INTERNATIONAL SYMPOSIUM
POTABLE REUSE
AUSTIN, TEXAS | JANUARY 22–23, 2018

The limitations of common molecular techniques for water reuse microbiology

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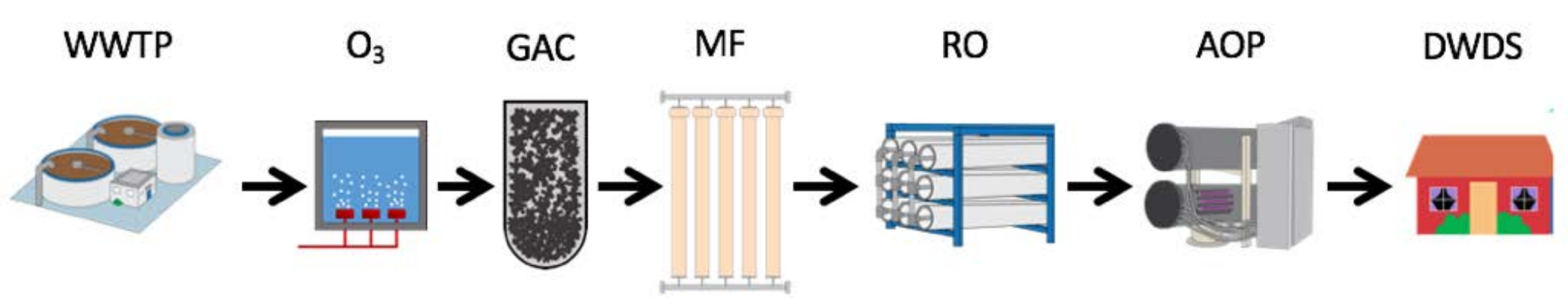
Talk outline

1. Motivation: why do we care about bacteria?
2. Methods: what tools are available?
3. Amplicon sequencing: the juicy details
4. Results from sequencing at advanced treatment pilot
5. Complementary methods

Motivation: potable reuse microbiology



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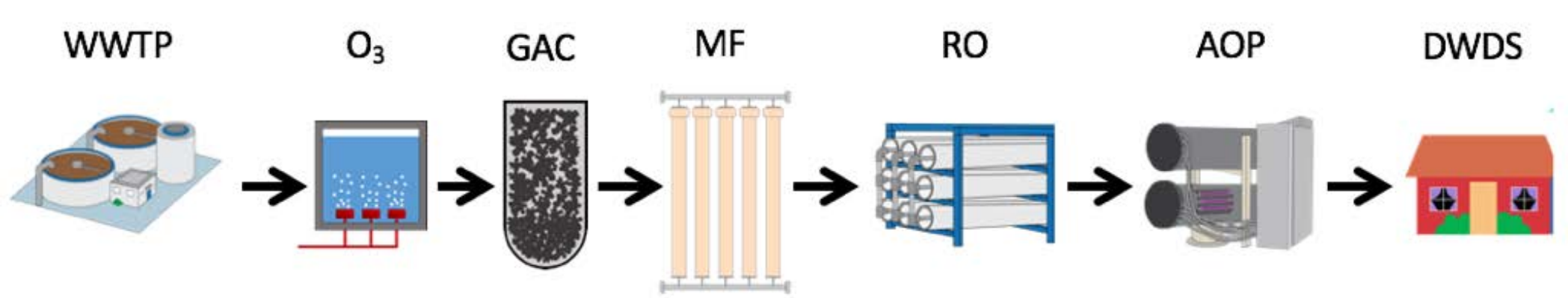
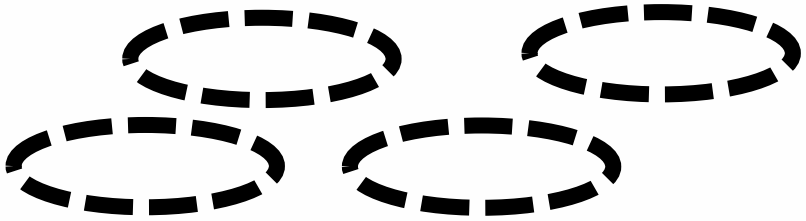


- What types of microorganisms are present and in what quantities?
- Where might the bacteria come from? (source tracking)

Non-molecular methods



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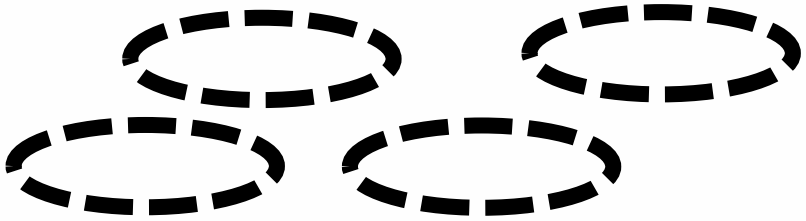
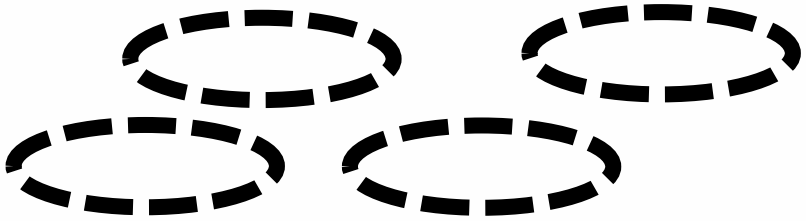


- Total coliforms
- Heterotrophic plate count
- ATP
- Flow cytometry

Non-molecular methods



- Total coliforms
- Heterotrophic plate count
- ATP
- Flow cytometry

Molecular methods

- PCR and qPCR
- 16S rRNA gene amplicon sequencing
- Metagenomics (shotgun sequencing)

Molecular methods

Targeted quantification
Low quantities of DNA

←

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- 16S rRNA gene amplicon sequencing
- Metagenomics (shotgun sequencing)

Molecular methods

Targeted quantification
Low quantities of DNA

← • PCR and qPCR

Broad identification
Low quantities of DNA

← • 16S rRNA gene amplicon sequencing

• Metagenomics (shotgun sequencing)

Molecular methods

Targeted quantification
Low quantities of DNA ← • PCR and qPCR

Broad identification
Low quantities of DNA ← • 16S rRNA gene amplicon sequencing

Broadest identification
Higher quantities of DNA ← • Metagenomics (shotgun sequencing)

