Advanced methods for microbial analyses of drinking water

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3/15/18

Question 1:

True or False:

Most microorganisms in water are pathogens

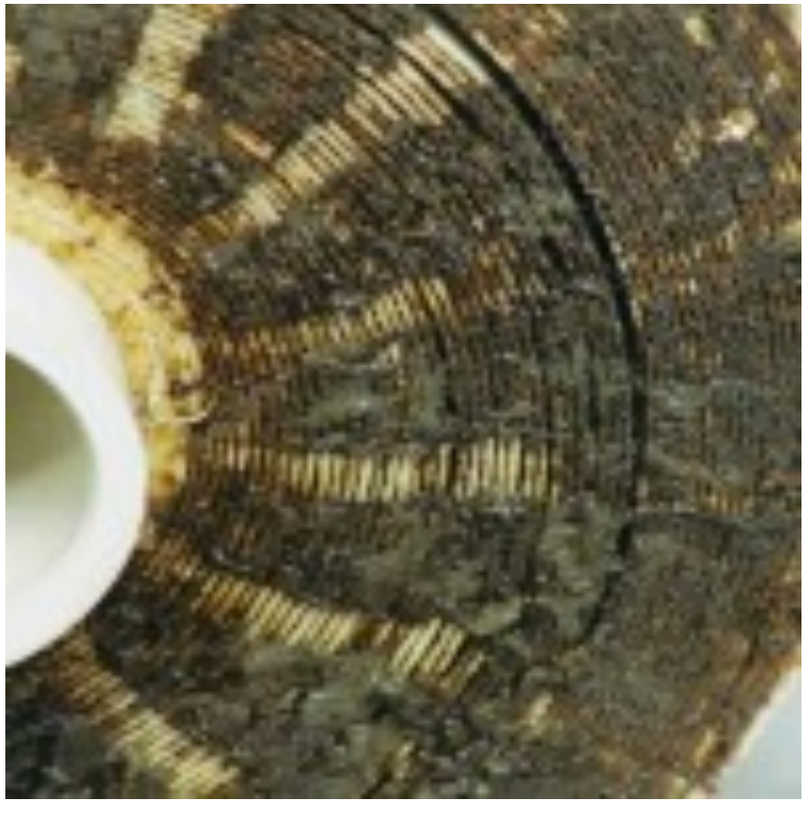
Question 2:

Why are non-pathogenic bacteria in drinking water a concern?

