Microbial Source Tracking (MST) in South Carolina: Lessons Learned and Future Directions

JA Gooch, JR Stewart, LF Webster,
C Johnston, BJ Robinson, BC Thompson, MH Fulton

National Oceanic and Atmospheric Administration (NOAA)
National Ocean Service (NOS)
Center for Coastal Environmental Health and
Biomolecular Research (CCEHBR)
Charleston, SC 29412

What is Microbial Source Tracking (MST)?

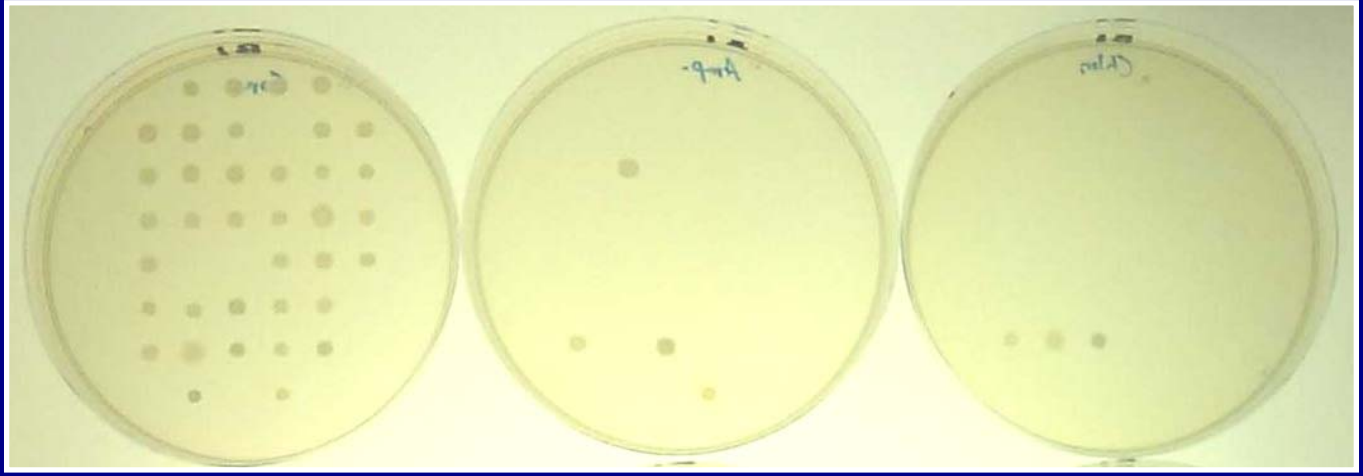
Library Dependent Source Tracking Methods

- Target organism must be cultured
- Requires a known source library of microorganisms to compare unknown isolates with for classification
  - Need “enough” isolates in the library
  - Library very expensive & time consuming to create

Multiple Antibiotic Resistance (MAR) Testing of *E. coli*

E. coli colonies in the (a) absence of antibiotics and presence of (b) ampicillin and (c) chlortetracycline

(a) (b) (c)



Control plate with no antibiotics

Resistant isolates on ampicillin plate

Resistant isolates on chlortetracycline plate

10 Antibiotics Used in MAR:

Ampicillin 10 ug/mL

Chlortetracycline 25 ug/mL

Kanamycin 25 ug/mL

Naladixic acid 25 ug/mL

Neomycin 50 ug/mL

Oxytetracycline 50 ug/mL

Penicillin G 75 U/mL

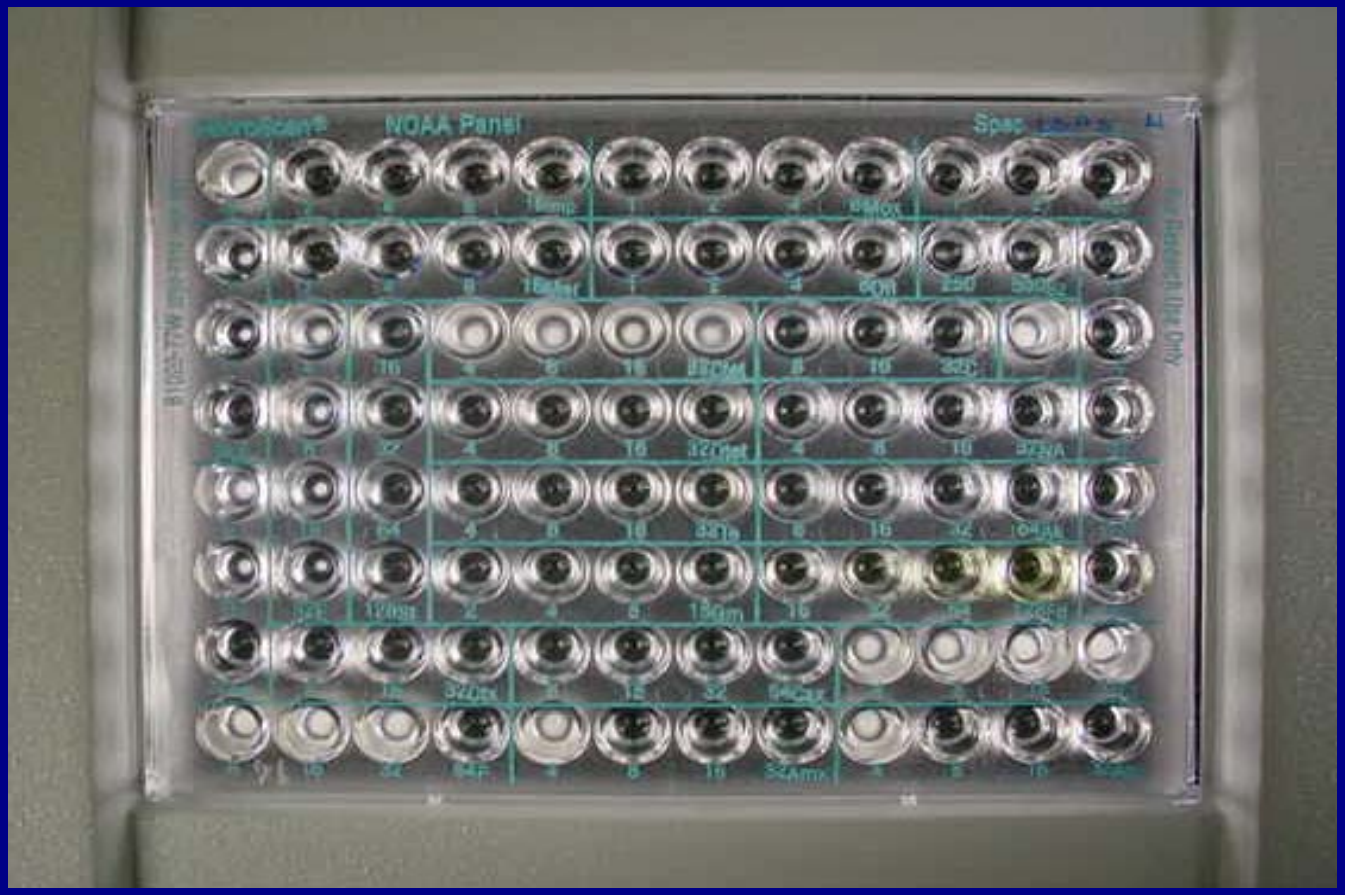
Streptomycin 12.5 ug/mL

Sulfathiazole 500 ug/mL

Tetracycline 25 ug/mL

Parveen *et al*., 1997

Antibiotic Resistance Analysis (ARA)