Molecular methods

Targeted quantification
Low quantities of DNA ← • PCR and qPCR

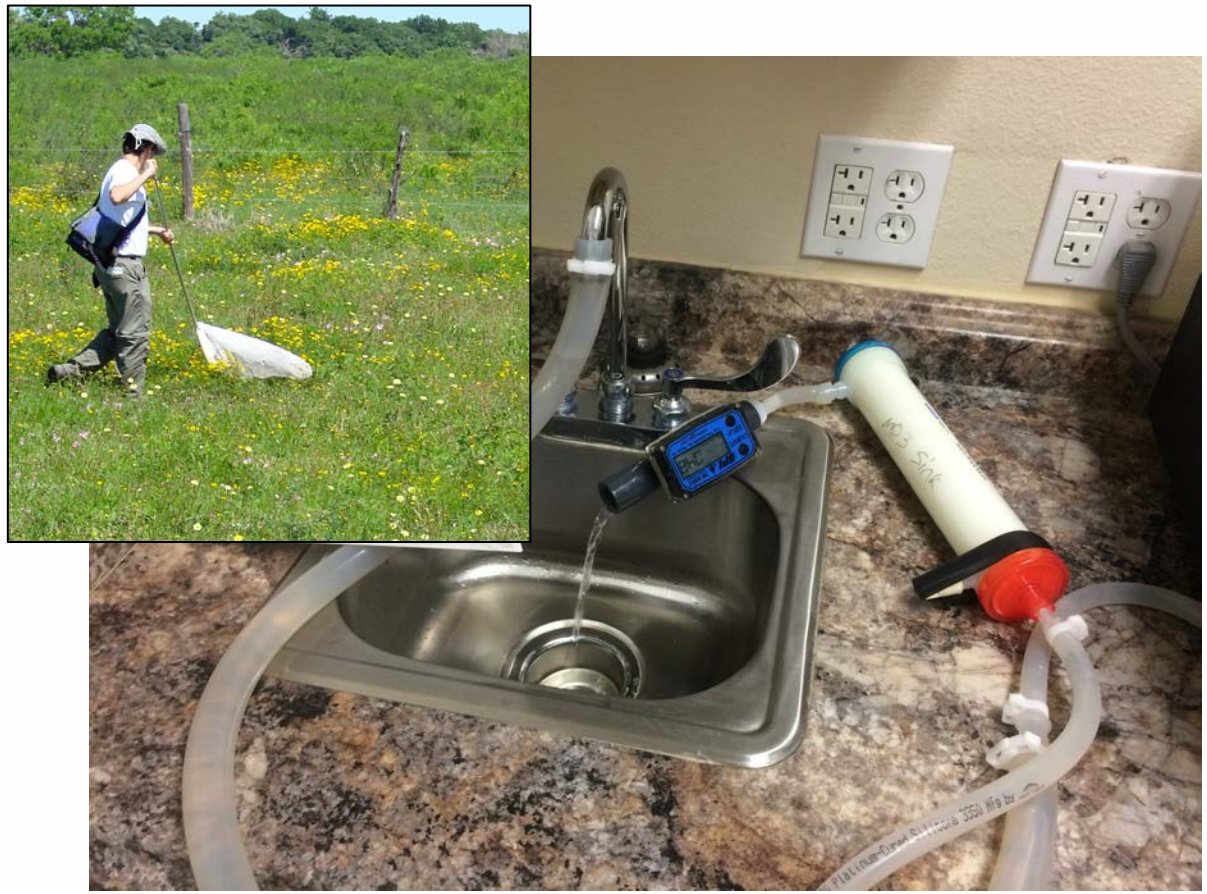
Broad identification
Low quantities of DNA ← **• 16S rRNA gene amplicon sequencing**

Broadest identification
Higher quantities of DNA ← • Metagenomics (shotgun sequencing)

Amplicon sequencing is like bug collecting

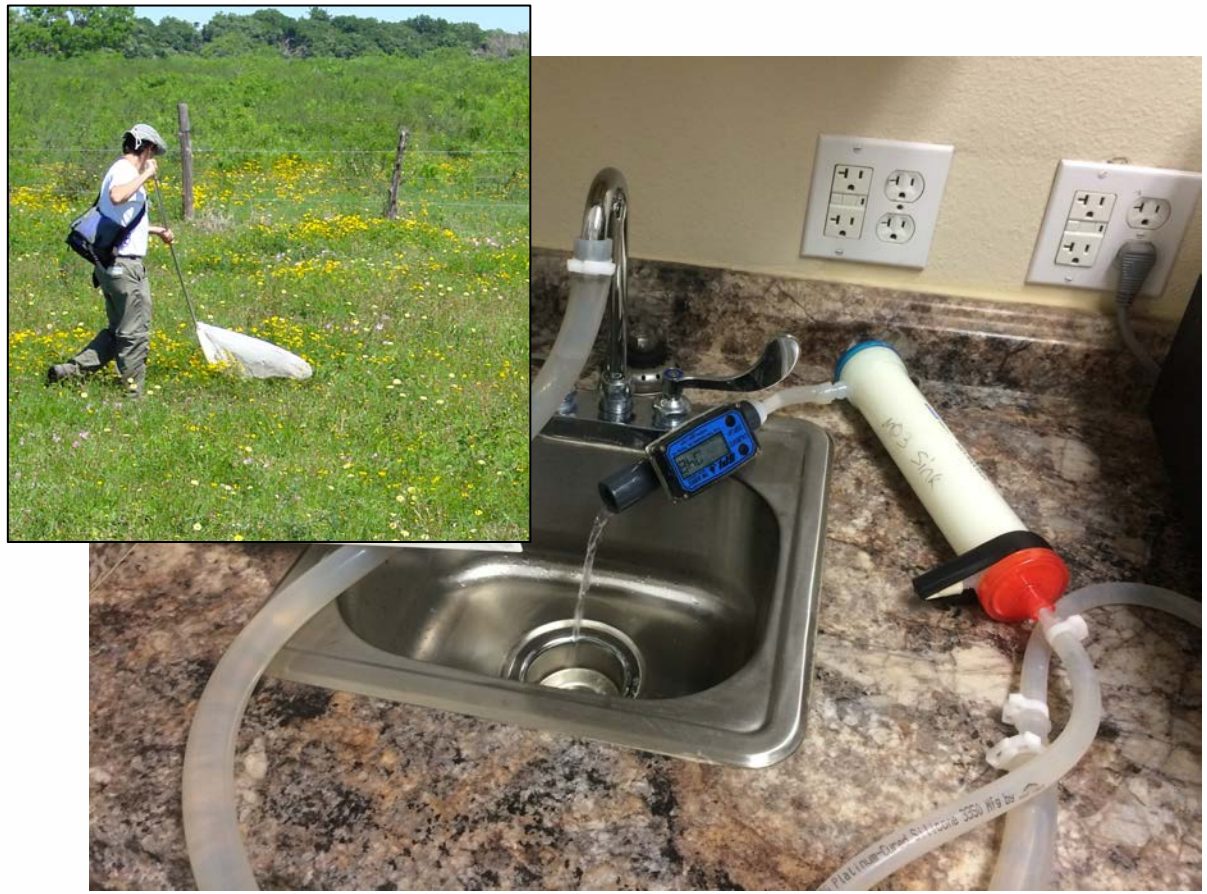
https://6legs2many.wordpress.com/techniques/tools-for-insect-collecting/
https://www.bugsdirect.com/products/real-butterfly-collection-all-natural-butterflies-mounted-under-glass-in-a-wall-hanging-frame-taxidermy-butterfly-art-804

Amplicon sequencing is like bug collecting

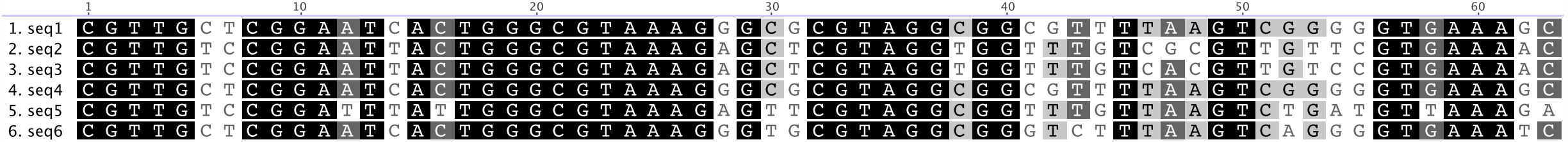


?

