Small Successes: Library-Independent Methods to Identify Microbial Pollution Sources

Von Sigler

Department of Environmental Sciences
University of Toledo

Presentation outline

- Background of library independent methods (LIM)
  - Contrast with library-dependent methods
- Examples of LIM
  - Gene/host-specific PCR
  - Community analyses
- Case studies
  - Testing DGGE as a LIM
  - *E. coli* associated with sediment transport
  - Identifying the geographic source of bacterial pollution
- Conclusions
  - Advantages and disadvantages of LIM
  - Outlook

Library-dependent vs. library-independent

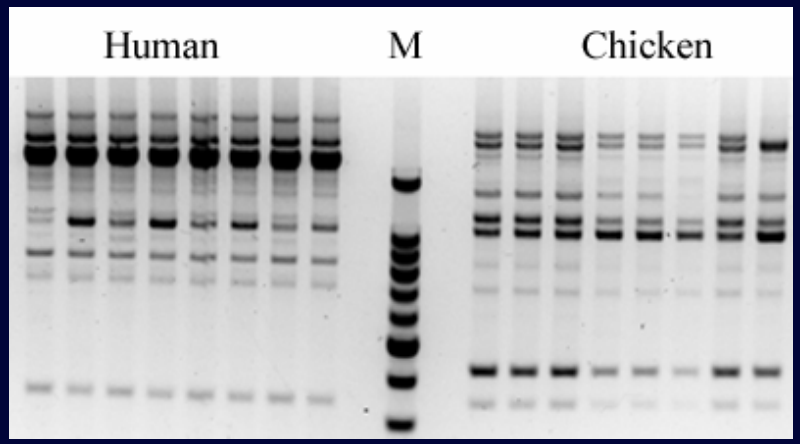
- **Dependent** – the occurrence of phenotypic or genotypic traits in fecal isolates from the sink is compared to those in source library
  - Based on the culture of a library of indicator organisms

Library-dependent vs. library-independent

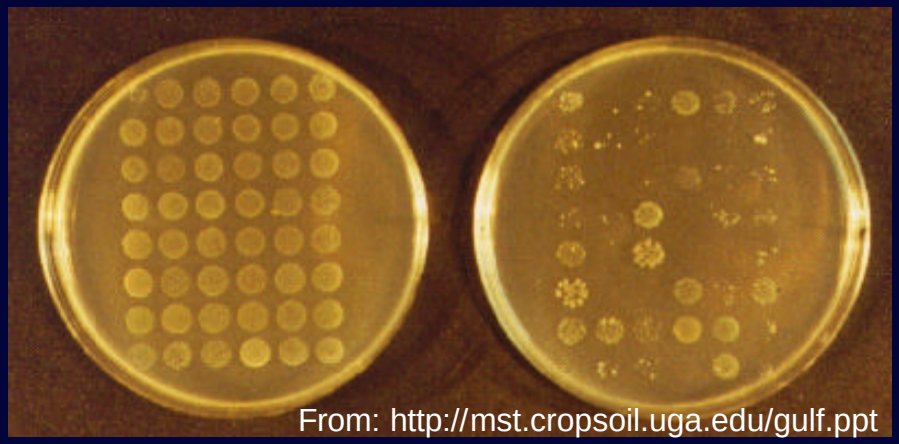
- **Dependent** – the occurrence of phenotypic or genotypic traits in fecal isolates from the sink is compared to those in source library
  - Based on the culture of a library of indicator organisms
  - Relies on further identification and a characterization assay
    - ARA, RAPD, PFGE, ribotyping, C-source utilization, rep-PCR, etc.

Library-dependent vs. library-independent

- **Dependent** – the occurrence of phenotypic or genotypic traits in fecal isolates from the sink is compared to those in source library
  - Based on the culture of a library of indicator organisms
  - Relies on further identification and a characterization assay
    - ARA, RAPD, PFGE, ribotyping, C-source utilization, rep-PCR, etc.



BOX-PCR of *E. coli* isolates



ARA of *E. coli* isolates

Library-dependent vs. library-independent

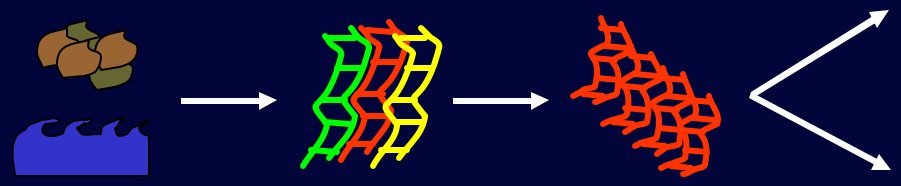
- **Independent** –Involve targeting signature sequences in DNA isolated from the environment

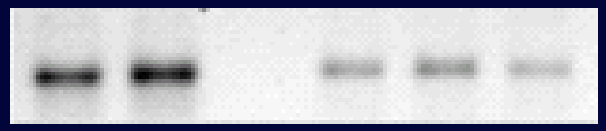
Library-dependent vs. library-independent

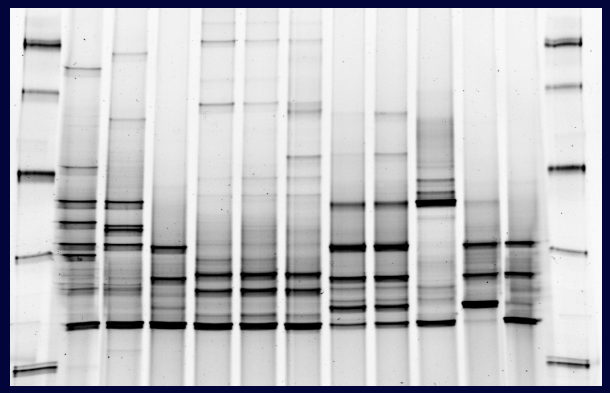
- **Independent** –Involve targeting signature sequences in DNA isolated from the environment
  - PCR

Library-dependent vs. library-independent

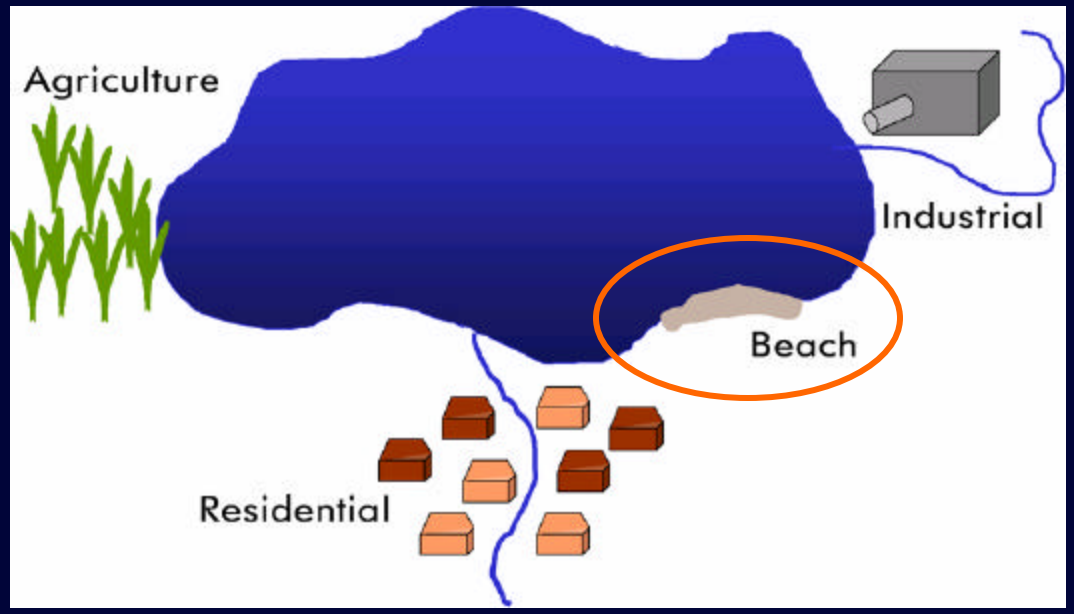
- **Independent** –Involve targeting signature sequences in DNA isolated from the environment
  - PCR





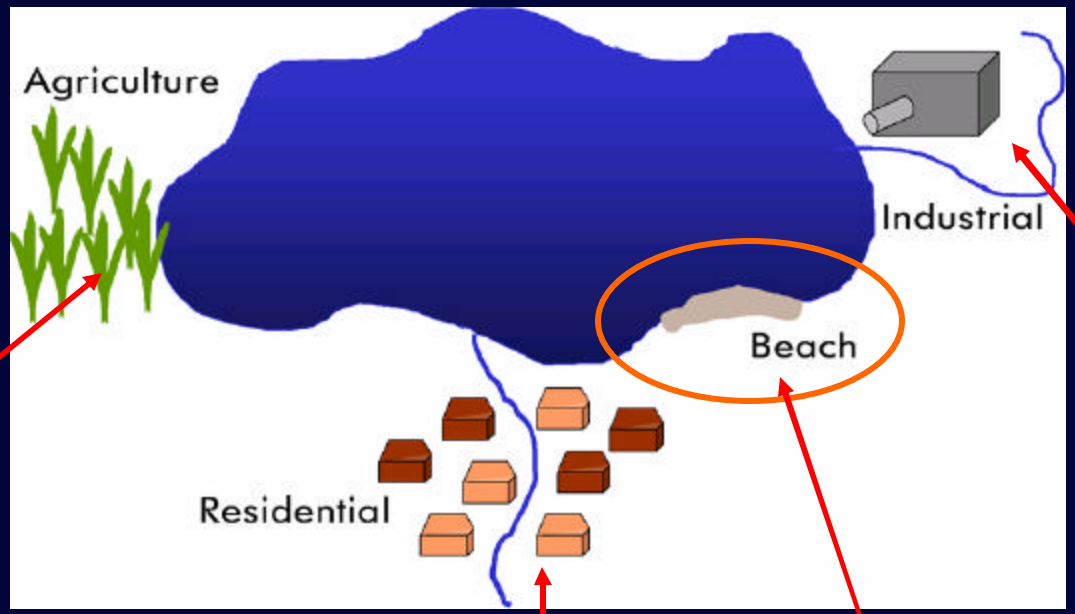


What is your question?



What is the <u>host origin</u> of the pollution?

What is your question?



Pig

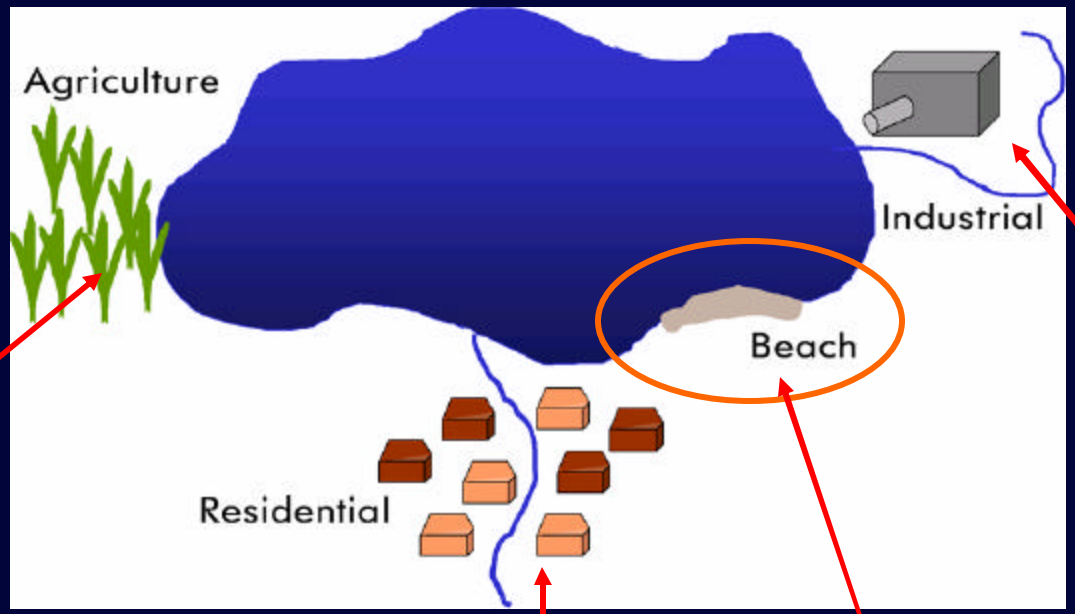
WWTP effluent*

Birds

Dogs/Cats

What is the <u>host origin</u> of the pollution?

What is your question?



Pig

WWTP effluent*

Birds

Dogs/Cats

What is the <u>host origin</u> of the pollution? = Gene/host-specific PCR

Categories of LIM

- Gene/host-specific PCR
  - Diagnostic genes can identify the host origin of bacterial pollution
    - Human, horse, pig, gull, etc.

Categories of LIM

- Gene/host-specific PCR
  - Diagnostic genes can identify the host origin of bacterial pollution
    - Human, horse, pig, gull, etc.

TABLE 7.1 Species of the genera *Enterococcus* and *Streptococcus* of faecal origin

Species	*Intestinal origin*
Enterococcus	
faecium	Man, cattle, pigs, birds
faecalis	Man, cattle, pigs, birds
durans	Man, pigs, birds
hirae	Man, pigs, birds
avium	Man, cattle, pigs, birds
gallinarum	Man, birds
cecorum	Cattle, pigs, birds
columbae	Cattle, pigs, birds
Streptococcus	
bovis	Man, cattle, pigs
equinus	Man, cattle, pigs
alactolyticus	Pigs, birds
hyointestinalis	Pigs
intestinalis	Pigs
suis	Pigs

Source: Godfree *et al.* (1997).