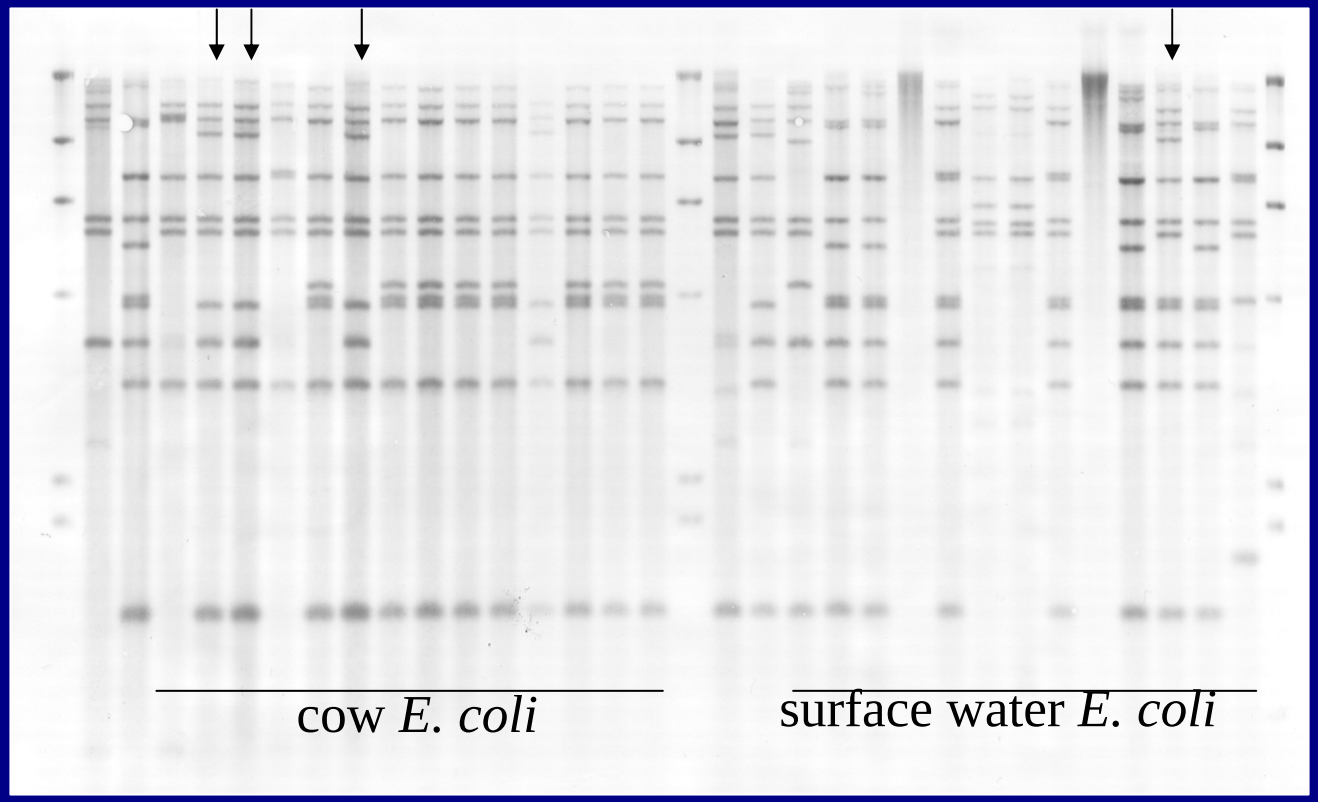
Custom antibiotic panels manufactured by Dade Behring

26 different antibiotics—determine MIC value for each

26 Antibiotics Used in ARA

- Amikacin
- Amoxicillin
- Ampicillin
- Apramycin
- Azithromycin
- Cefoxitin
- Ceftriaxone
- Cephalothin
- Chloramphenicol
- Ciprofloxacin
- Erythromycin
- Gentamicin
- Imipenem
- Meropenem
- Moxifloxacin
- Nalidixic Acid
- Nitrofurantoin
- Ofloxacin
- Oxytetracycline
- Penicillin
- Streptomycin
- Sulfathiazole
- Tetracycline
- Trimethoprim
- Trimethoprim/Sulfamethoxazole

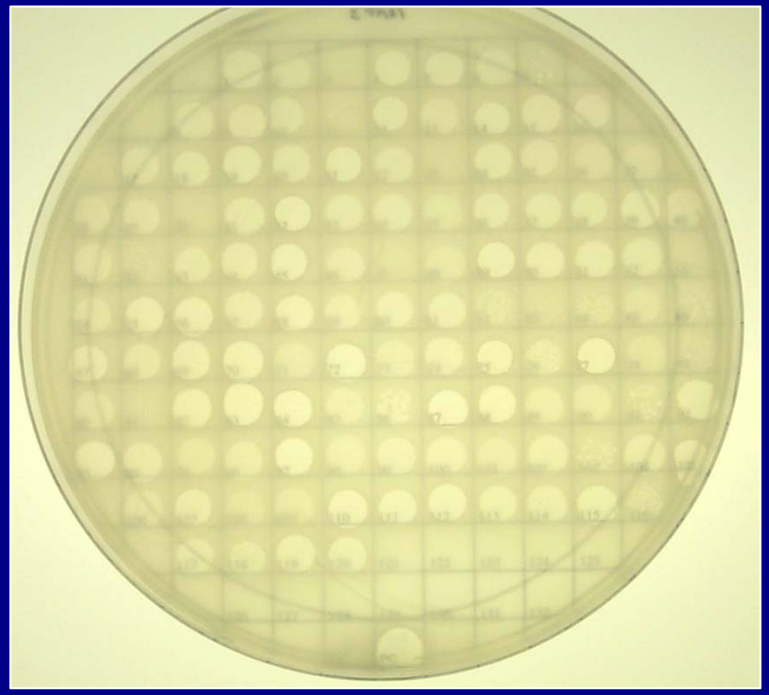
Ribotyping *E. coli*



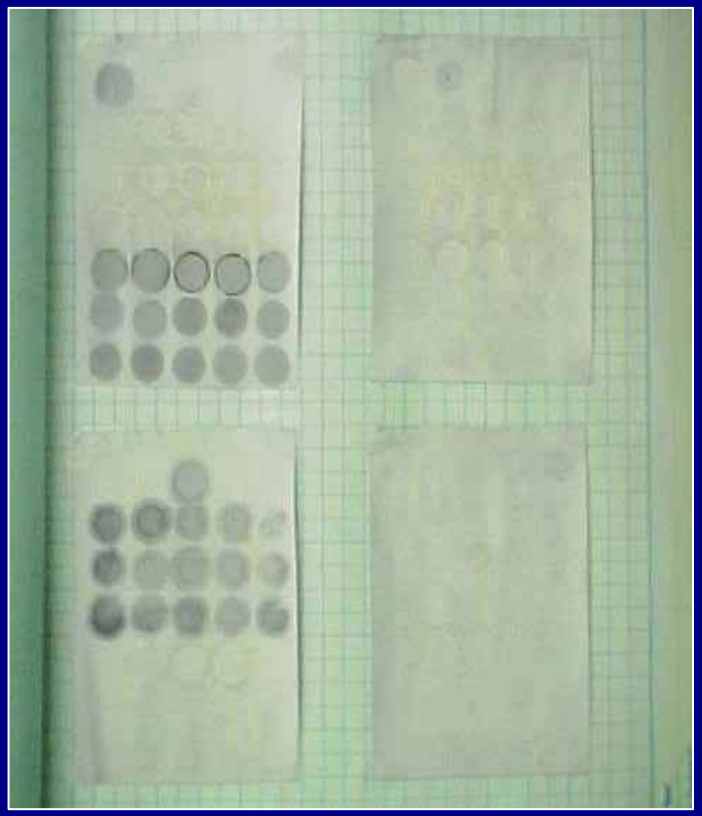
Matching patterns observed for cow and water *E. coli*

