Removal of Microorganisms and Antibiotic Resistance Genes in AWTFs

Scott Miller, PhD and Rose Kantor, PhD

CWEA Water Reuse Workshop
Emerging Issues in Potable Reuse

January 15, 2020

Outline

- Background on antibiotic resistance and removal by advanced treatment

- Our recent findings on removal of bacteria and resistance genes from two AWTPs

Background: Resistance is part of a bacterial arms race

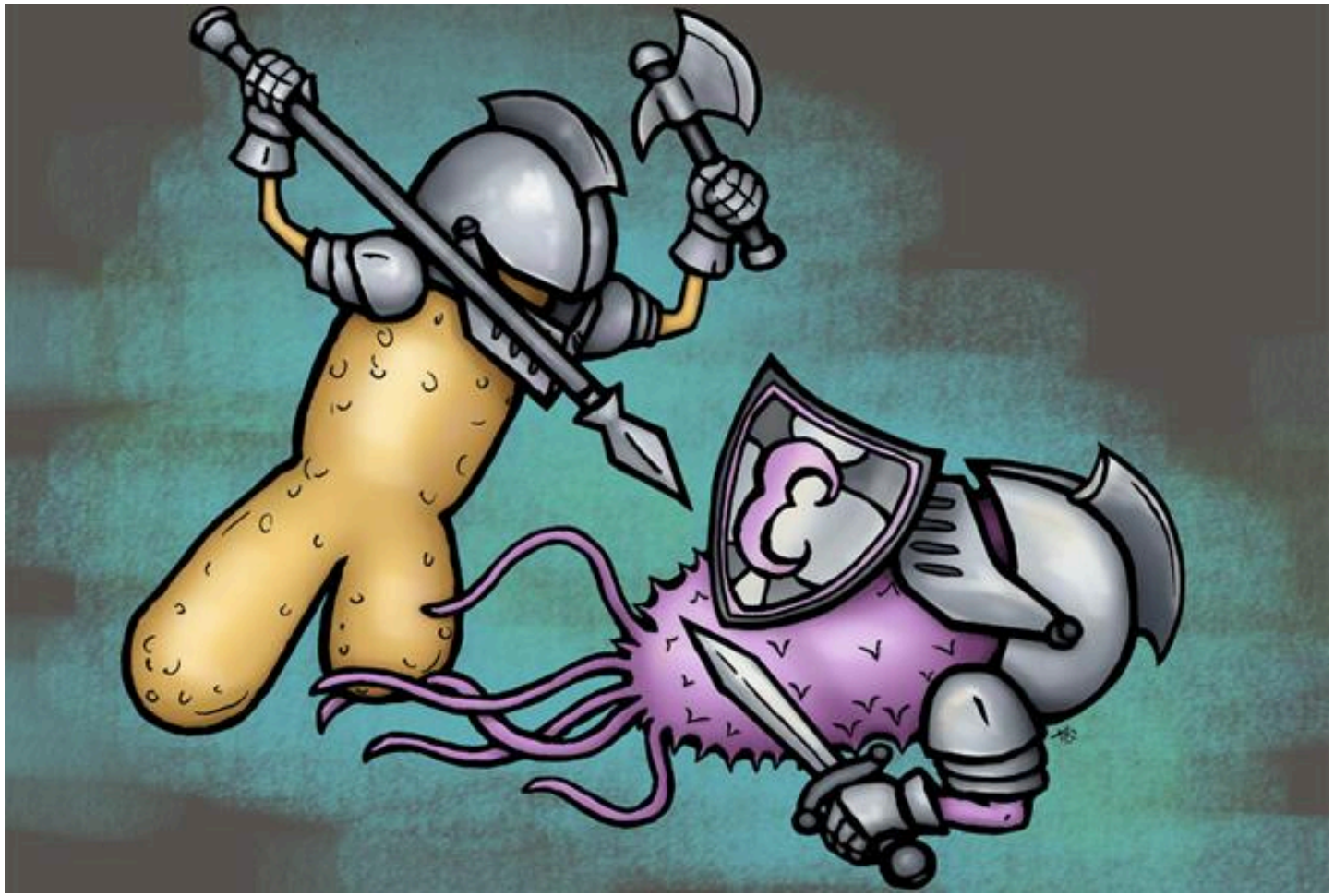

Image credit: Alice C. Gray, https://newsroom.uw.edu/

- Bacteria and fungi make antibiotics against each other
- Resistant bacteria are more fit
- Resistance is found in natural environments

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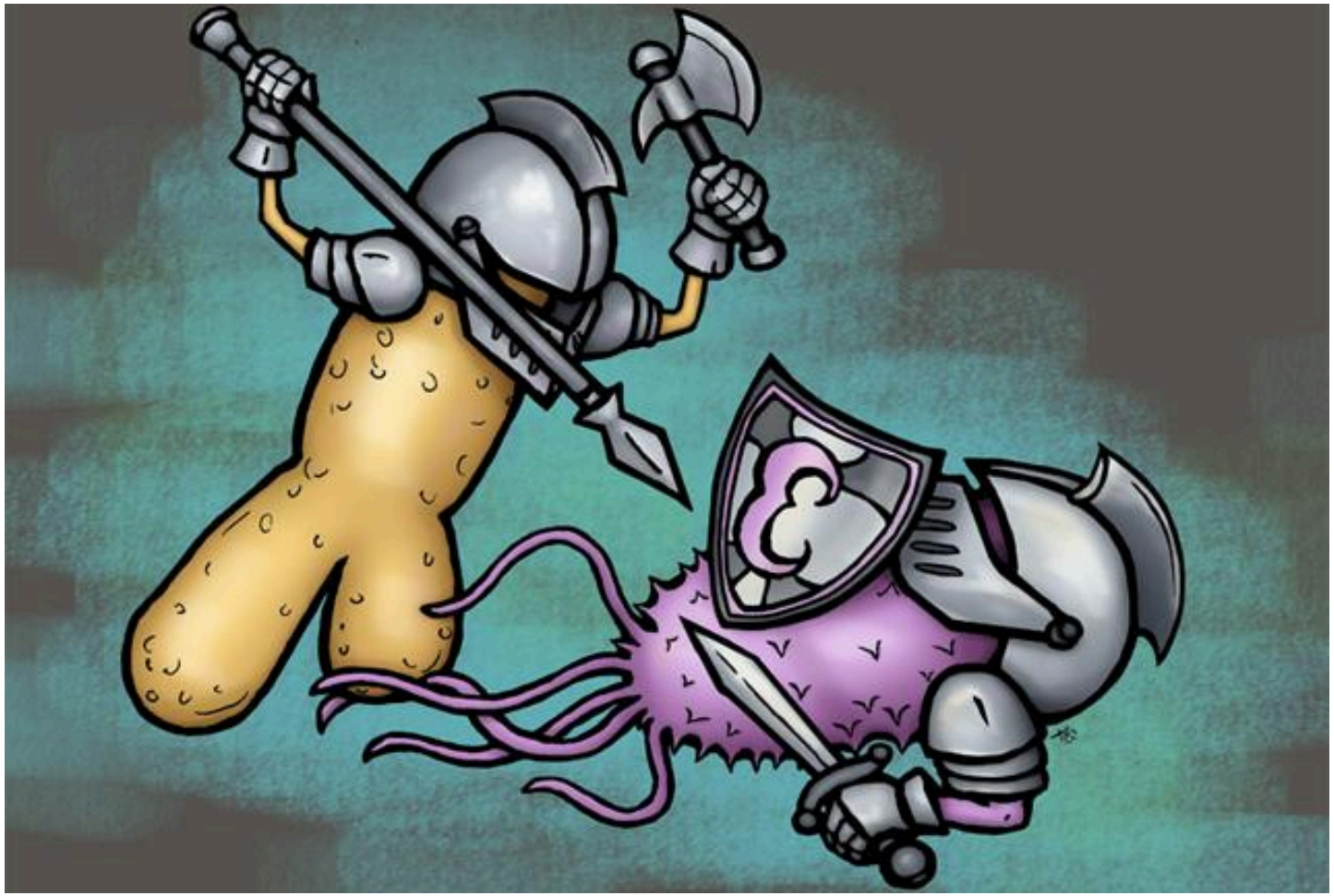

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Van Goethem *et al. Microbiome* (2018) 6:40
https://doi.org/10.1186/s40168-018-0424-5

Microbiome

RESEARCH Open Access

A reservoir of 'historical' antibiotic resistance genes in remote pristine Antarctic soils

Marc W. Van Goethem[1†], Rian Pierneef[2†], Oliver K. I. Bezuidt[1], Yves Van De Peer[1,3,4,5], Don A. Cowan[1] and Thulani P. Makhalanyane[1*]

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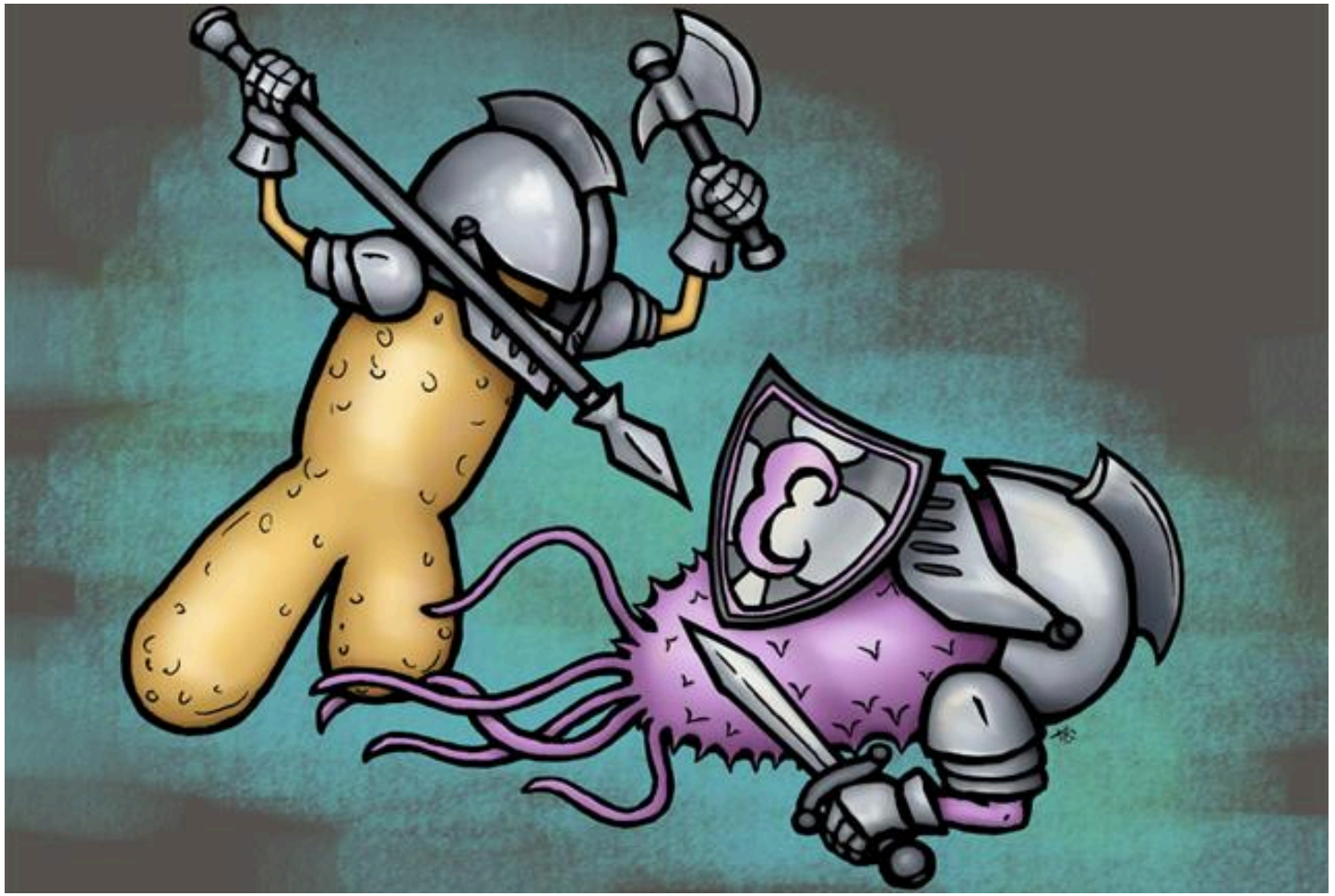

Image credit: Alice C. Gray, https://newsroom.uw.edu/

- Bacteria and fungi make antibiotics against each other
- Resistant bacteria are more fit
- Resistance is found in natural environments

- Humans are now part of the arms race
- Our battleground is hospitals and clinical settings
- Concern is resistant pathogens, NOT all bacteria

Background: How does antibiotic resistance arise?