Non-pathogens in drinking water

Bio-corrosion

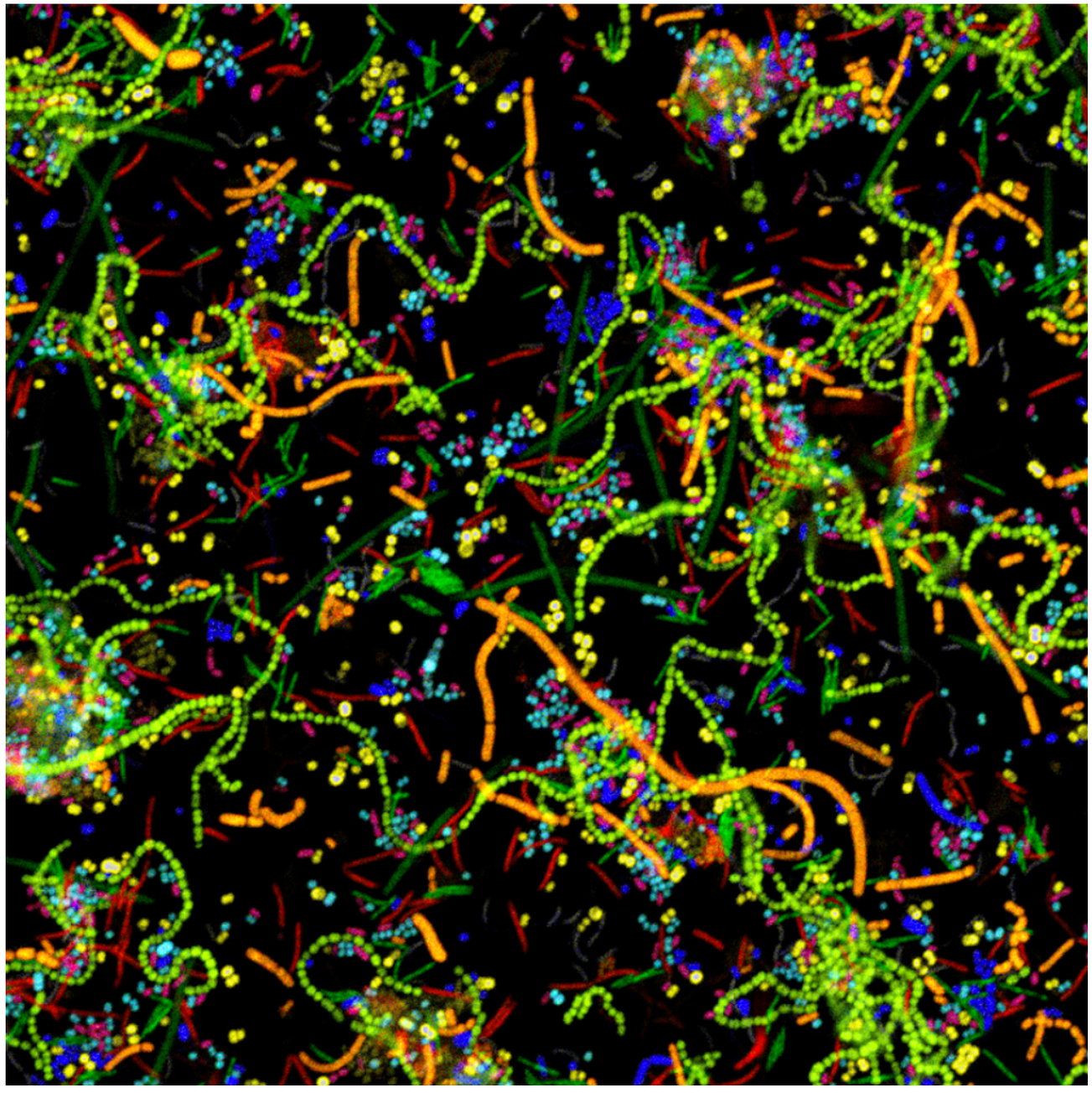
Biofouling



Biofilm

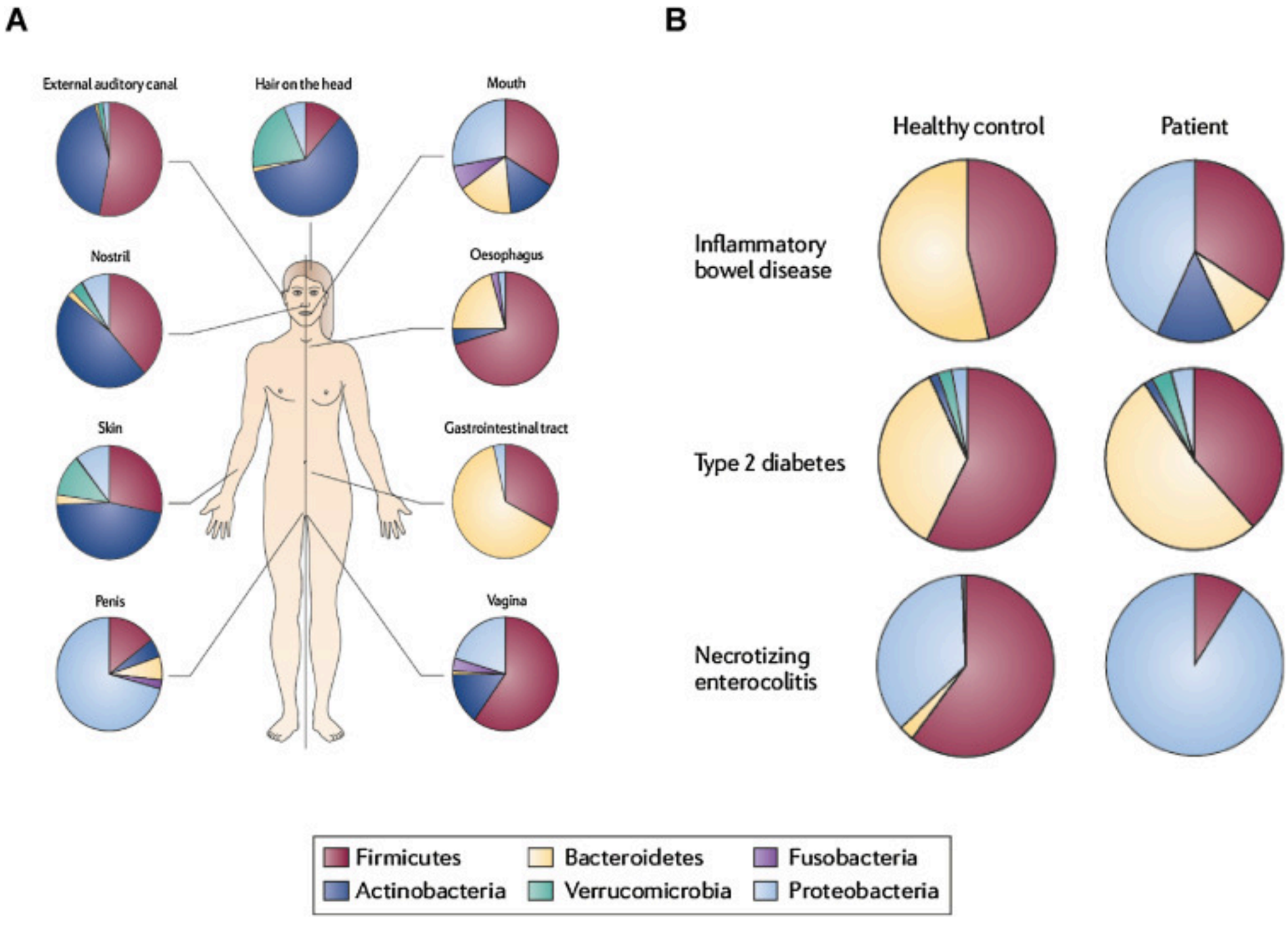
Resistance to disinfectants
Bacterial growth
Sharing antibiotic resistance genes?

