– Session 3: New Methods to Assess Water Quality–

Advances in Methods to Assess Microbial Water Quality

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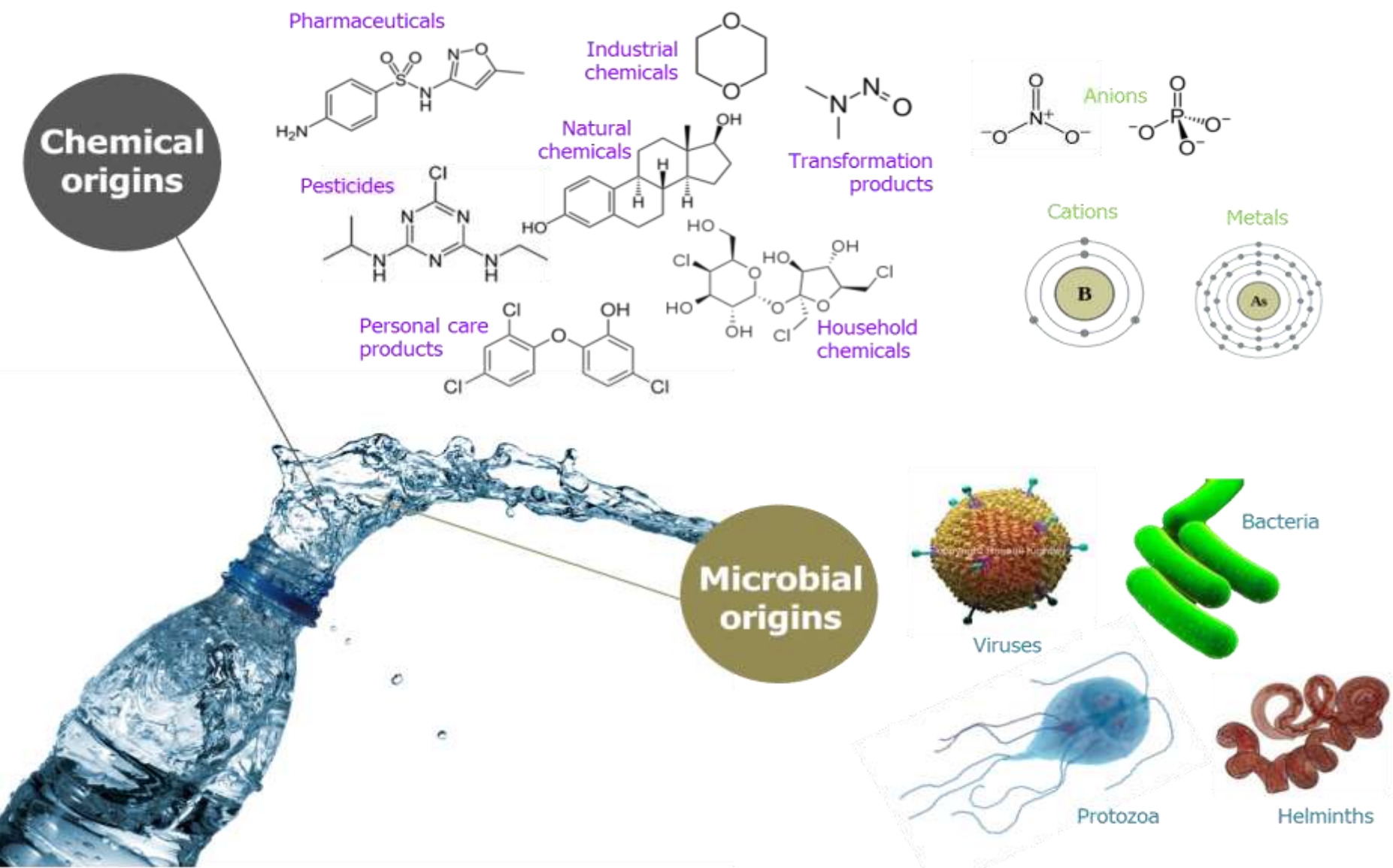
Clarke Prize Award Ceremony and Conference| Newport Beach| November 2016

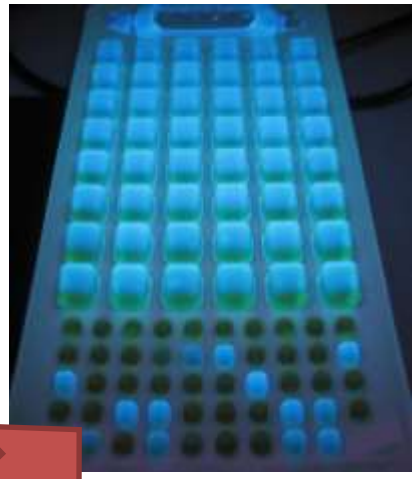
Potential Contaminants

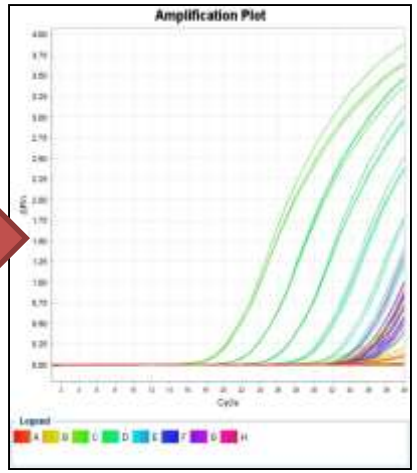


Questions to Water Quality Problems

- Source water pollution
  - Who is causing the pollution?
- Biofilm formation
  - Who is there? What are they doing?
- Disease in the community
  - Where did the disease start? Who started it? How virulent is it?
- Efficiency of wastewater treatment
  - Who is there? How are they treating water?

Methods for Microbial Water Quality Analysis





Culture Based *E.coli* Methods

- IDEXX Colilert
- ENDETEC TECTA-B16™
- BACTcontrol

- Total coliform bacteria and *E.coli* in water by enrichment
- Chromogenic media and automated evaluation
- Real-time fluorescence monitoring

Problems with the Methods?

- Current methods used for the detection of *E.coli* and other indicators were designed for **drinking water**
- Treated wastewater may contain substances that affect the ability of these methods to accurately detect *E.coli*
- Previous work by our group suggested **false-positive rates** upwards of **45%** in methods commonly used by the industry for *E.coli* detection

Beyond *E.coli* Culture Methods

- Biological Molecule Assays
  - Adenosine Triphosphate (ATP)
- Molecular Biological Assays
  - PCR and qPCR
  - Droplet Digital PCR
  - Pyrosequencing
- Immunological Assays
  - Enzyme -linked Immunosorbent Assay (ELISA)
- Biosensors and Immunosensors
  - Optical (fluorescence), electrochemical (surface plasmon resonance)
  - Light scattering

QCM (Qsense)

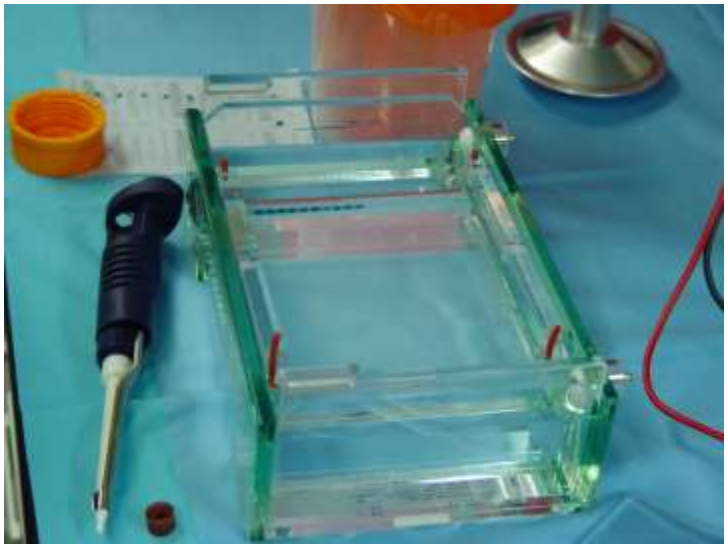
Genetic Techniques

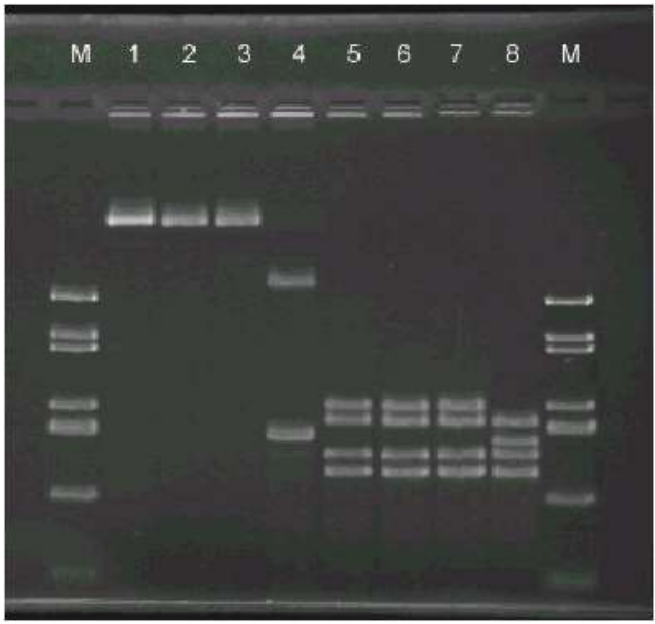
- Advancements in genetic techniques can be used to answer environmental questions not answered by traditional cultural methods.

- Disadvantages to cultural methods
  - Rely on growth of organism
  - Time consuming
  - Cost
  - Detection limit (# of organisms)
  - Must know who you are looking for….

Polymerase Chain Reaction

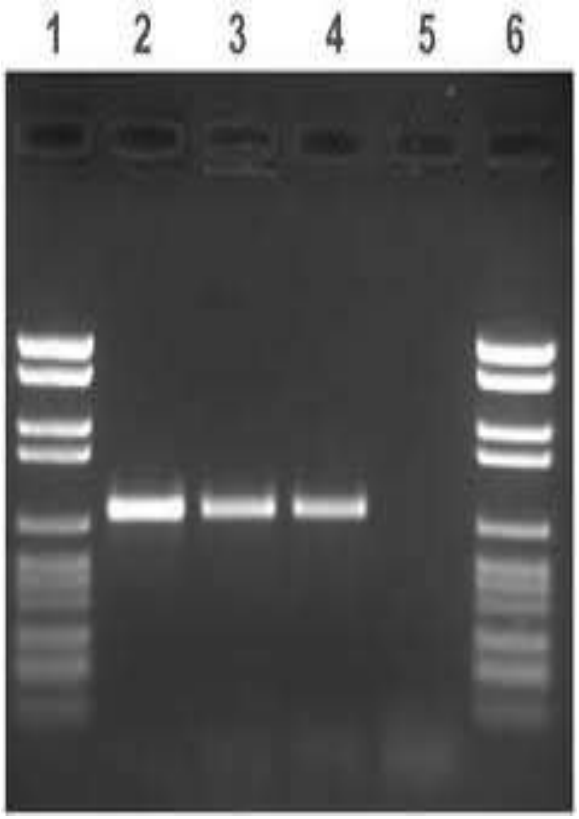
- Method of generating a “DNA Fingerprint”
- Different strains of bacteria have different fingerprints
- Polymerase Chain Reaction (PCR) amplifies the genetic material or DNA/RNA of the specific target organism





PCR is the work horse of molecular biology.