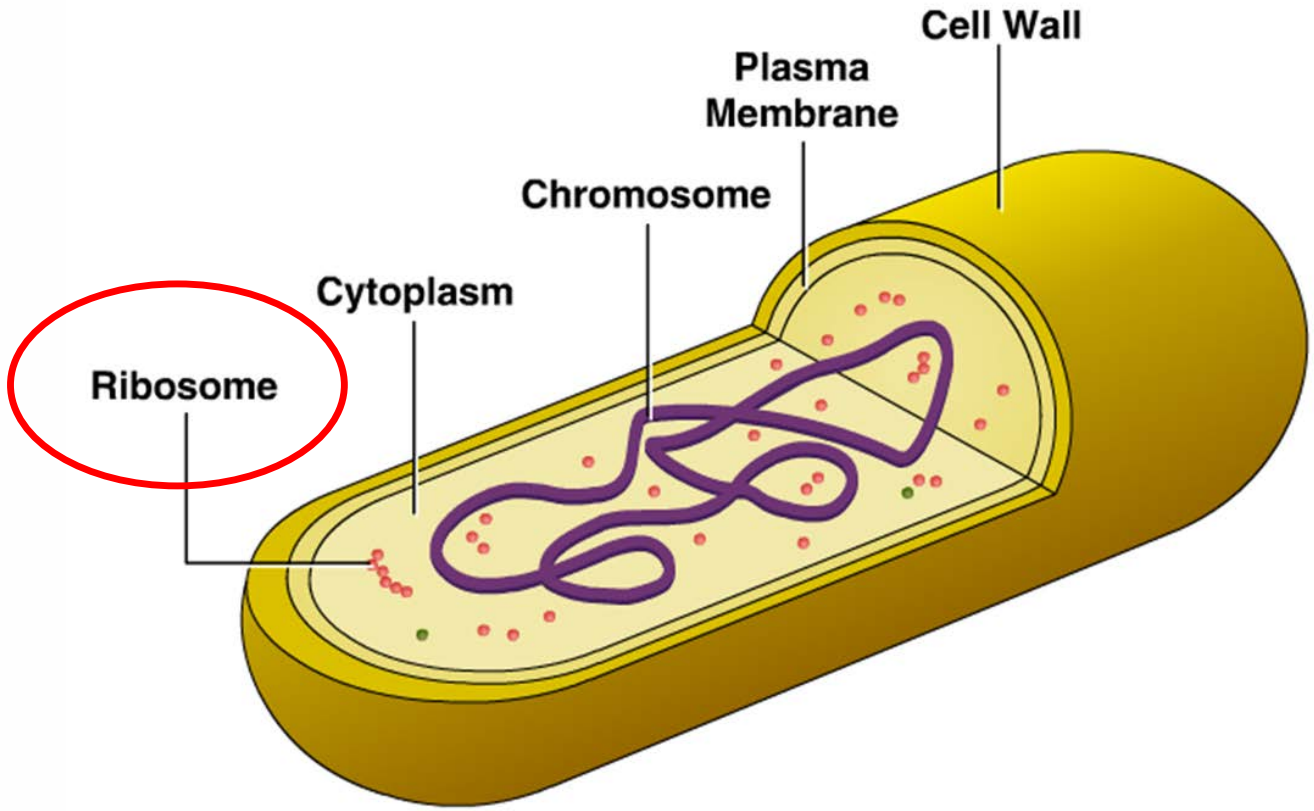
Amplicon sequencing is like bug collecting



DNA sequences can be used for identification



Amplicon sequencing is based on ribosomal RNA genes



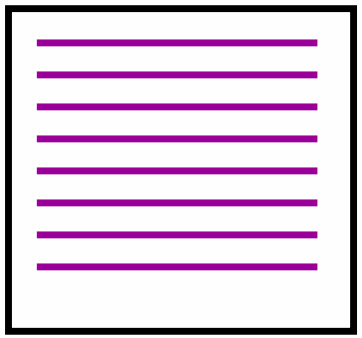
http://innovicmedical.blogspot.com/2013/05/bacterial-cell-structure.html