Microbial communities



http://comm.archive.mbl.edu/news/press_releases/2011/2011_pr_02_15.html

Microbiome



https://www.sciencedirect.com/science/article/pii/S189611261730038X

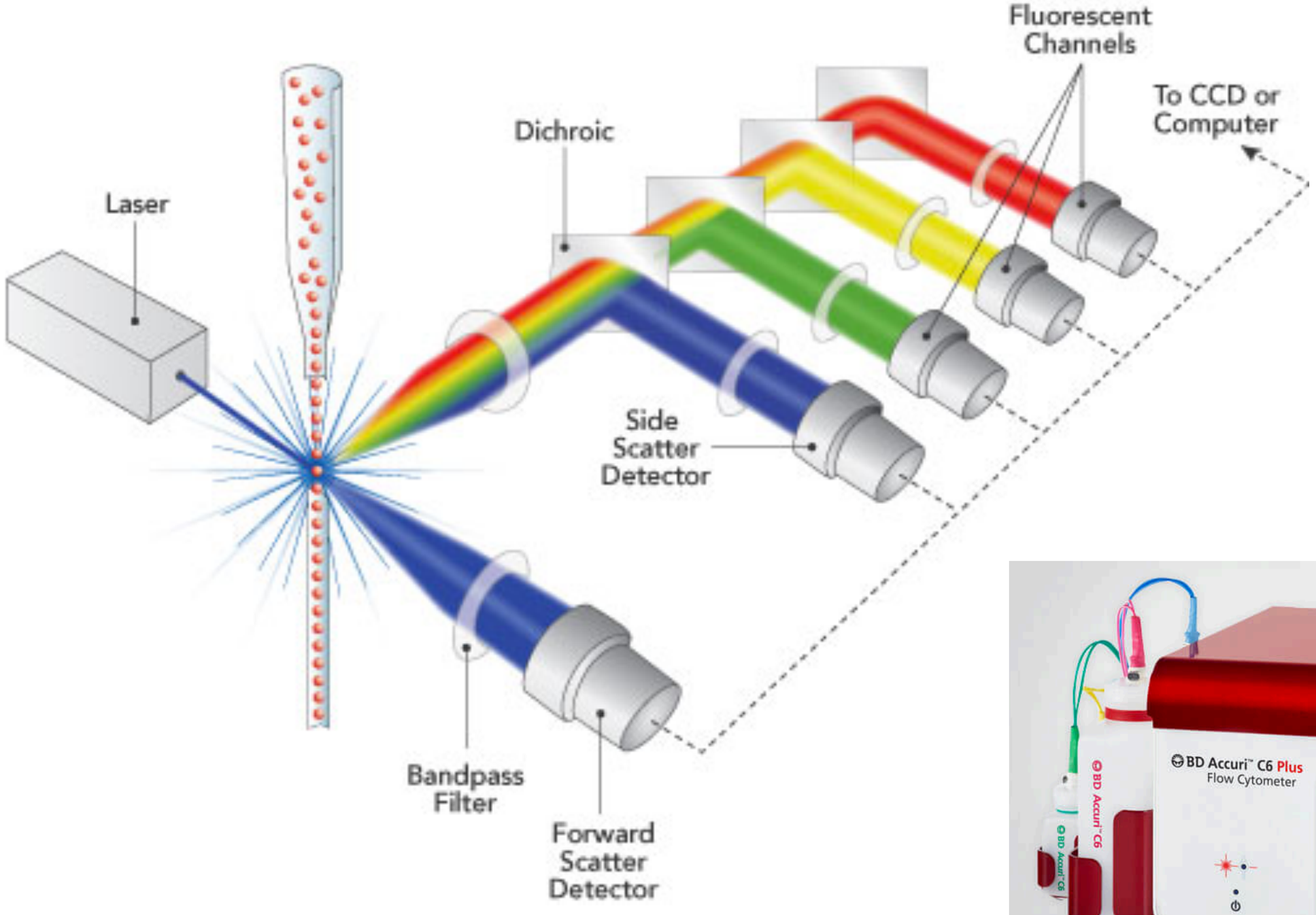
Question 3:

How many bacteria per mL are found in tap water?