- >30 different applications/techniques
  - Extremely powerful tool
- USEFUL for molecular identification and detection
  - Low detection limit
  - Faster and cheaper than culture
- NOT useful for things you don't know about
  - Hard to say if viable or not
  - False positives and negatives



Quantitative PCR

- Specific Primers and Probes (short pieces of DNA) are designed to “bind” only to the DNA sequence from the organism of interest (lock and key)
- Special dyes are used to indicate if the Primers “bind” to DNA in the sample
- If the binding occurs the dyes emit a fluorescence signal that is then detected by a machine in real time
- This signal increases in direct proportion to the amount of DNA/RNA in a reaction

Specificity

Human

Bovine

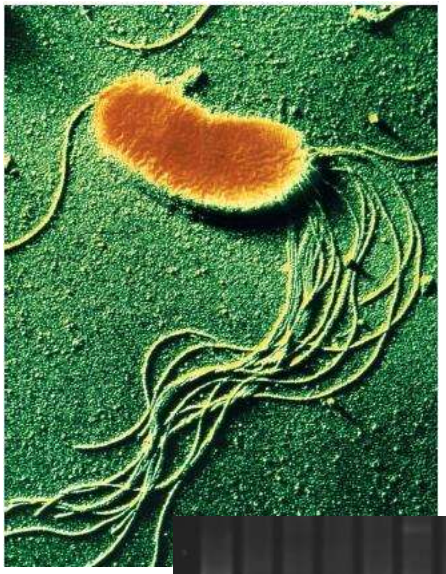
Specificity

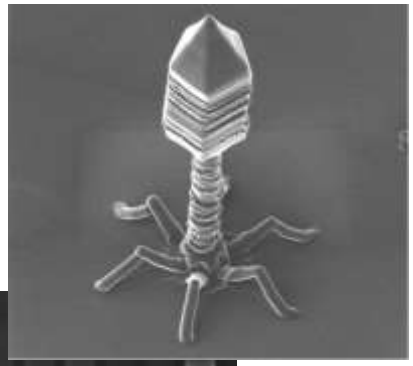
Human

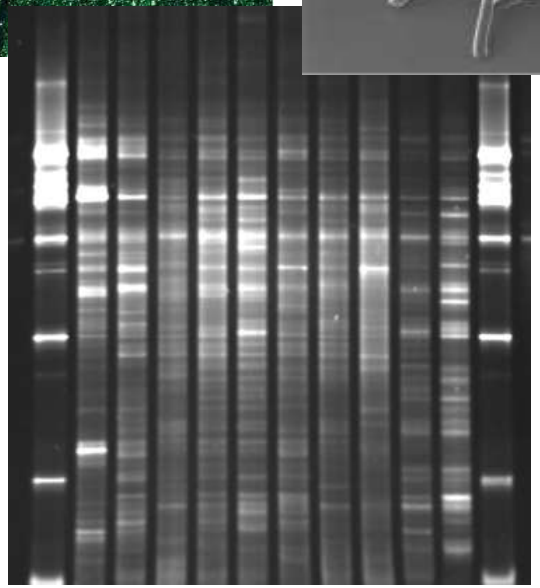
Bovine

Source Tracking Using Molecular Microbial Markers

- Fecal organism carries a molecular “signature” that is unique to its source
- Fingerprinting techniques: Ribotyping, PFGE, DGGE
- Repetitive DNA Sequences
- Host-specific 16S rDNA genetic markers



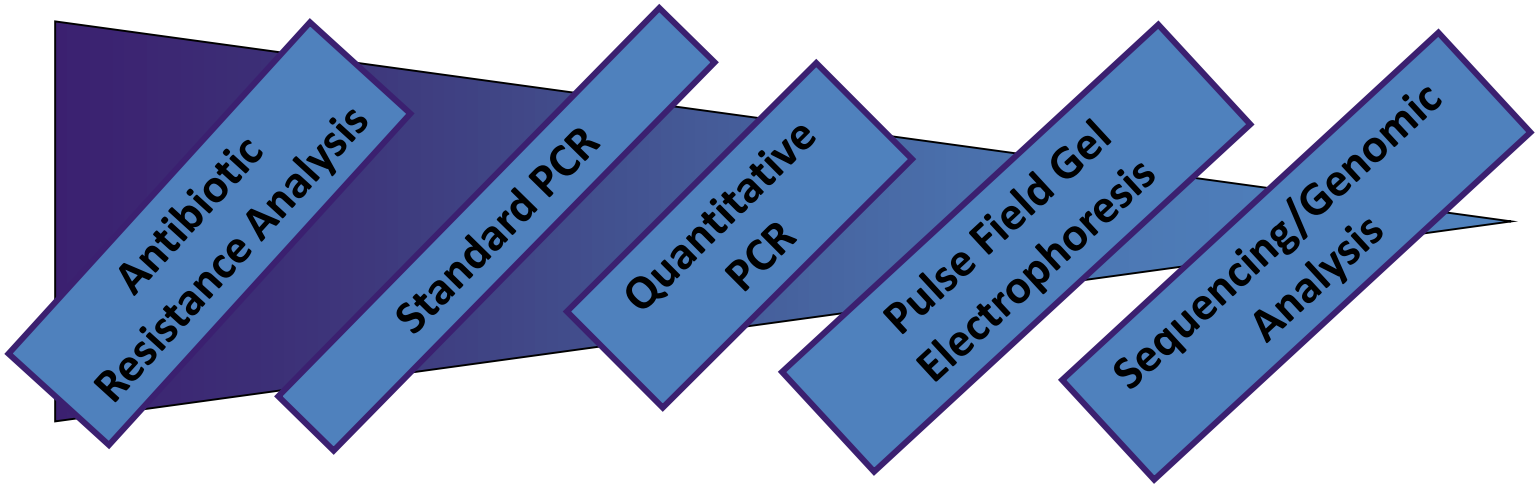




Ability of Methods to Discriminate Differences Between Bacterial/Viral Strains

Lowest Discrimination

Highest Discrimination



Which method or combination is best?

Host-specific Bacterial PCR

- Extraction, amplification, and analysis of bacterial DNA using PCR or qPCR.

- *Bacteroides*
- *Enterococcus*
- *Methanobrevibacter smithii*

Host-specific Viral PCR

- Viruses are isolated from water sources, DNA extracted, and amplified using PCR techniques.

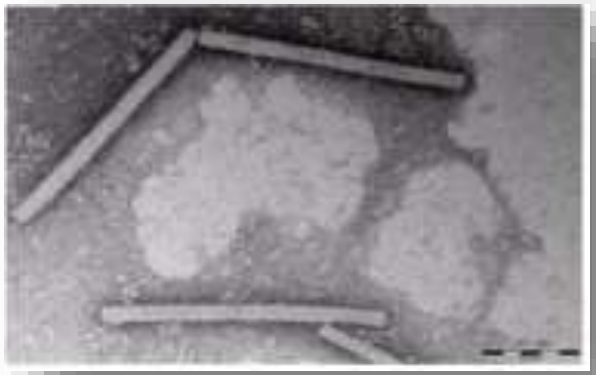
- Human specific adenoviruses, enteroviruses, Aichi, PMMV, Sapovirus = ***human fecal contamination***

- Bovine and procine adenoviruses = ***livestock contamination***

Alternative Viral Indicators and Surrogates

- Bacteriophages
  - Easy to detect but no “perfect” indicator
- Pathogens
  - Molecular methods: infectivity?
  - WRRF 14-17 “White Paper on the Application of Molecular Methods for Pathogens for Potable Reuse”
- Aichi, Calici, & Pepper Mild Mottle Virus (PMMoV)
  - Abundant in wastewater; limited seasonality
  - Not effectively removed in WWTP

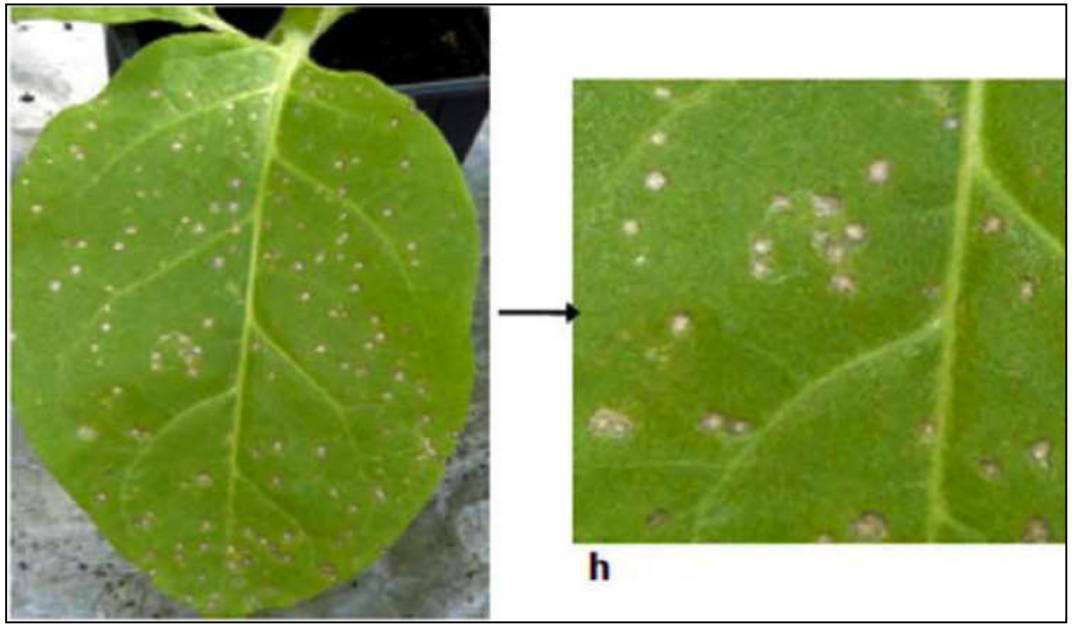
Aichi virus *(Springer Images)*



PPMoV virus isolated from Tabasco sauce *(Colson et al, 2010)*

What is Pepper Mild Mottle Virus?

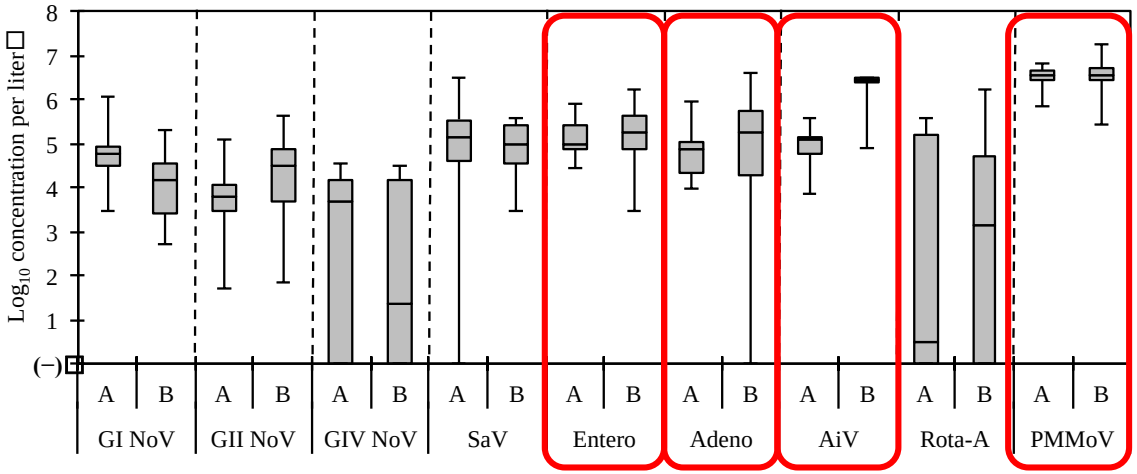
- Plant virus that infects hot, bell, and ornamental peppers and tobacco plants.
  - Causes mosaic diseases ("mild mottles")
  - Does NOT cause infection in humans.
  - Dietary origin (up to 10^7 virus/ml in Tabasco sauce)