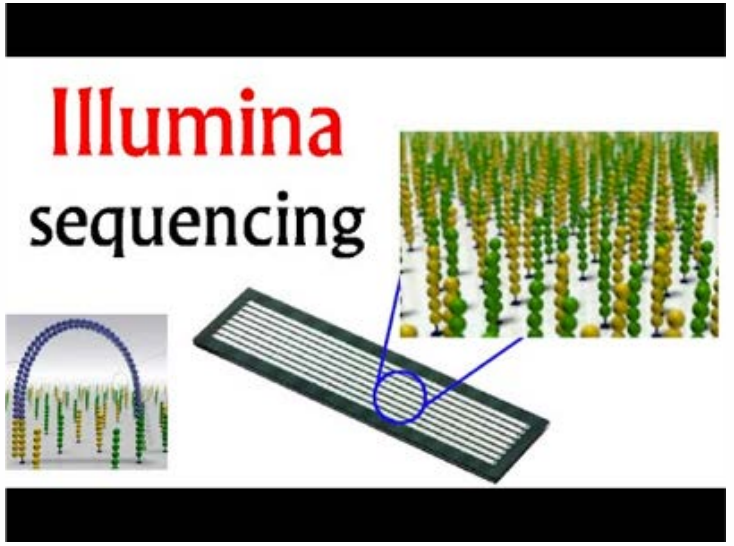
Amplicon sequencing process

Water sample

DNA

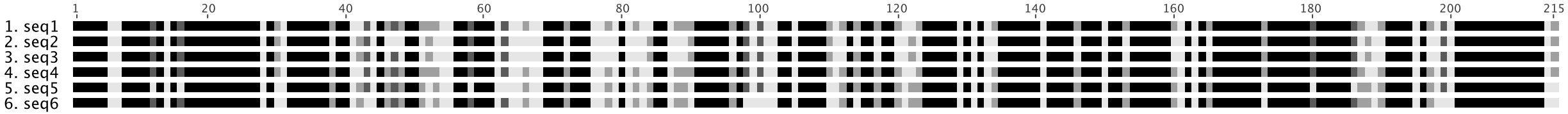
Amplicons



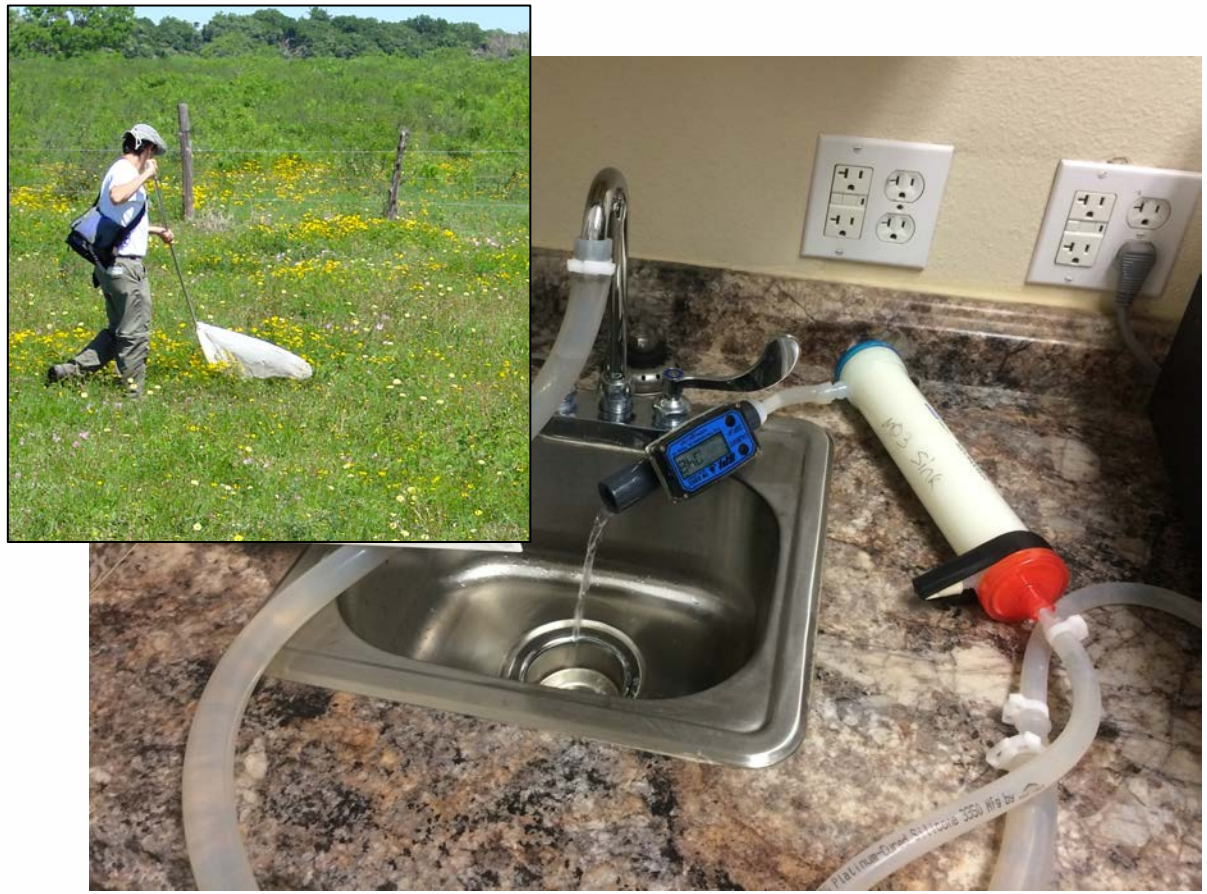


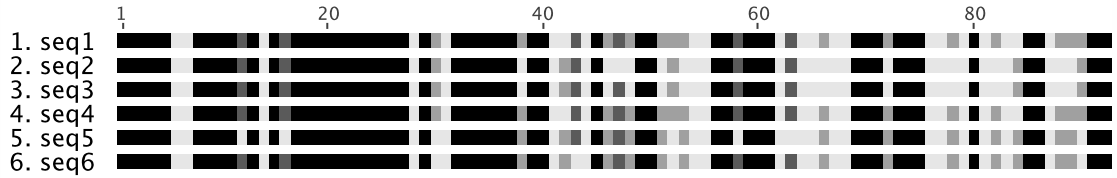
Data analysis

16S rRNA genes show relatedness of bacteria

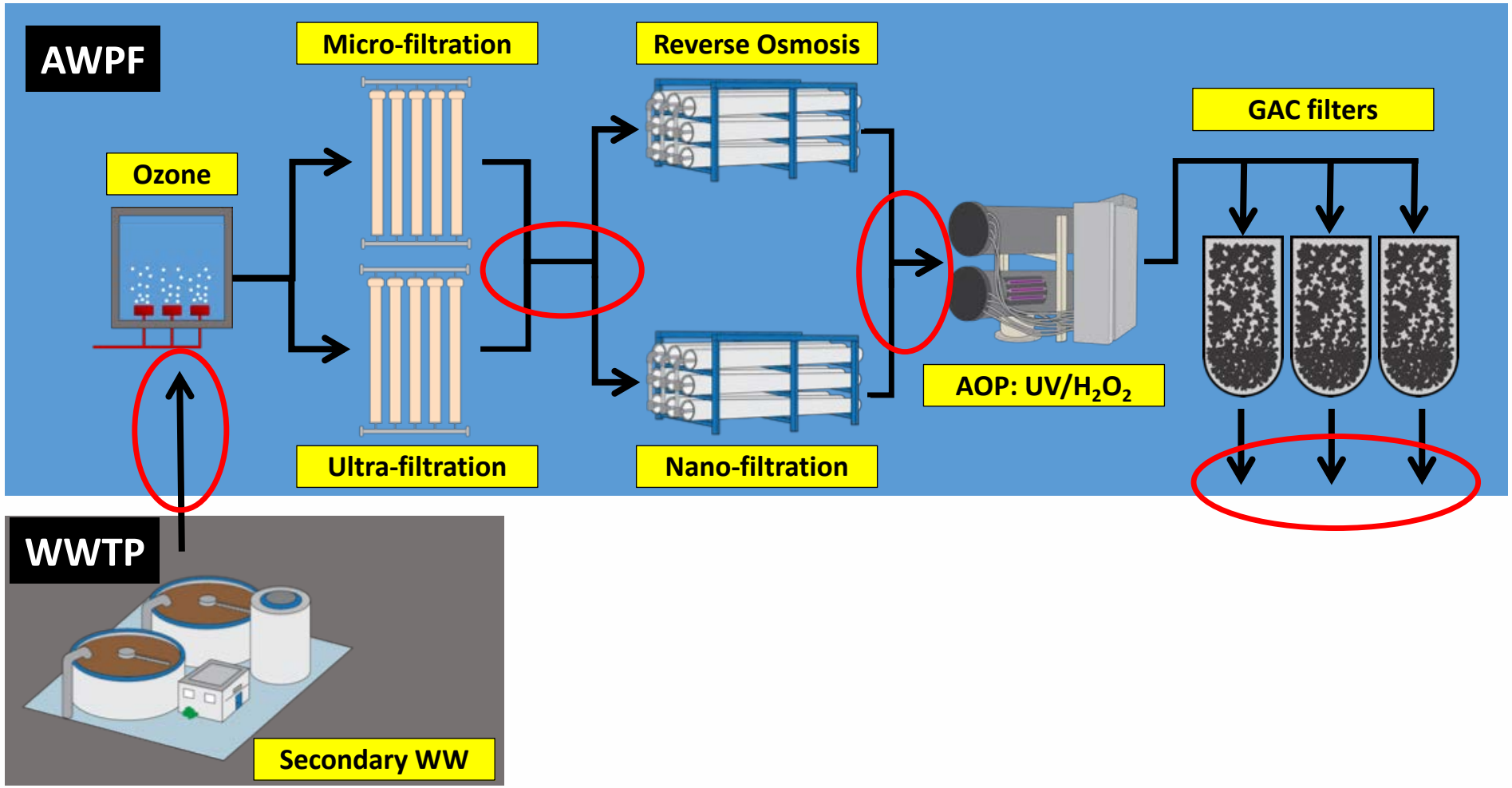


Identification via amplicon sequencing

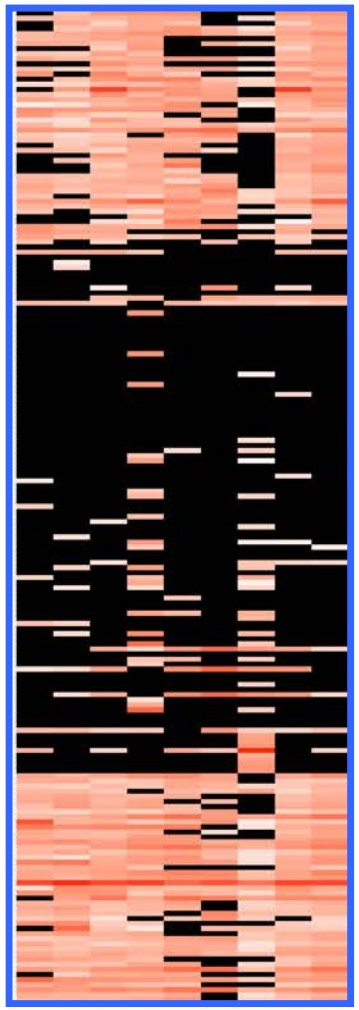