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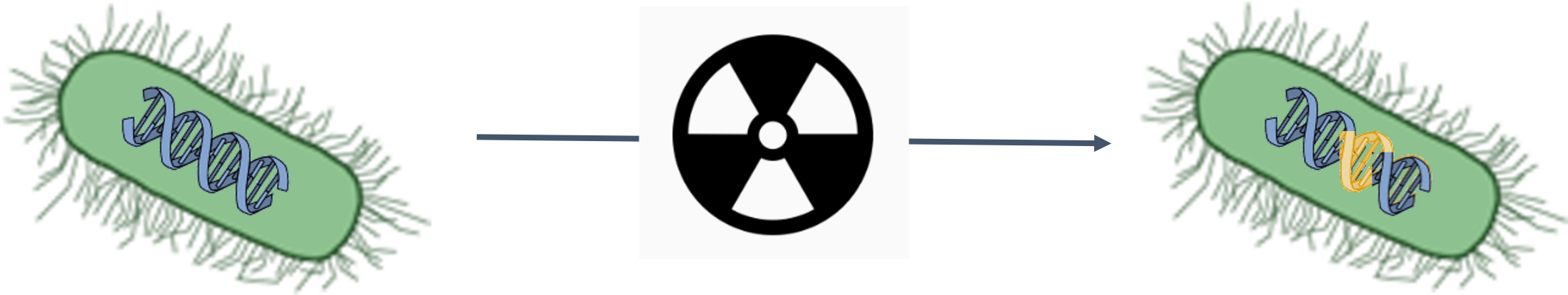
1) Genetic mutation



Bacteria image credit to Lauren Kennedy, UC Berkeley

Background: How does antibiotic resistance arise?

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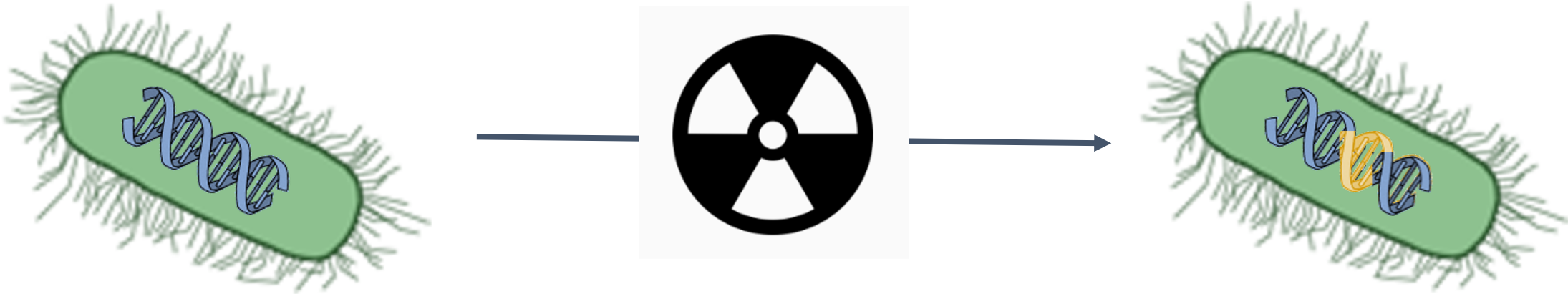
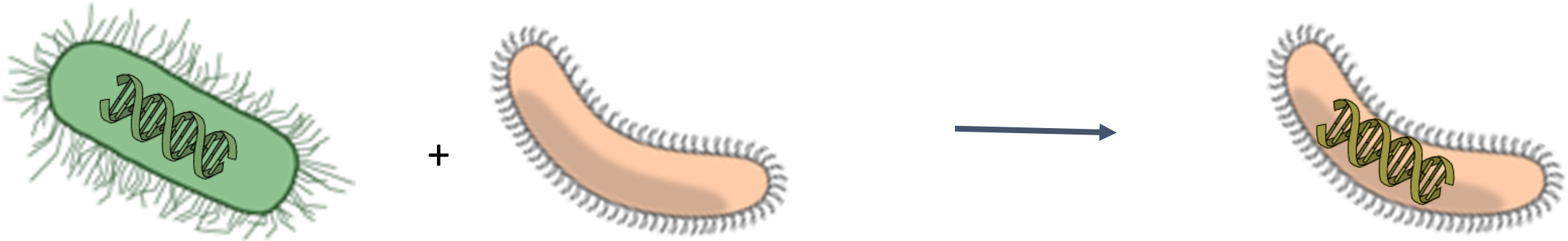
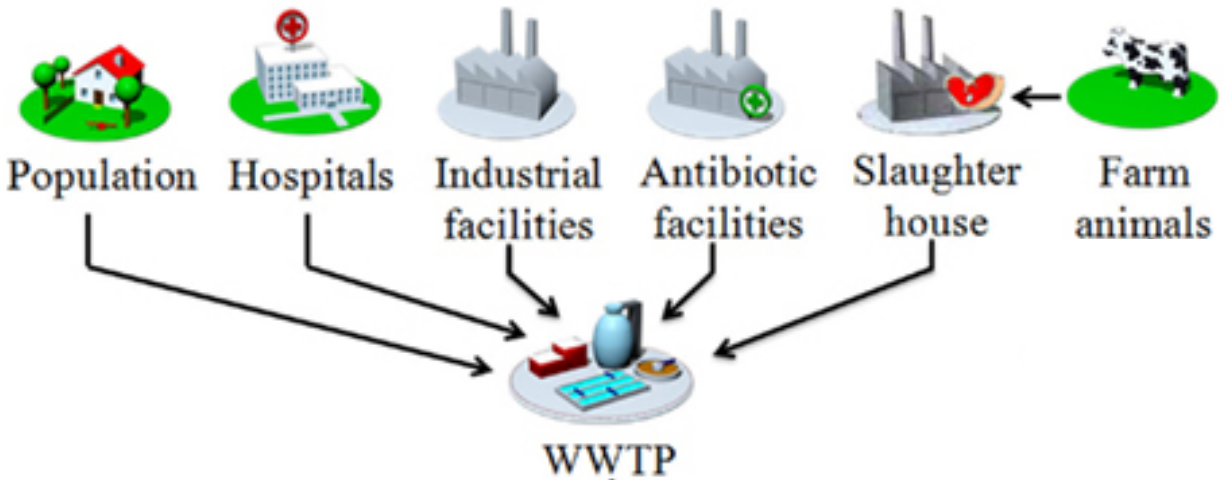


2) Horizontal gene transfer



Bacteria image credit to Lauren Kennedy, UC Berkeley

Background: Wastewater as a hotspot for ARGs



ELSEVIER

Water Research

Volume 123, 15 October 2017, Pages 468-478

Metagenomic analysis reveals wastewater treatment plants as hotspots of antibiotic resistance genes and mobile genetic elements

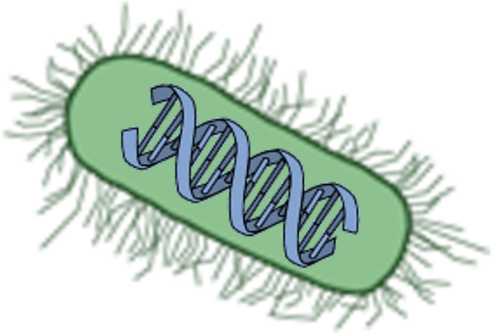
Jianhua Guo [a], Jie Li [b], Hui Chen [a], Philip L. Bond [a], Zhiguo Yuan [a]

- High density of human-associated bacteria
- Lots of opportunity for horizontal gene transfer
- High concentrations of antibiotics that select for resistance

Image credit: Stalder 2012. Front. Micro.

Background: Removal during advanced treatment

ARB = Antibiotic Resistant Bacteria

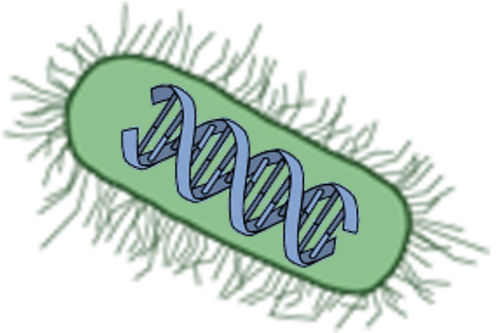


ARG = Antibiotic Resistance Genes

Background: Removal during advanced treatment

ARB = Antibiotic Resistant Bacteria

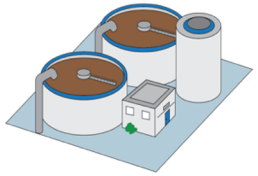
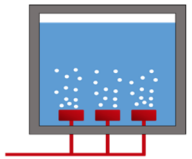
Treat these the same as removal of all bacteria

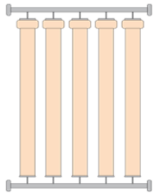
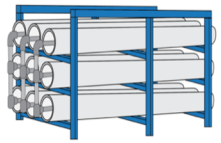
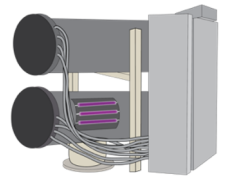


ARG = Antibiotic Resistance Genes

Treat these the same as removal of organic chemical constituents

Background: Removal during advanced treatment processes

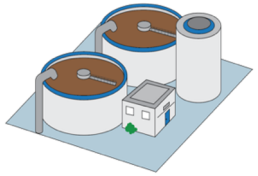
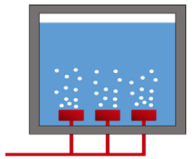



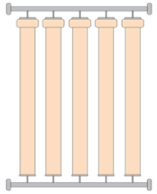
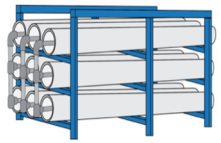
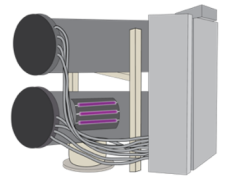