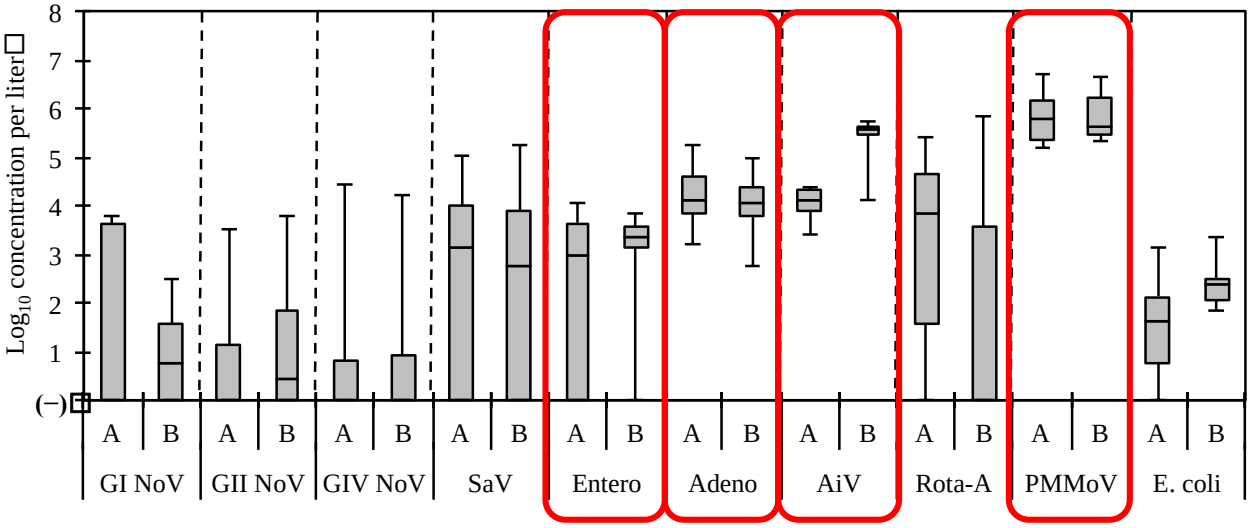
Colson *et al.*, 2010

Virus Occurrence in Wastewater

Influent

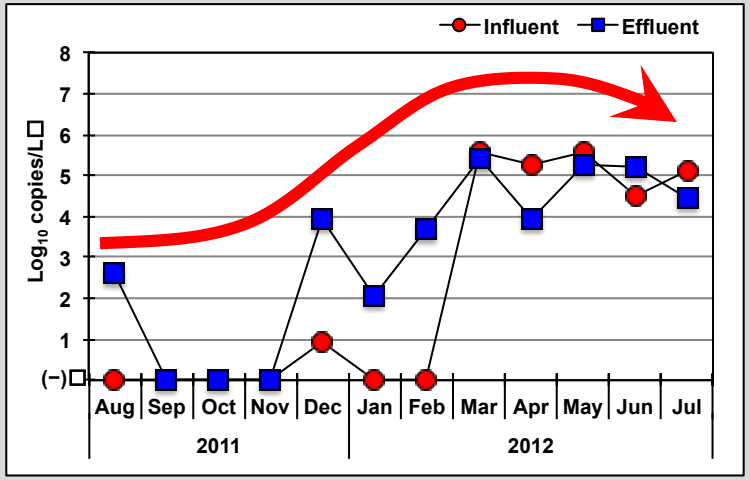
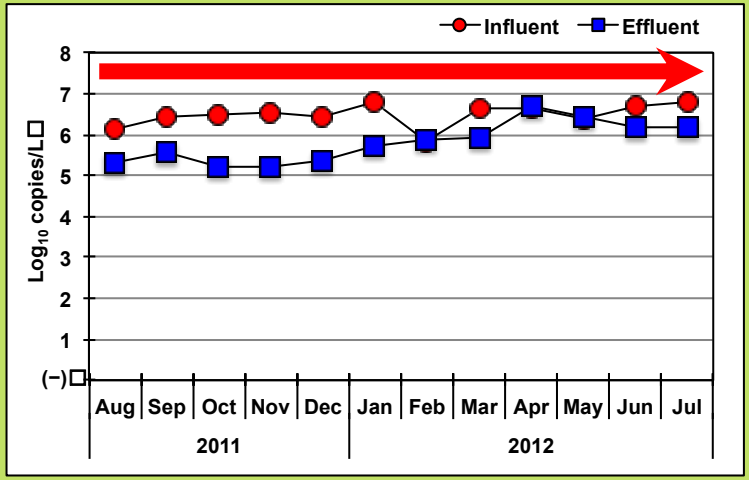
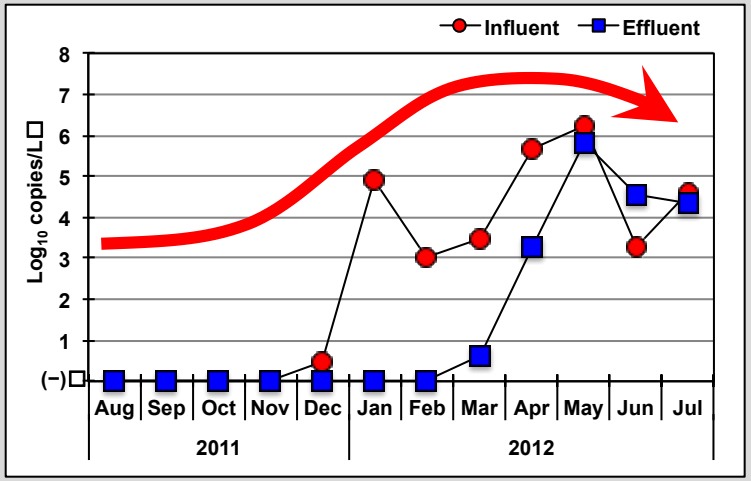
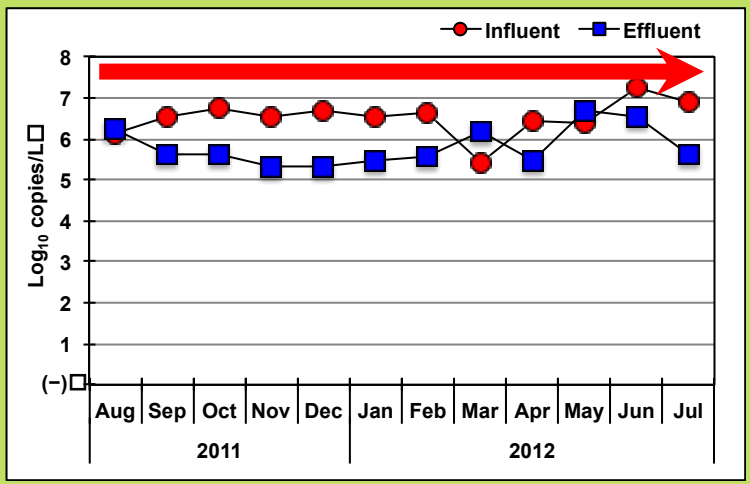


Effluent



Gerba, Kitajima, Betancourt, Regnery, and Drewes

Pepper Mild Mottle Virus is highly abundant in municipal wastewater

	Group A Rotavirus (Human gastroenteritis virus)	**Pepper Mild Mottle Virus**
Tucson WWTP-A		
Tucson WWTP-B		

Viral Concerns

- Although unable to replicate outside of their host, viruses have a greater ability to persist in treated water than bacteria due to
  - their small size (which hinders physical removal)
  - the resistance of some viruses to certain disinfection processes (e.g., ultraviolet [UV] resistance of adenovirus).

Concentration of Viruses from Water

Drinking Water
< 1 virus/100,000 /L
This is the Goal

Surface Water
1 - 10 virus/L

Waste Water
10^4 Virus /L

Low numbers of viruses

↓

As a result, viruses must be concentrated

↓

Filtration

Virus Detection in Water

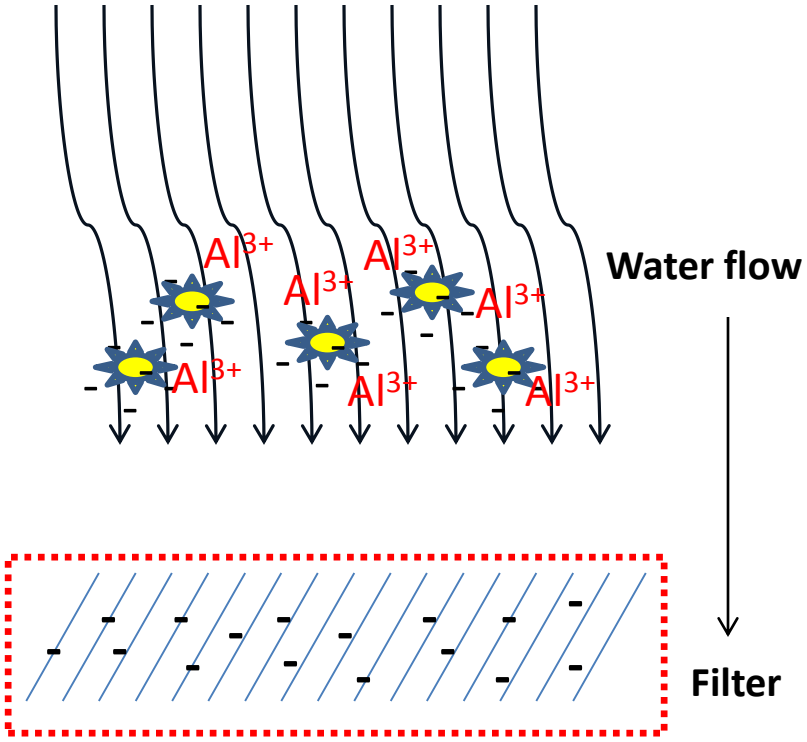
Concentration → **Recovery (elution)** → **Secondary Concentration** → **Detection**

Current Virus Concentration Methods

VIRADEL Procedure (VIRus ADsorption and ELution)

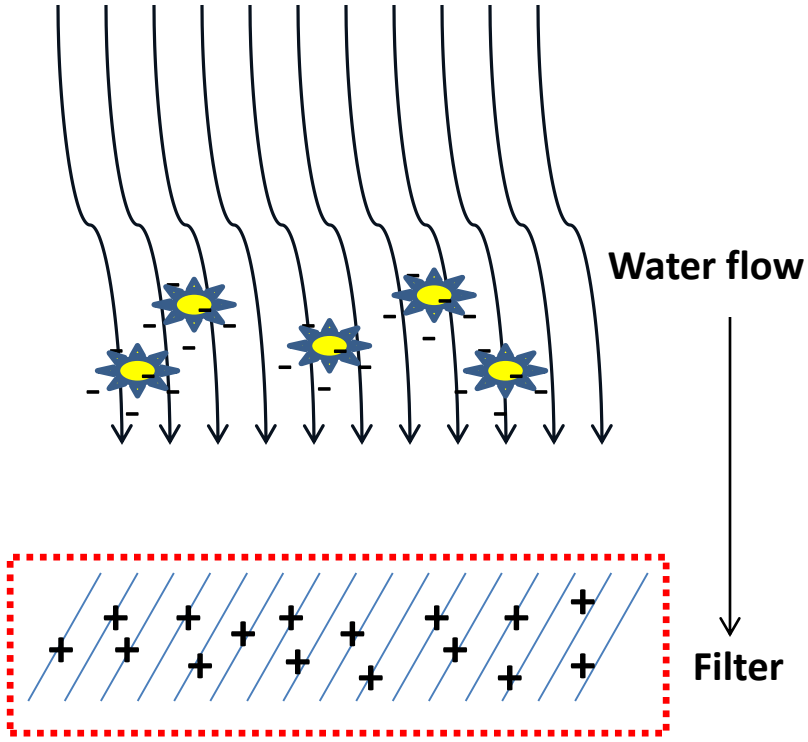
Negatively-charged filters:
Millipore HA, Filterite

Water samples require pre-conditioning: (multivalent salts (e.g. $AlCl_3$) and acidification to pH 3.5).