Categories of LIM

- Gene/host-specific PCR
  - Diagnostic genes can identify the host origin of bacterial pollution
    - Human, horse, pig, gull, etc.

TABLE 7.1 Species of the genera *Enterococcus* and *Streptococcus* of faecal origin

Species	*Intestinal origin*
Enterococcus	
faecium	Man, cattle, pigs, birds
faecalis	Man, cattle, pigs, birds
durans	Man, pigs, birds
hirae	Man, pigs, birds
avium	Man, cattle, pigs, birds
gallinarum	Man, birds
cecorum	Cattle, pigs, birds
columbae	Cattle, pigs, birds
Streptococcus	
bovis	Man, cattle, pigs
equinus	Man, cattle, pigs
alactolyticus	Pigs, birds
hyointestinalis	Pigs
intestinalis	Pigs
suis	Pigs

Source: Godfree *et al.* (1997).

- *Bacteroides spp.*
  - ~30% of fecal microflora
  - Limited survival in secondary environment
    - 16S rRNA gene primers can discriminate between humans, bovine, swine, equine and canine
    - Successful in natural waters (Bernard and Field, 2000)

Gene/host-specific PCR - examples

Gene	Bacterium	Host
16S rRNA	Bacteroidetes group	Humans, Bovine, Swine, Equine and Canine
STIb	*E. coli* heat stable toxin	Human
LTIIa	*E. coli* heat labile toxin	Bovine
Esp	*Enterococcus faecium* surface protein	Human, Bovine
16S rRNA	Bifidobacteria group	Human

Gene/host-specific PCR - examples

Gene	Bacterium	Host
16S rRNA	Bacteroidetes group	Humans, Bovine, Swine, Equine and Canine
STIb	*E. coli* heat stable toxin	Human
LTIIa	*E. coli* heat labile toxin	Bovine
Esp	*Enterococcus faecium* surface protein	Human, Bovine
16S rRNA	Bifidobacteria group	Human

- Overall, detection of theses genes might indicate the presence of pollution originating from a specific host

Gene/host-specific PCR - examples

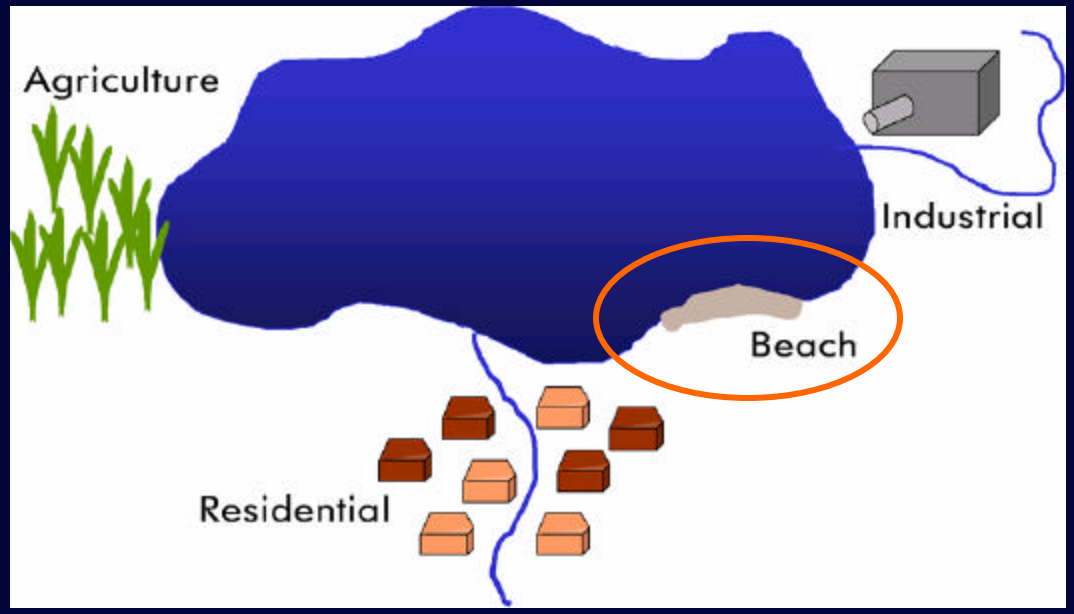
Gene	Bacterium	Host
16S rRNA	Bacteroidetes group	Humans, Bovine, Swine, Equine and Canine
STIb	*E. coli* heat stable toxin	Human
LTIIa	*E. coli* heat labile toxin	Bovine
Esp	*Enterococcus faecium* surface protein	Human, Bovine
16S rRNA	Bifidobacteria group	Human

- Overall, detection of theses genes might indicate the presence of pollution originating from a specific host
  - Genes must exhibit adequate genetic diversity among hosts
  - Geographically and temporally stable bacteria
  - Abundant in host

Categories of LIM

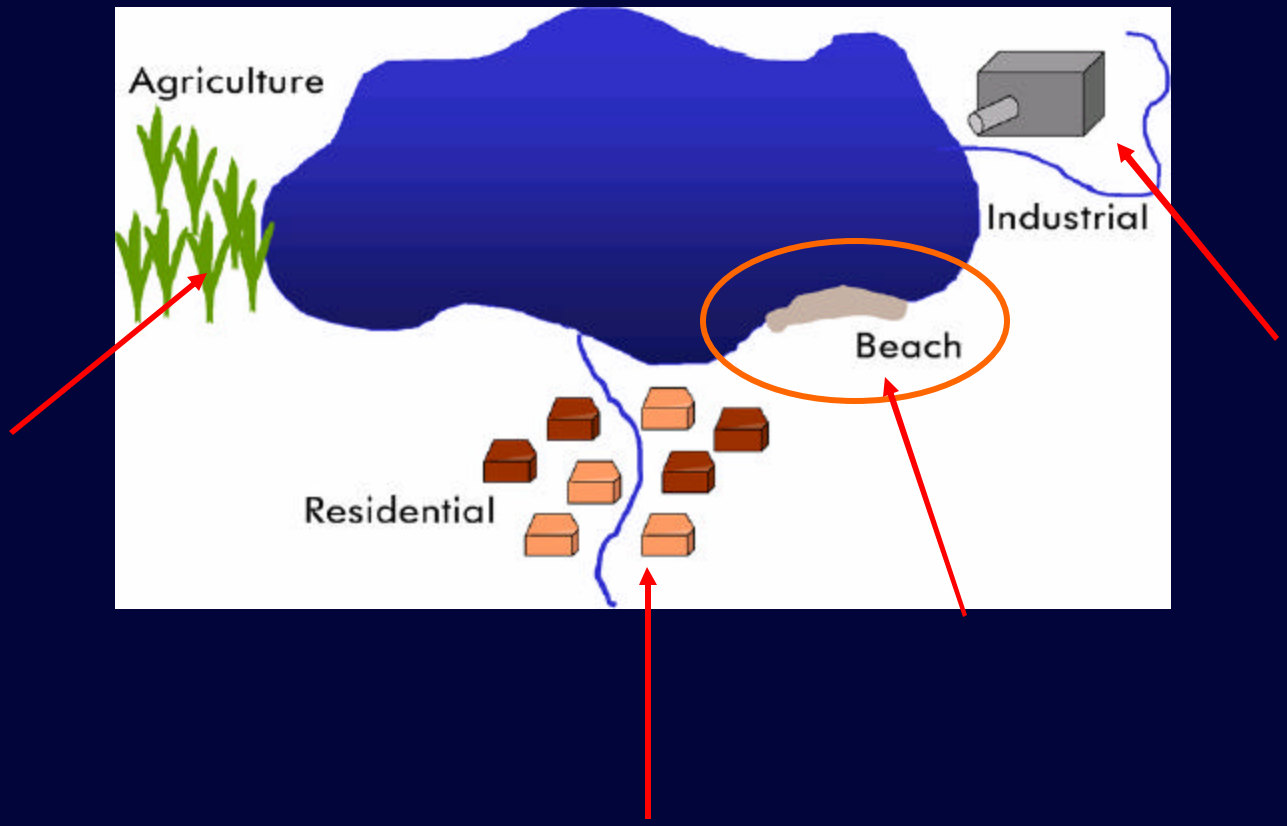
- Community analyses
  - Identify the geographic source of the bacterial pollution
    - Creek, drainage ditch, sand, etc.
  - Fingerprinting

What is your question?



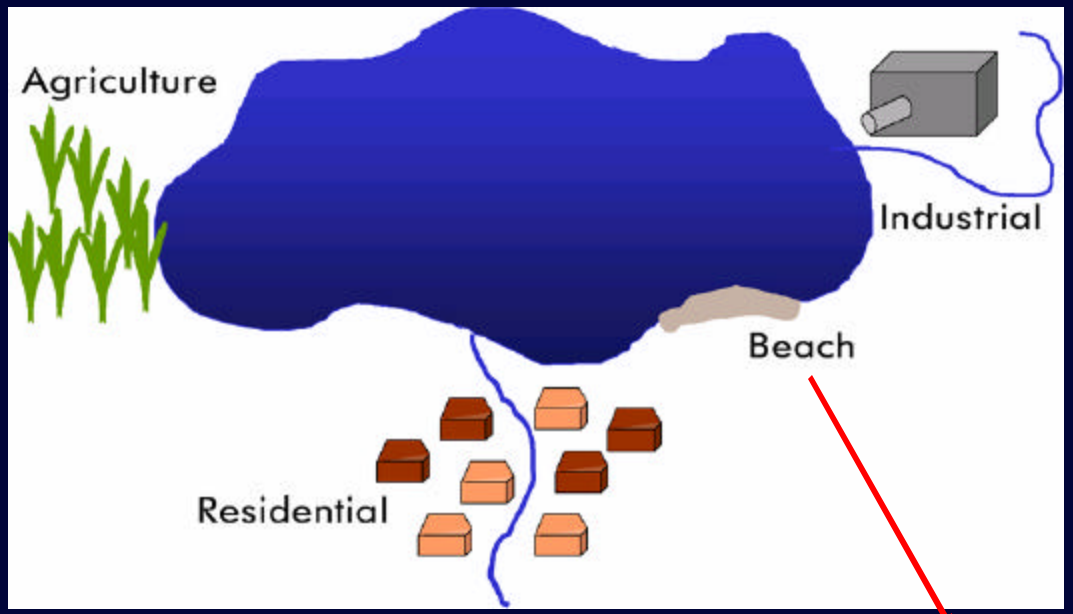
Where is the pollution coming from?

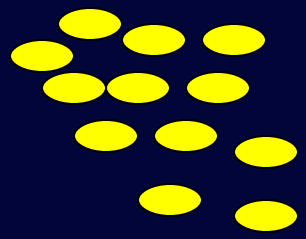
What is your question?



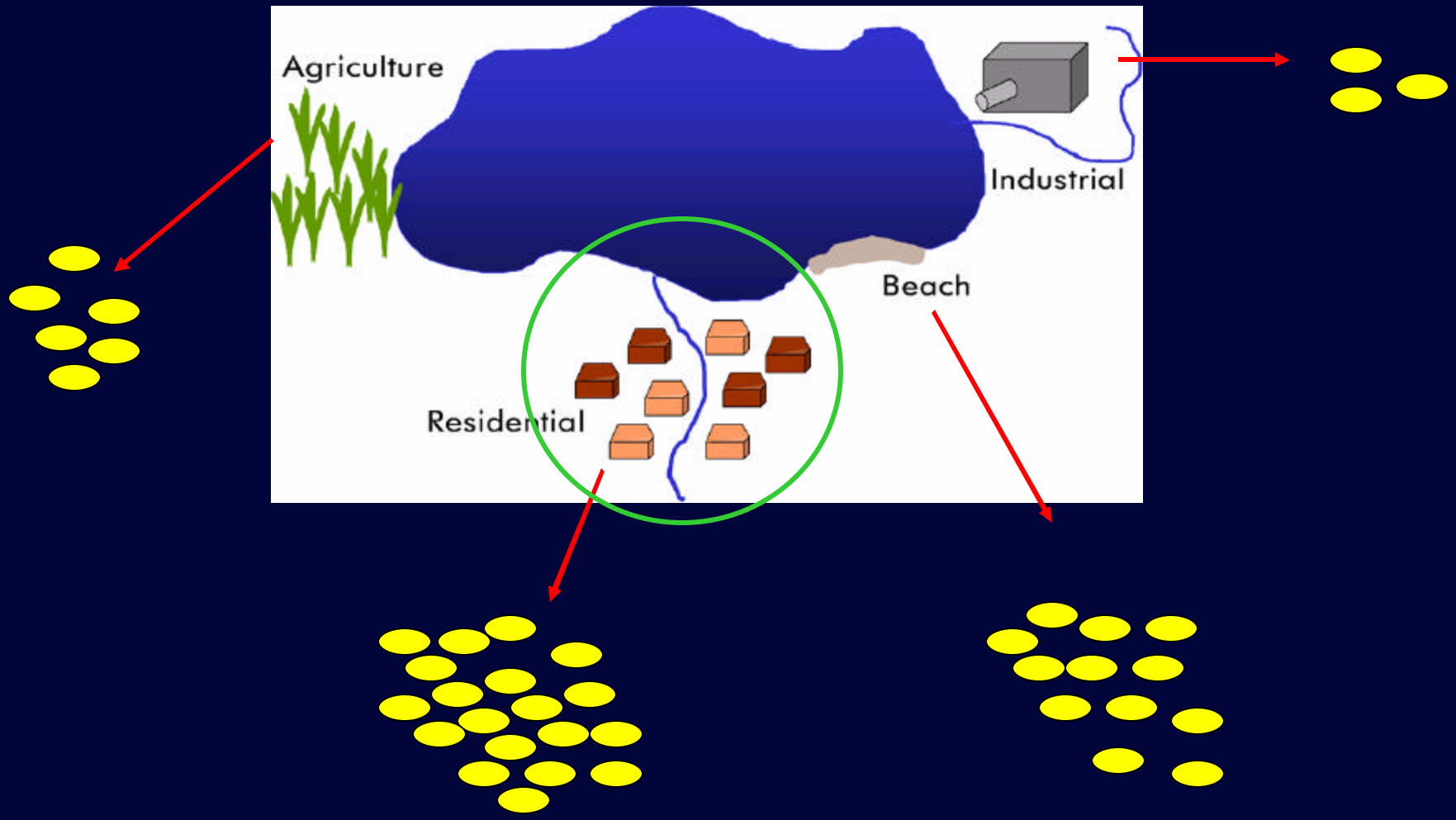
Where is the pollution coming from? = Community analyses

Why not simply count isolates?