Process	WWTP	Ozone	BAC	MF	RO	UV-AOP

Slide modified from Dr. Brian Pecson, Trussell Technologies
Advanced treatment image credit to Emily Marron and Dr. Daniel Daft, Stanford University

Background: Removal during advanced treatment processes

Process	WWTP	Ozone	BAC	MF	RO	UV-AOP
Treatment Mechanism	Biological Physical Removal	Oxidation	Biological Physical Removal	Physical Removal	Physical Removal	Oxidation Irradiation

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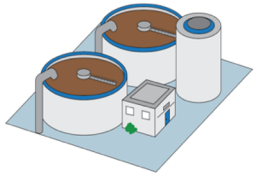
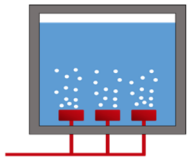

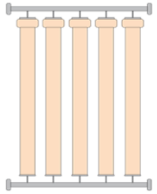
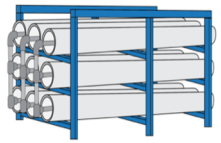
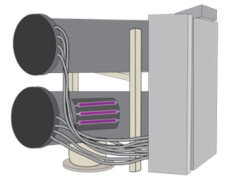

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Bacteria	+	+		+	+	+
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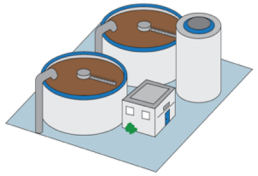
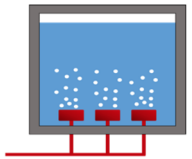

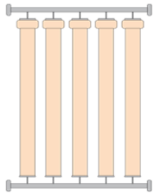
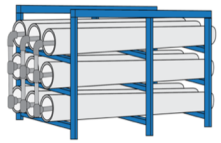
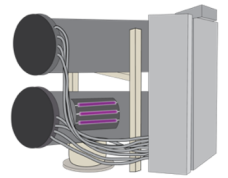

Process	WWTP	Ozone	BAC	MF	RO	UV-AOP
Bacteria	+	+		+	+	+
Free DNA	+	+	+		+	+
Treatment Mechanism	Biological Physical Removal	Oxidation	Biological Physical Removal	Physical Removal	Physical Removal	Oxidation Irradiation

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Key questions in our research

1. How well does advanced treatment remove bacteria?
2. How well does advanced treatment remove ARGs?
3. Are the SAME bacteria present before and after treatment?

Study sites

Two pilot treatment trains sampled

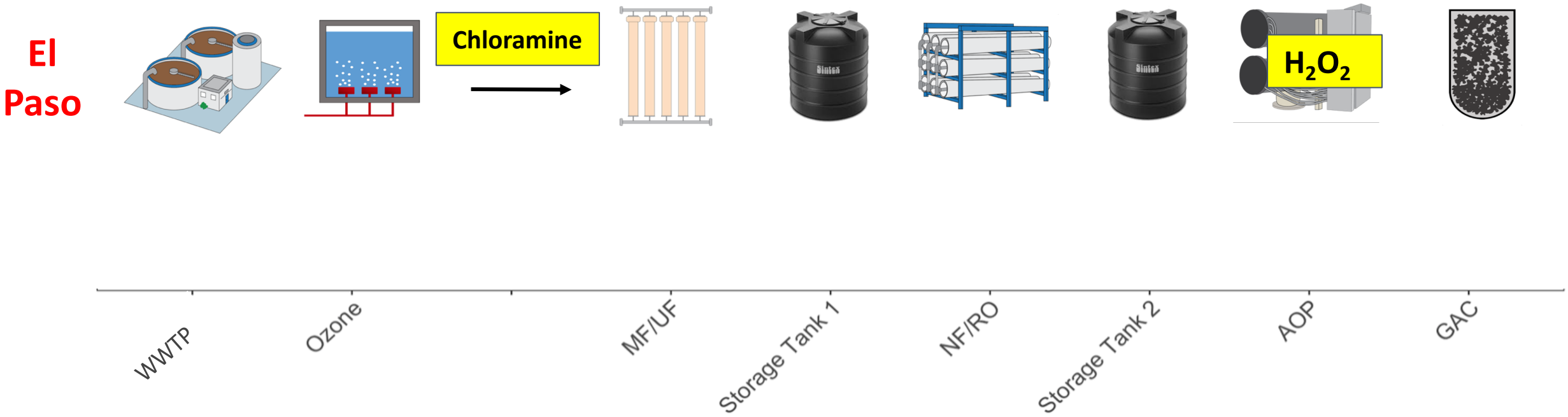

Image credit: Emily Marron and Daniel Daft

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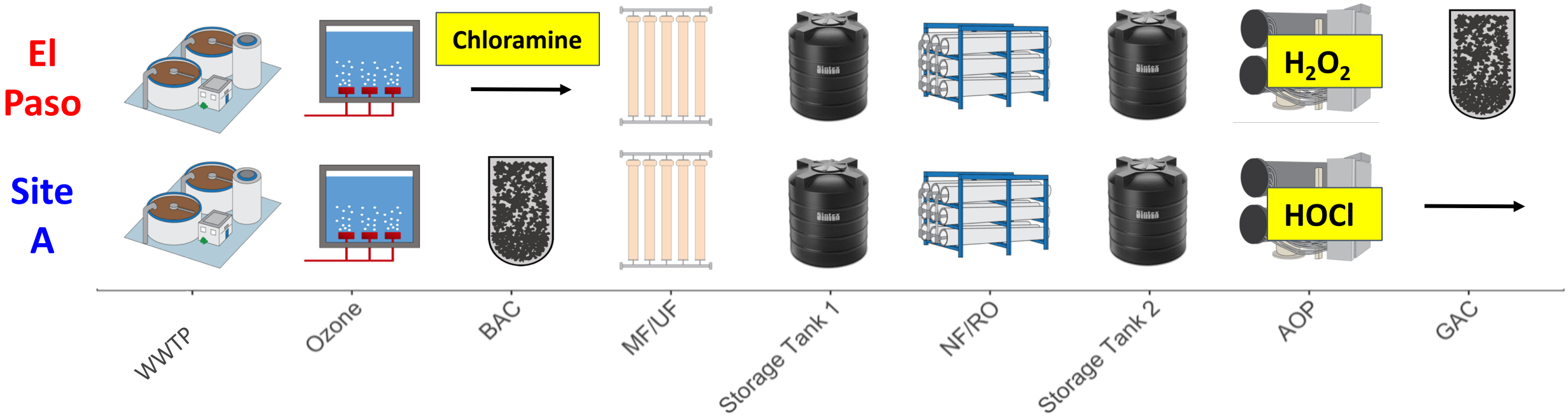

Image credit: Emily Marron and Daniel Daft

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Quantification of TOTAL cells using flow cytometry

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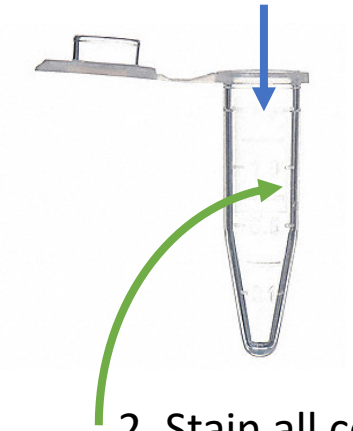
Quantification of TOTAL cells using flow cytometry

1. Grab sample



2. Stain all cells with DNA-binding green fluorescent dye

3. Pump known volume of sample through instrument

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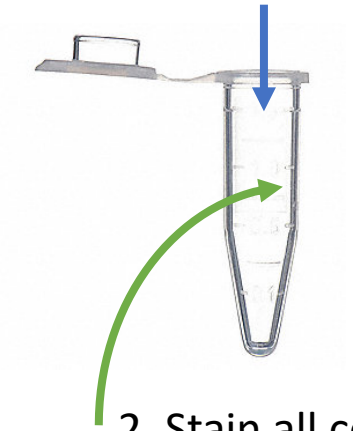

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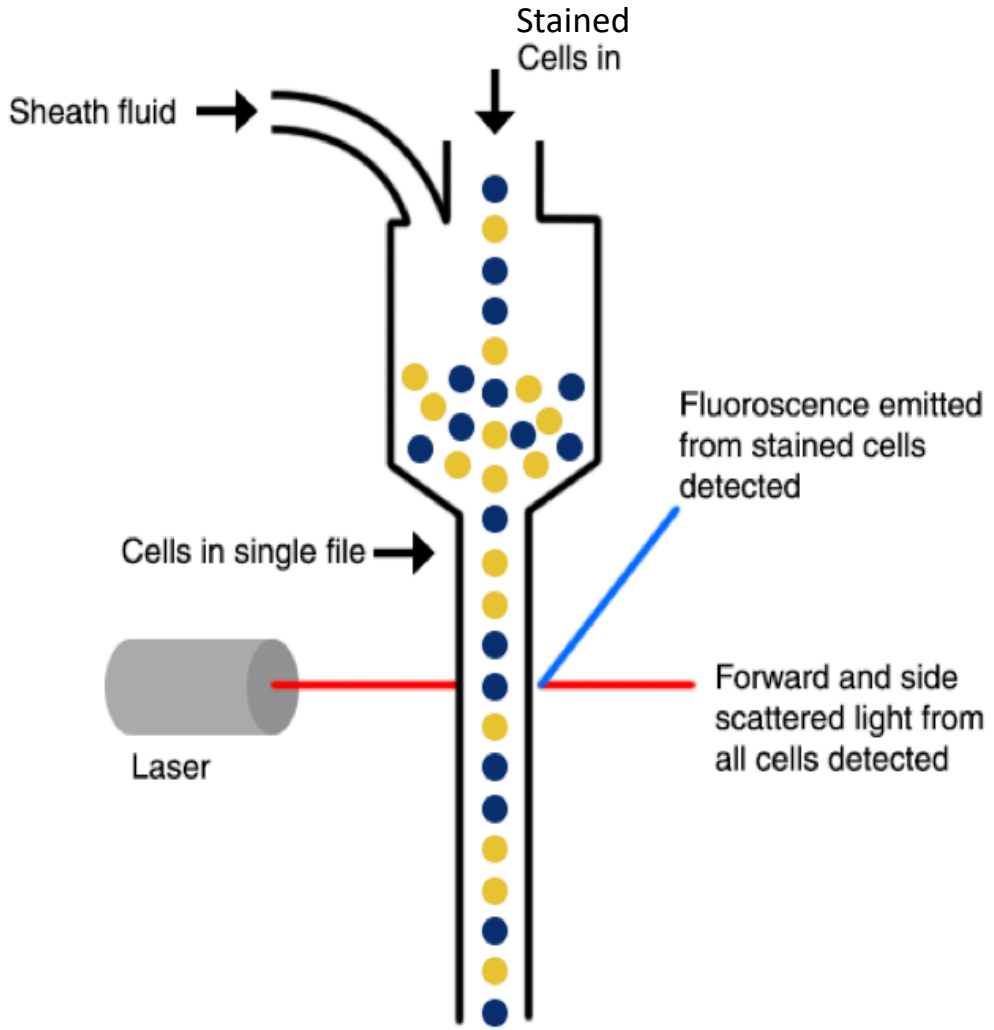

Image credit https://www.abbexa.com/flow-cytometry-info

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Quantification of INTACT cells using flow cytometry