Positively-charged filters:
Virosorb 1MDS, NanoCeram

Water samples *do not* require pre-conditioning.



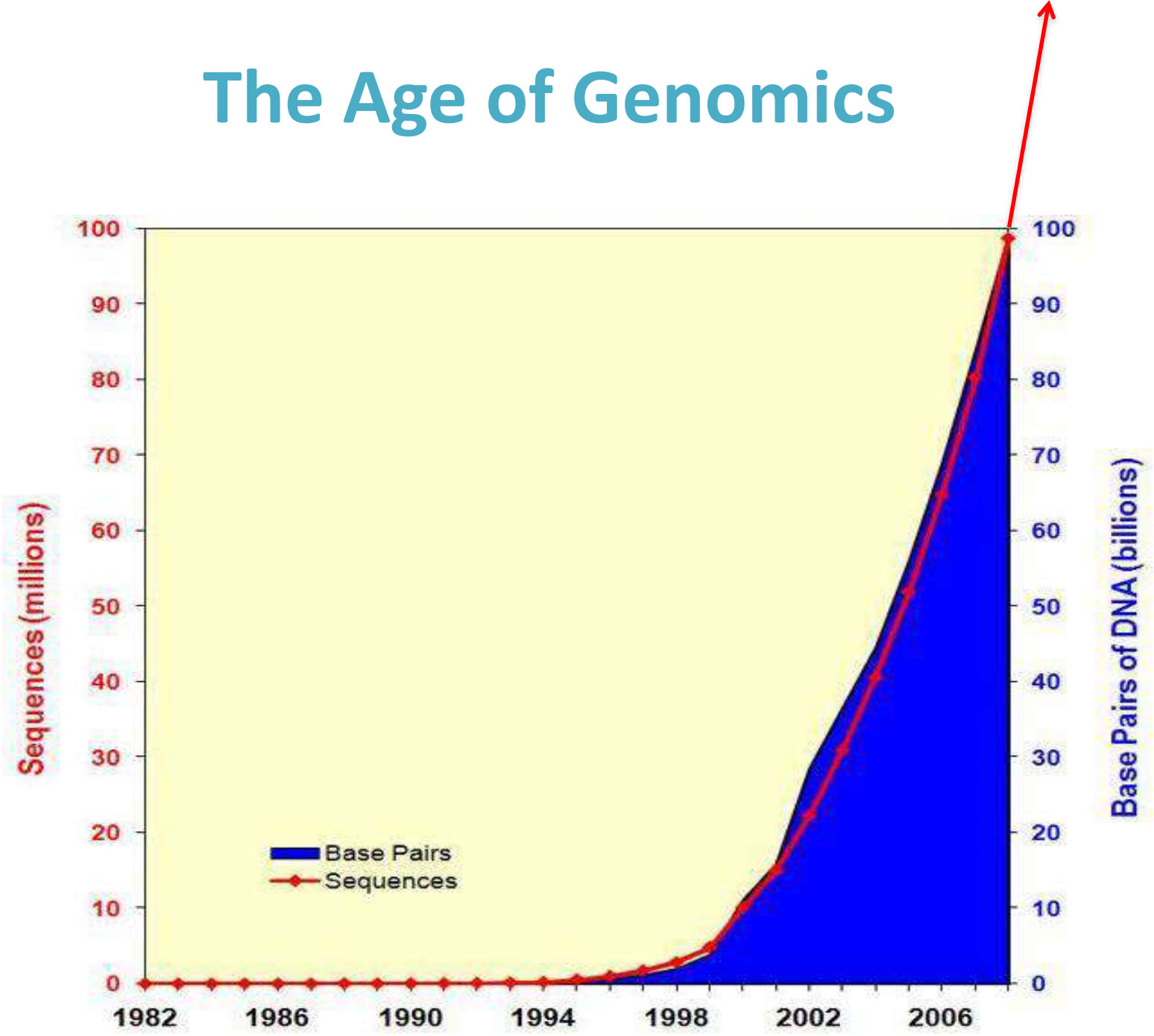
Slide Credit: Ikner, 2010

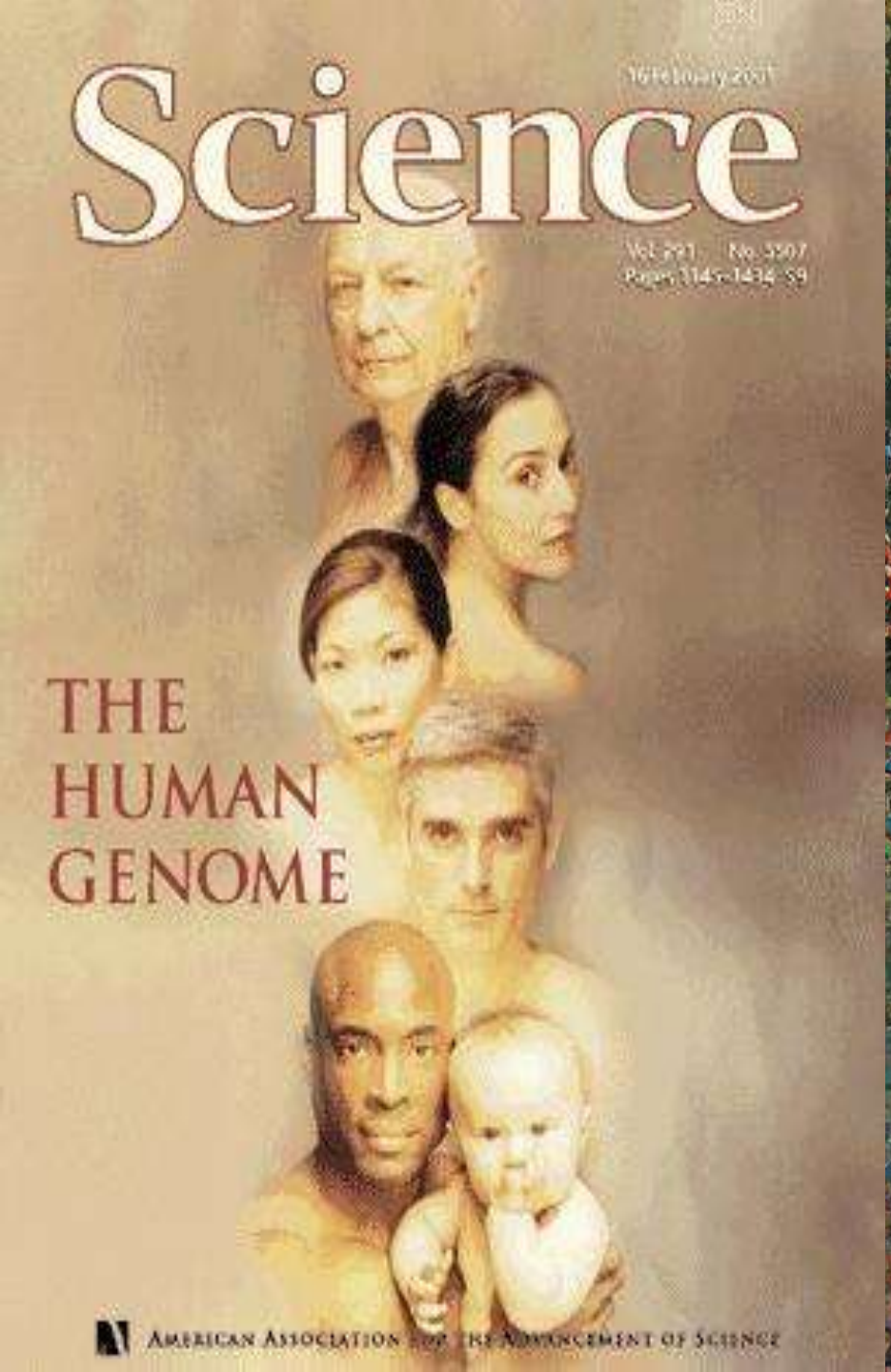
Challenges of MST and Genetic Methods

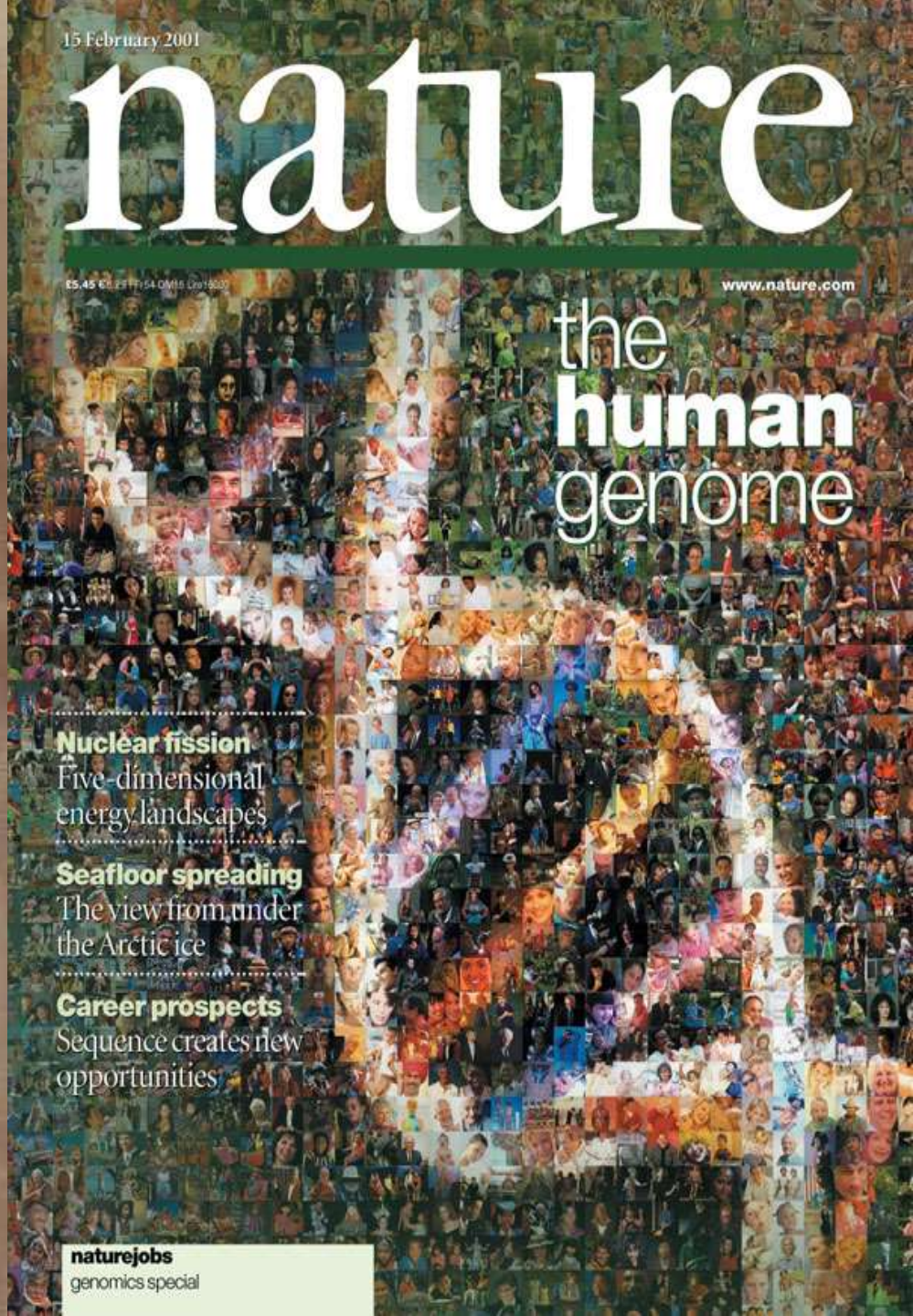
- Because the isolates analyzed from collected environmental samples represent a small portion of the total population, the results might not represent the actual relative presence of sources in the environment.