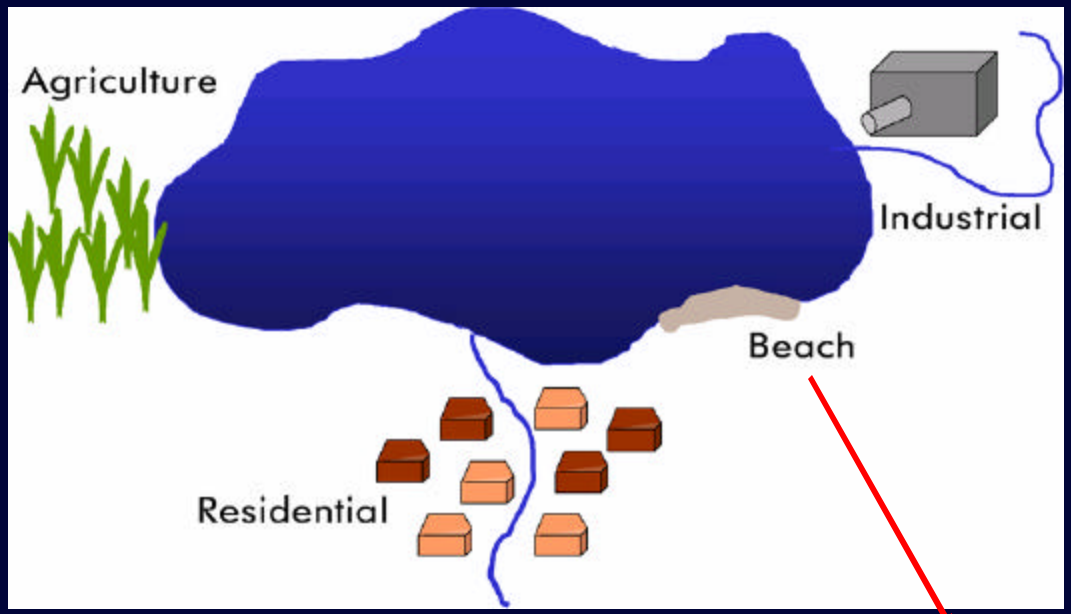


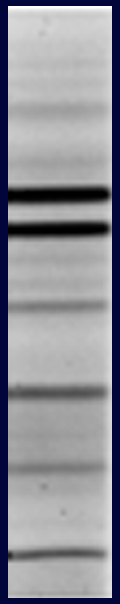


Counting isolates

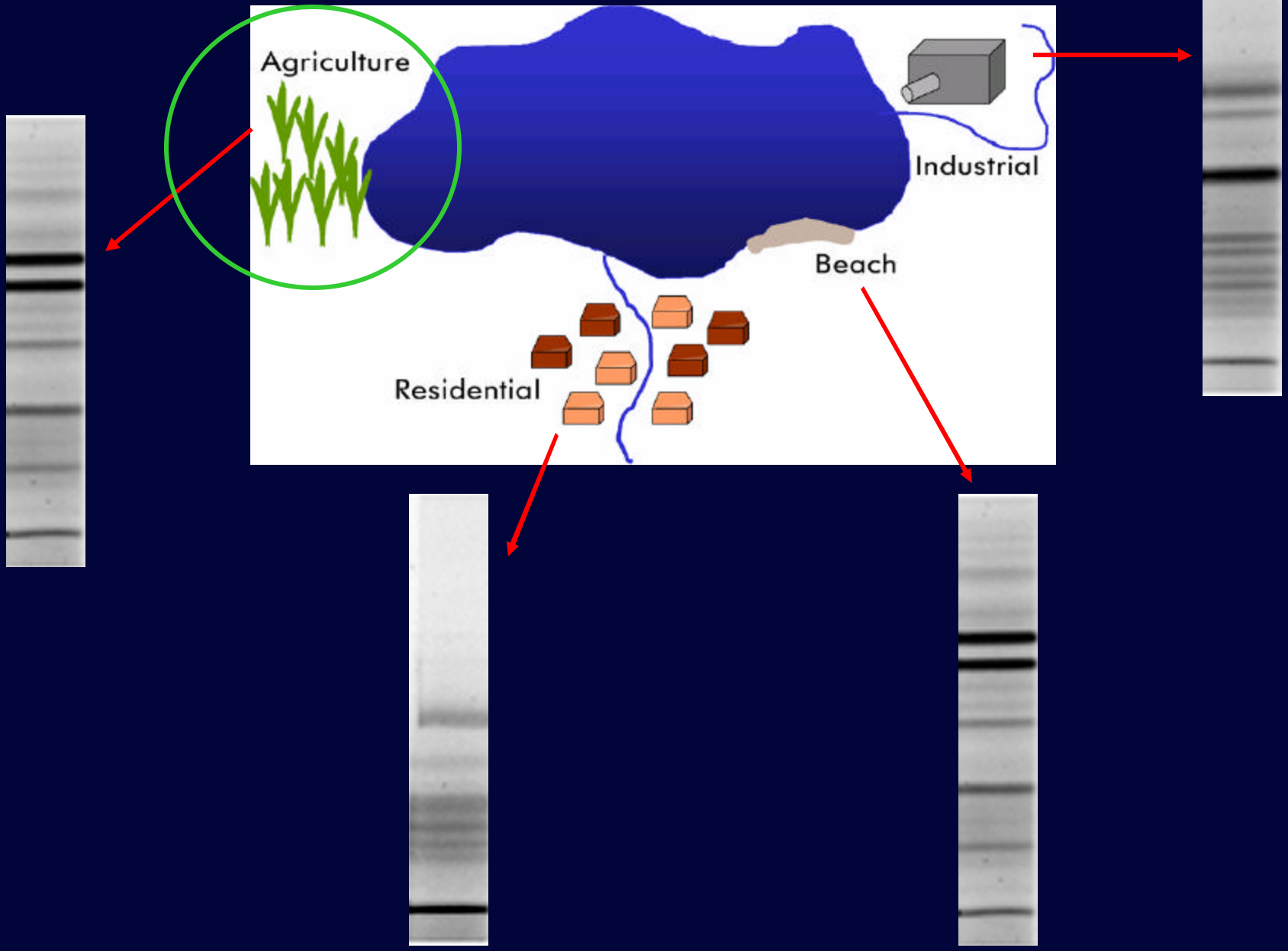


Library independent fingerprinting



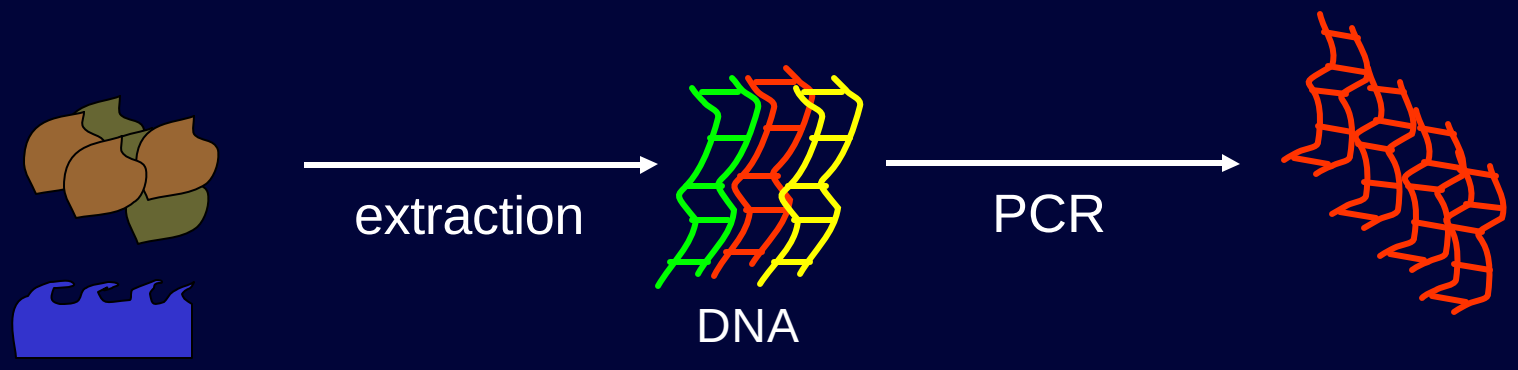


Library independent fingerprinting

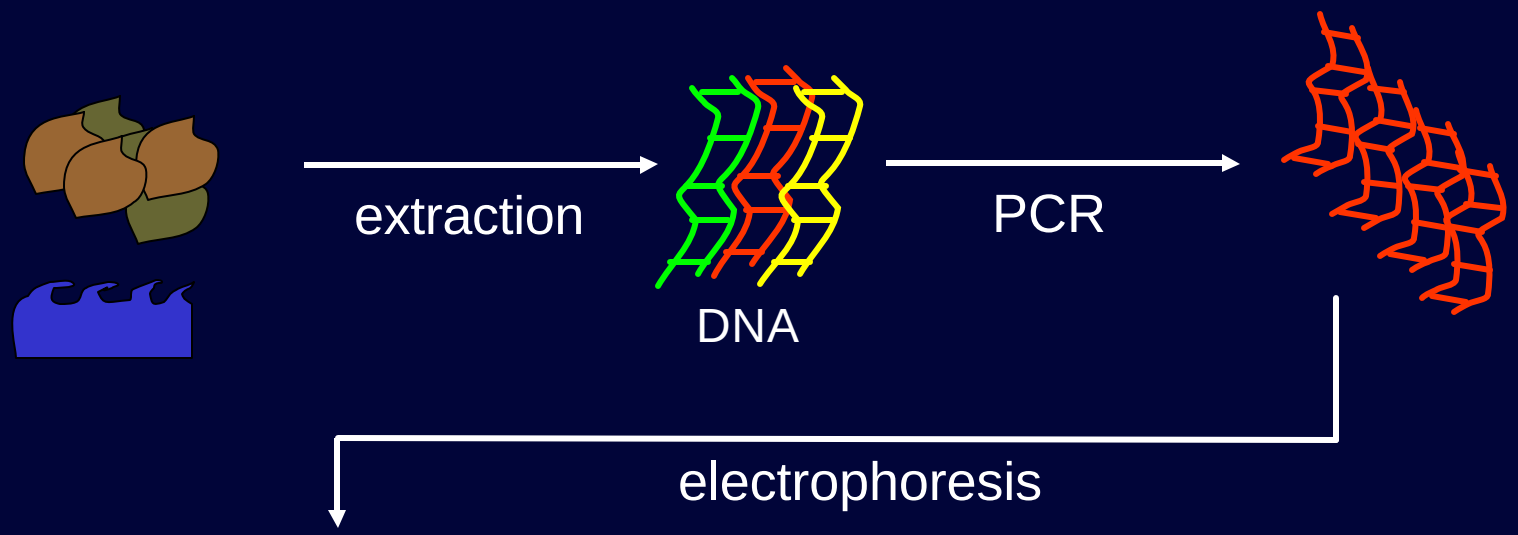


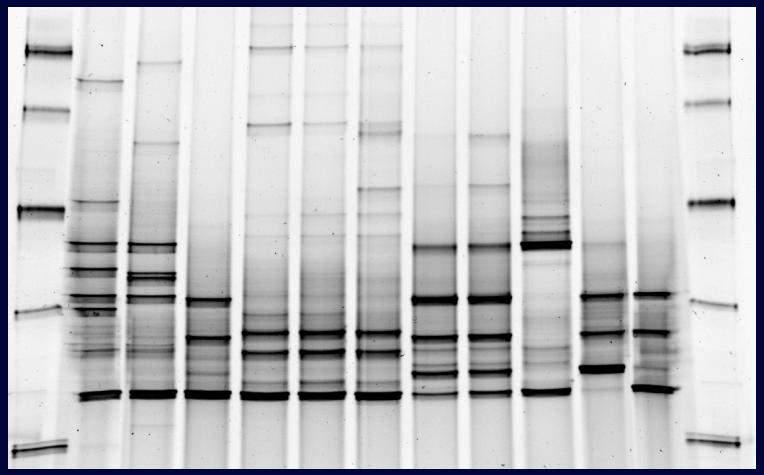
Denaturing gradient gel electrophoresis (DGGE)

Denaturing gradient gel electrophoresis (DGGE)



Denaturing gradient gel electrophoresis (DGGE)



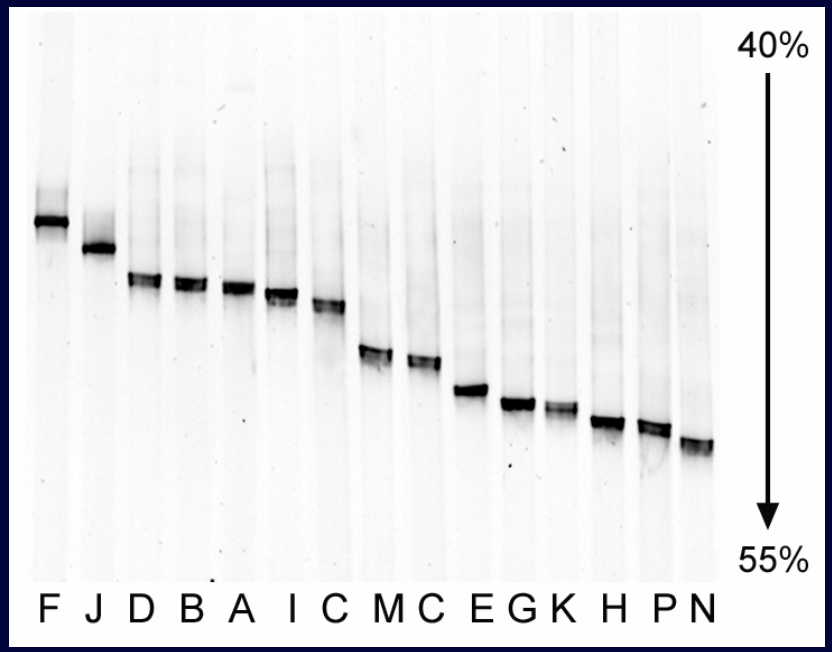


Does DGGE differentiate unique *E. coli* communities?