PART I: Methods to *quantify* microbial communities

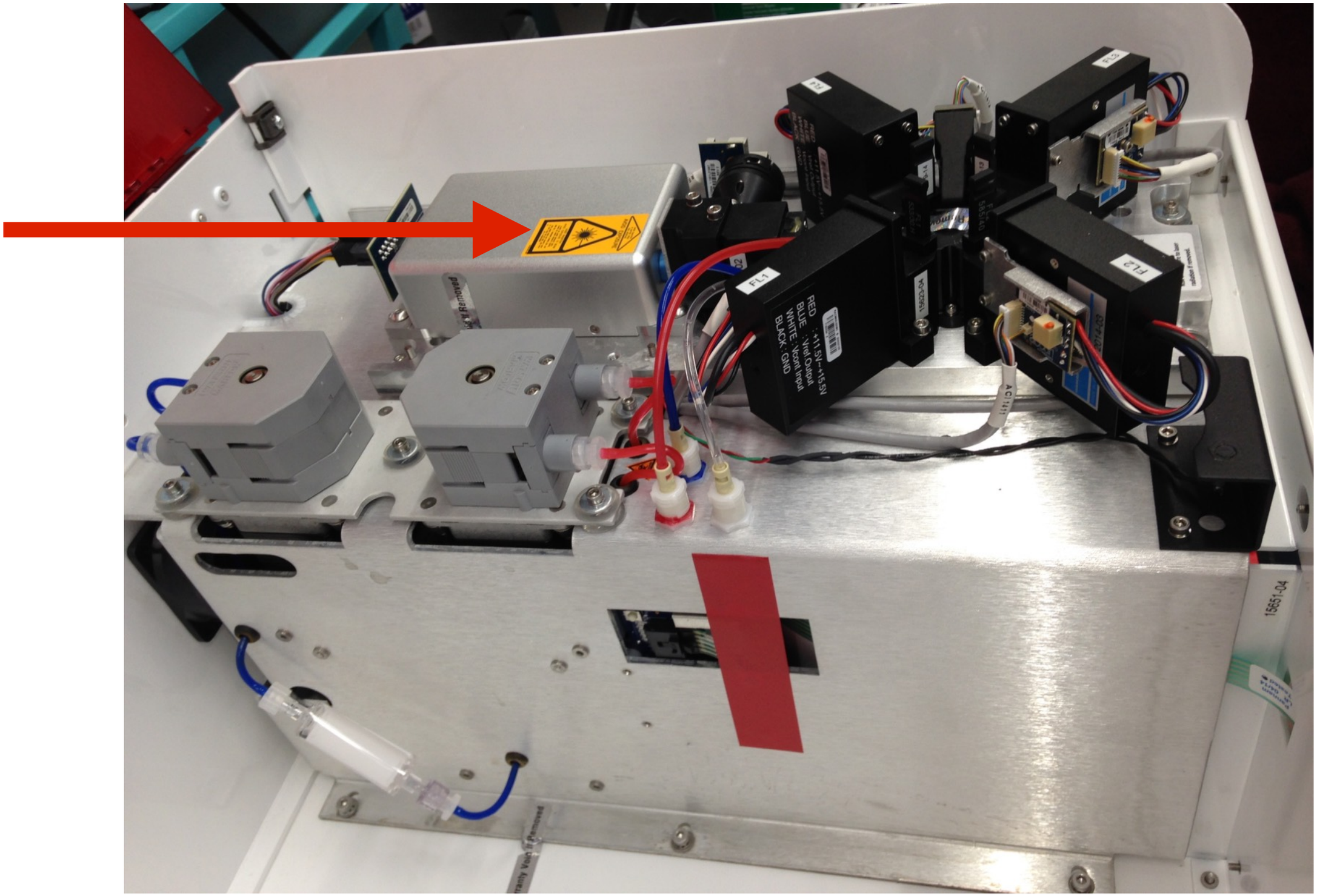
- Flow cytometry
- qPCR

Flow cytometry



https://www.semrock.com/flow-cytometry.aspx

Inside a flow cytometer



Flow cytometry

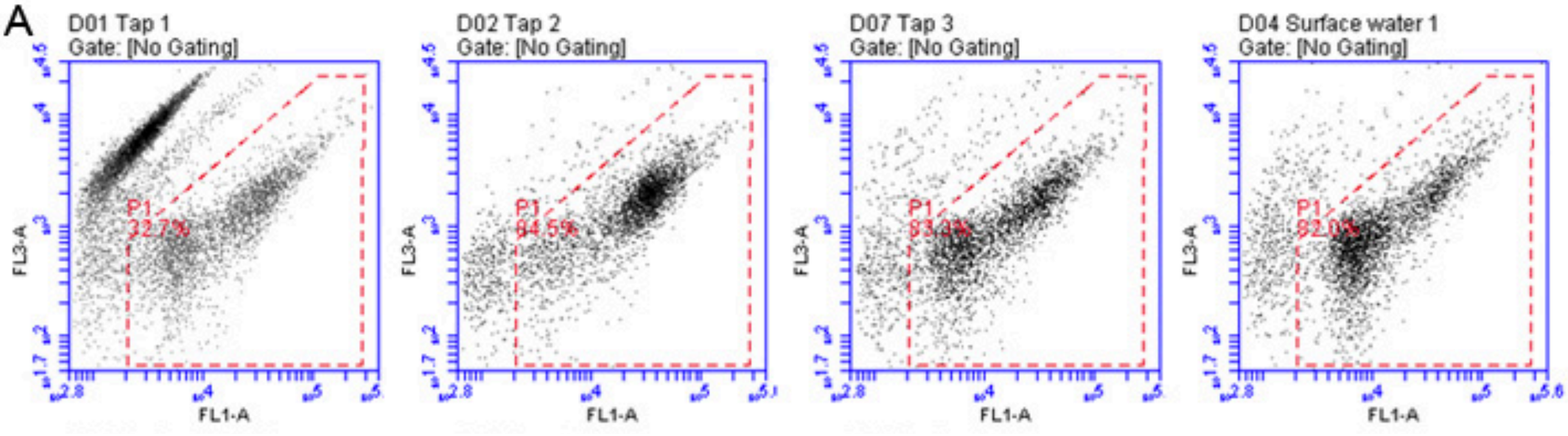
- How many cells?
- How big are cells?
- Fluorescent stains/dyes:
  - *Sybr Green*: stains all DNA, distinguishes debris vs. cells
  - *Propidium iodide*: stains only cells with permeable membranes (**not viable**)
  - *Immuno-stains*: specific for organism of interest

Flow cytometry: steps

- Collect grab sample
- Pre-filter and/or dilute
- Stain with fluorescent dye(s)
- Place sample on cytometer
- Run a known volume of sample

Flow cytometry output

Different water types stained with Sybr Green (all DNA)