An intact cell membrane is an indicator of potential viability

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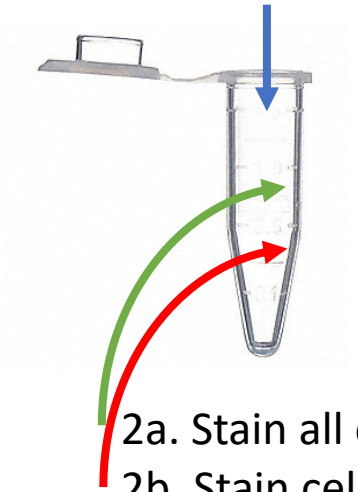
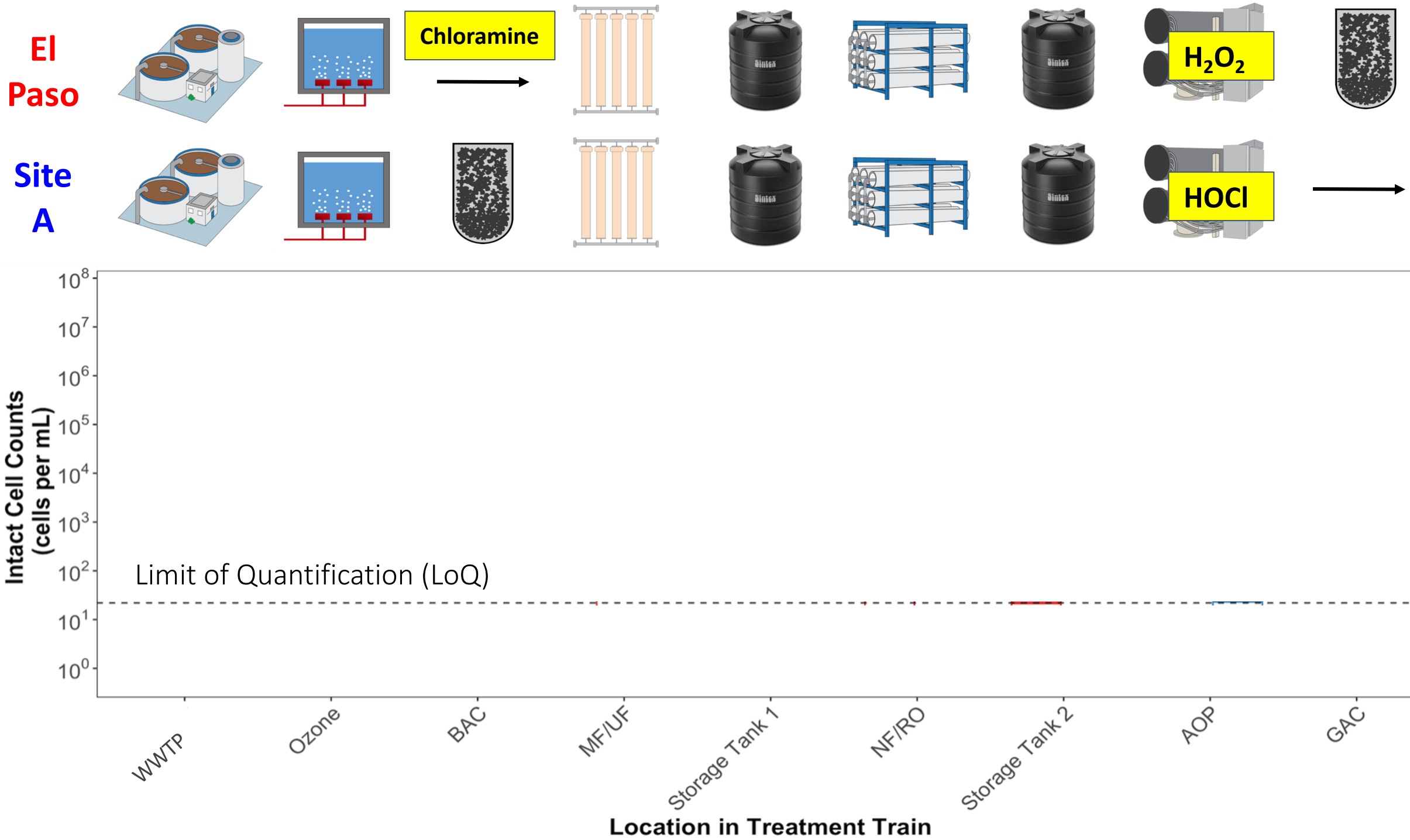
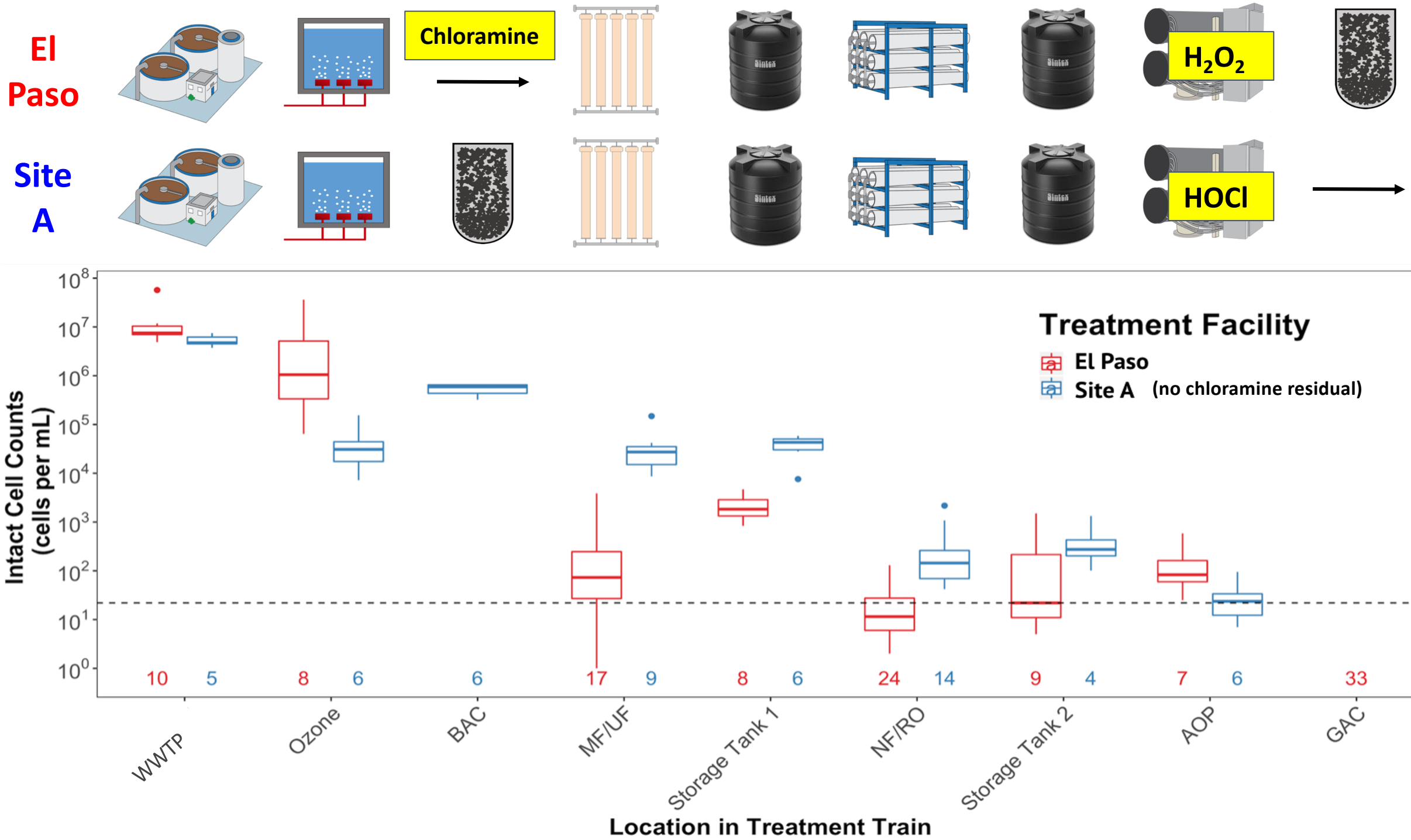


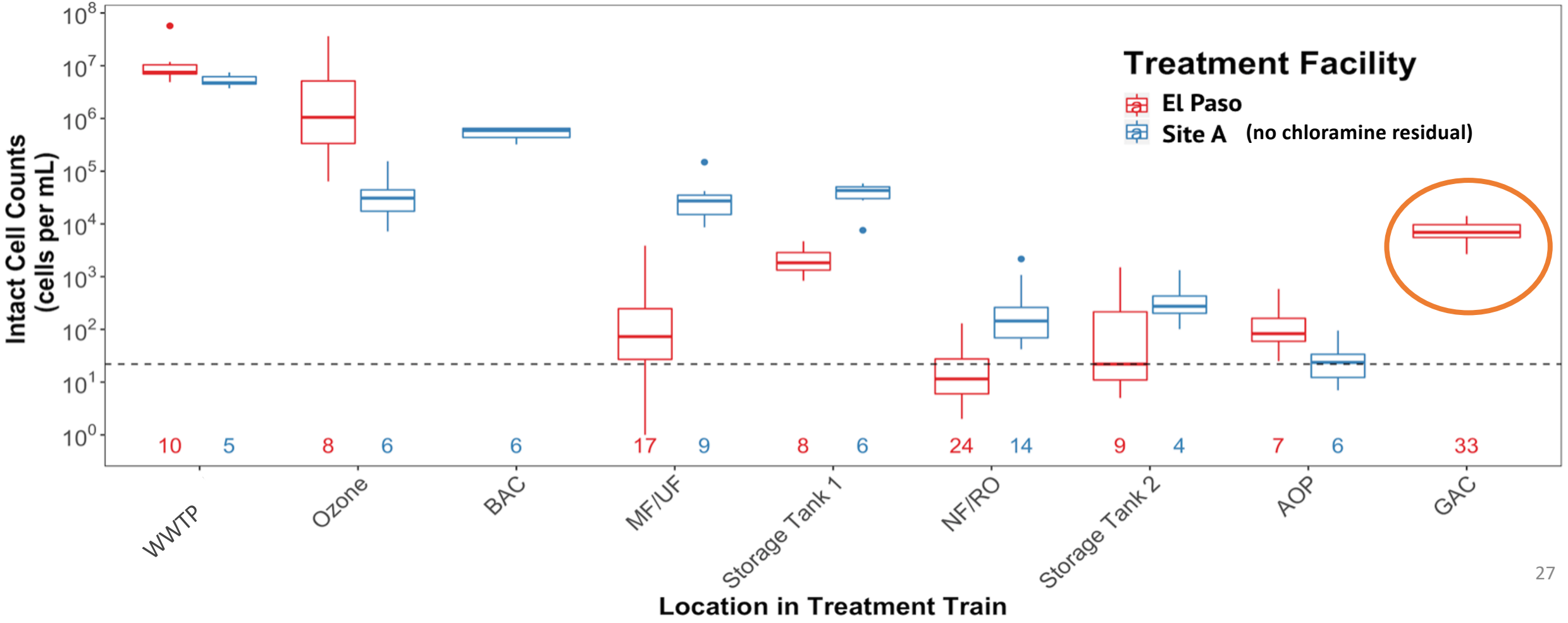
2a. Stain all cells with DNA-binding green fluorescent dye
2b. Stain cells with broken membranes with DNA-binding red fluorescent dye