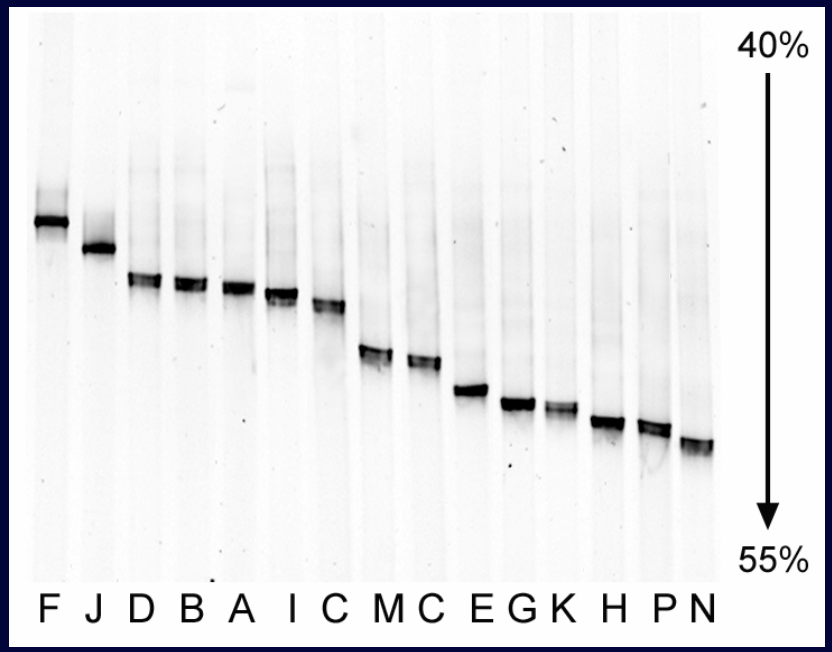
- Maumee Bay State Park (OH)
  - Water
- 184 confirmed *E. coli*
  - Modified m-TEC
  - EMB agar
  - 16S rRNA PCR

- Determine the number of distinct *E. coli* phylotypes detected by DGGE
  - PCR-amplify the *uidA* gene from each *E. coli* isolate
    - *uidA* exhibits sequence polymorphism (Farnleitner et al., 2000)

- Determine the number of distinct *E. coli* phylotypes detected by DGGE
  - PCR-amplify the *uidA* gene from each *E. coli* isolate
    - *uidA* exhibits sequence polymorphism (Farnleitner et al., 2000)
- DGGE of each isolate resulted in detection of <u>18</u> unique phylotypes



- Determine the number of distinct *E. coli* phylotypes detected by DGGE
  - PCR-amplify the *uidA* gene from each *E. coli* isolate
    - *uidA* exhibits sequence polymorphism (Farnleitner et al., 2000)
- DGGE of each isolate resulted in detection of <u>18</u> unique phylotypes



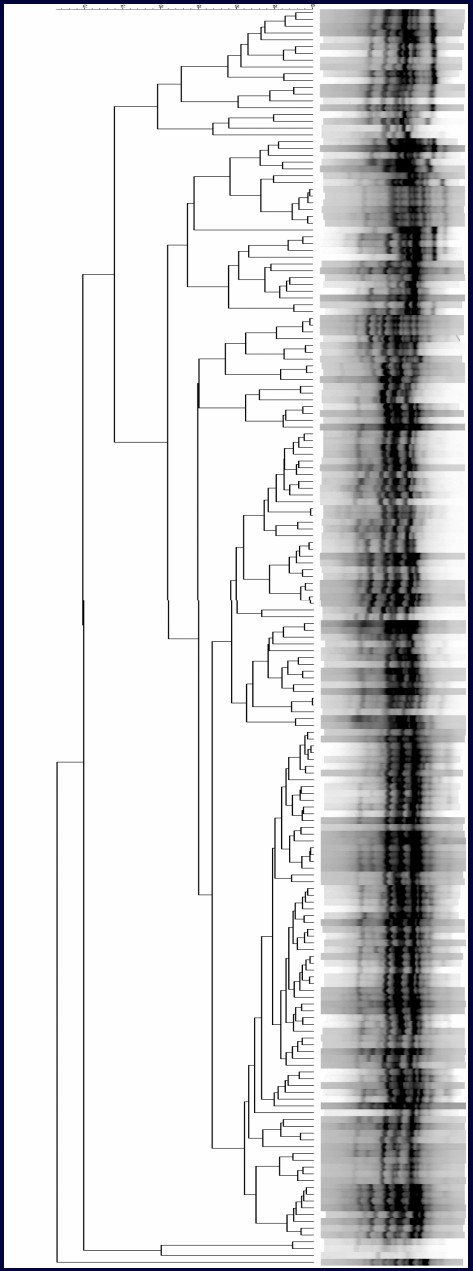
Good or bad?

DGGE vs. BOX-PCR

- BOX-PCR performed on each *E. coli* isolate
  - Fingerprints were compared to determine phylotype similarity

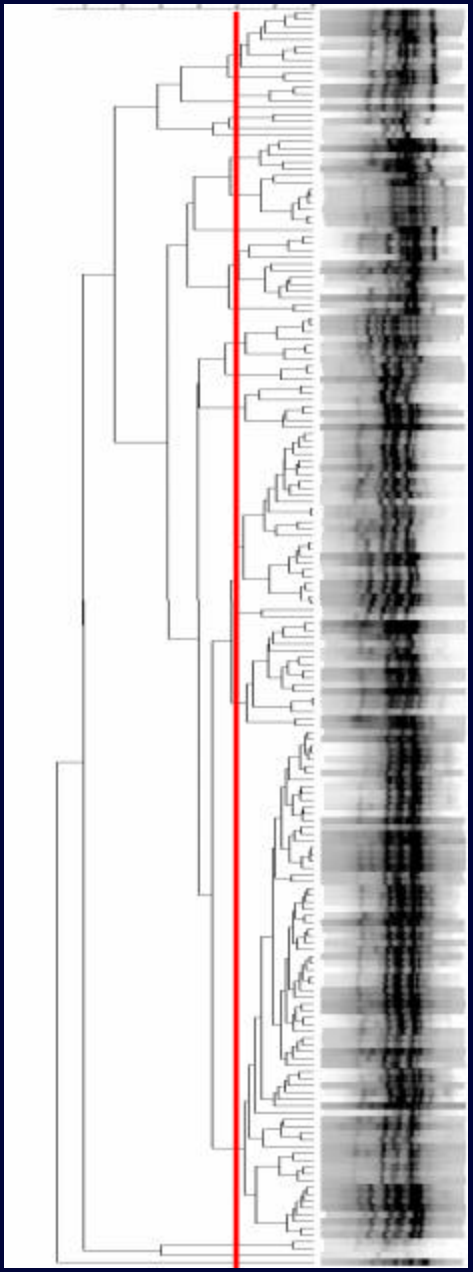
DGGE vs. BOX-PCR

- BOX-PCR performed on each *E. coli* isolate
  - Fingerprints were compared to determine phylotype similarity



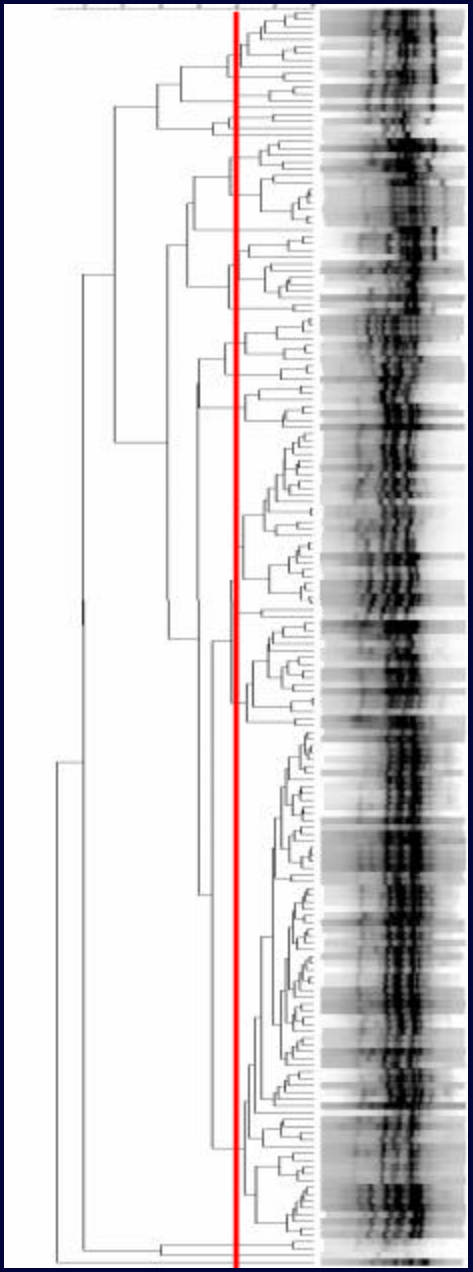
DGGE vs. BOX-PCR

- BOX-PCR performed on each *E. coli* isolate
  - Fingerprints were compared to determine phylotype similarity



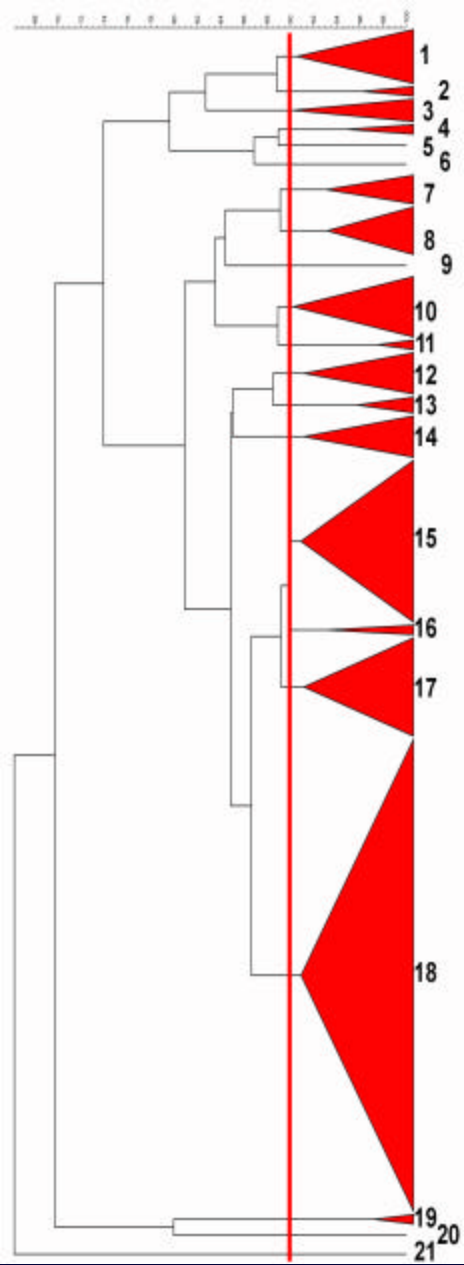
DGGE vs. BOX-PCR

- BOX-PCR performed on each *E. coli* isolate
  - Fingerprints were compared to determine phylotype similarity
  - Clusters that formed above 90% similarity were collapsed to yield phylotype number



DGGE vs. BOX-PCR

- BOX-PCR identified 21 *E. coli* phylotypes
  - Compare with 18 phylotypes detected by DGGE of *uidA* from isolates