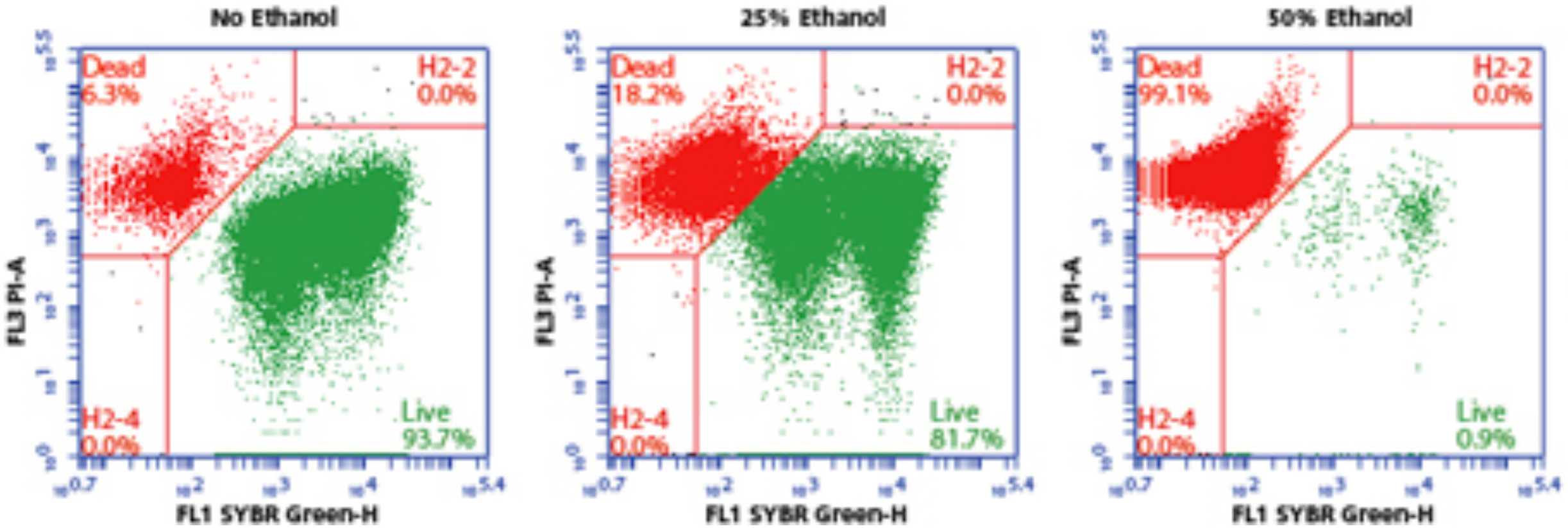


Every point is an “event” (something that passed the detector)