Library Independent MST Method
F^+RNA Coliphage Analysis

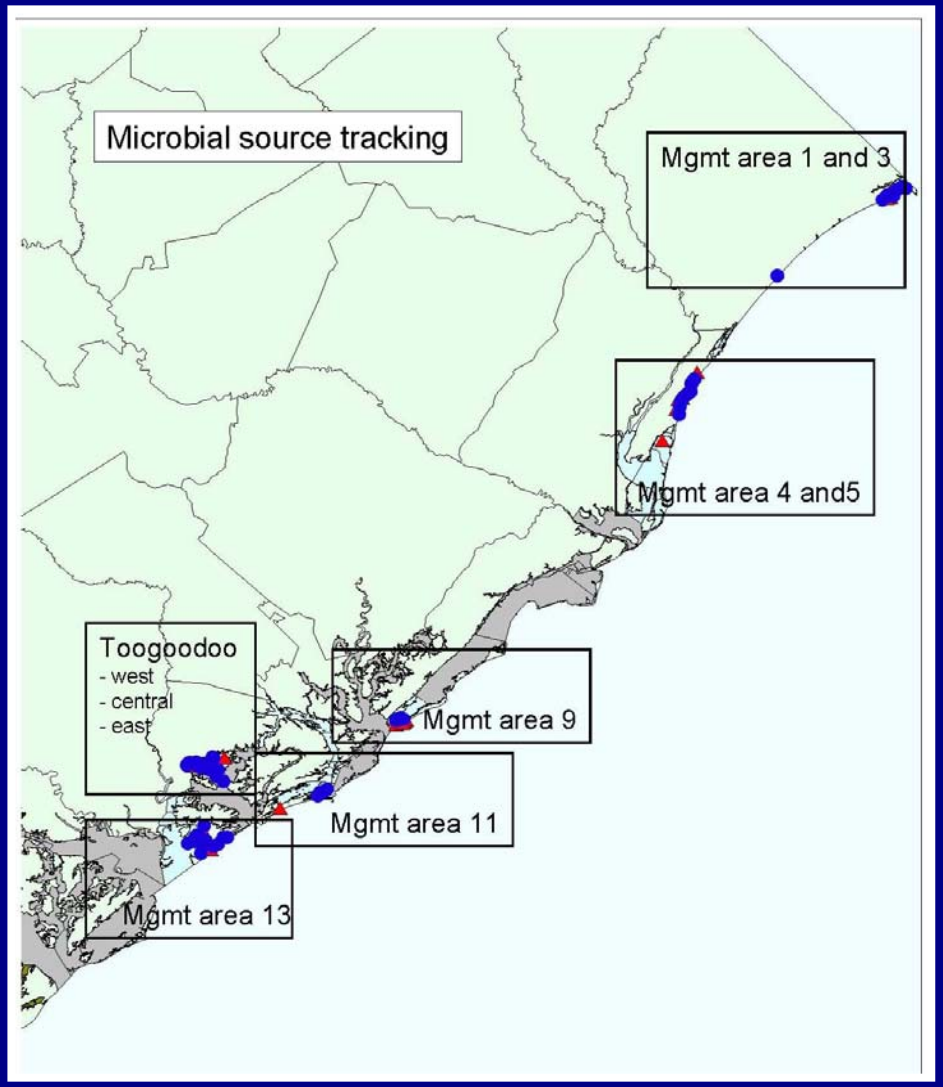
Serotyping



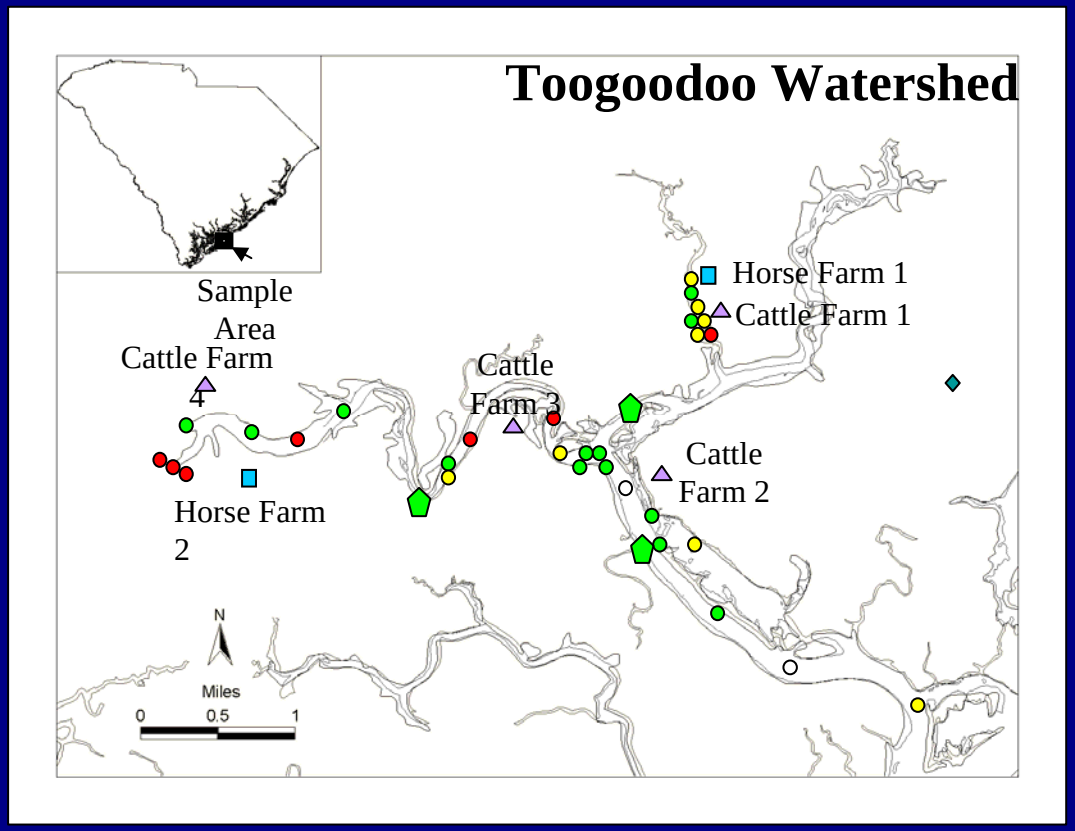
Genotyping



Study of Closed Shellfish Harvesting Waters



Multiple Antibiotic Resistance Analysis Results



- Pump Station
- Horse Farms
- Cattle Farms
- Surface Water Samples
- No Antibiotic Resistance
- 1-3 Isolates with Antibiotic Resistance
- >3 Isolates with Antibiotic Resistance
- No Bacteria to Test

Mud and Manhole Samples not Shown.