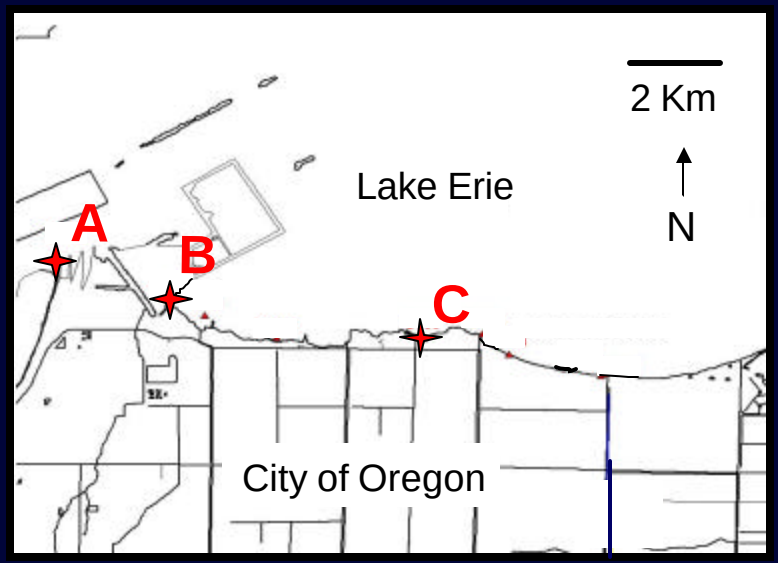


Case study: *E. coli* transport

- Can sediments transport novel *E. coli* populations throughout Maumee Bay (OH)?
  - Lake Erie Beach is posted with advisories ~25% of the swim season
  - Western Basin is shallow and hydrologically dynamic

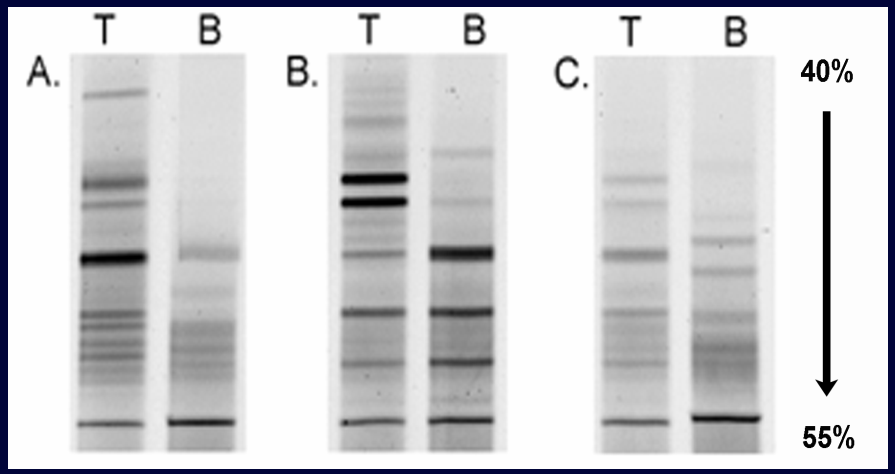
Case study: *E. coli* transport

- Can sediments transport novel *E. coli* populations throughout Maumee Bay (OH)?
  - Lake Erie Beach is posted with advisories ~25% of the swim season
  - Western Basin is shallow and hydrologically dynamic

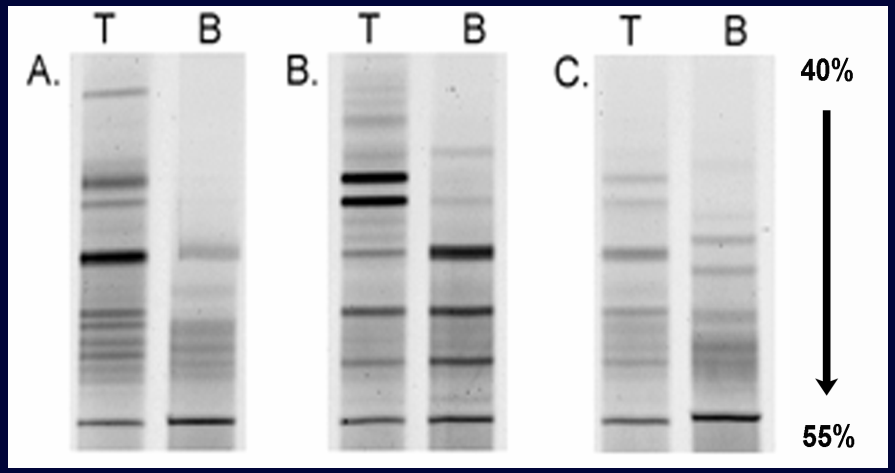


- Isolate DNA
  - Trapped sediment
  - Bottom sediment
- PCR-DGGE of *uidA*

- Isolate DNA
  - Trapped sediment
  - Bottom sediment
- PCR-DGGE of *uidA*
- *E. coli* communities in trapped sediments are different than those in bottom sediments
  - Supports sediment-mediated transport
  - Trapped sediments appear to harbor a greater number of *E. coli* phylotypes



- Isolate DNA
  - Trapped sediment
  - Bottom sediment
- PCR-DGGE of *uidA*
- *E. coli* communities in trapped sediments are different than those in bottom sediments
  - Supports sediment-mediated transport
  - Trapped sediments appear to harbor a greater number of *E. coli* phylotypes



Older, less viable inputs

Newer, more viable inputs

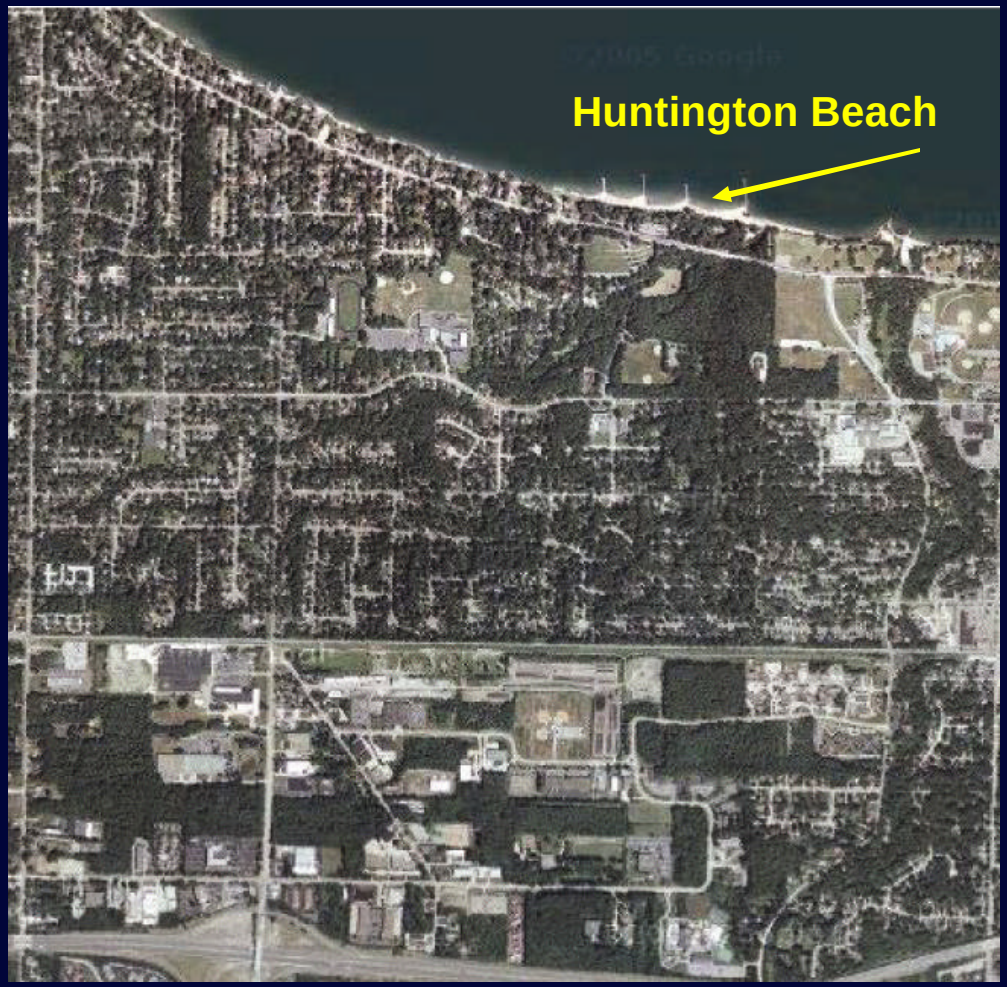
Case study: Beach Pollution

Identifying the source of bacterial pollution is the most important measure toward mitigating the problem.

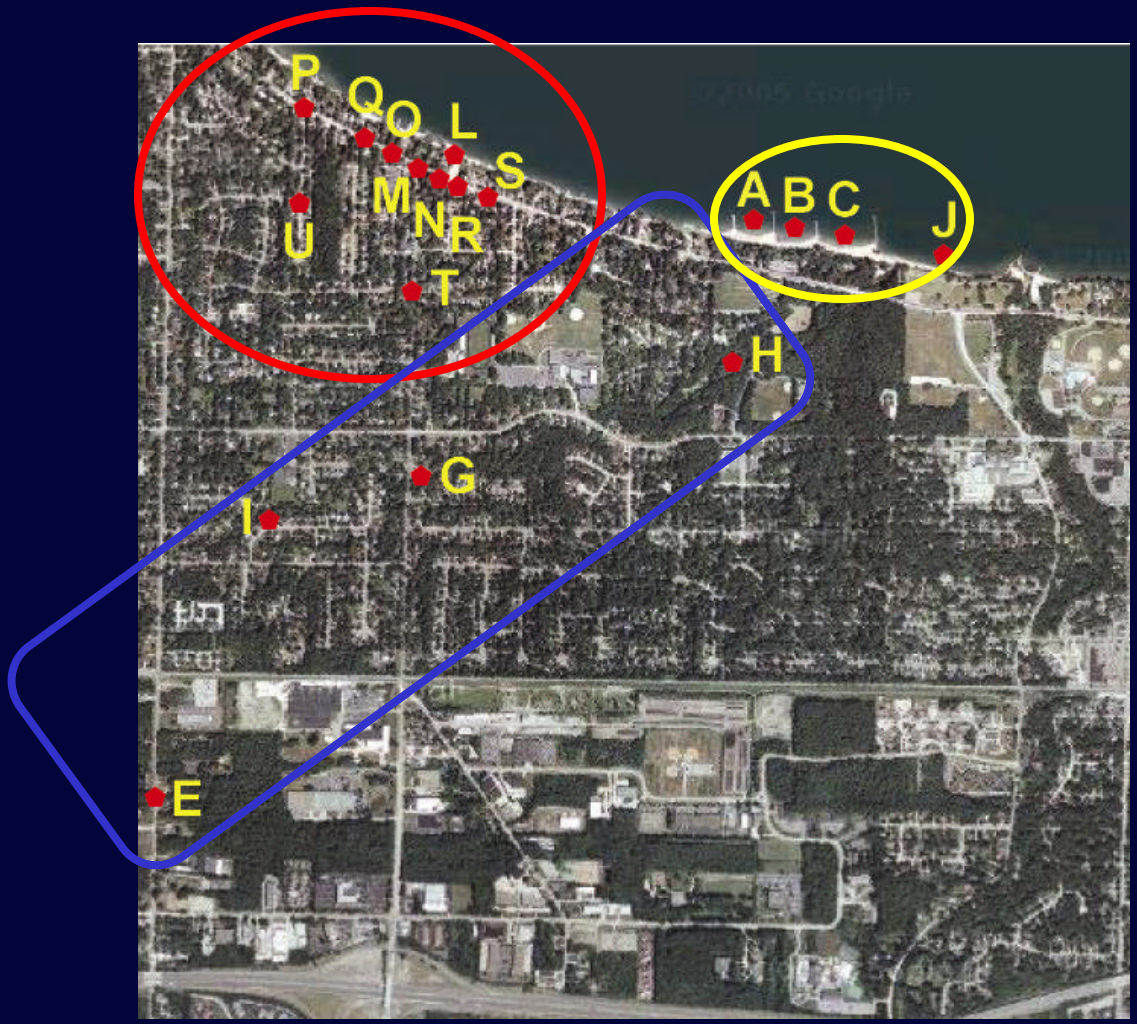
Case study: Huntington Beach…Ohio

- Huntington beach was posted with an advisory 18 times (20%) during the 2006 swim season.