Amplicon seq. on El Paso DPR treatment train



Results: amplicon seq. across treatment train



Unique sequences

Samples

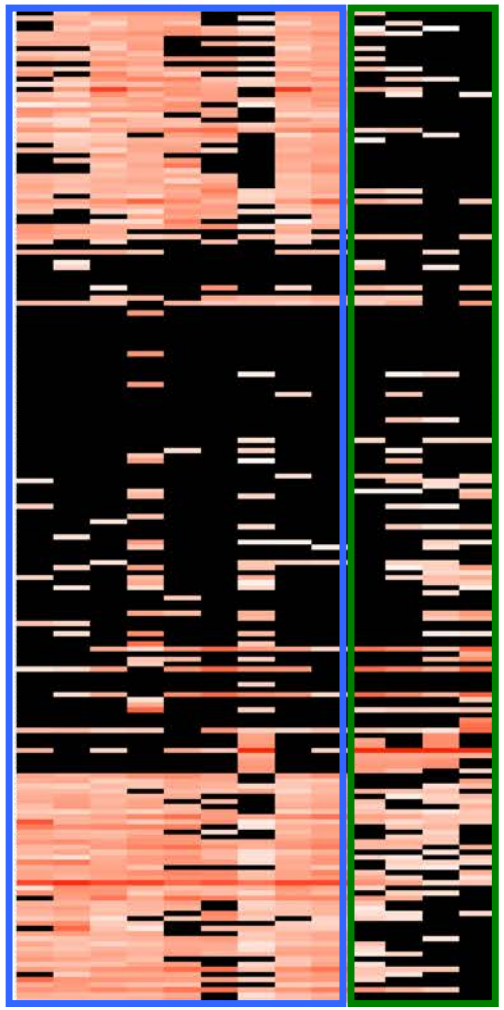
Influent samples

Relative abundance

High

Low

Results: amplicon seq. across treatment train



Unique sequences

Microfiltration effluent

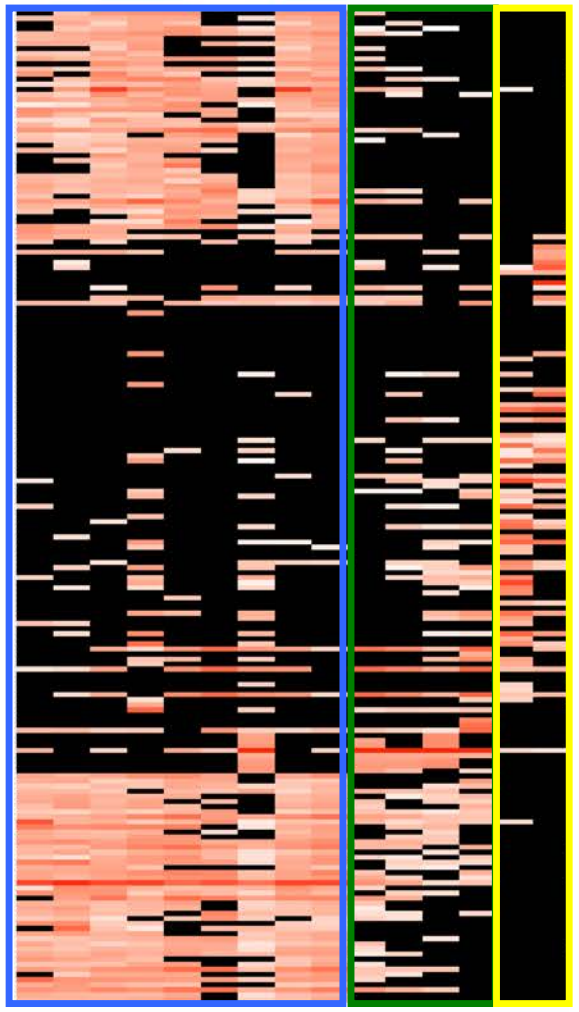
Relative abundance

High

Low

Samples

Results: amplicon seq. across treatment train