3. Pump known volume of sample through instrument


El Paso
Site A
Chloramine
H_2O_2
HOCl
Intact Cell Counts (cells per mL)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Limit of Quantification (LoQ)
WWTP
Ozone
BAC
MF/UF
Storage Tank 1
NF/RO
Storage Tank 2
AOP
GAC
Location in Treatment Train


El Paso
Site A
Chloramine
H_2O_2
HOCl
Treatment Facility
El Paso
Site A (no chloramine residual)
Intact Cell Counts (cells per mL)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10 5 8 6 6 17 9 8 6 24 14 9 4 7 6 33
WWTP
Ozone
BAC
MF/UF
Storage Tank 1
NF/RO
Storage Tank 2
AOP
GAC
Location in Treatment Train

1. Treatment removes nearly all bacteria but there is growth after treatment



2. How well does advanced treatment remove ARGs?

Quantification of ARGs using qPCR

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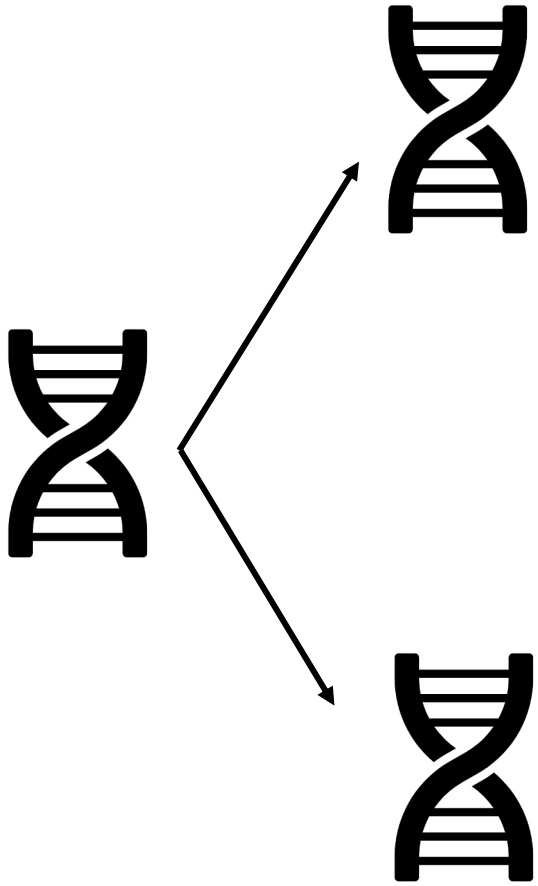

Repeatedly copy DNA

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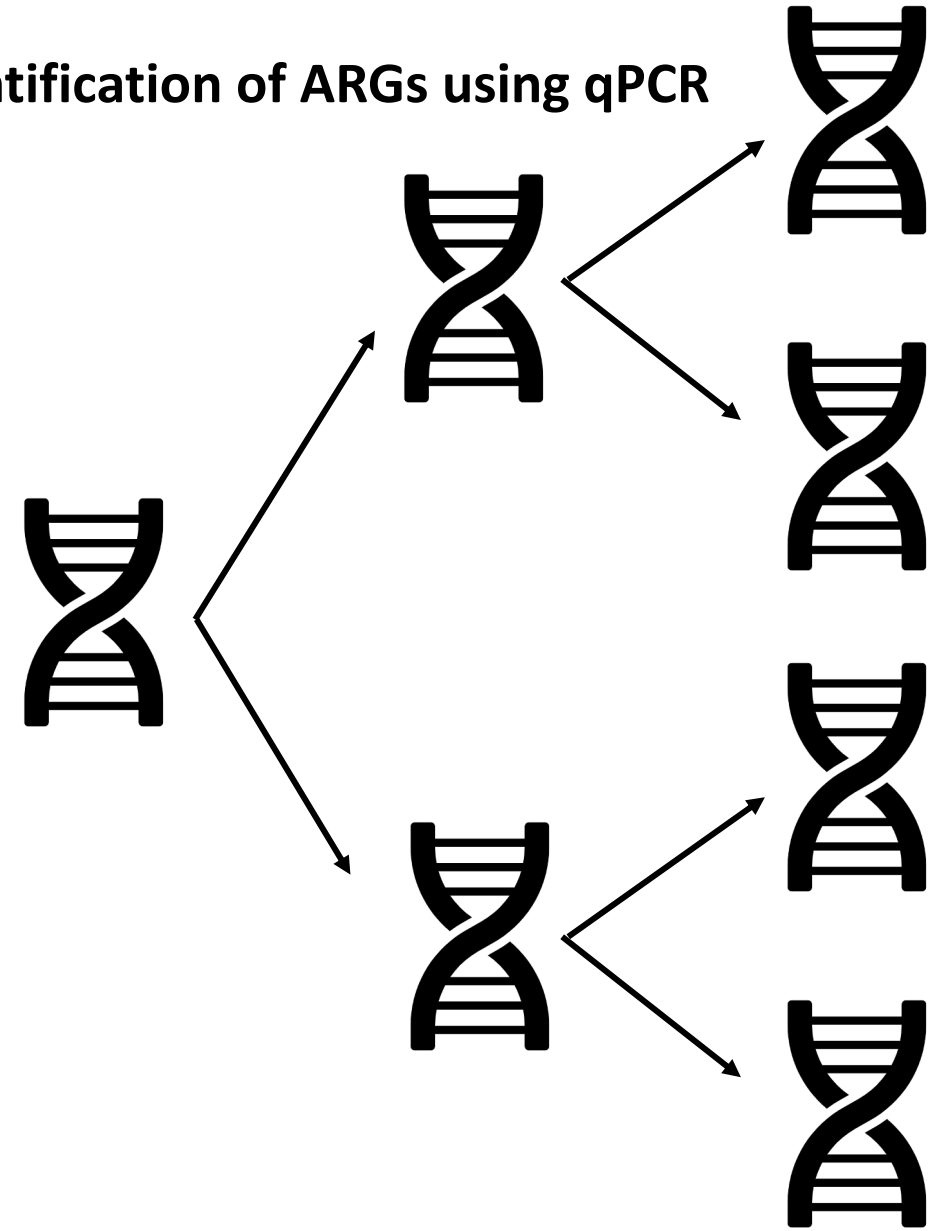
Quantification of ARGs using qPCR



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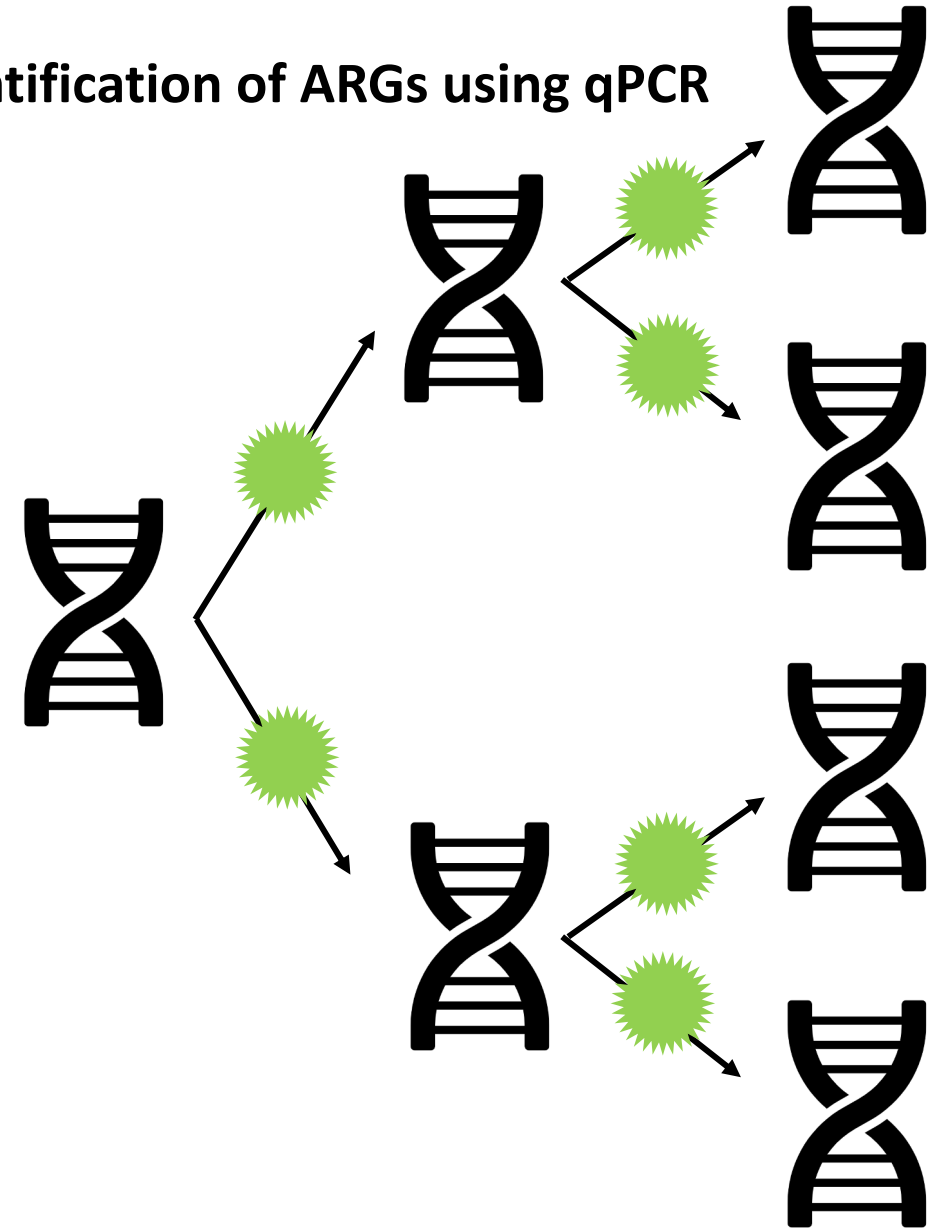
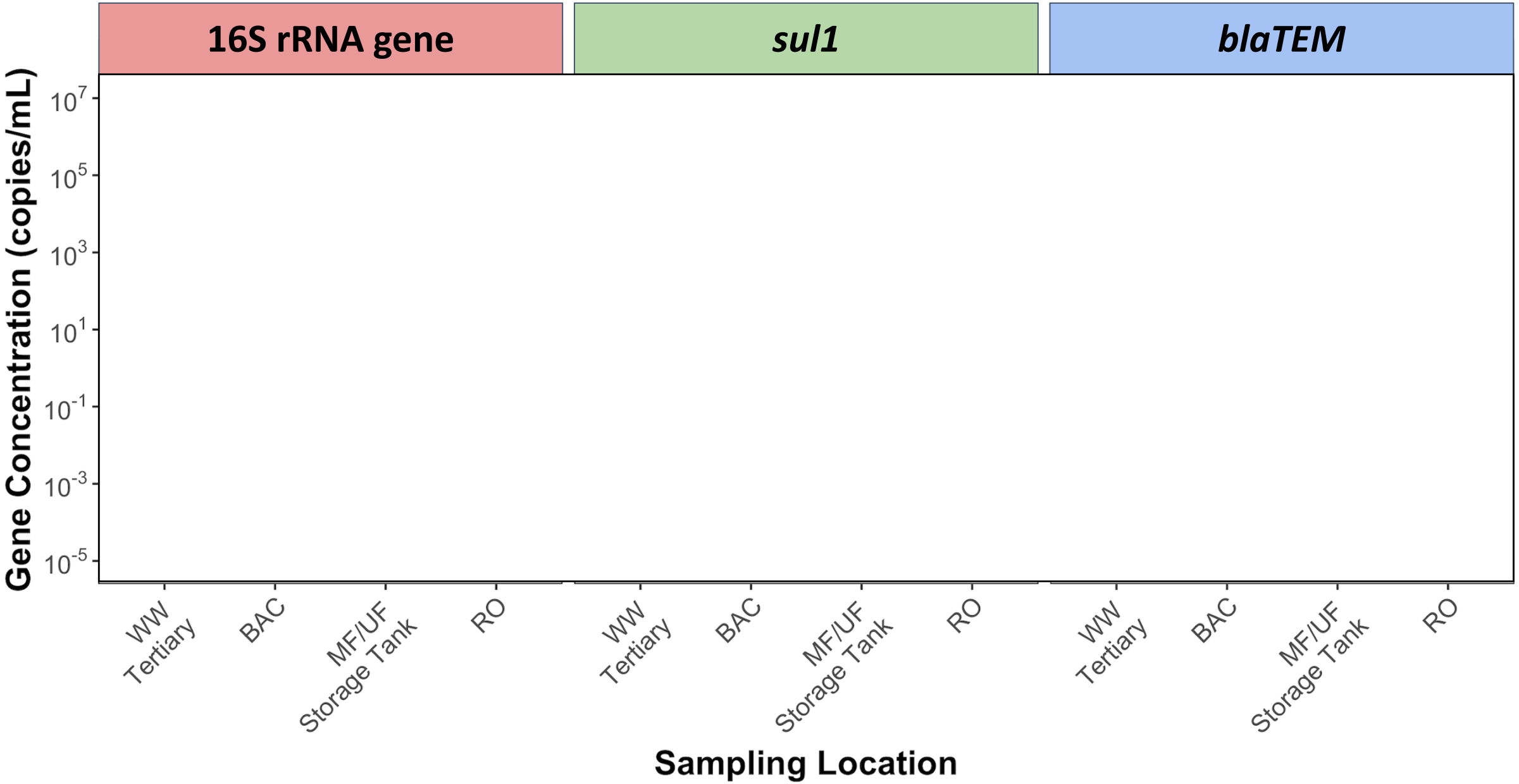