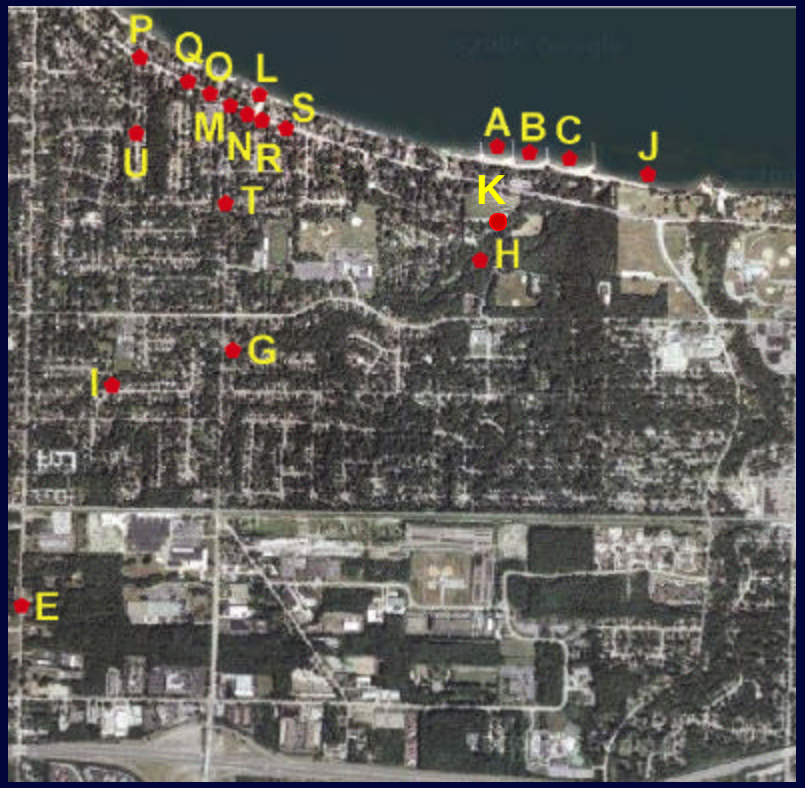
Identifying geographic bacterial pollution sources impacting Huntington Beach

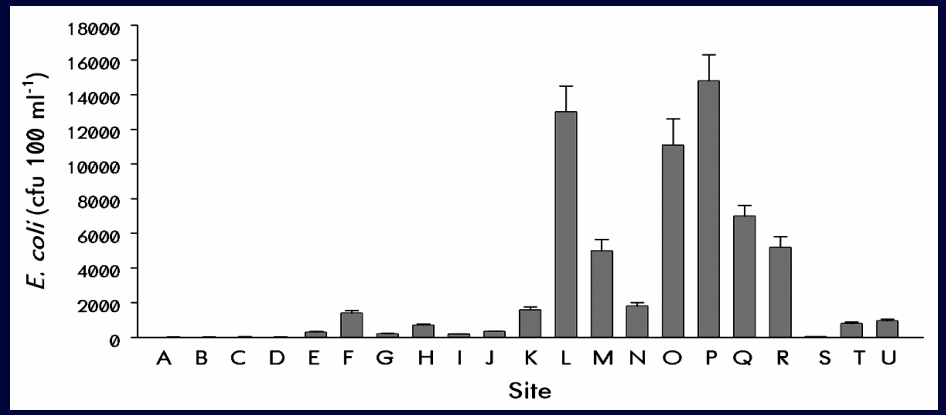


2005

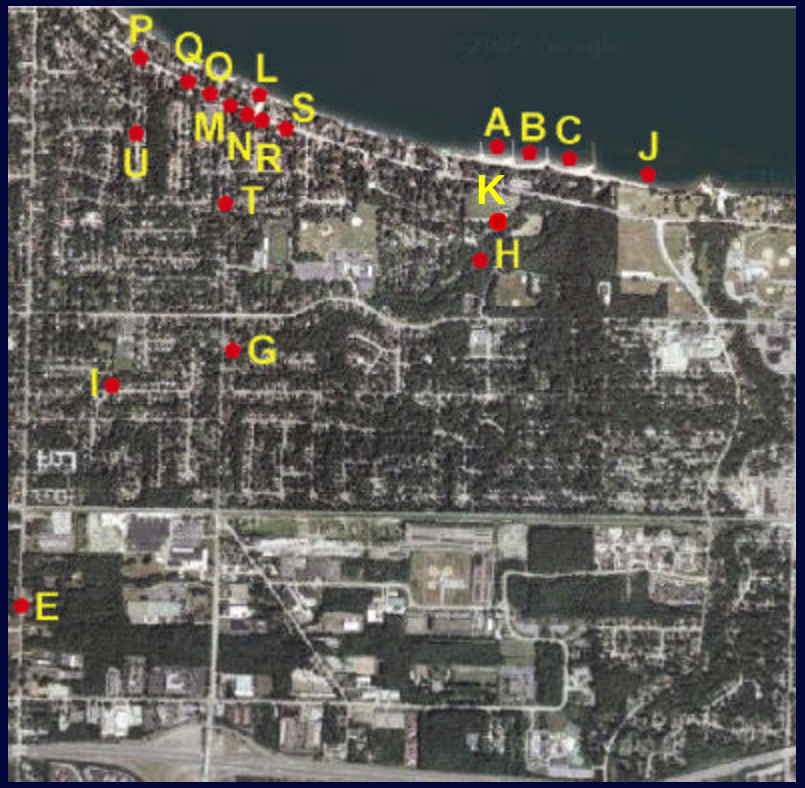


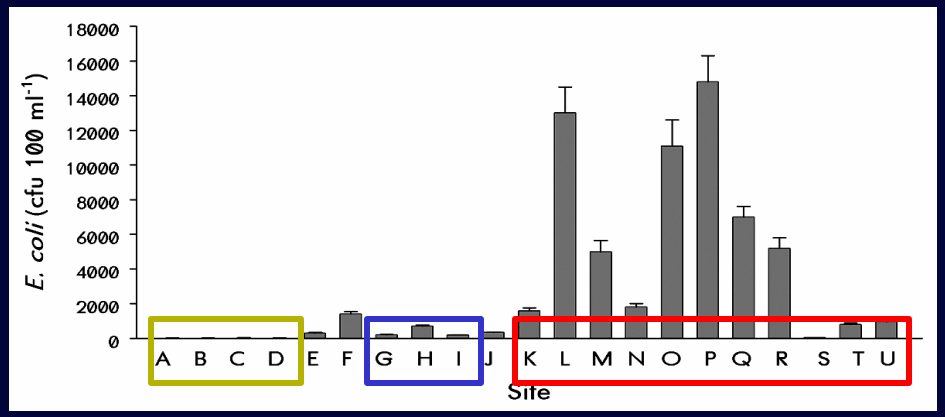
2005


P
Q
O
L
S
M
N
R
U
T
A
B
C
J
K
H
G
I
E

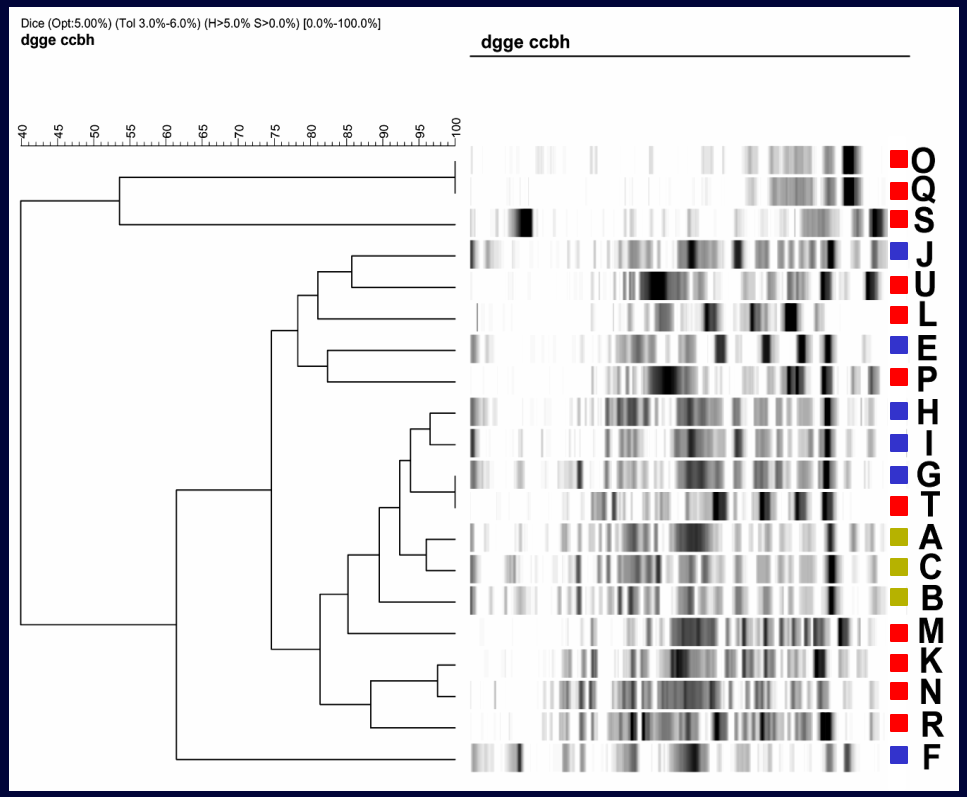

E. coli (cfu 100 ml-1)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
A B C D E F G H I J K L M N O P Q R S T U
Site

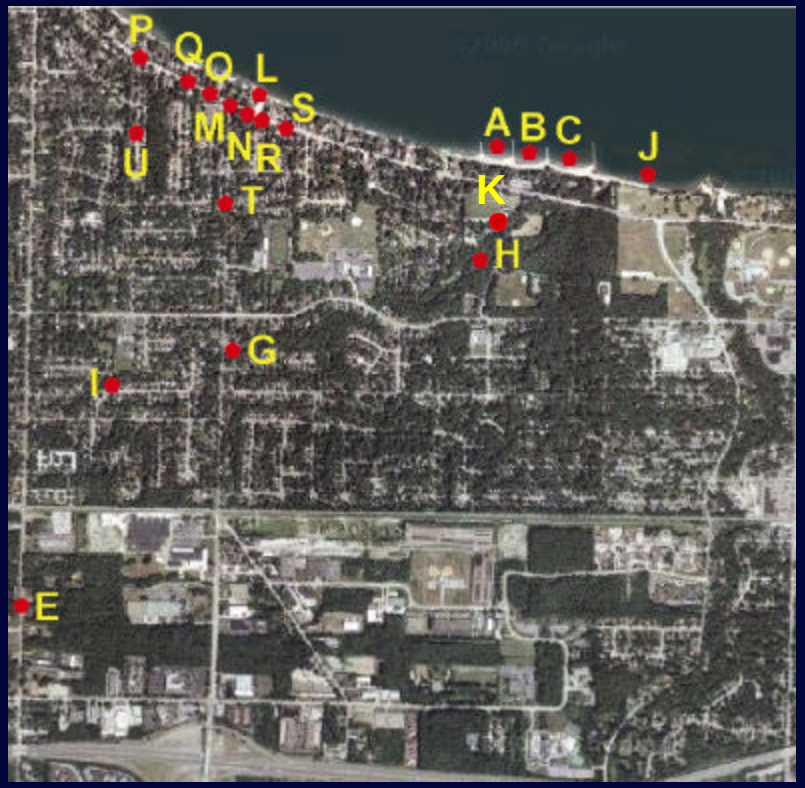
2005

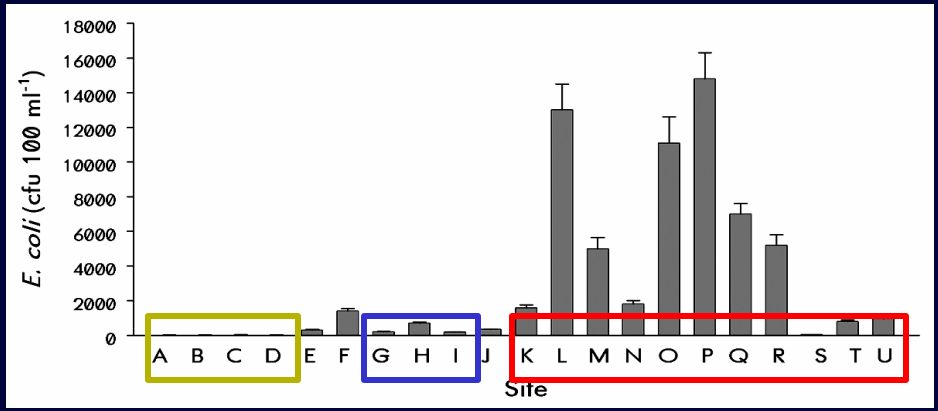

P
Q
O
L
S
M
N
R
U
T
A
B
C
J
K
H
G
I
E


E. coli (cfu 100 ml-1)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
A B C D E F G H I J K L M N O P Q R S T U
Site

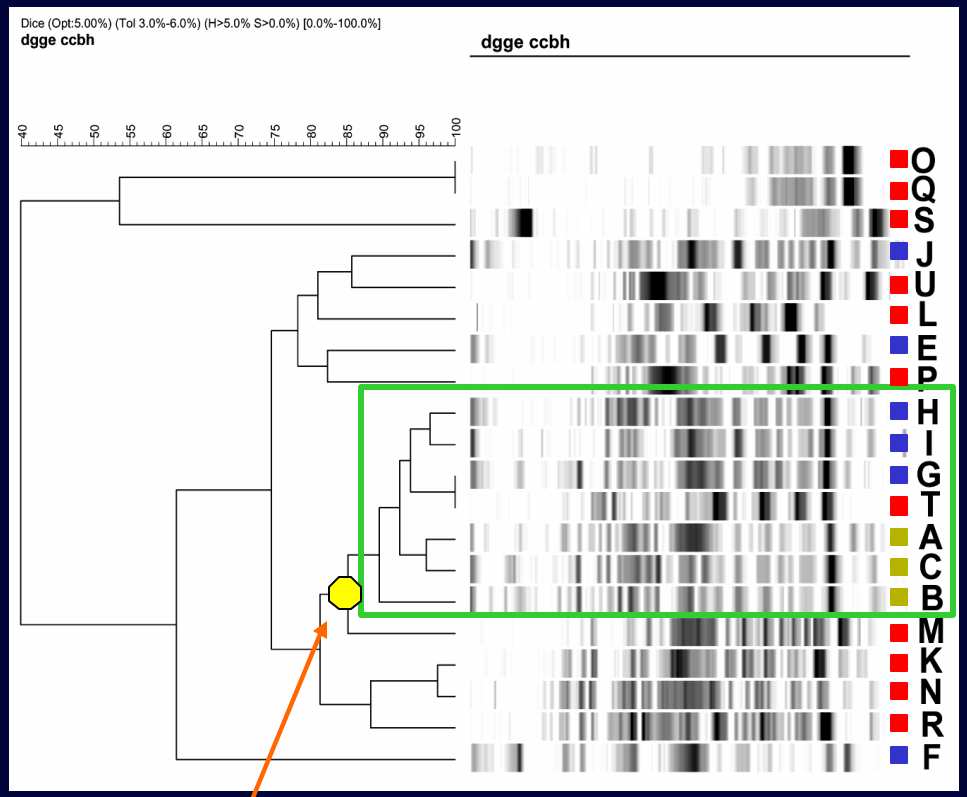
2005


Dice (Opt:5.00%) (Tol 3.0%-6.0%) (H>5.0% S>0.0%) [0.0%-100.0%]
dgge ccbh
dgge ccbh
40 45 50 55 60 65 70 75 80 85 90 95 100
O
Q
S
J
U
L
E
P
H
I
G
T
A
C
B
M
K
N
R
F