- There is no standardized techniques genetic methods
  - Reproducibility
  - No EPA Approved Methods
  - Constantly evolving (faster, longer, cheaper)

The Age of Genomics




Science
16 February 2001
Vol. 291 No. 5507
Pages 1145–1434 $9
THE HUMAN GENOME
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE


15 February 2001
nature
www.nature.com
the human genome
Nuclear fission
Five-dimensional energy landscapes
Seafloor spreading
The view from under the Arctic ice
Career prospects
Sequence creates new opportunities
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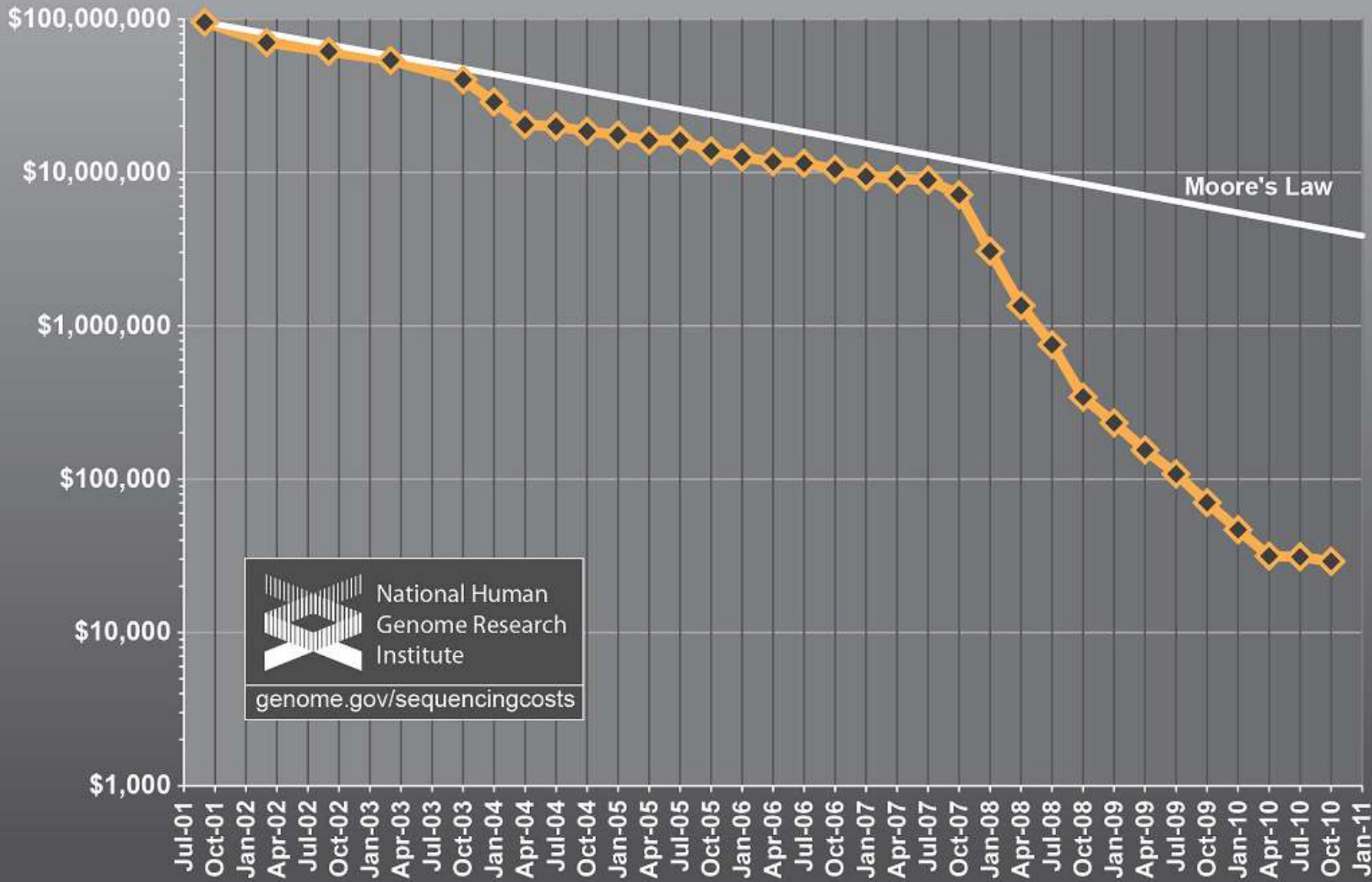
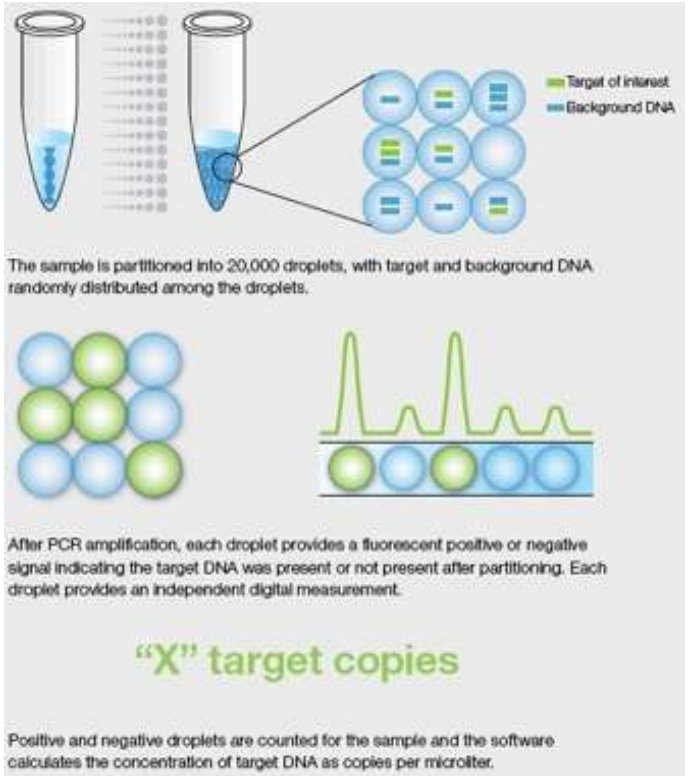
genomics special

Cost per Genome



Innovative Sequencing and Digital Technologies

- Roche 454 “pyrosequening”
  - Sequence by synthesis
  - Long sequences ~800bps
- Illumina HiSeq/MiSeq
  - 600 GB of DNA
  - Accuracy 99.6%
  - Personal genome analyzers
- Digital Droplet PCR (ddPCR)
  - Sample partitioning in 20,000 droplets



CASE STUDY - ARG

AR in the Media

New superbug found in two patients here

Four more infected by new superbug

They are likely to have caught the bug locally; more cases may surface

02 SINGAPORE HEALTH MAY/JUN 2011

News

The rise of the superbug

SGH's Director of Infection Control clues u
of simple hygiene habits to combat the su

BY JACQUELINE CHIA

HE WOKE UP COUGHING, FEELING WEAK AND WITH BODY ACHES. Just the flu, he

The study, done between and early 2008, also found infected patients stayed longer in hospital and faced higher hospital-related costs, More of these patients faced tions, requiring readmission within six months after disc ing to higher costs for outp

theguardian

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News › Society › Antibiotics

Antibiotic-resistant diseases pose 'apocalyptic' threat, top expert says

Chief medical officer Dame Sally Davies tells MPs issue should be added to national risk register of civil emergencies

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Ian Sample, science correspondent
The Guardian, Wednesday 23 January 2013 14.41 EST
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Local Attention

Flagstaff weighs action on resistant bacteria find

Posted: Apr 08, 2013 11:18 AM
Updated: Apr 22, 2013 11:18 AM

Posted by Dawn Alexander - email

NEW STUDY FINDS ANTIBIOTIC-RESISTANT BACTERIA GENES IN FLAGSTAFF'S RECLAIMED WATER

Posted By Linda Dailey Paulson On Sep 19, 2012 In Water In America, Water Research & Reports

October 11, 2012

Wastewater Snow-Making 'Could Breed Super-Bacteria'

NATURE | NEWS

Antibiotic resistance racing downriver

A river that runs through Colorado's plains carries two different genes that protect microbes from antibiotics.

Naomi Lubick

17 October 2012

The pristine Platte river picks up microbial genes for antibiotic resistance as it flows past human settlements.

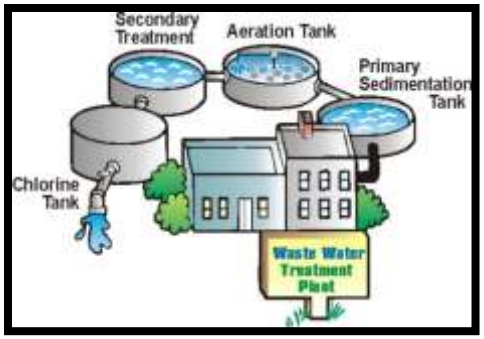
PHILIP NEALEY/GETTY

Are AR bacteria a concern for water reuse?

- New emerging contaminant?
- Are current treatment technologies sufficient for their removal/disinfection?
- Can they confer resistance to native bacteria in the environment?
- Human health impacts? Risk assessment?

Shifting Research Objectives

Multi-millions of antibiotics prescribed daily

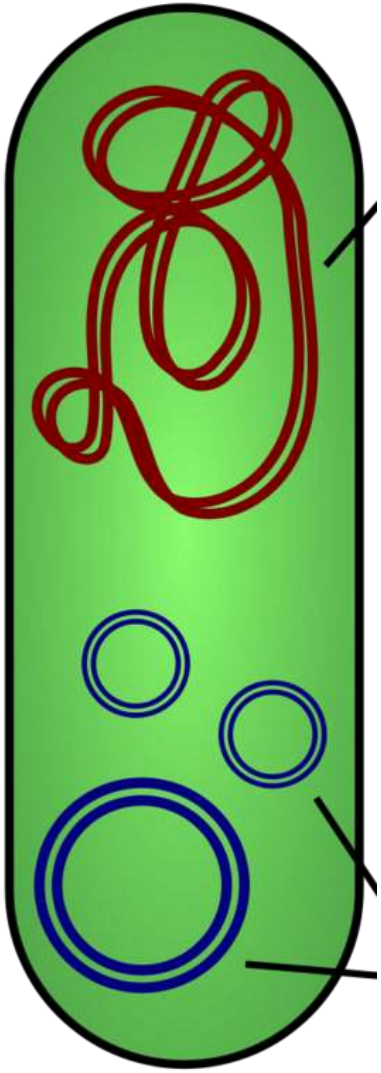


Wastewater treatment plants discharge treated water into the environment

Can treated wastewater transport antibiotic resistant bacteria and their resistance genes?