Points inside the red box are likely bacteria

https://www.bdbiosciences.com/sg/instruments/accuri/applications/microbio.jsp

Flow cytometry output

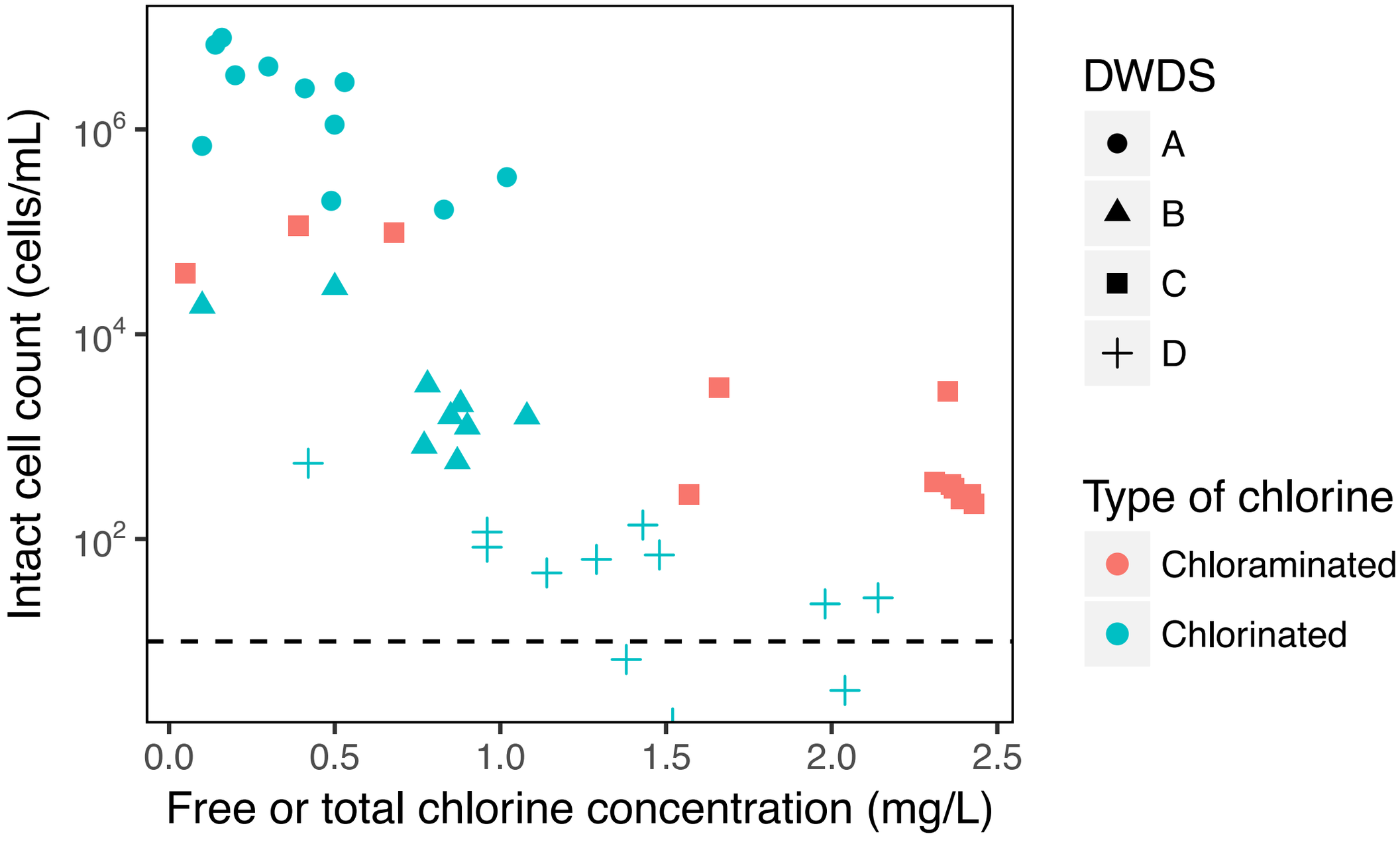


Ethanol —→

Green = viable
Red = dead

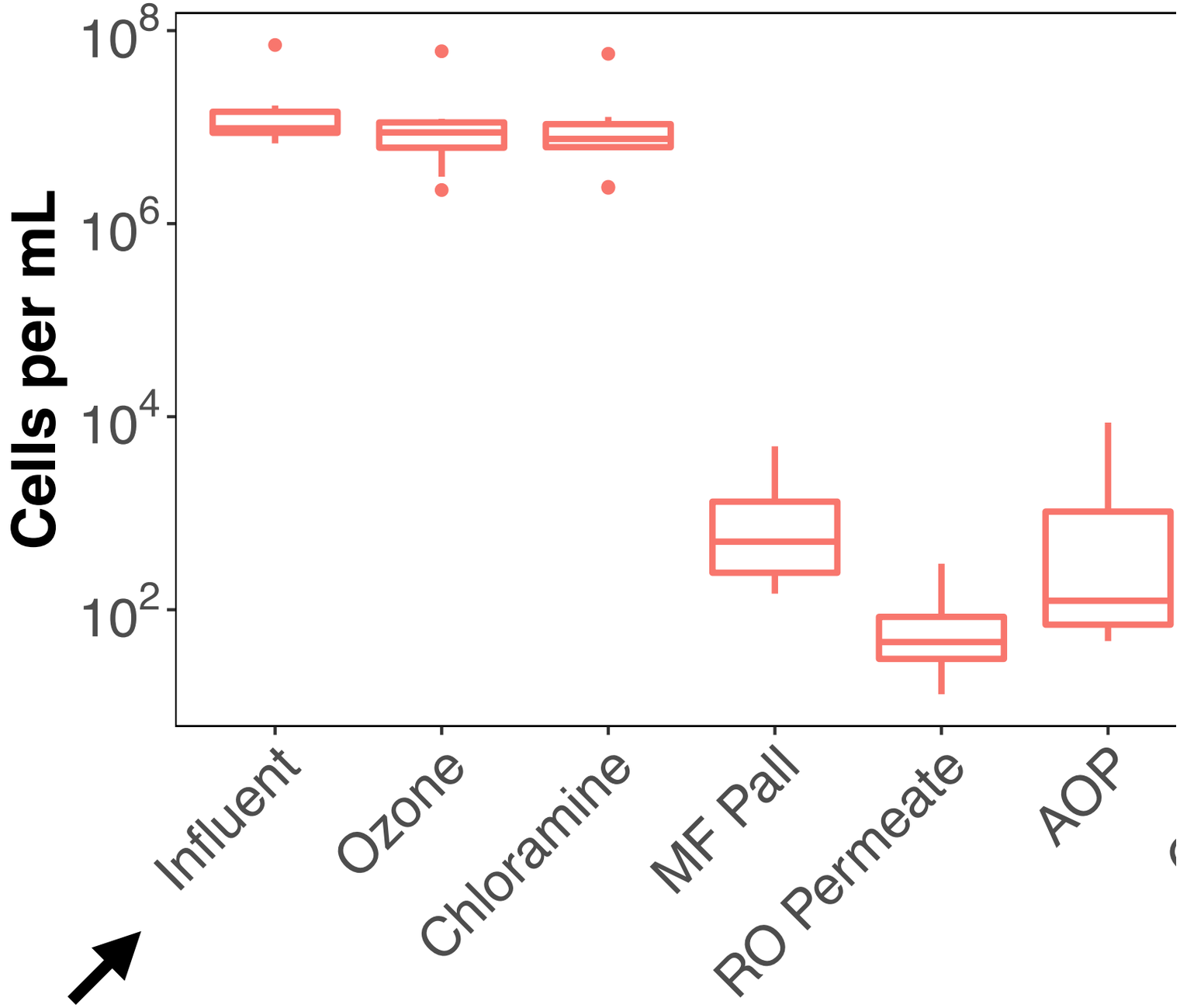
https://www.bdbiosciences.com/sg/instruments/accuri/applications/microbio.jsp

Bacteria in drinking water distribution systems in the Bay Area



Data from Scott Miller and Lauren Kennedy

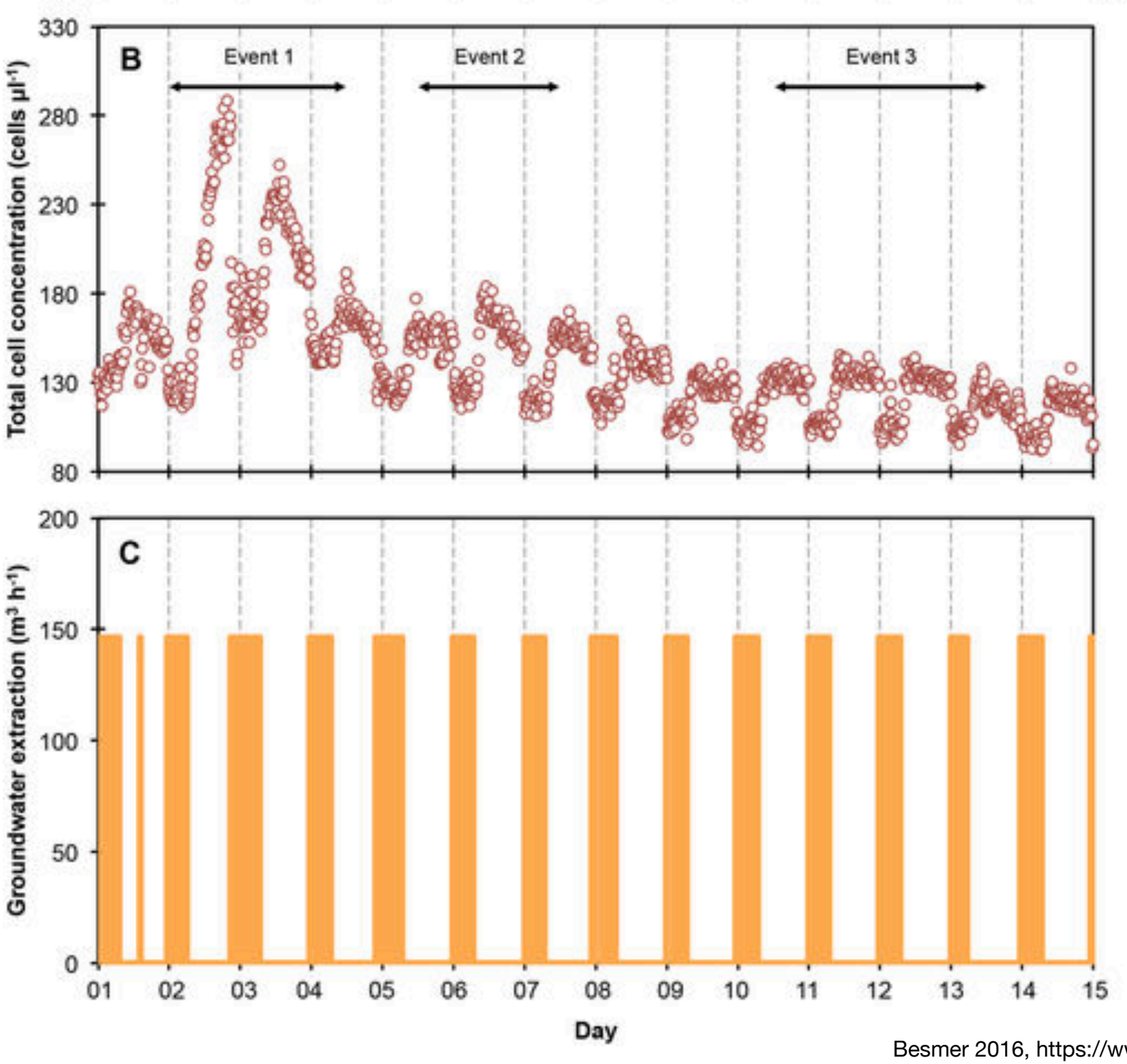
From flow cytometry: advanced treated wastewater