1. During copying, use a fluorescent dye to quantify how much DNA is present.
2. Measure fluorescence after each cycle of copying.
3. Count how many cycles it takes to reach a threshold fluorescence value.
4. Use known standards to calculate gene copies per mL of sample

2. How well does advanced treatment remove ARGs? (Site A)



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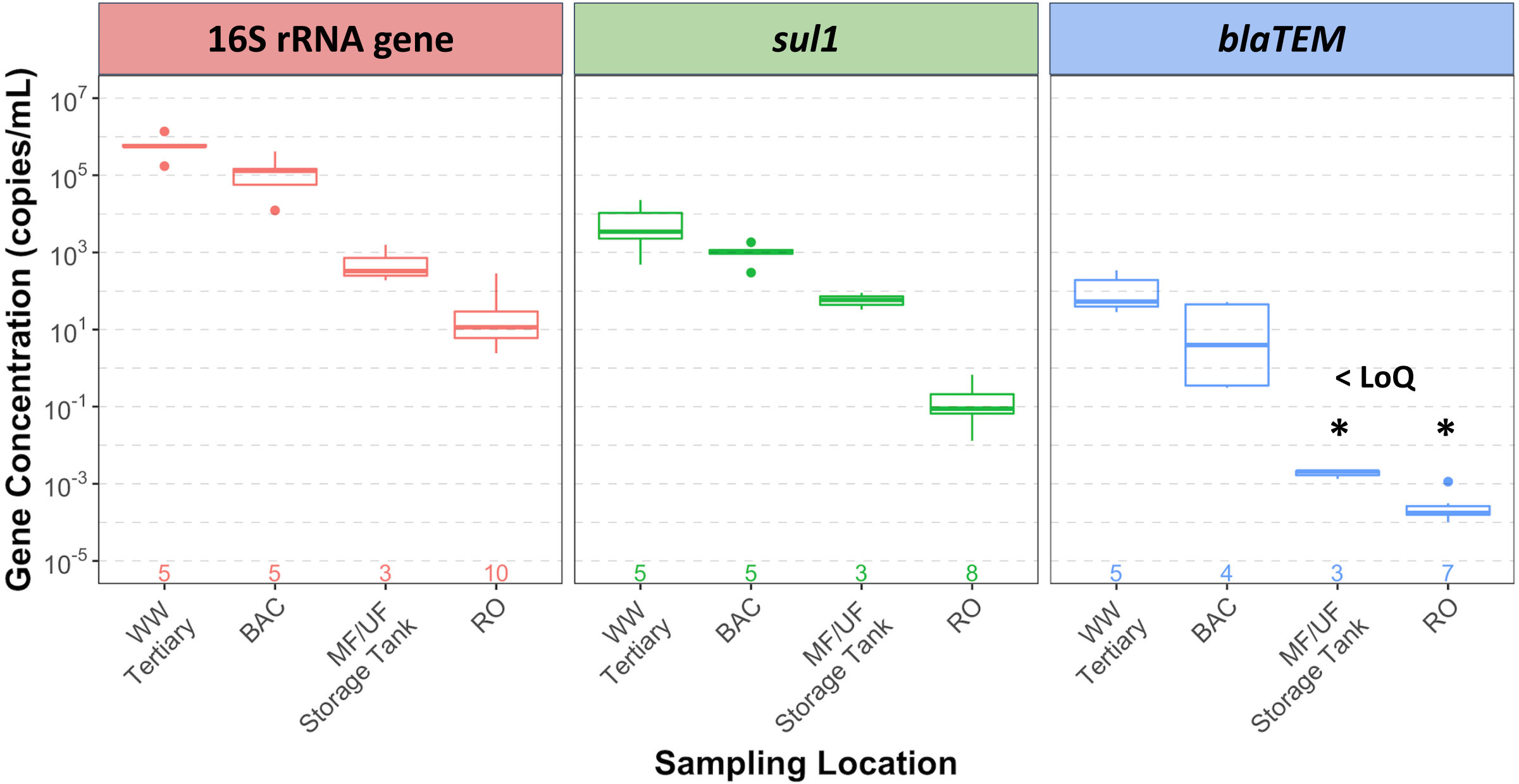

3. Are the SAME bacteria present before and after treatment?

Inventorying bacteria with DNA sequencing

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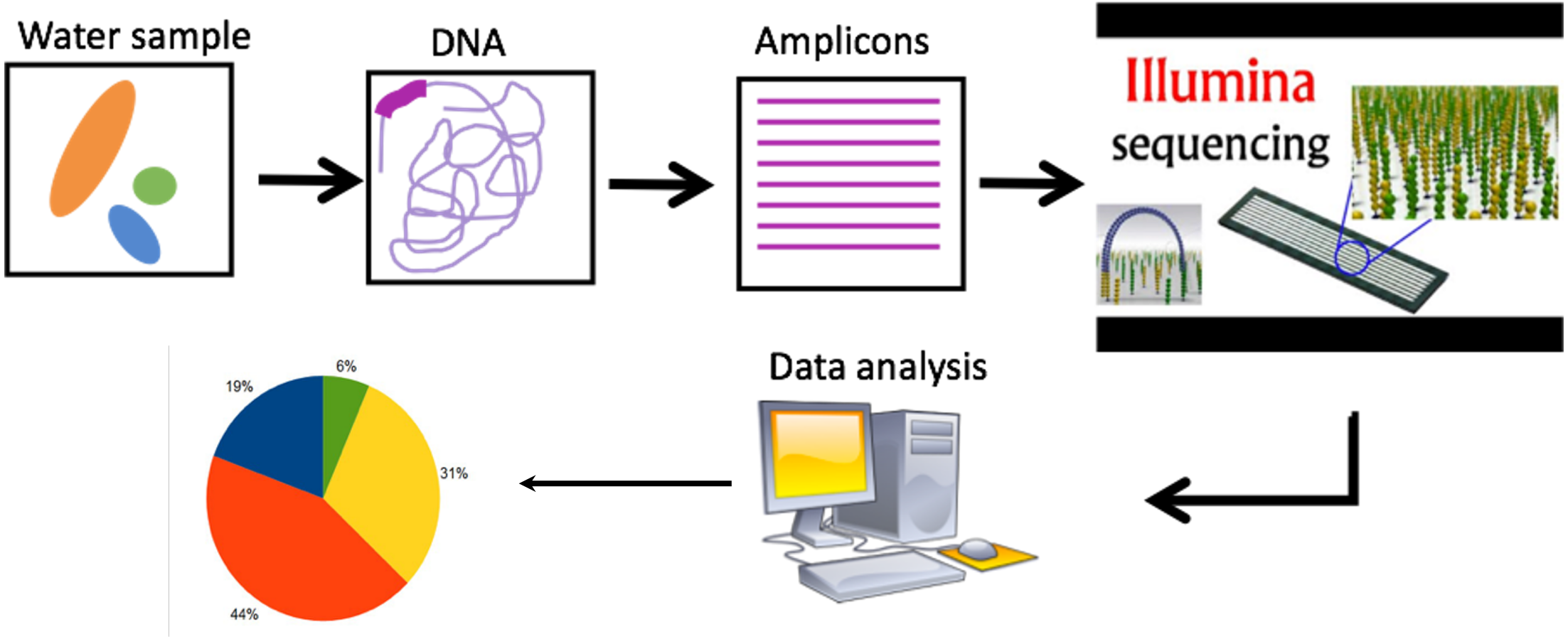