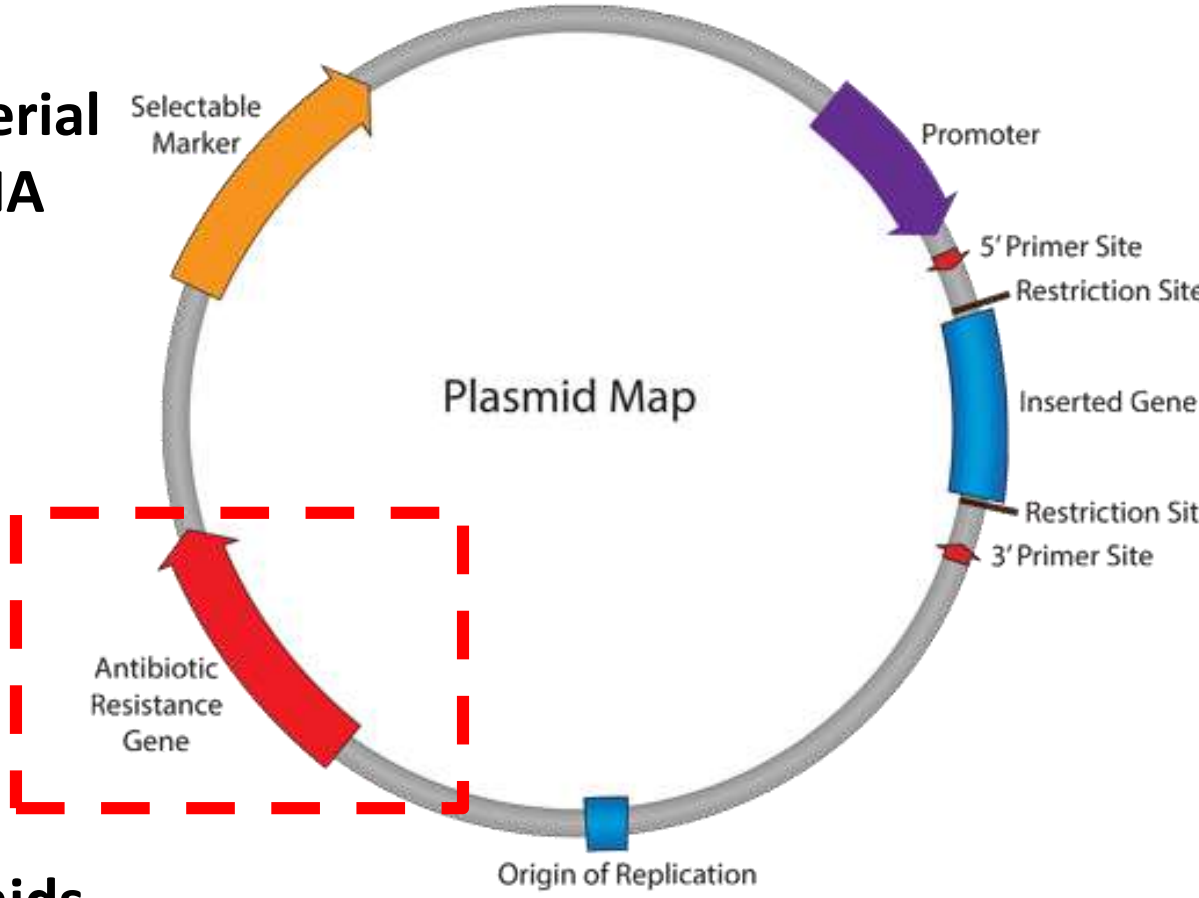
Optimization of the treatment process may lower the spread of antibiotic resistance throughout the environment.

Antibiotic Resistance Genes (ARG) and AR Bacteria



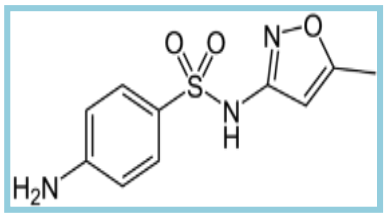
Bacterial DNA

Plasmids

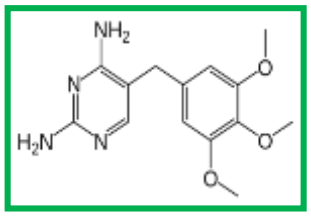


www.addgene.org

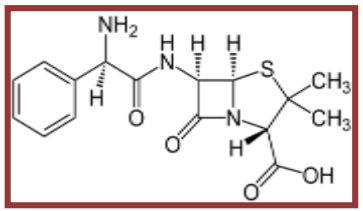
Clinically Relevant Antibiotics



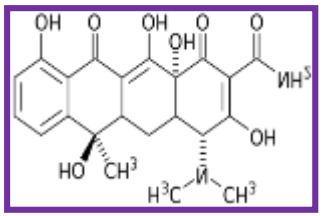
sulfamethoxazole



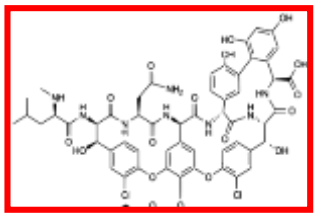
trimethoprim



ampicillin



tetracycline



vancomycin

***sul*(I) & *sul*(II)**

***dfr*1A**

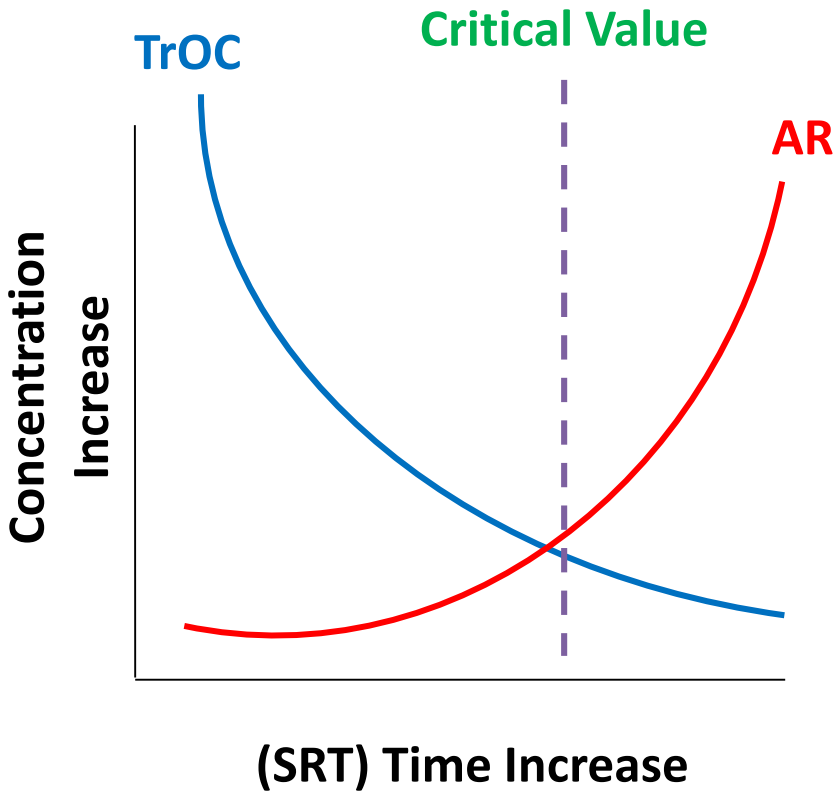
***amp*C**

***tet*W**

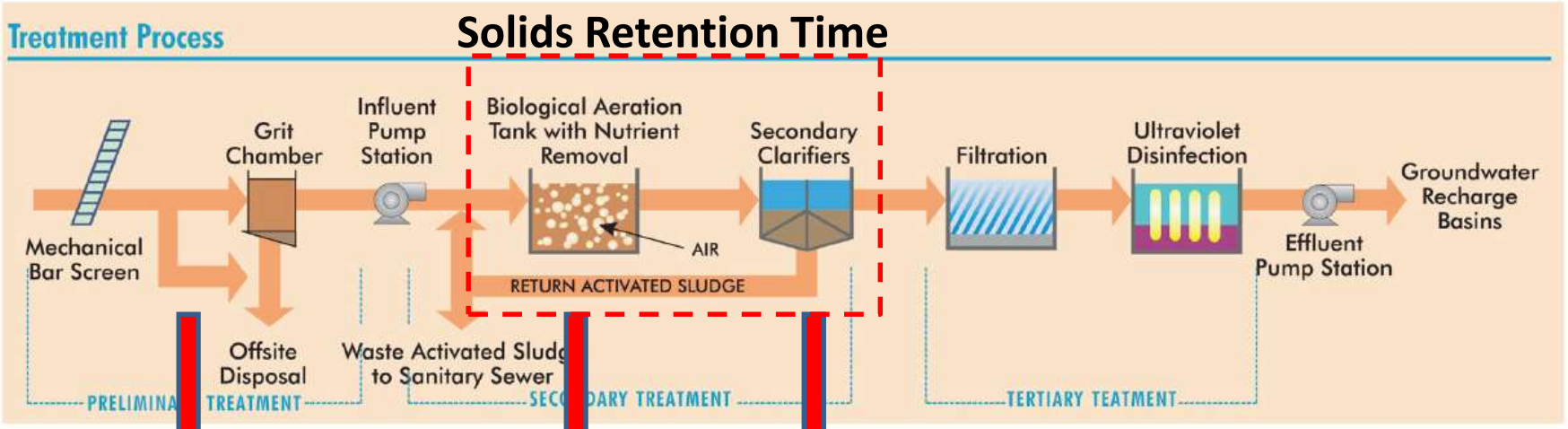
***van*A**

Effect of Solids Retention Time (SRT)

- Though **increased** solids retention time (SRT) has been correlated with **reductions** in trace antibiotics, higher SRTs also provide prolonged exposure of bacteria to influent antibiotic levels, potentially increasing the development of antibiotic resistance

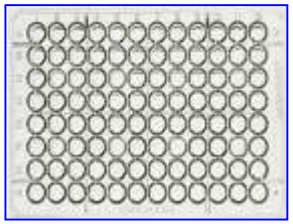


Methods

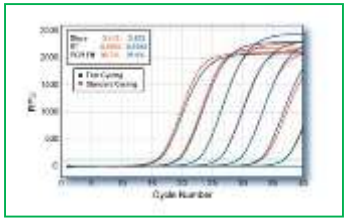


Molecular & Cultural Analysis

Chemical Analysis



Bacterial Isolates (MIC)