- 351 source *E. coli* isolates and 310 surface water isolates were assayed from the Toogoodoo watershed
- The most common antibiotic resistance pattern observed, to penicillin only, was common among all source groups and could not be used to distinguish sources
- Surface water sites in proximity to cattle farms 2 & 3 had *E. coli* with resistance patterns that matched that of cattle *E. coli*

Multiple Antibiotic Resistance (MAR) Overlapping Patterns

	Source		
Pattern*	**Sewage**	**Chicken**	**Hog Lagoon**
O-St-T	2	1	0
C-O-T	14	16	5
O-T	1	1	2
St	1	10	0
St-Su	1	8	0

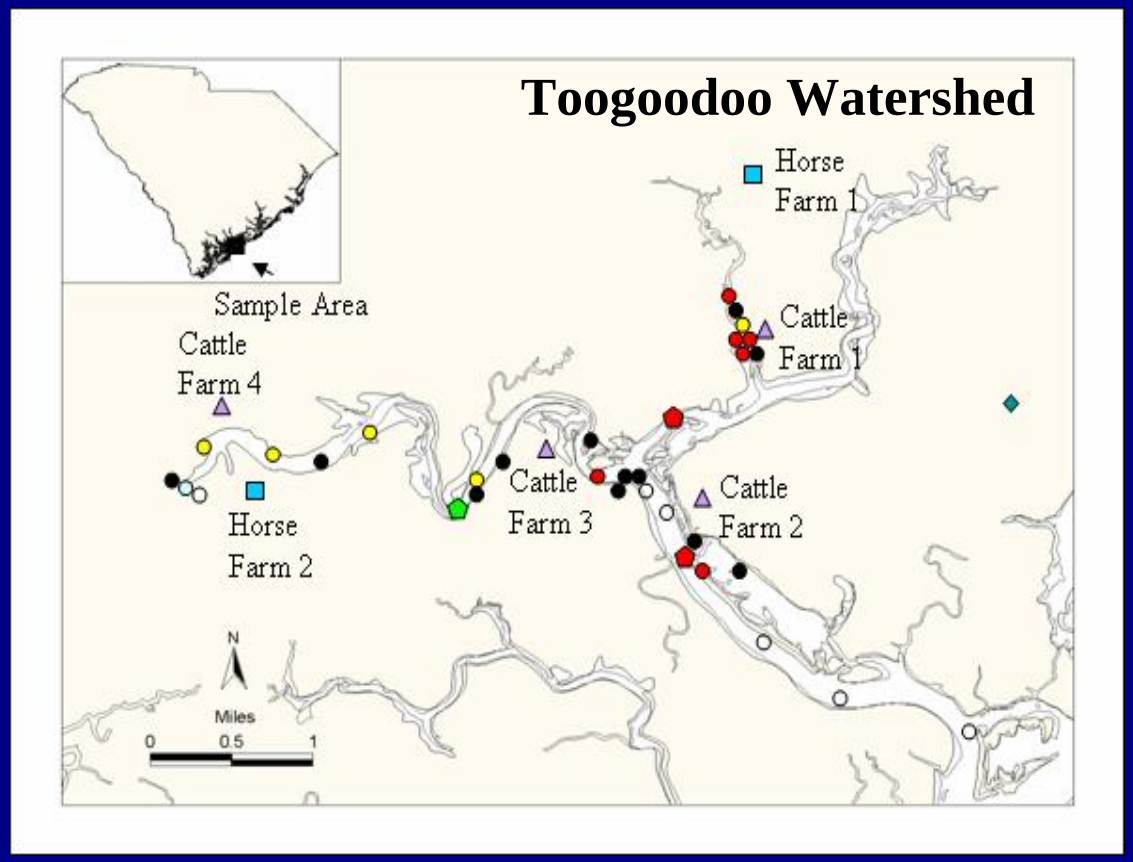
* C = chlortetracycline, O = oxytetracycline, St = streptomycin, Su = sulfathiazole, T = tetracycline

Antibiotic Resistance Analysis (ARA) Overlapping Patterns

	Source			
Pattern*	**Sewage**	**Dog**	**Cattle**	**Horses**
Cf-E	1	9	2	0
Cf-E-P	8	3	1	0
Cf-P	1	0	1	0
E-P	4	10	5	0
P	1	0	1	0

*Cf = cephalothin, E = erythromycin, P = penicillin

Ribotyping Results



- Pump Station
- Horse Farms
- Cattle Farms
- Rain Event Surface Water Sample
- No Ribotyping Pattern Match
- Ribotyping Pattern Match to Wildlife
- Ribotyping Pattern Match to Cattle
- Ribotyping Pattern Match to cattle and wildlife
- Ribotyping Pattern Match to sewage, cattle and wildlife
- Not tested or could not be ribotyped

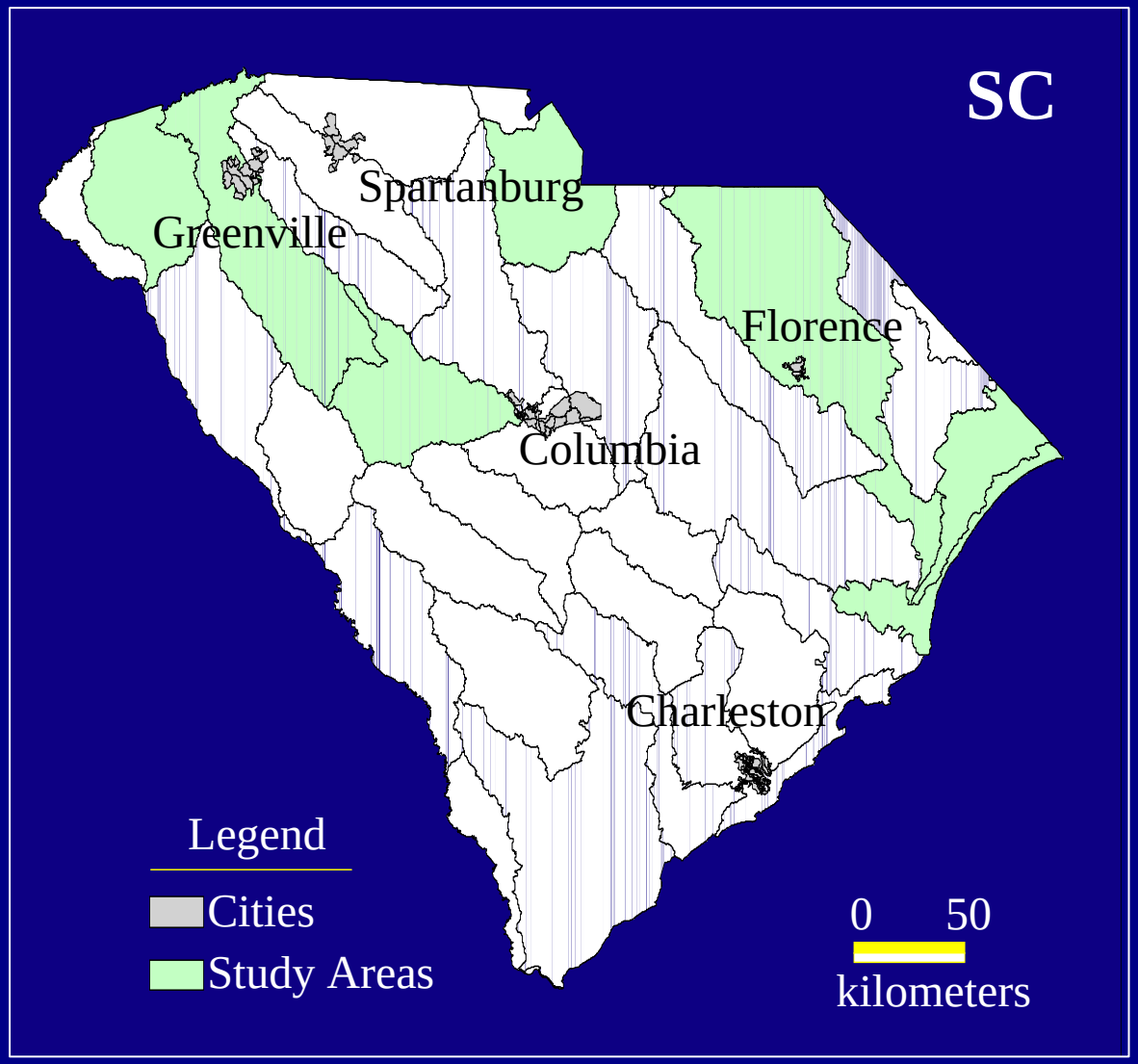
- 526 *E. coli* isolates: SW total (n=253) Known Sources total (n= 273)
- 41% of SW samples matched a Library Source (at 90% Similarity)
- 96% of matched samples were either Raccoon (44%) or Cattle (52%)
- Only one SW sample matched sewage