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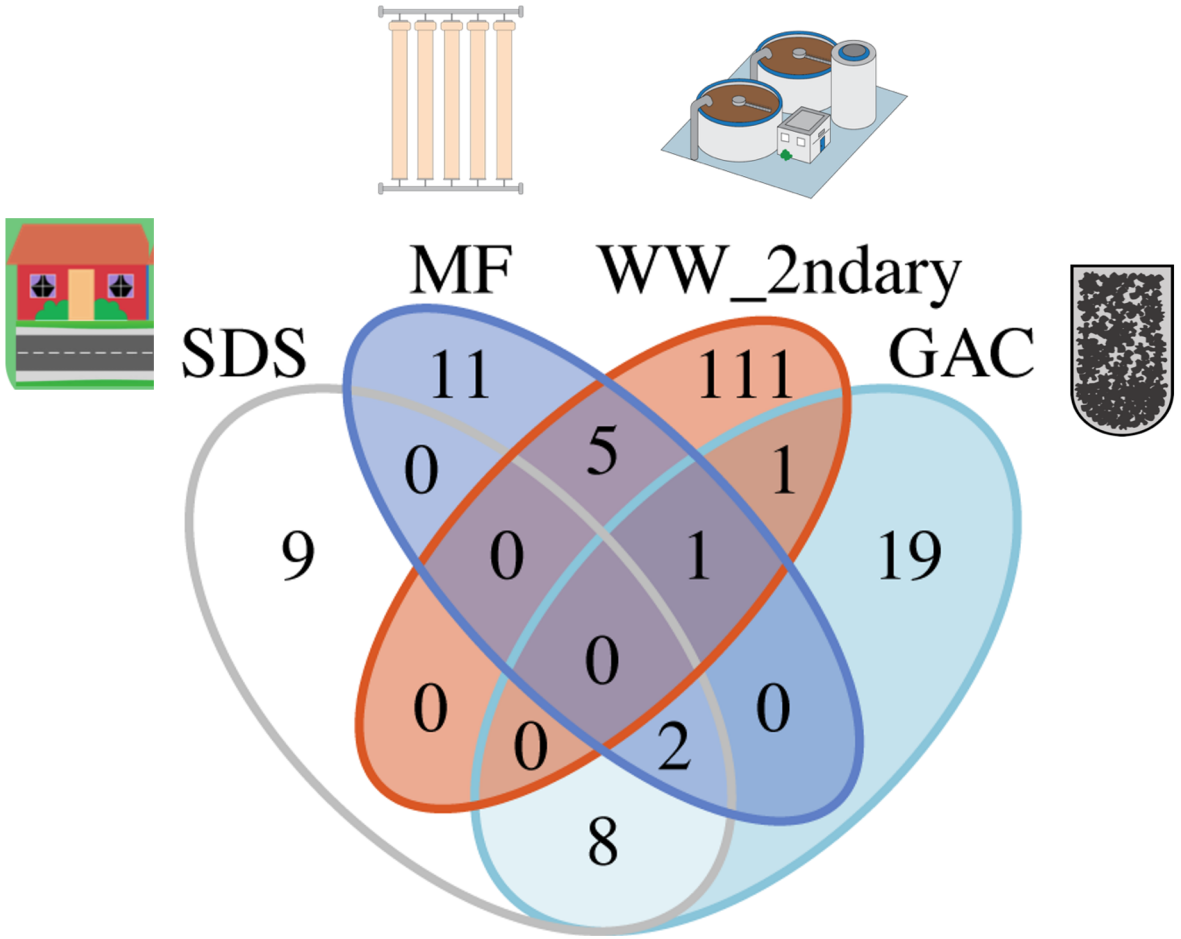

El Paso core community overlaps

Kantor et al. 2019

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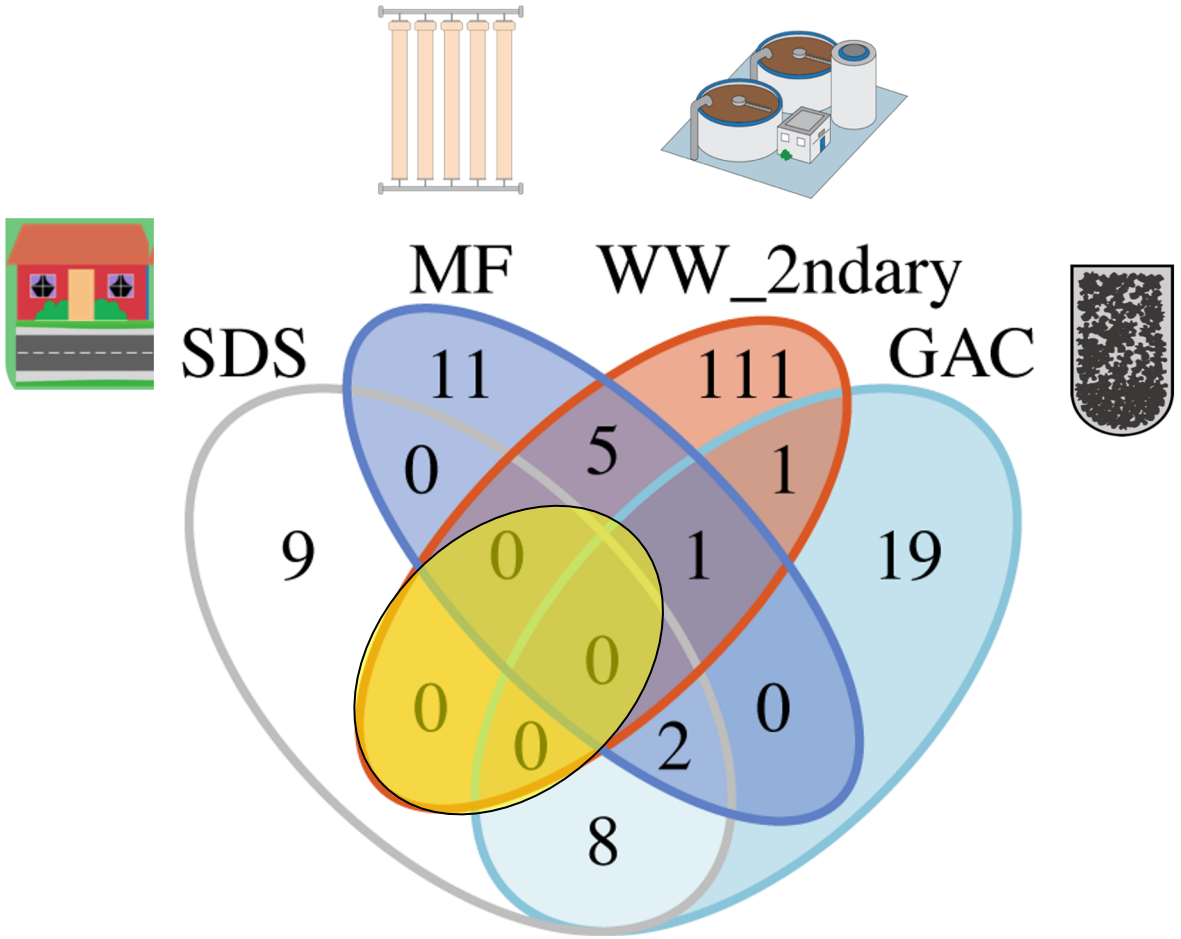

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Kantor et al. 2019

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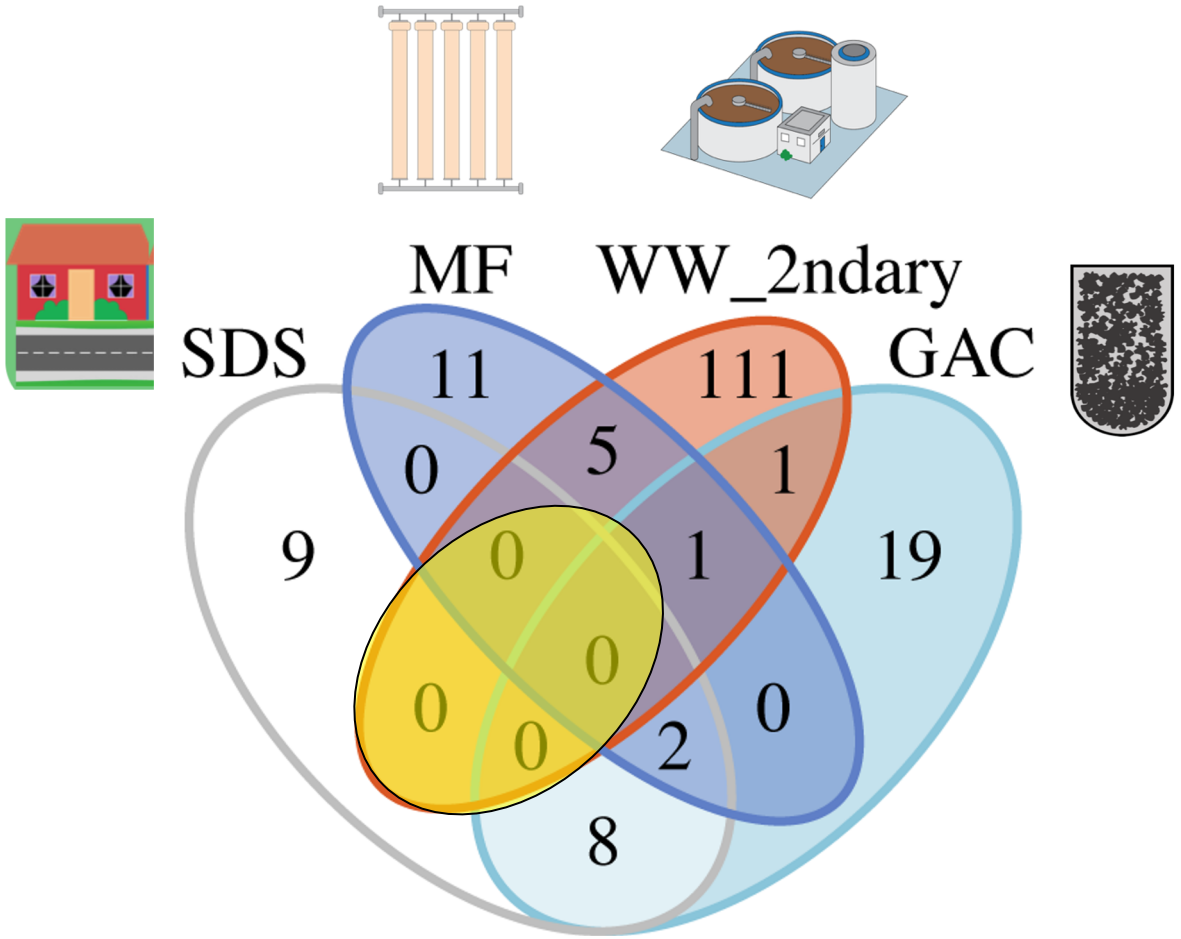
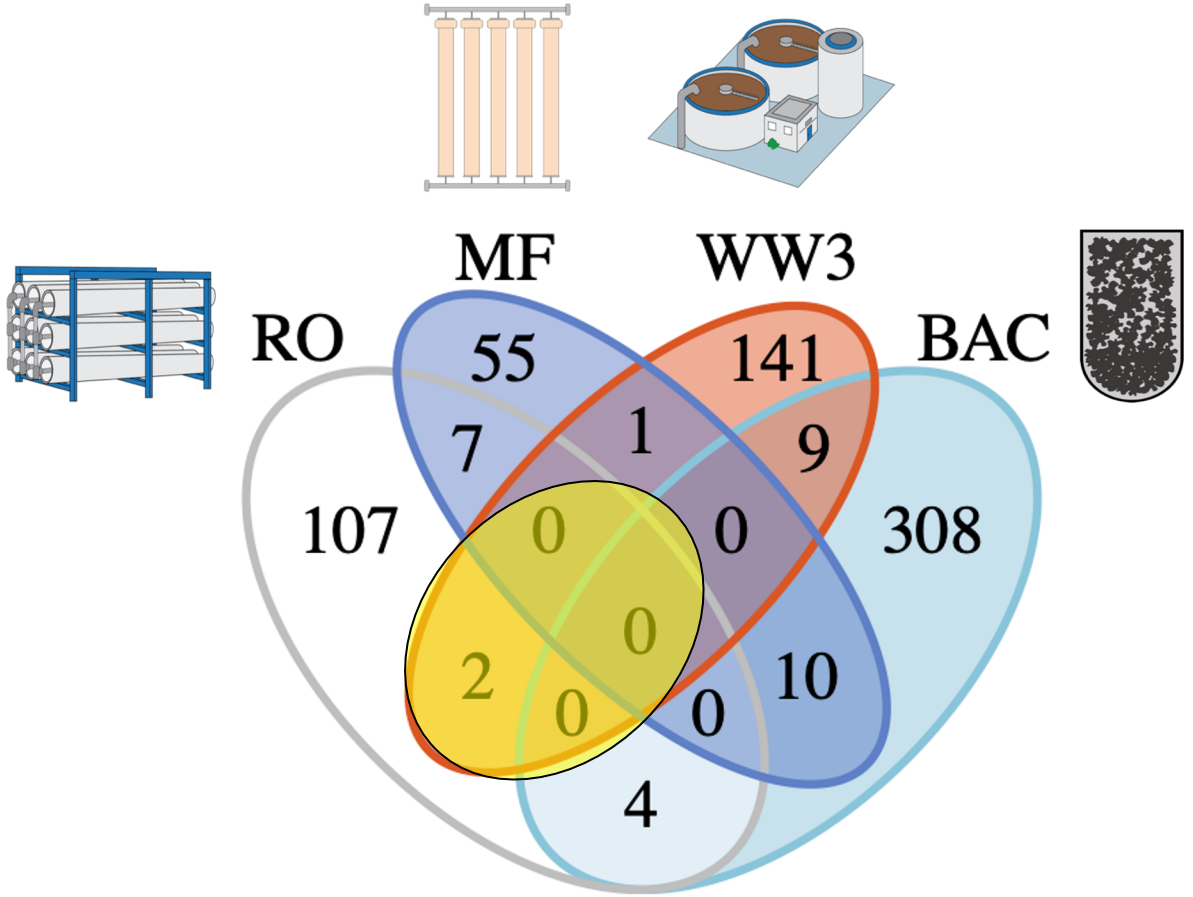