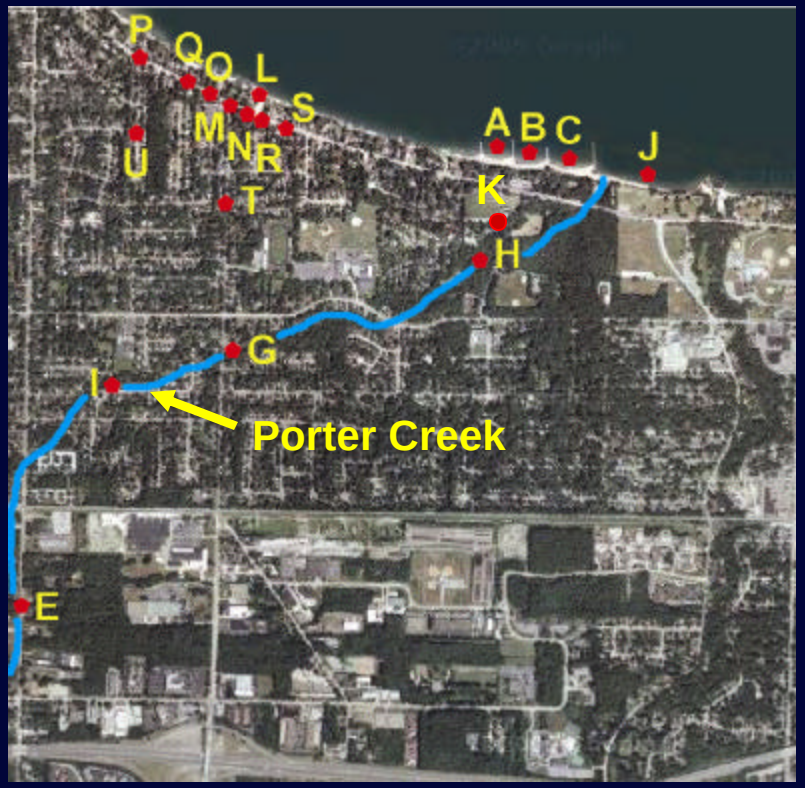

P
Q
O
L
S
M
N
R
U
T
A
B
C
J
K
H
G
I
E

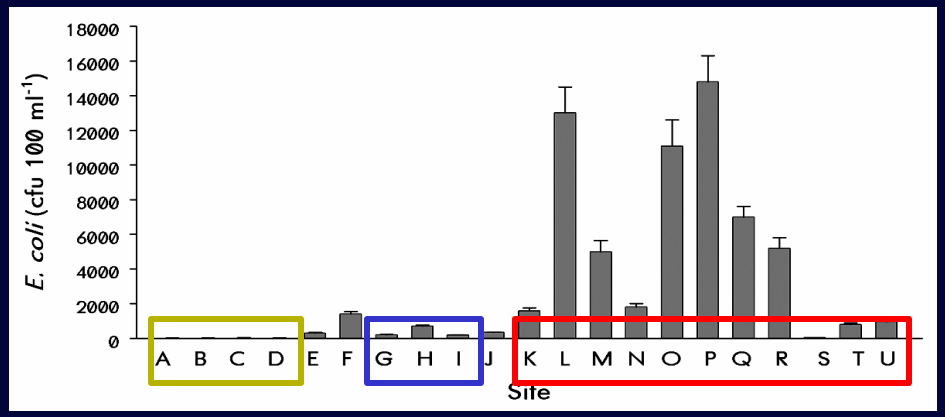

E. coli (cfu 100 ml-1)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
A B C D E F G H I J K L M N O P Q R S T U
Site

2005


Dice (Opt:5.00%) (Tol 3.0%-6.0%) (H>5.0% S>0.0%) [0.0%-100.0%]
dgge ccbh
dgge ccbh
40 45 50 55 60 65 70 75 80 85 90 95 100
O
Q
S
J
U
L
E
P
H
I
G
T
A
C
B
M
K
N
R
F

80%

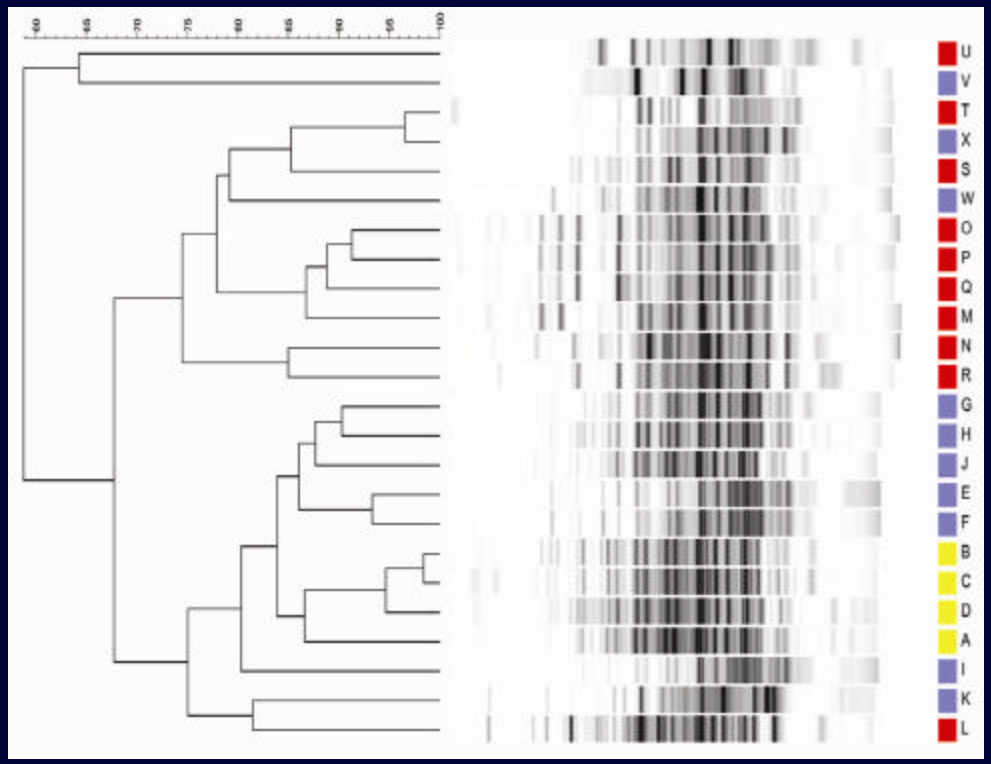

P
Q
O
L
S
M
N
R
U
T
A
B
C
J
K
H
G
I
Porter Creek
E

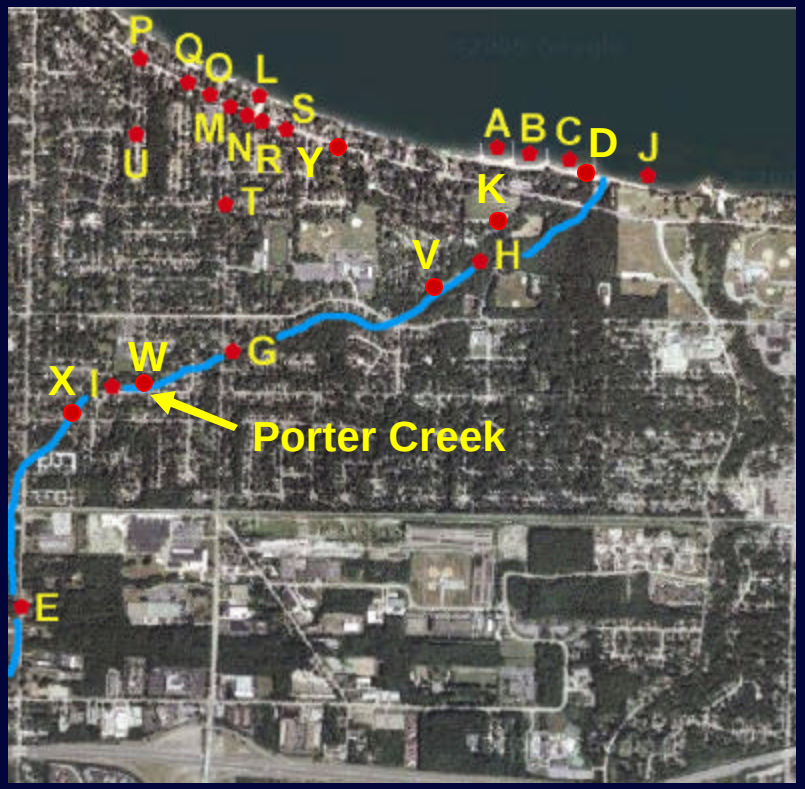

E. coli (cfu 100 ml-1)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
A B C D E F G H I J K L M N O P Q R S T U
Site

2006 sampling

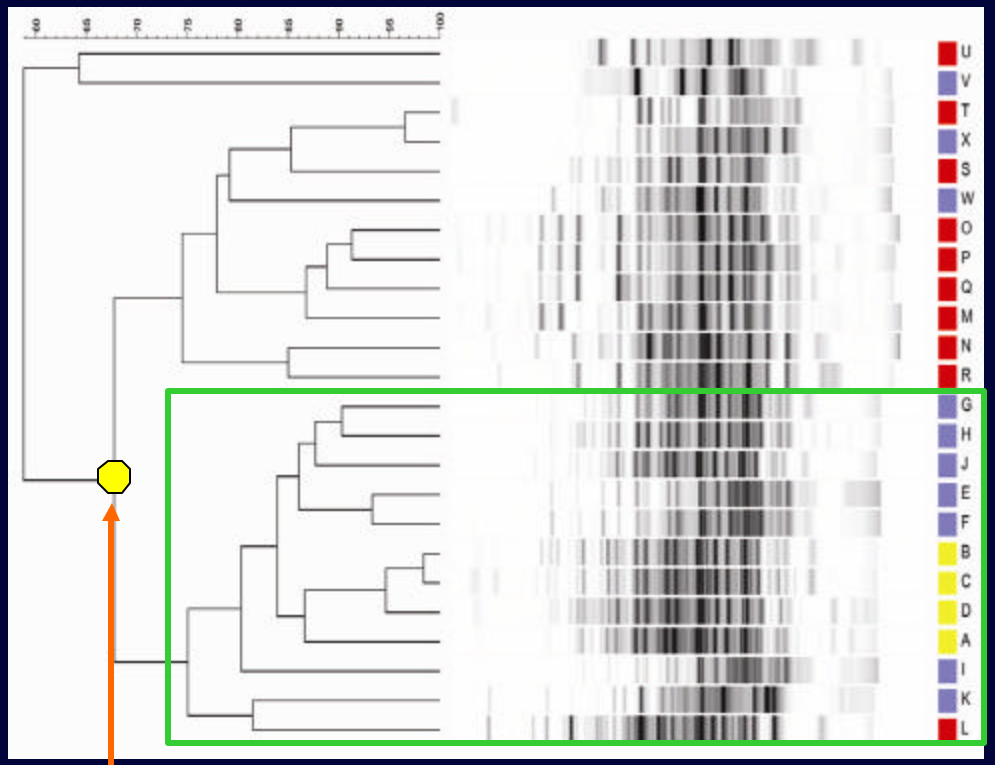
- Necessary to confirm the results observed in 2005
- Dry weather vs. wet weather sampling
  - Dry weather – prior week was free of precipitation
  - Wet weather – collected following approximately two inches of rain
- Additional sites were sampled