Overlapping Ribotype Patterns

(From Jumping Run Creek, NC Study)

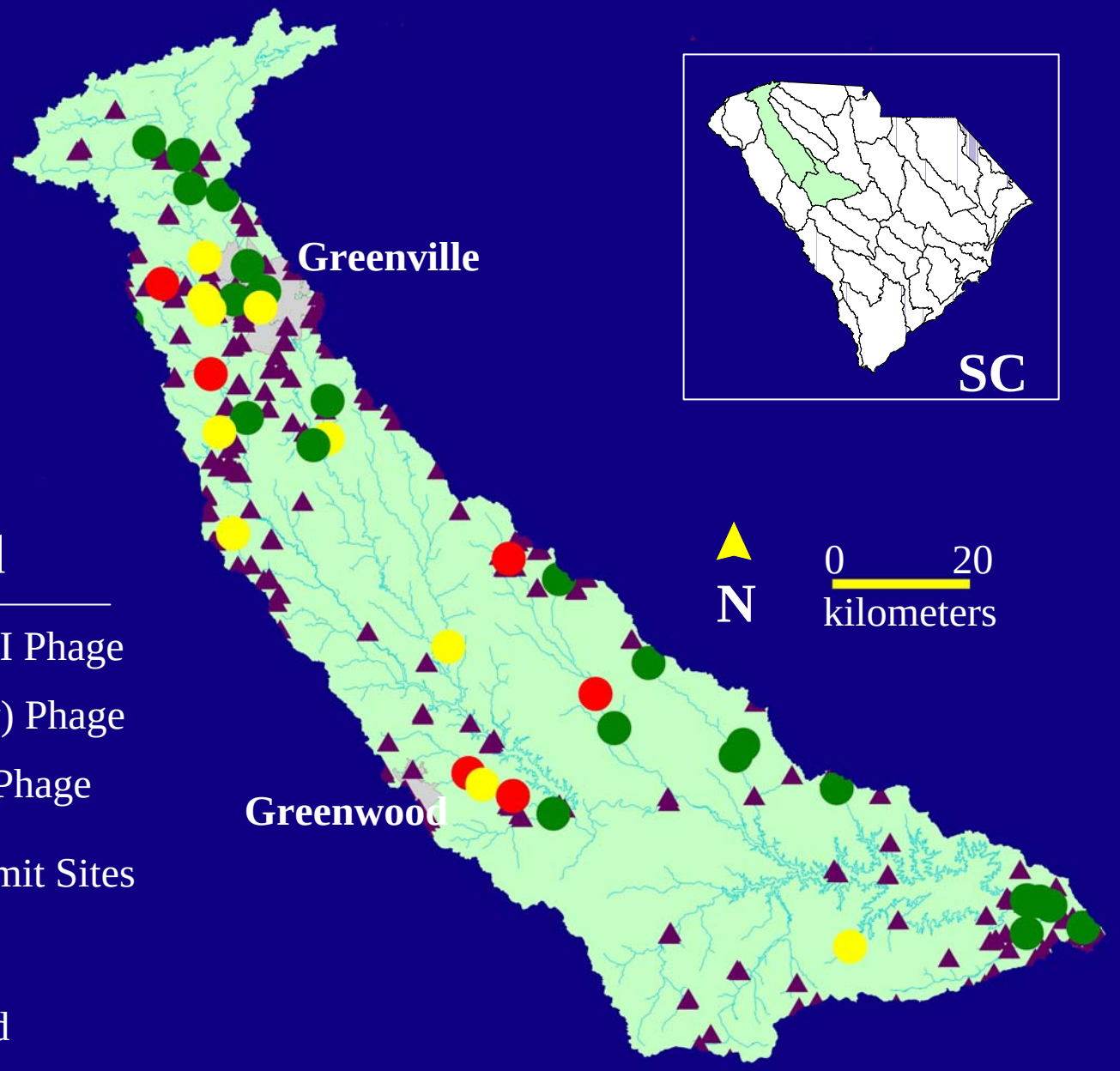
Isolate	Water Sample Site	Ribotyping Pattern Match (Source)	% Similarity
A438 #1	water JRC4	Cat / ibis / opossum / otter / owl / raccoon / septic / squirrel	92.3%
A438 #3	water JRC4	Otter	92.3%
A438 #4	water JRC4	Gull / muskrat	100%
A439 #1	water JRC4A	muskrat	100%
A439 #5	water JRC4A	Cat / dog / opossum / owl / septic	100%
A440 #1	water JRC5	septic	100%
A440 #2	water JRC5	Duck / ibis / goose / opossum / septic	91.7%
A440 #3	water JRC5	septic	100%
A440 #4	water JRC5	Duck / goose / ibis / opossum / otter / septic	91.7%
A440 #5	water JRC5	ibis	100%

Impaired Surface Water Study



- 96 surface water stations were chosen for study based on coliform data and were located within five “priority” watersheds.