El Paso core community overlaps

Kantor et al. 2019

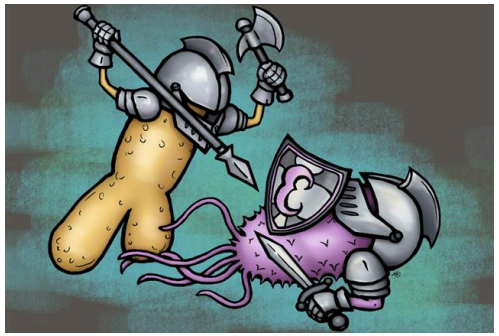


Site A core community overlaps

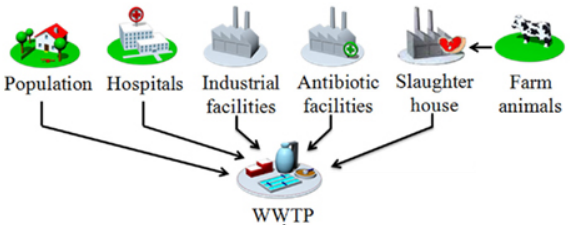
Kantor et al. unpublished

Summary

- Rise of antibiotic resistance (arms race)
  - ARGs are present at background levels in pristine environments
  - We are most concerned when they are in pathogens

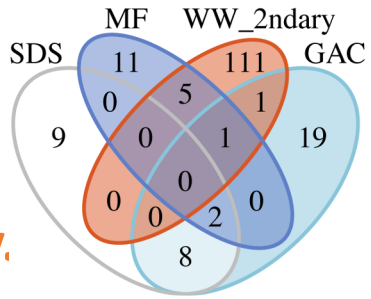


- Wastewater is a “hotspot” for ARGs



- Methods: flow cytometry, qPCR, DNA sequencing

- Removal of bacteria and DNA through treatment:
  - How well does treatment remove bacteria? **Very well.**
  - How well does treatment remove ARGs? **Very well.**
  - Are the SAME bacteria present before and after treatment? **No/not many.**



- Concluding thought: drinking water distribution is not sterile

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--Arcadis U.S.--
Priscilla Sandoval

--Carollo--
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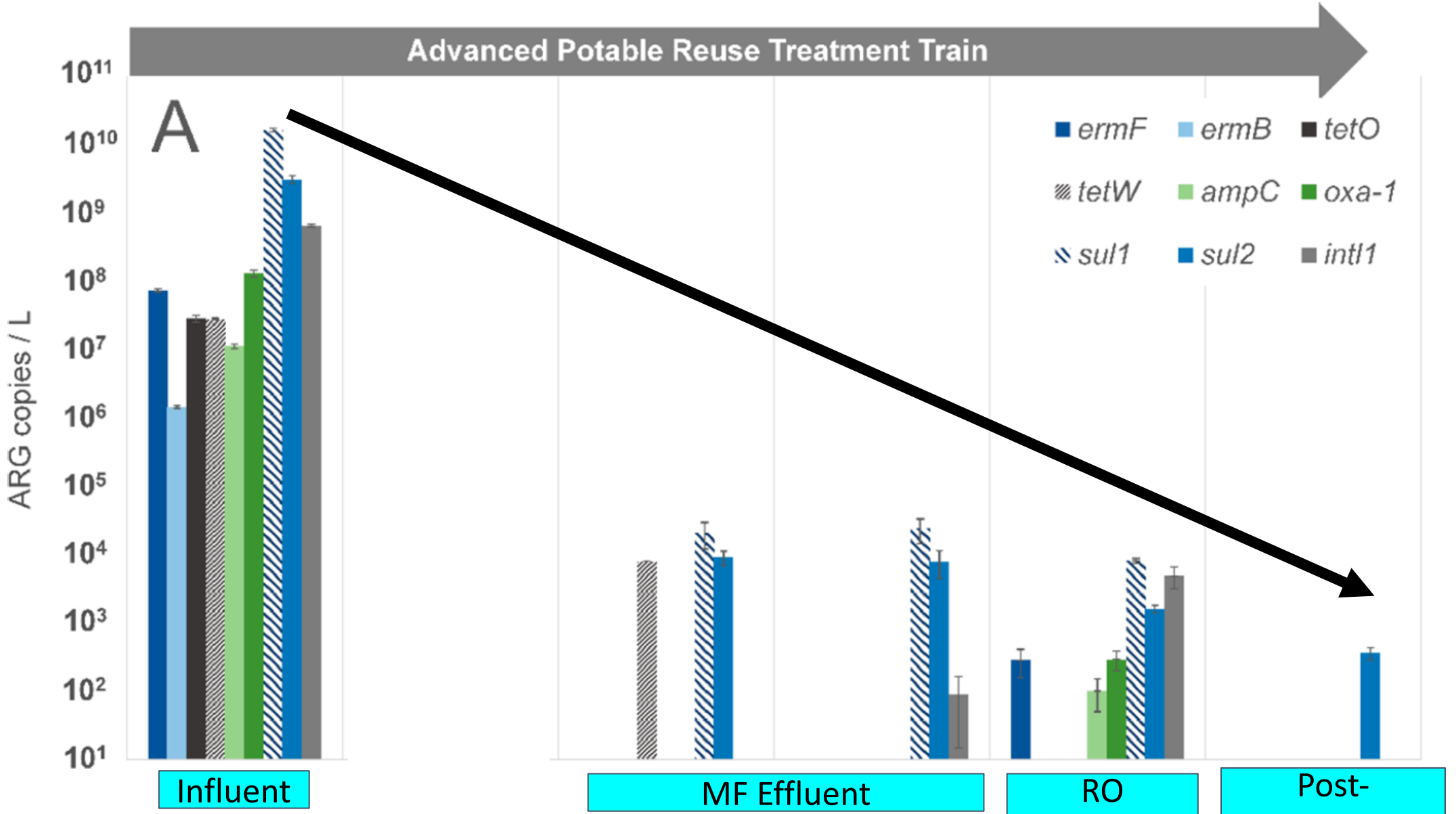
Corin Marron

Questions?

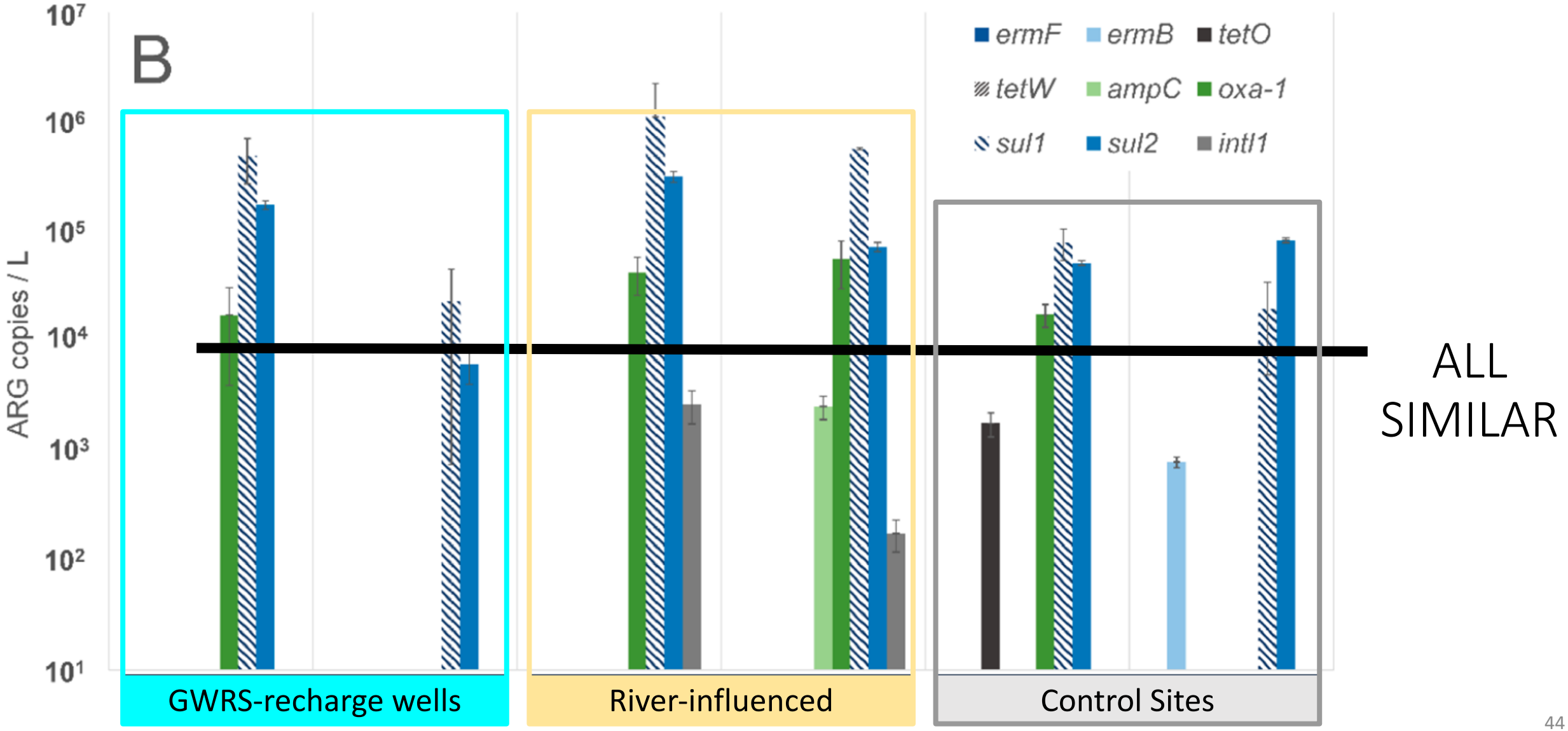
Appendix

ARGs through Orange County GWRS



Harb et al. 2019 (OCWD)

ARGs through Orange County GWRS



Harb et al. 2019 (OCWD)