2006 - Dry

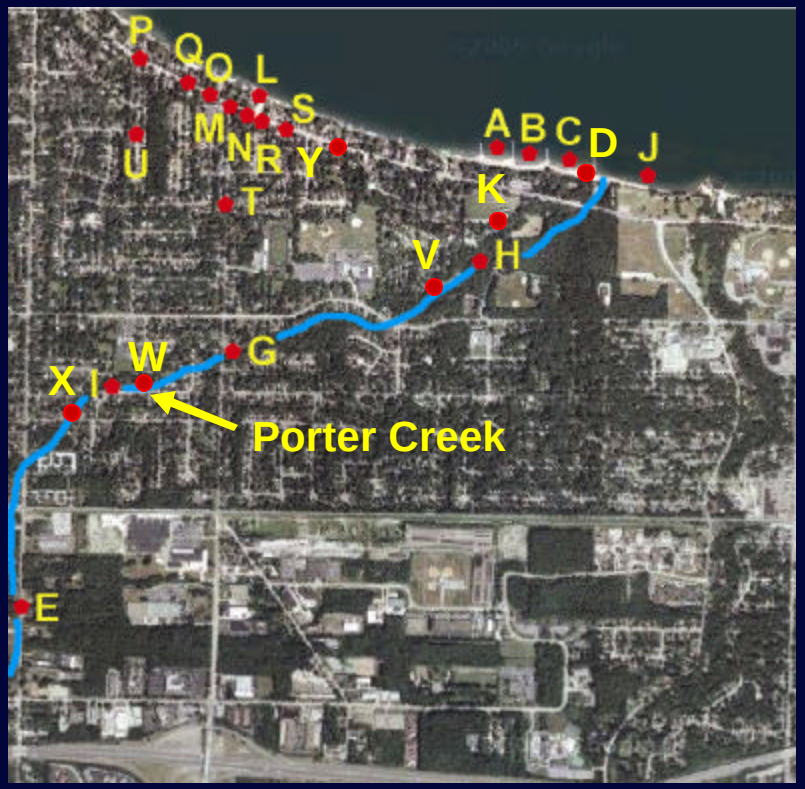

U
V
T
X
S
W
O
P
Q
M
N
R
G
H
J
E
F
B
C
D
A
I
K
L


P
Q
O
L
S
M
N
R
Y
U
T
A
B
C
D
J
K
V
H
W
G
X
I
Porter Creek
E

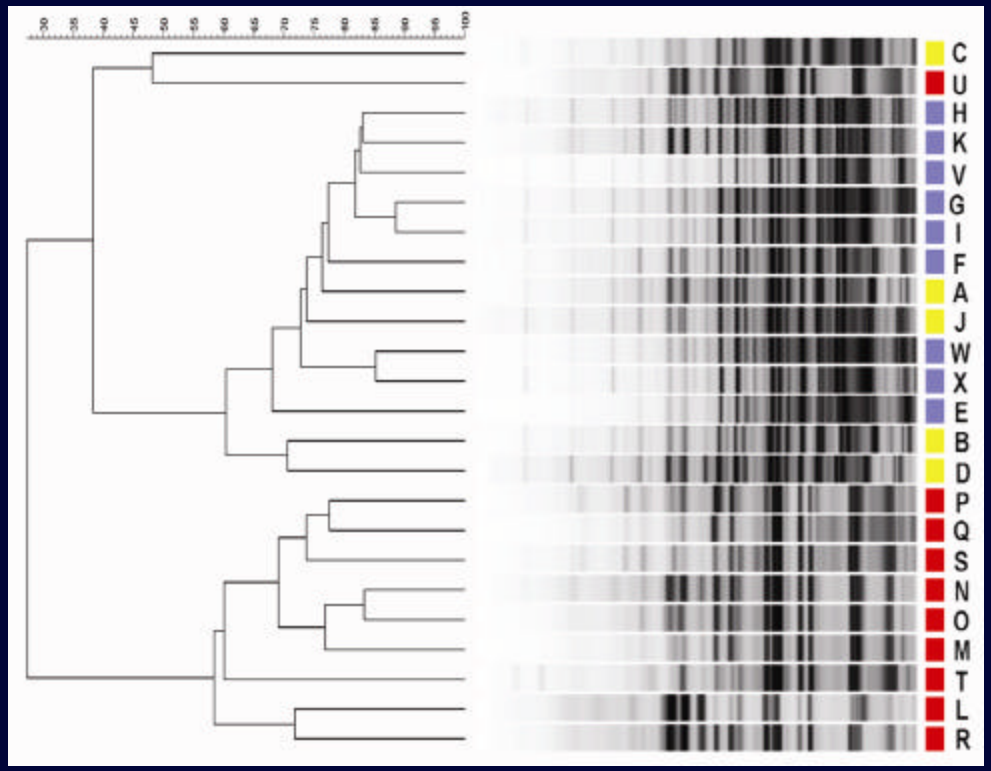
2006 - Dry

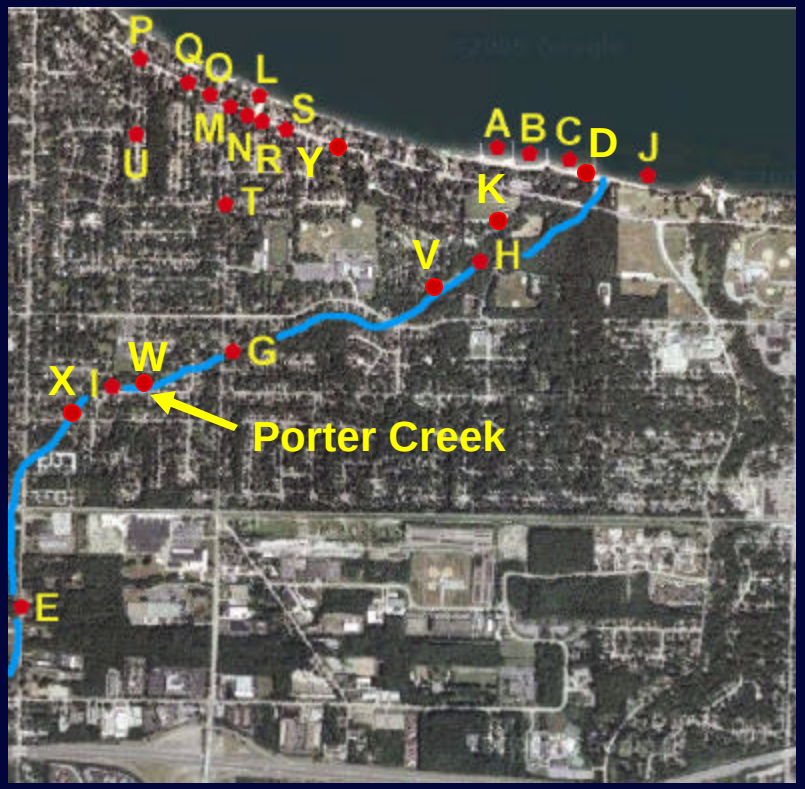

U
V
T
X
S
W
O
P
Q
M
N
R
G
H
J
E
F
B
C
D
A
I
K
L

68%

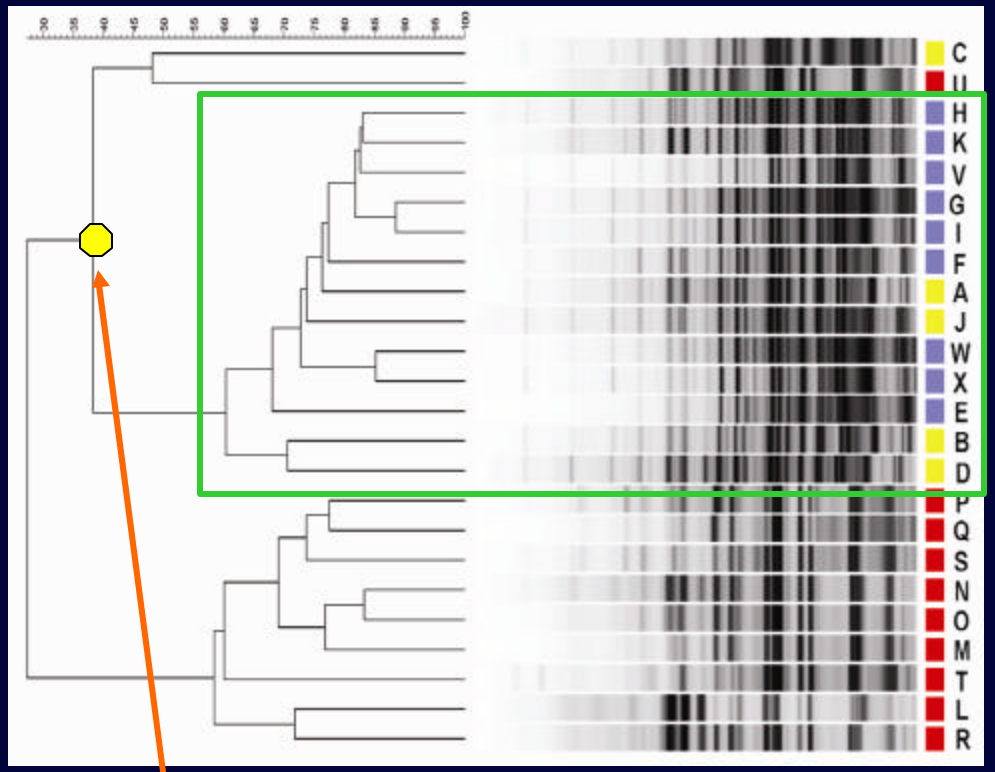

P
Q
O
L
S
M
N
R
Y
U
T
A
B
C
D
J
K
V
H
W
G
X
I
Porter Creek
E

2006 - Wet

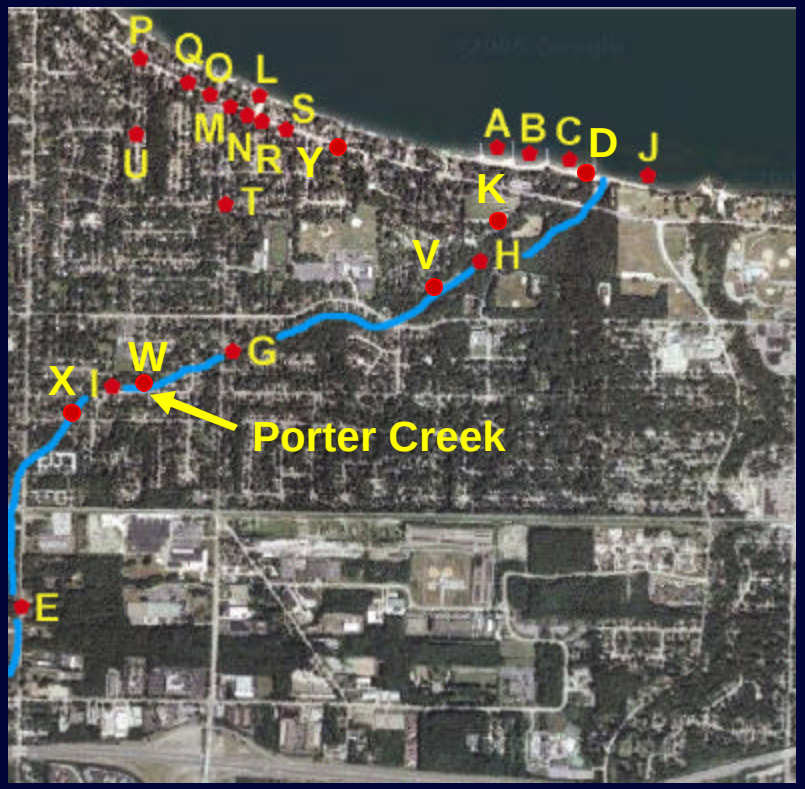

C
U
H
K
V
G
I
F
A
J
W
X
E
B
D
P
Q
S
N
O
M
T
L
R


P
Q
O
L
S
M
N
R
Y
U
T
A
B
C
D
J
K
V
H
G
W
X
I
Porter Creek
E

2006 - Wet


C
U
H
K
V
G
I
F
A
J
W
X
E
B
D
P
Q
S
N
O
M
T
L
R

38%


P
Q
O
L
S
M
N
R
Y
U
T
A
B
C
D
J
K
V
H
G
W
X
I
Porter Creek
E

Advantages of LIM vs. library-dependent methods

- No isolate library
  - Resource consuming – time, money and people
    - Wiggins et al. (2003) suggest that at least 2300 fecal enterococci were necessary for a true representation of watershed enterococci populations (ARA)
  - Some isolates are cosmopolitan or transient

Advantages of LIM vs. library-dependent methods

- No isolate library
  - Resource consuming – time, money and people
    - Wiggins et al. (2003) suggest that at least 2300 fecal enterococci were necessary for a true representation of watershed enterococci populations (ARA)
  - Some isolates are cosmopolitan or transient
- Potentially faster
  - Same day data
  - “Rapid” screening

Advantages of LIM vs. library-dependent methods

- No isolate library
  - Resource consuming – time, money and people
    - Wiggins et al. (2003) suggest that at least 2300 fecal enterococci were necessary for a true representation of watershed enterococci populations (ARA)
  - Some isolates are cosmopolitan or transient
- Potentially faster
  - Same day data
  - “Rapid” screening
- The numerically dominant bacteria in fecal material are difficult to culture…
  - …DNA relatively easy to isolate

Disadvantages of LIM vs. library-dependent methods

- Resolution
  - Fingerprinting will not capture the diversity of the target organism
    - Finite number of band positions

Disadvantages of LIM vs. library-dependent methods

- Resolution
  - Fingerprinting will not capture the diversity of the target organism
    - Finite number of band positions
- No archived bacteria, just DNA
  - Further biochemical testing not possible

Disadvantages of LIM vs. library-dependent methods

- Resolution
  - Fingerprinting will not capture the diversity of the target organism
    - Finite number of band positions
- No archived bacteria, just DNA
  - Further biochemical testing not possible
- Statistics are not totally worked out

Conclusions and outlook

- Library independent methods are effective…
  - Host origin
  - Rapid screening
  - Pathogen ecology

  …if you ask the right question

Conclusions and outlook

- Library independent methods are effective…
  - Host origin
  - Rapid screening
  - Pathogen ecology

  …if you ask the right question
- Future research
  - Focus on the pathogens in addition to the indicators
  - Identify pathogen reservoirs
  - Emerging, community-acquired pathogens

Acknowledgements

- Hilary Nightingale - Research Technician
- Malak Esseili - Ph.D. student
- Xixi Huang - M.S. student
- Environmental Pathogens Information Network (EPI-Net)