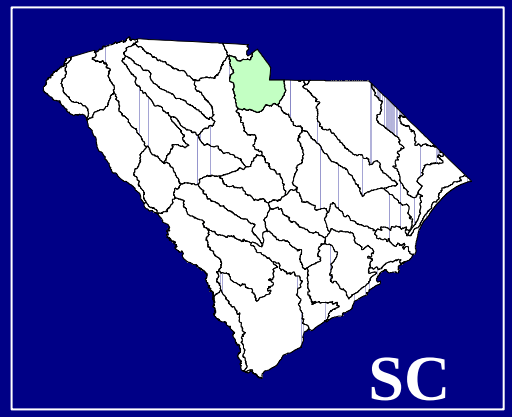
Saluda Watershed

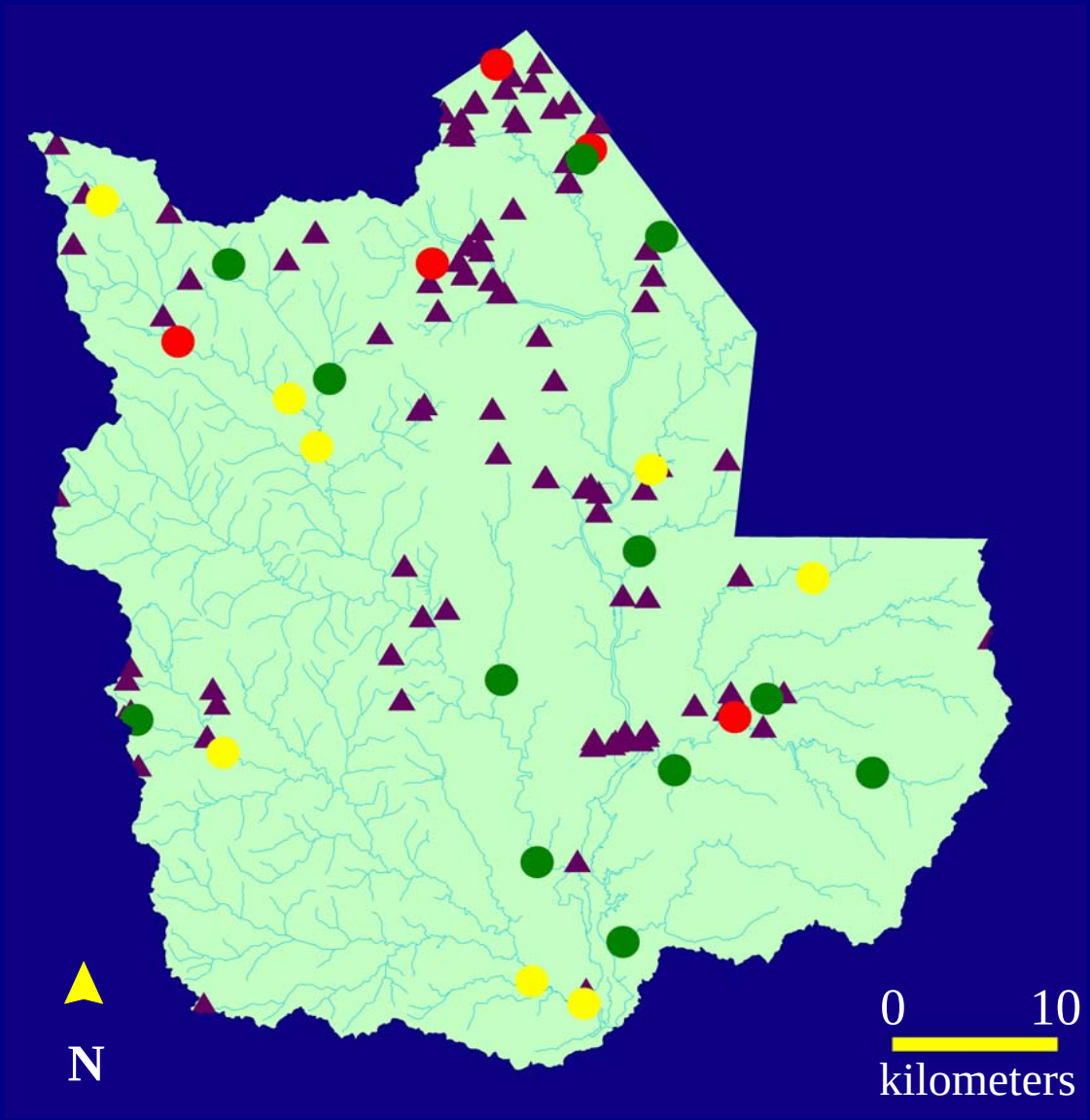


Legend

- Type II or III Phage
- Type I (only) Phage
- No F^+RNA Phage
- NPDES Permit Sites
- Streams
- Watershed

Catawba Watershed





Legend

- Type II or III Phage
- Type I (only) Phage
- No F^+RNA Phage
- NPDES Permit Sites
- Streams
- Watershed

Source Tracking Lessons Learned

- Each tested method has unique advantages and disadvantages
  - Discriminatory power
  - Ease
  - Cost
  - Stability of signal
- Library-dependent methods, in general, require large source databases that are geographically and temporally specific. Library-independent methods are more user friendly
- A “toolbox” approach is recommended

Library Independent Source Tracking Methods

- Does not require a library
- Direct analysis of the genome or indirect analysis after amplification by Polymerase Chain Reaction (PCR)—can have problem with inhibition of the reaction
- May or may not require cultivation of the target organism

