1.1 DRY	Oct-03	BLACK DUCK
#6 of 1.30wfiltercce	1-W3001	HASHAM STA 1.1 DRY	Oct-03	BIRD
#7 of 1.30wfiltercce	1-W3001	HASHAM STA 1.1 DRY	Oct-03	BIRD

Results For Upper Sag Harbor Cove: D.E.C. Station 9

Isolate Label	Sample Number	Sample Location	Date	Predicted Source
#13 of 1.29wcce	1-W2194	UP. SAG. HBR. CV. STA.9WET	Oct-03	MUSKRAT
#14 of 1.29wcce	1-W2194	UP. SAG. HBR. CV. STA.9WET	Oct-03	MUSKRAT
#11 of 2.49wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	CORMORANT
#12 of 2.49wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	NOT HUMAN,NOT WILDLIFE
#13 of 2.49wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	CORMORANT
#14 of 2.49wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	CORMORANT
#9 of 2.49wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	NOT HUMAN, POSSIBLE BLACK DUCK
#10 of 2.50wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	CORMORANT
#14 of 2.50wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	DEER
#11 of 2.50wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	DEER
#6 of 2.51wcce	2-W3003	UP. SG. HRB. COVE STA. 9 DRY	Oct-03	GBBG
#10 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#11 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#12 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#13 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#3 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	DOMESTIC, LIKELY DOG
#5 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#6 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#7 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#9 of 1.38wcce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#9 of 1.39wfiltercce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT
#10 of 1.39wfiltercce	1-W2194	UP. SG. HRB. COVE STA. 9 WET	Oct-03	MUSKRAT

Results For Northwest Creek: D.E.C. Station 12

Isolate Label	Sample Number	Sample Location	Date	Predicted Source
#12 of cce3.33w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#13 of cce3.33w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#14 of cce3.33w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#10 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#11 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#12 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#13 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#2 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#3 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#4 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#5 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG
#6 of cce3.34w	3-W3130	NW CREEK STA. 12 Wet	Nov-05	CG

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