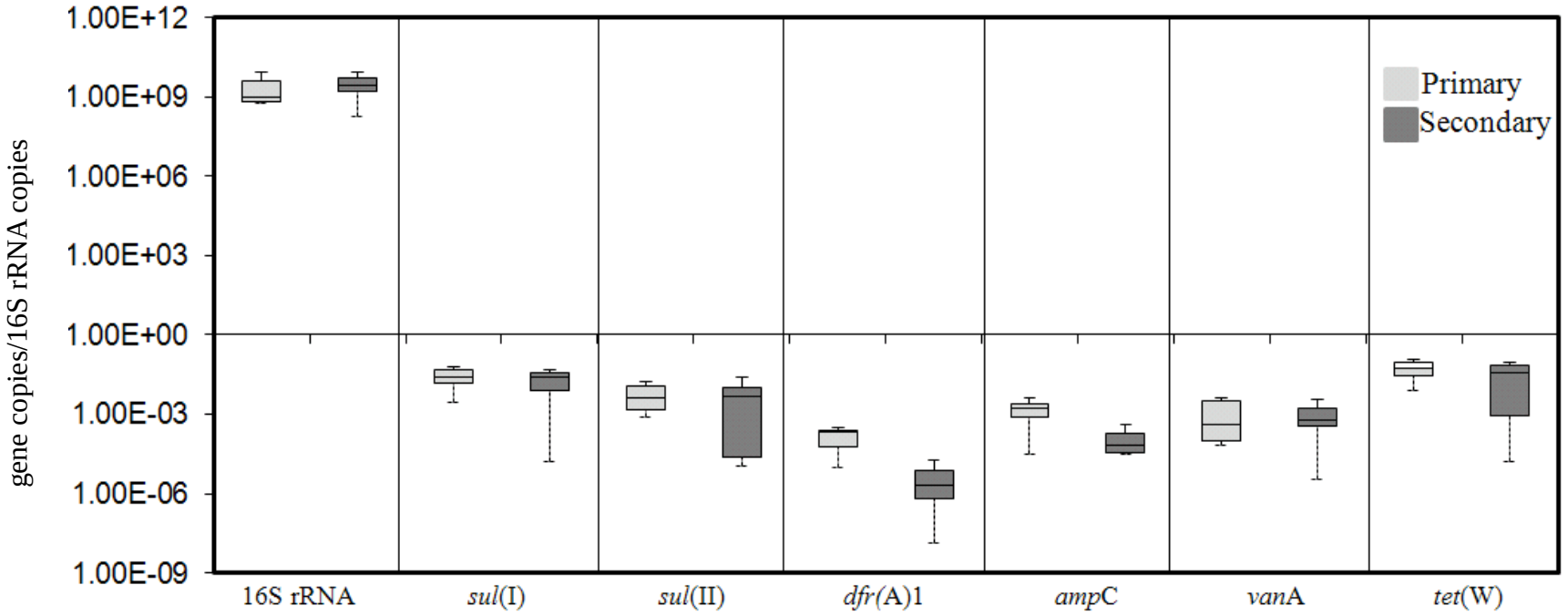


Quantitative PCR

SPE

LC-MS/MS

Antibiotic Resistance Genes



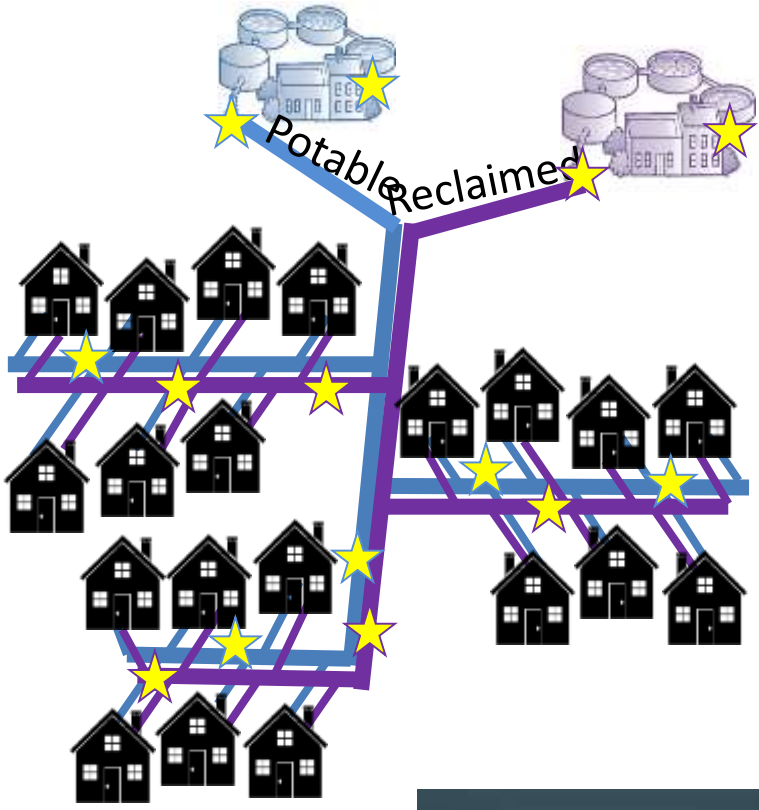
UNLV

Daniel Gerrity

Jean McLain

WRRC
WATER RESOURCES RESEARCH CENTER

Recycled vs Potable Water Distribution System Survey



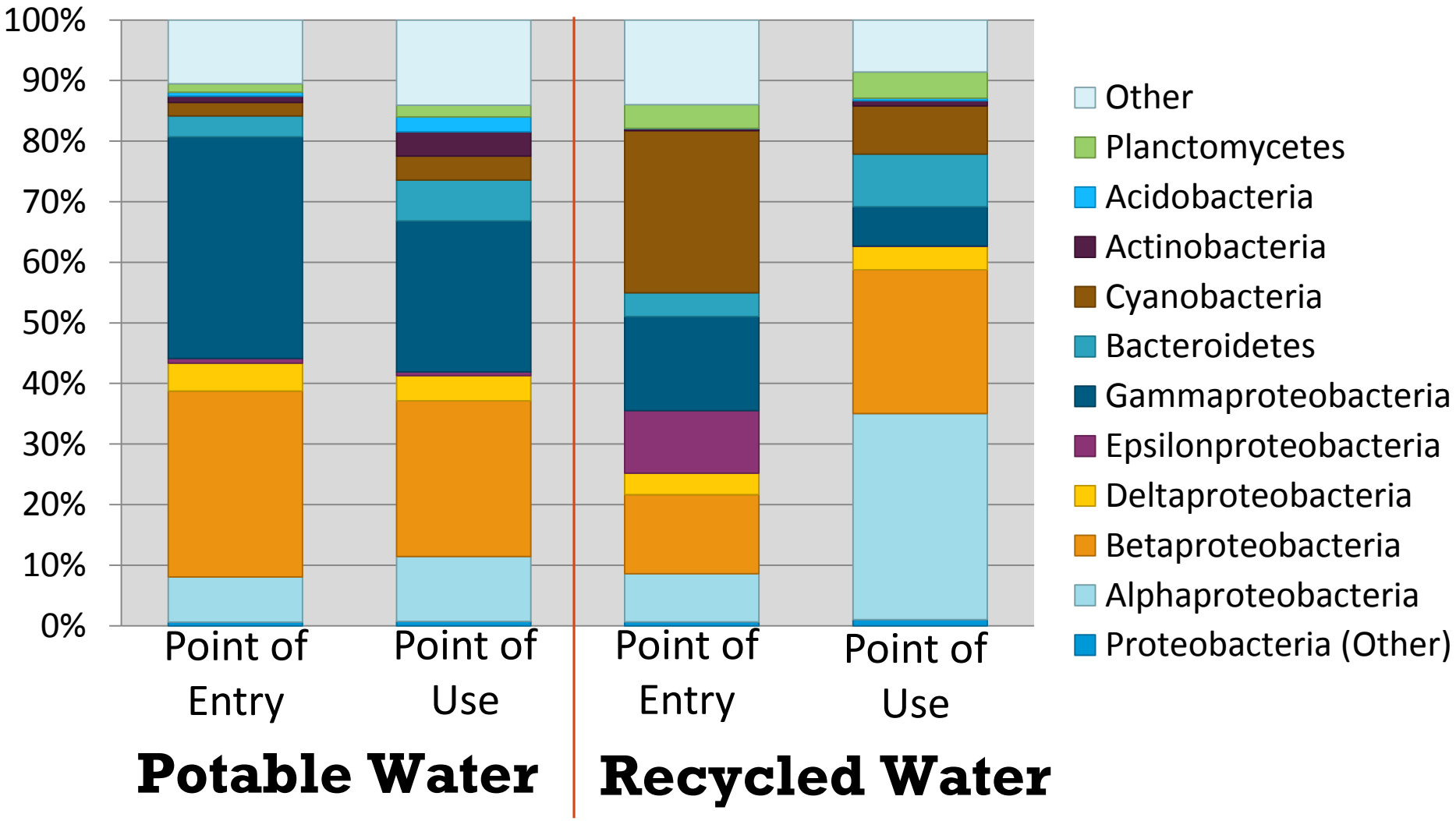
- Before treatment
- Point of Entry (POE)
- 5 Distribution Systems

VirginiaTech
Global Change Center

Amy Pruden

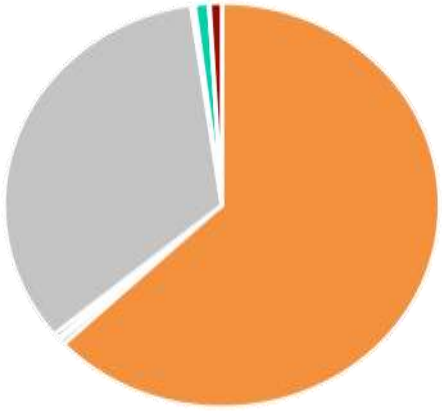
Emily Garner

Microbial community shifts with water age to a greater extent in recycled than in drinking water.

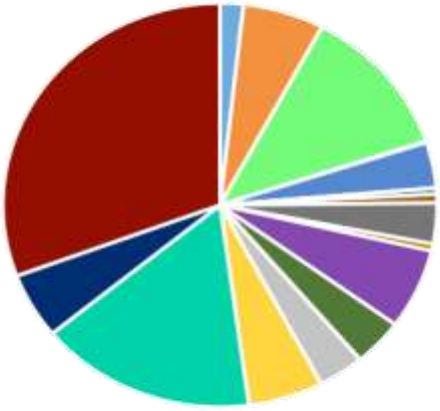


Metagenomics: Average composition of ARGs by class (recycled systems)

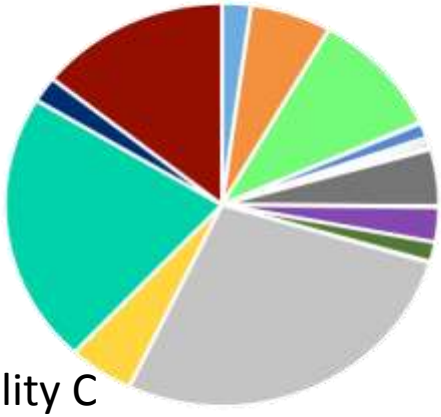
Utility A



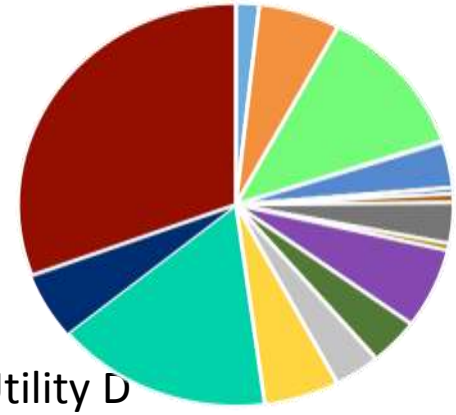
Utility B



Utility C



Utility D



- aminocoumarin
- aminoglycoside
- beta-lactam
- chloramphenicol
- fluoroquinolone
- fosfomycin
- glycopeptide
- lincosamide
- macrolide
- peptide
- polymyxin
- rifampin
- streptogramin
- streptothricin
- sulfonamide
- tetracycline
- trimethoprim
- other
- multidrug

Simulated Direct Potable Reuse (DPR)