Secondary treated wastewater

Credit to Scott Miller

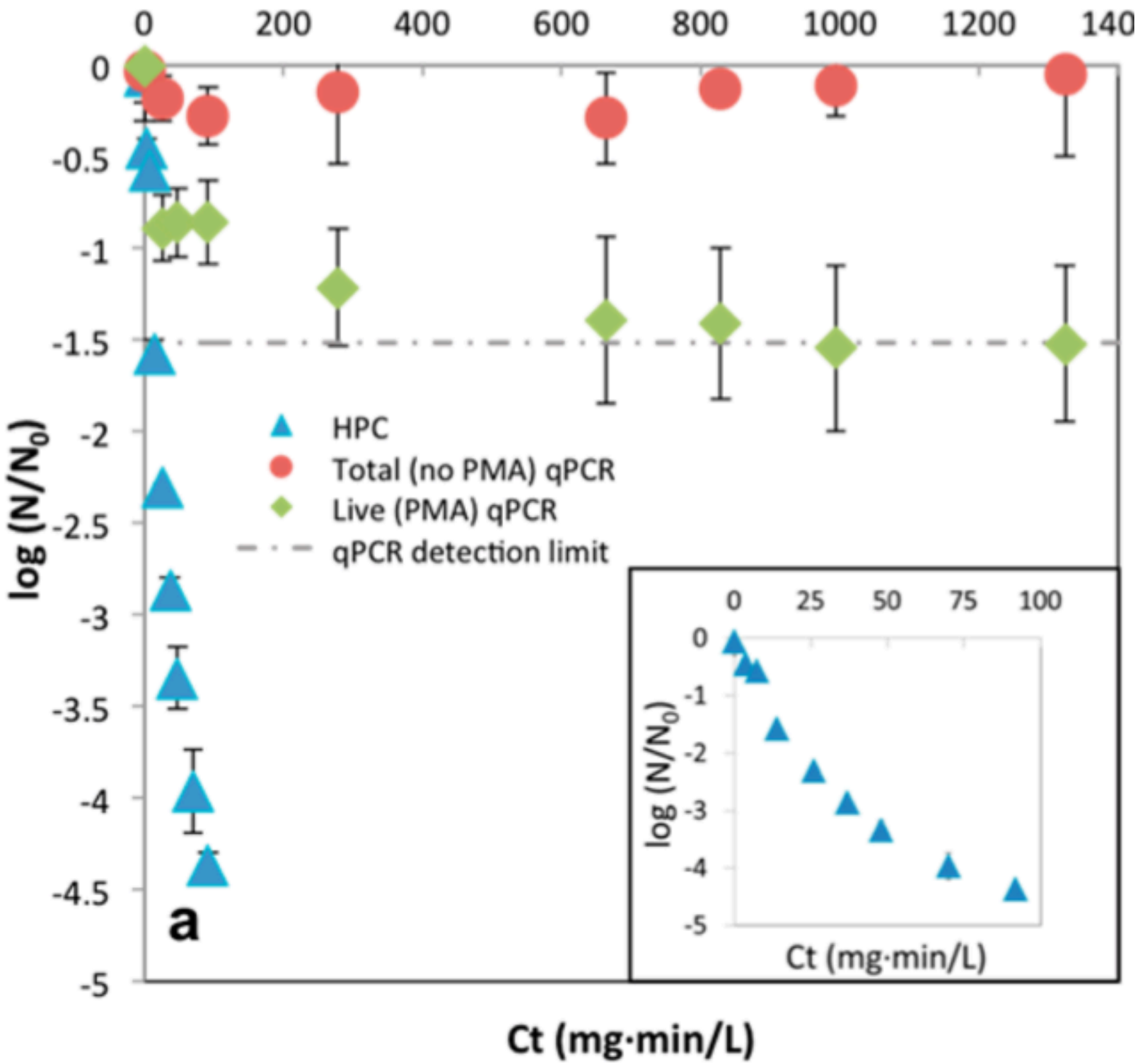
Bonus: Flow cytometry in real time



Besmer 2016, https://www.nature.com/articles/srep38462

Other ways to measure bacteria in drinking water?