Current MST Methods

F+ RNA coliphage, including genotyping

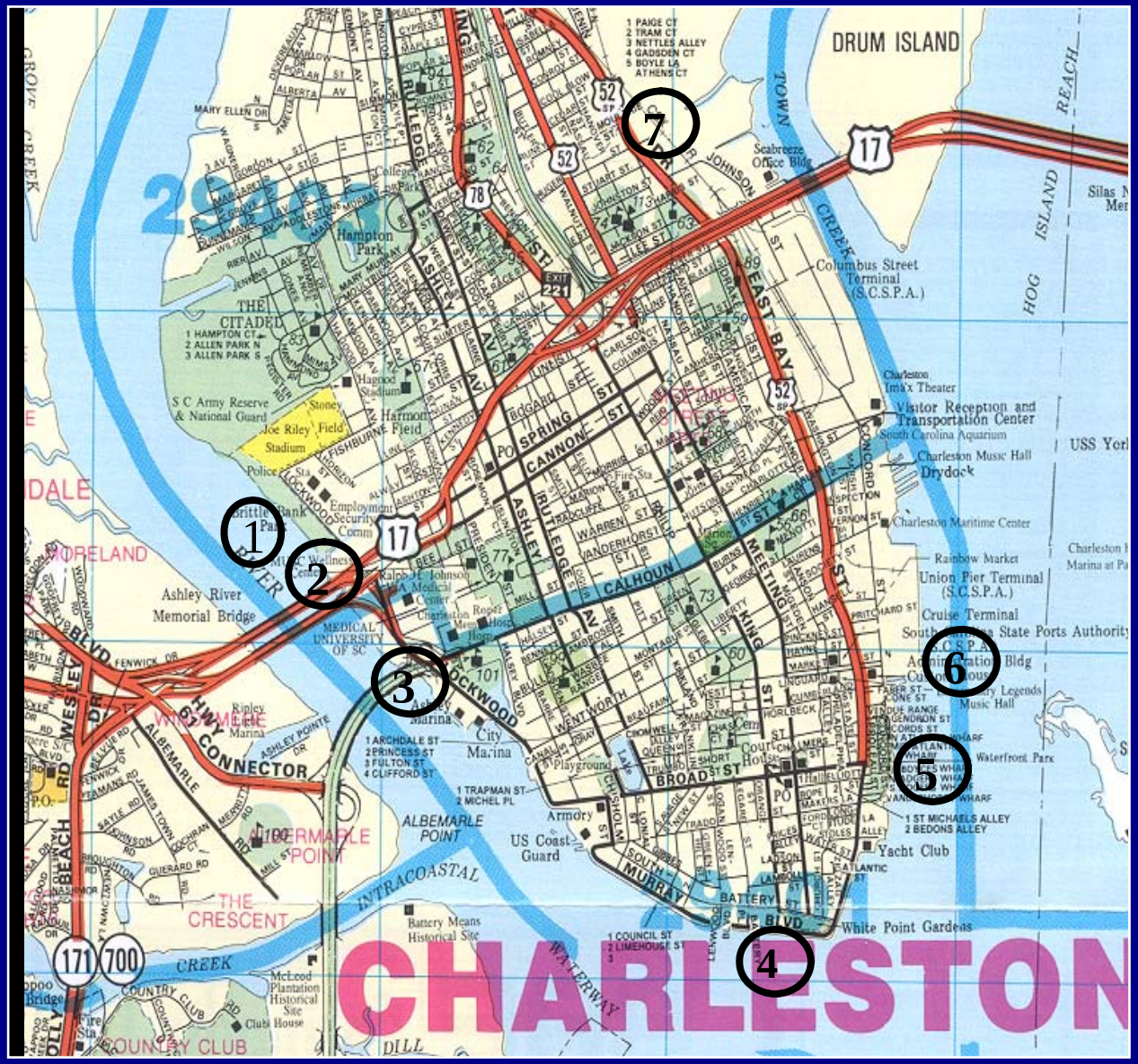
Enterovirus

Norovirus

Methanobrevibacter smithii

Bacteroides spp.--human, bovine, canine

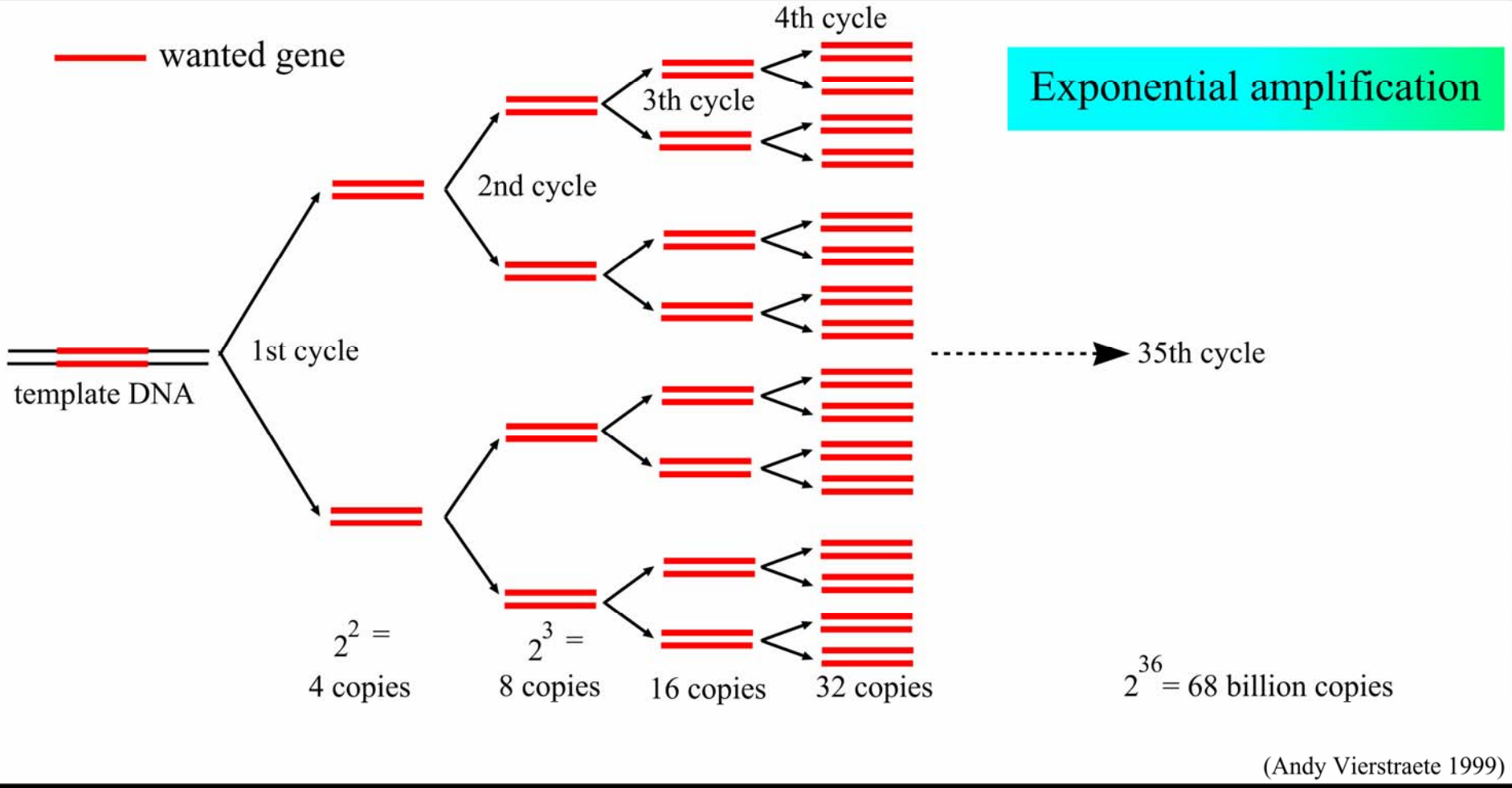
Charleston Stormwater Study



Charleston Stormwater Study

Sample	group II or III F^+RNA coliphages	*Methanobrevibacter smithii*	human-source contamination detected
Calhoun St.	-	-	no
King St.	-	-	no
Market St.	+	+	yes
Spring St.	-	-	no
New Market Ck.	+	-	yes
Ashley River	-	-	no
Cooper River	-	-	no