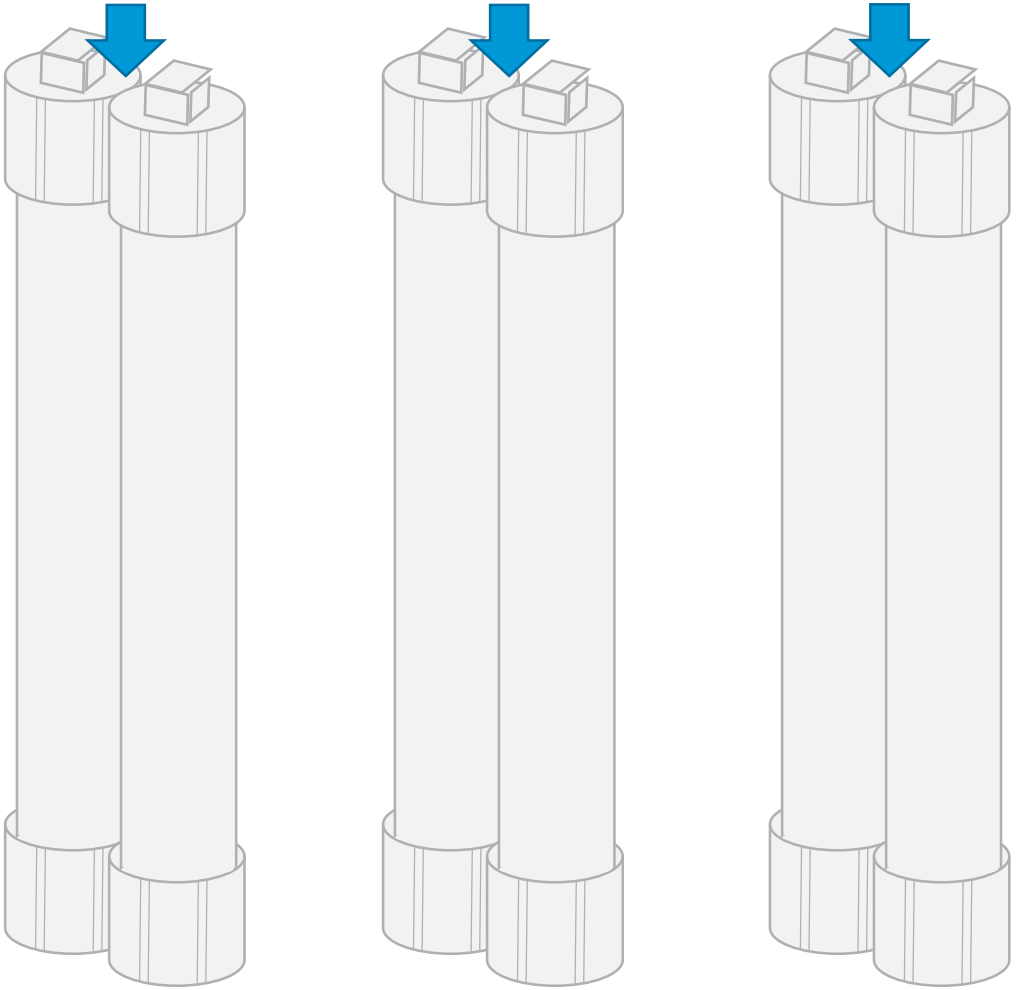
Water Research Foundation

Project 4536

10% DPR
90%
Potable

10% DPR w/
Biofiltration
90% Potable

100%
Potable



Water
Change
3x / week

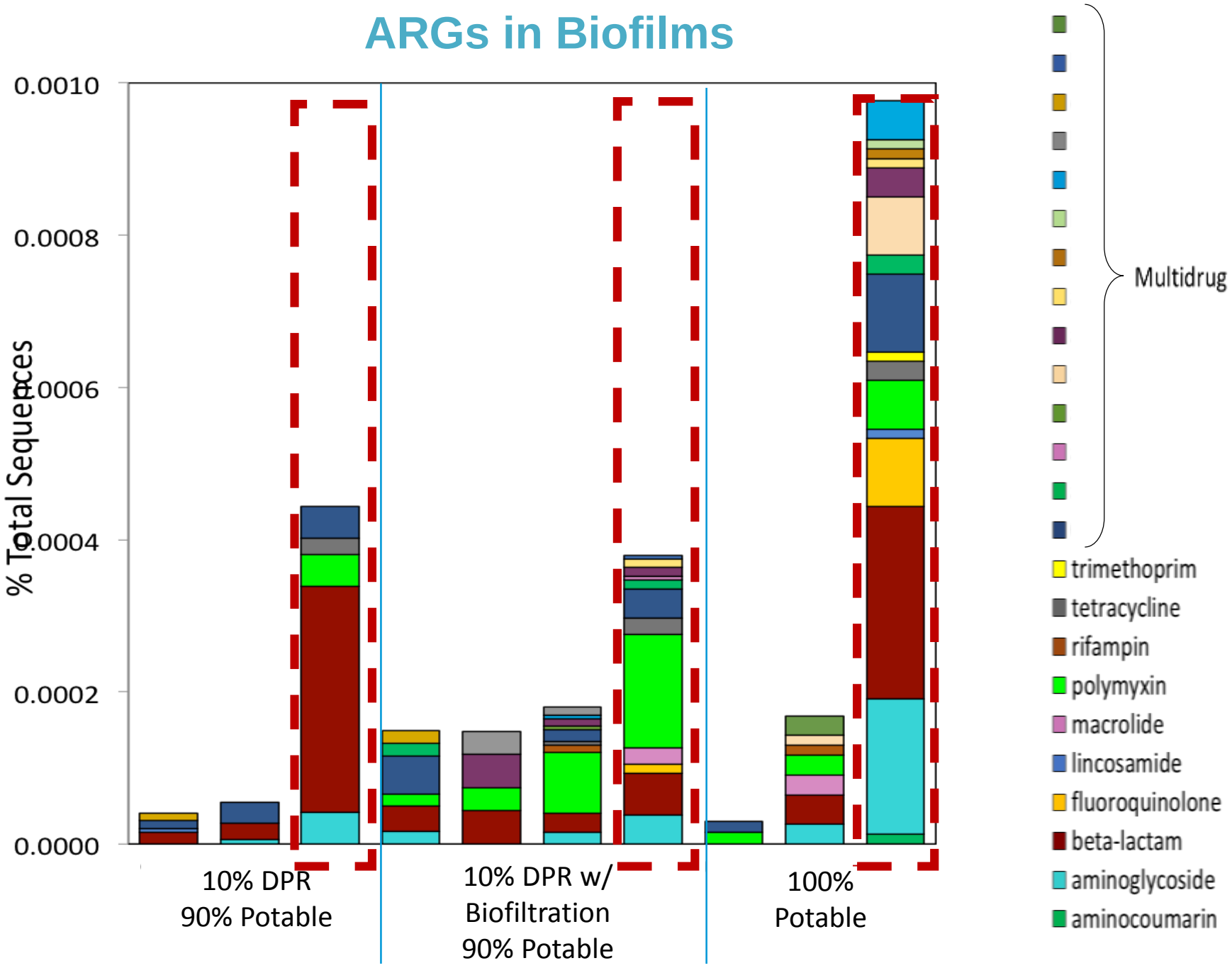
Sampled after
8 weeks
conditioning

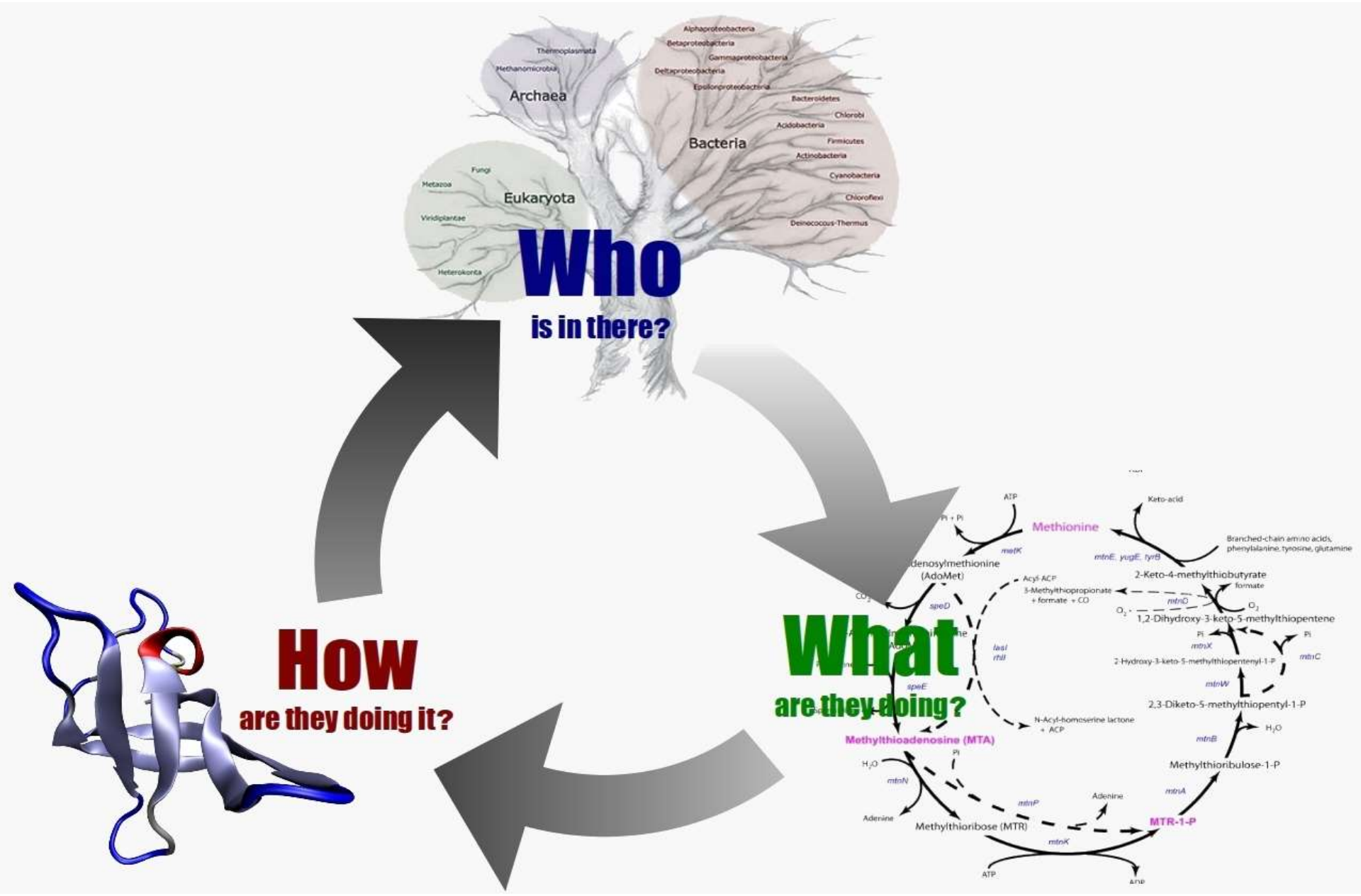
Emily Garner

Marc Edwards

Andy Salveson

ARGs in Biofilms




Archaea
Thermoplasmata
Methanomicrobia
Bacteria
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
Epsilonproteobacteria
Bacteroidetes
Chlorobi
Acidobacteria
Firmicutes
Actinobacteria
Cyanobacteria
Chloroflexi
Deinococcus-Thermus
Eukaryota
Fungi
Metazoa
Viridiplantae
Heterokonta
Who
is in there?
What
are they doing?
How
are they doing it?
ATP
Methionine
Keto-acid
Branched-chain amino acids, phenylalanine, tyrosine, glutamine
metK
mtnE, yugE, tyrB
(AdoMet)
2-Keto-4-methylthiobutyrate
formate
Acyl-ACP
3-Methylthiopropionate
+ formate + CO
mtnD
1,2-Dihydroxy-3-keto-5-methylthiopentene
speD
lasI
rhlI
mtnX
mtnC
2-Hydroxy-3-keto-5-methylthiopentenyl-1-P
speE
mtnW
2,3-Diketo-5-methylthiopentyl-1-P
N-Acyl-homoserine lactone
+ ACP
Methylthioadenosine (MTA)
mtnB
Methylthioribulose-1-P
mtnN
mtnA
Adenine
mtnP
Methylthioribose (MTR)
MTR-1-P
mtnK