Unique sequences

Nanofiltration / RO effluent

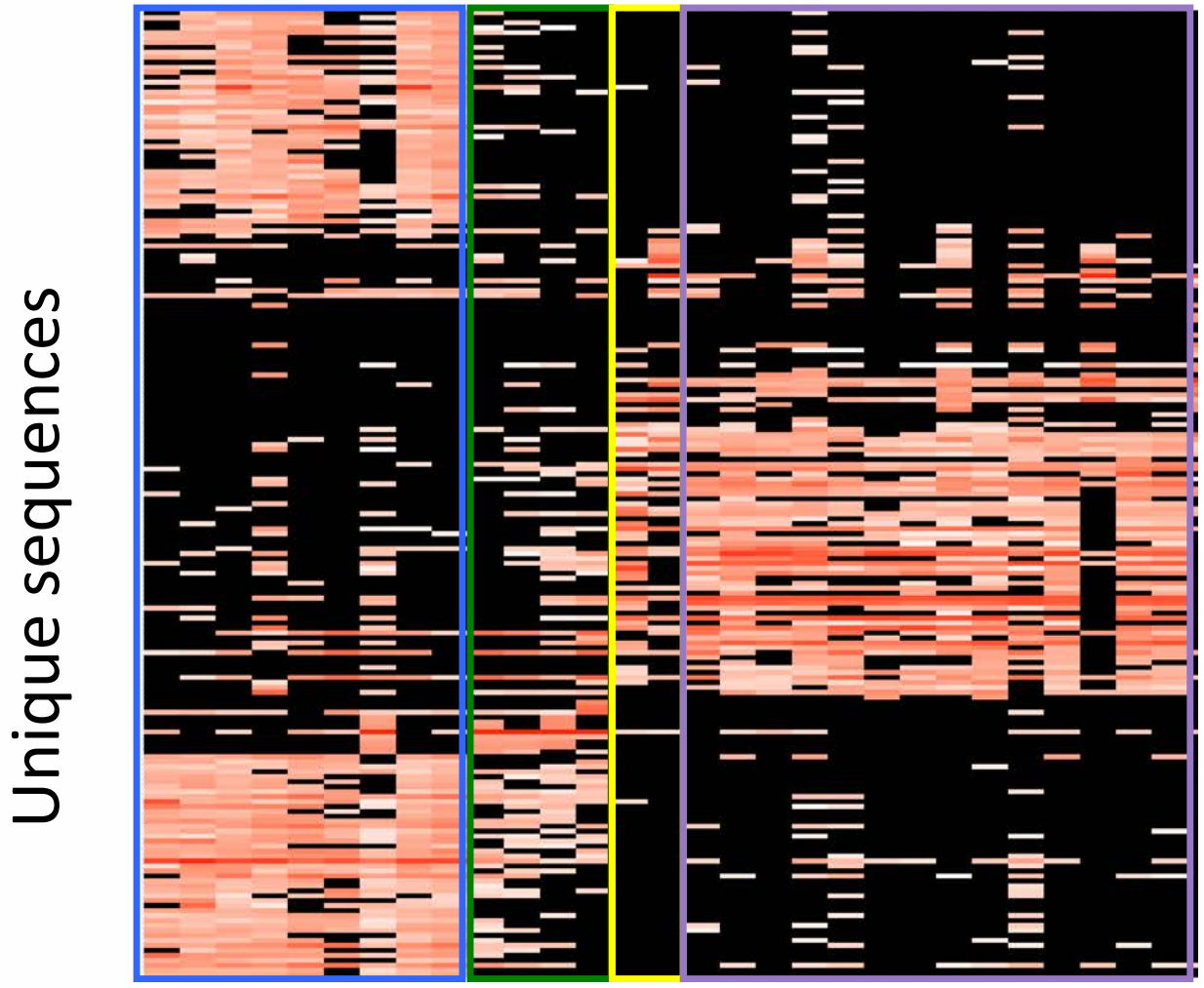
Relative abundance

High

Low

Samples

Results: amplicon seq. across treatment train



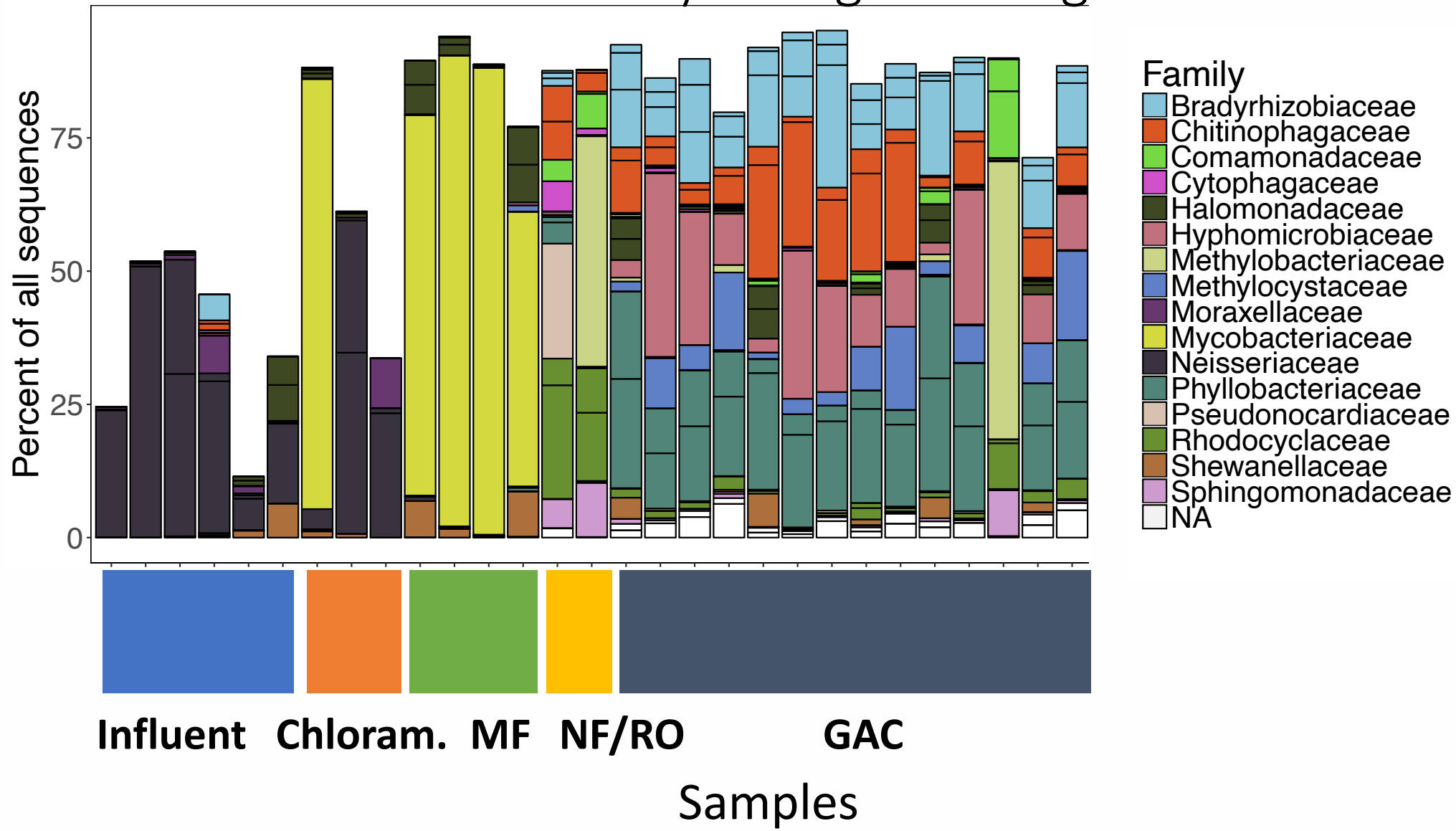
GAC filter effluent

Relative abundance

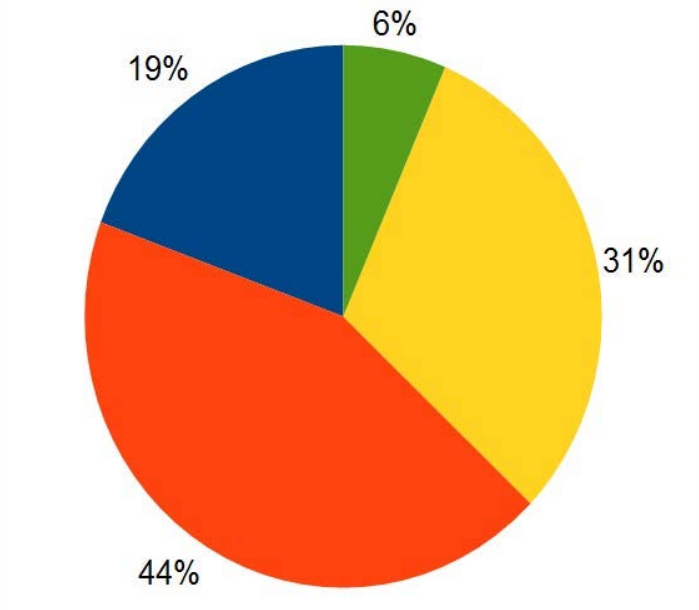
High

Low

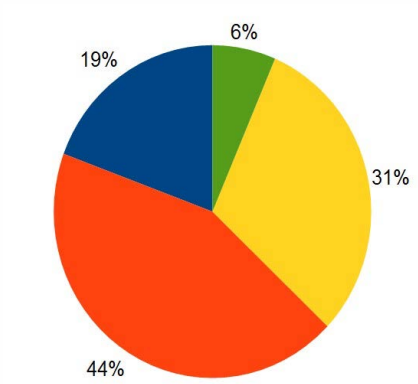
Results: microbial community changes during treatment