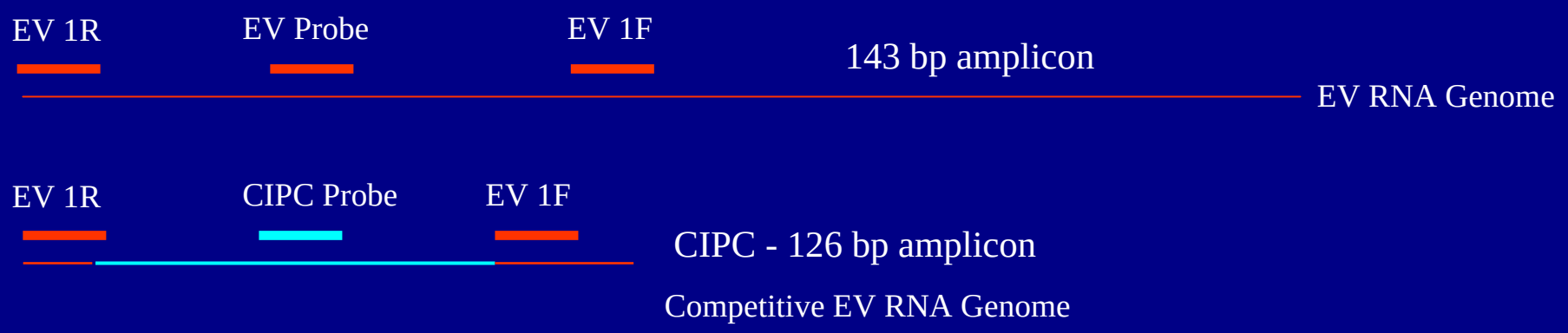
Polymerase Chain Reaction



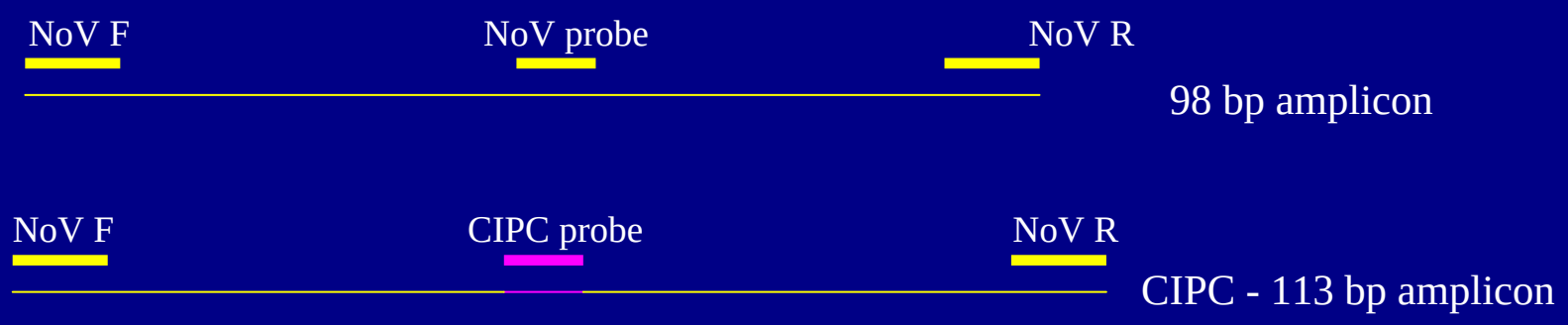
http://users.ugent.be/~avierstr/tif.html

Competitive Internal Positive Controls (CIPC)

Enterovirus CIPC (Gregory et al. 2006)



Norovirus CIPC (Gregory et al. *in preparation*)



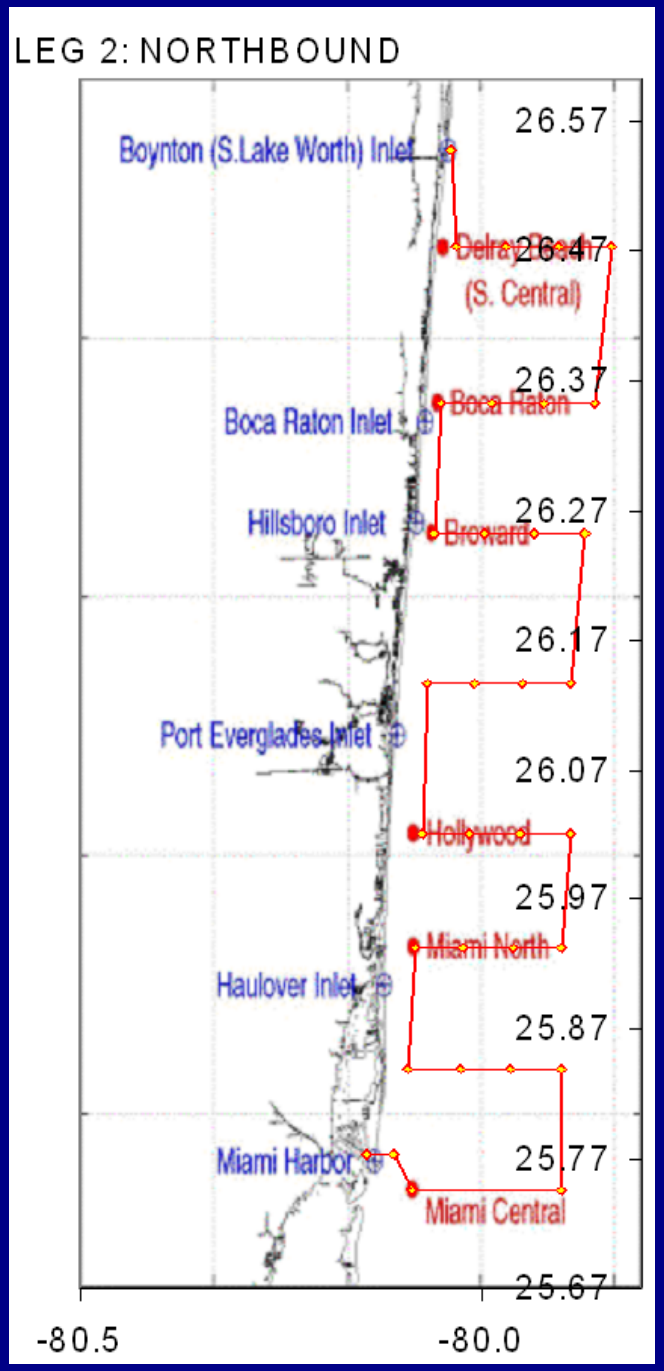
Detection of Norovirus in spiked and control seawater samples

Sample Location	Genomes Reaction^{-1}	Δ CIPC C_T	Correction factor $= (E+1)^{\Delta CT}$	Corrected Genomes Reaction $^{-1}$	Genome Equivalents 100 mls^{-1}
Imperial Beach wavewash		0.22			
Imperial Beach wavewash		5.02			
Offshore seawater		-0.09			
Offshore seawater		0.40			
Seawater with sewage 50 ent		0.73			
Seawater with sewage 50 ent	6	0.48	1.27	8	16
Seawater with sewage 150 ent		0.40			
Seawater with sewage 150 ent	23	0.30	0.84	20	40
Seawater with sewage 500 ent	10	0.03	1.02	11	22
Seawater with sewage 500 ent	10	0.27	1.21	12	24
Seawater with sewage 1,000 ent	19	0.71	1.64	30	60
Seawater with sewage 1,000 ent	66	0.17	1.14	80	160
Seawater with sewage 10,000 ent	458	0.23	1.17	535	1070
Seawater with sewage 10,000 ent	503	0.41	1.35	707	1414
Tijuana River	749	0.29	1.23	900	45000
Tijuana River	597	0.77	1.70	1005	50225
Doheny w/w with Doheny guano		0.43			
Doheny w/w with Doheny guano		0.14			

SCCWRP Methods Evaluation Study

Number of positive samples per number of samples analyzed

	(from SCCWRP)		Single Agar Layer		qPCR	Luminex (duplex)		RT-PCR	RT-PCR
	E. coli	ENT	F+ phage	F- phage	***M. smithii***	***M. smithii***	ENT	Enterovirus	Norovirus
True Negatives (PBS)	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
"Negatives“ Offshore Water	0/6	0/6	0/6	0/6	1/6	1/6	0/6	0/6	0/6
Ambient Water with Low Bacterial Counts	8/8	7/8	3/8	6/8	1/8	5/8	4/8	0/8	0/8
Sewage Spiked Offshore Water	9/9	9/9	6/8	8/8	6/10	9/10	8/10	2/10	8/10
Sewage Spiked Doheny Water	4/4	4/4	4/4	4/4	2/4	4/4	4/4	1/4	0/4
Dirty Ambient Water (Tijuana River)	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2



Florida Outfalls Survey (FACE)

Sample	***M. Smithii*** (GEU/100ml)	**Norovirus** (GEU/100ml)
Hollywood Boil	$3.0X10^5$	235
Boca Raton Boil	$2.7x10^4$	2.3
Broward Boil	$3.7x10^4$	6.3
Miami Central Boil	$3.4x10^5$	11
South Central Boil	$7.0x10^2$	-
North Miami Boil	$1.3x10^5$	347
Deep Water Control	-	-