Limitation 1: Relative vs. Absolute Abundance



Influent

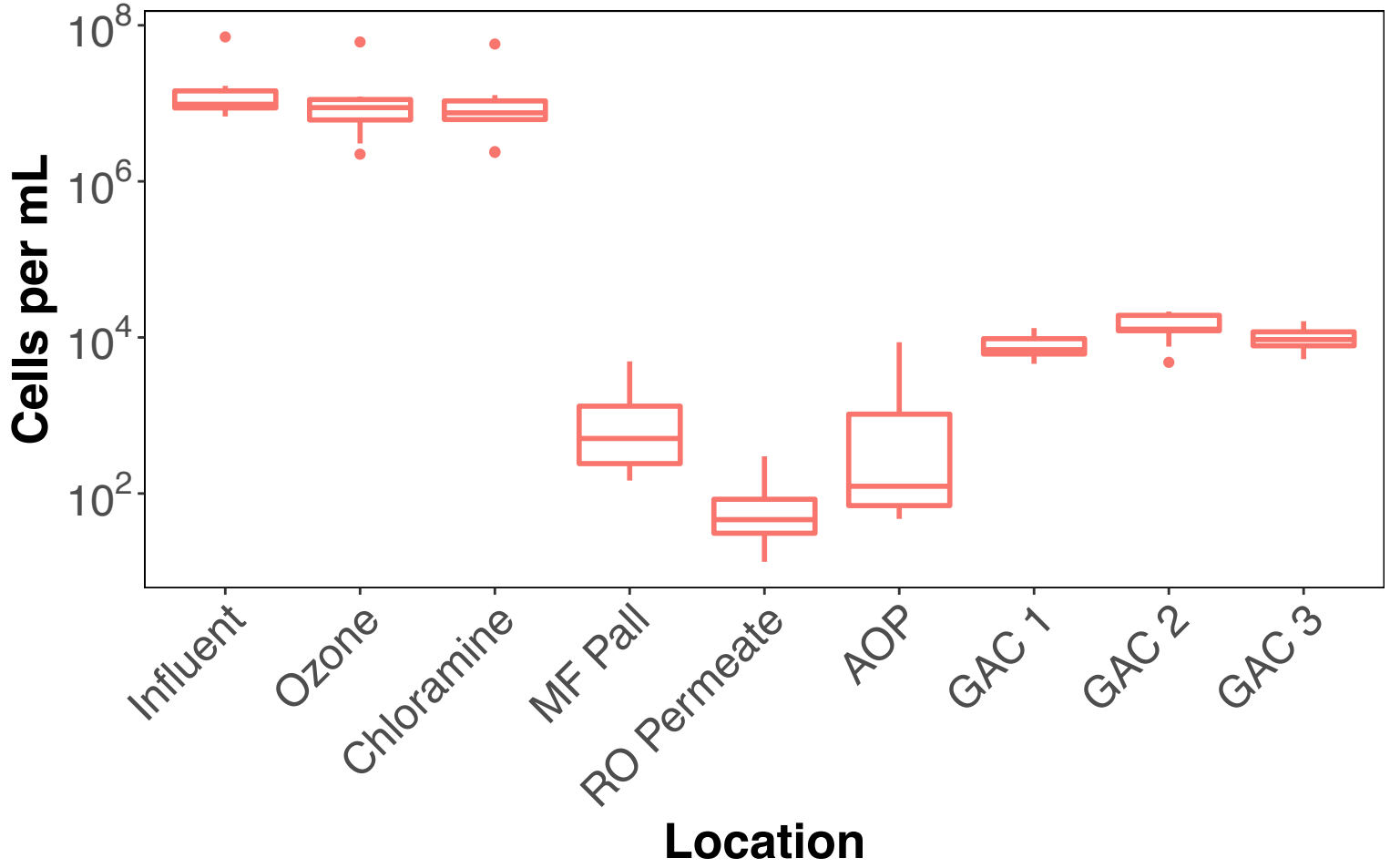


MF effluent

RO effluent

Absolute quantification

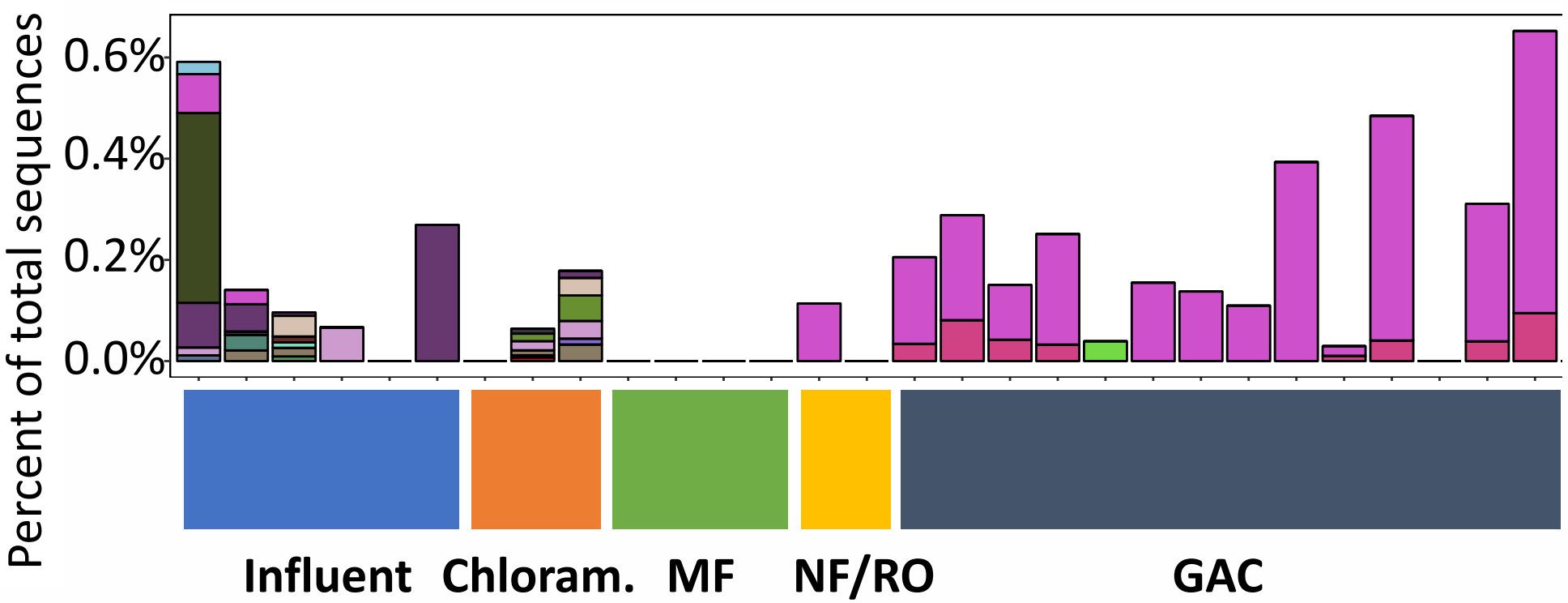
Cell counts via flow cytometry



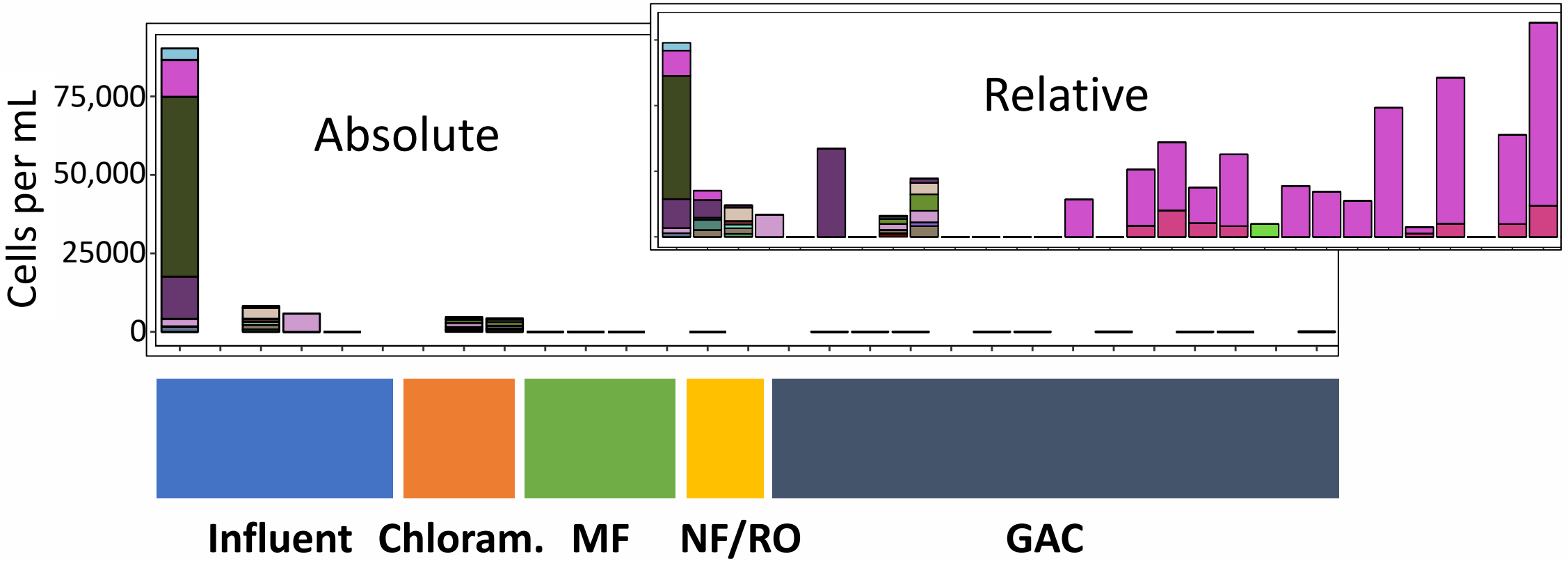
Limitation 2: Specificity

- Most data analysis programs don’t determine to species-level
- 16S amplicon sequencing is usually too short to distinguish **strains**
- Recommend qPCR (specific for pathogen)

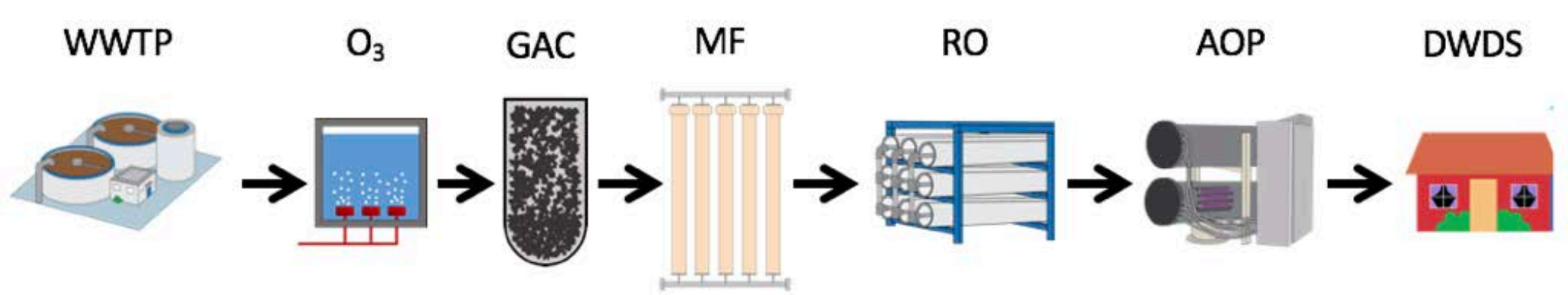
Legionella (all species): relative abundance



Legionella (all species): absolute abundance

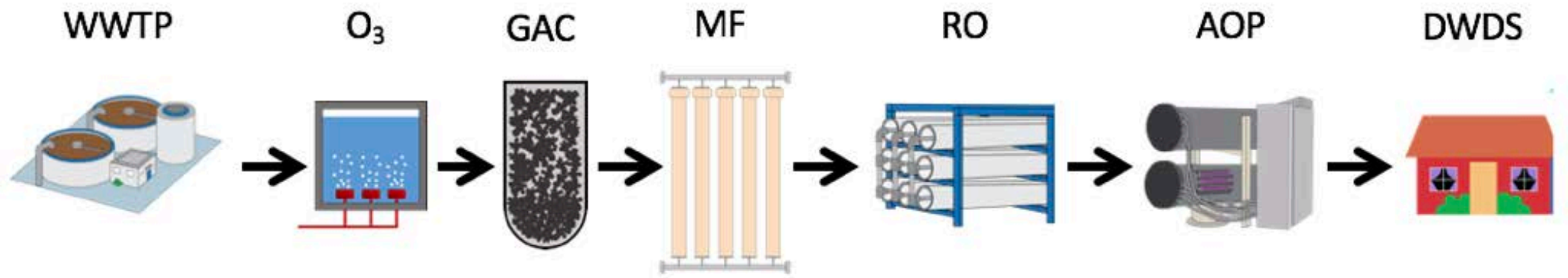


Motivation: potable reuse microbiology



- What types of microorganisms are present and in what quantities?
  - **Predominant bacteria *change* across treatment train**

Motivation: potable reuse microbiology



- Where might the bacteria come from? (source tracking)
  - **They may start out in place, *not sourced* from upstream**
  - **They may start out too rare to detect and then survive treatment and grow**

Molecular methods with highly purified water: Controls matter

- Want high signal:noise ratio to prevent contamination
- Negative / blank
- Positive / DNA from known microorganisms

Take-home messages

- Amplicon sequencing: insight into patterns within a system
- Controls are critical
- Can’t detect pathogens with specificity (use qPCR)
- Should also include an absolute measure of biomass and viability or growth assays
- For functional analyses, should use metagenomics

Costs

- Amplicon sequencing: $5-17 per sample (other groups' estimates)
  - For highly purified water, allow 25-50% repeat rate (or filter extensively)
  - **We estimated $18 per sample**
  - Sequenced in batches of 96 or 384, depending on desired detection limit
  - Include controls in budget
  - Consider triplicate runs per sample
- qPCR: ~$2 per reaction, but also need calibration curve & controls
  - One assay per pathogen/ARG/other gene of interest