qPCR vs HPC


0
200
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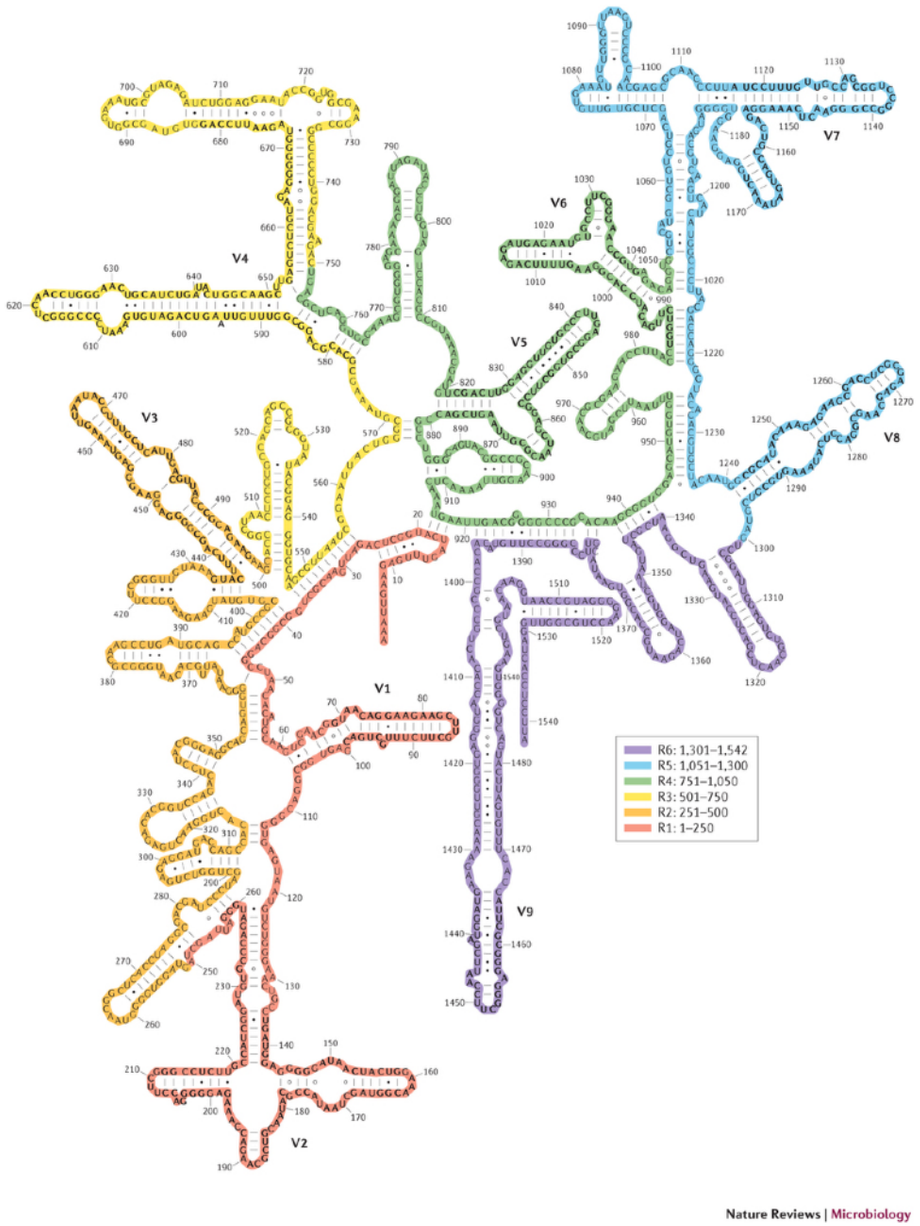
-2.5
-3
-3.5
-4
-4.5
-5
log (N/N0)
HPC
Total (no PMA) qPCR
Live (PMA) qPCR
qPCR detection limit
a
0
25
50
75
100
0
-1
-2
-3
-4
-5
log (N/N0)
Ct (mg·min/L)
Ct (mg·min/L)

qPCR for bacterial quantification uses the 16S ribosomal RNA gene

- Product is ribosomal RNA
- Found in ALL bacteria



Yarza *et al.* (2014) Nat. Rev. Micro.

But wait… we don't know who these bacteria are!

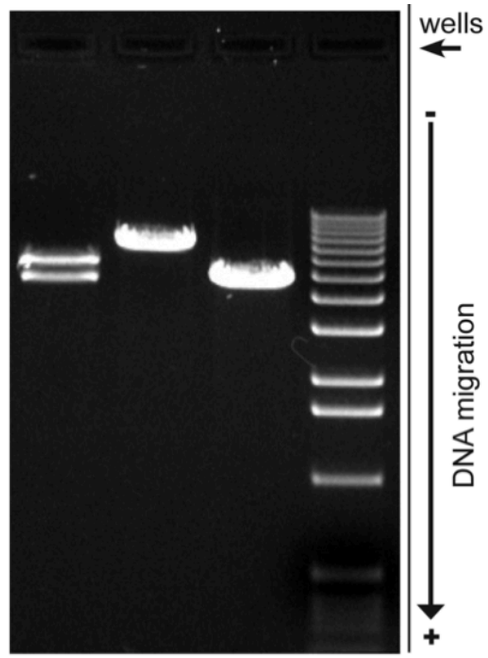
- How can you identify bacteria?
- How can you tell one species from another species?

PART II: Methods to *classify* microbial communities

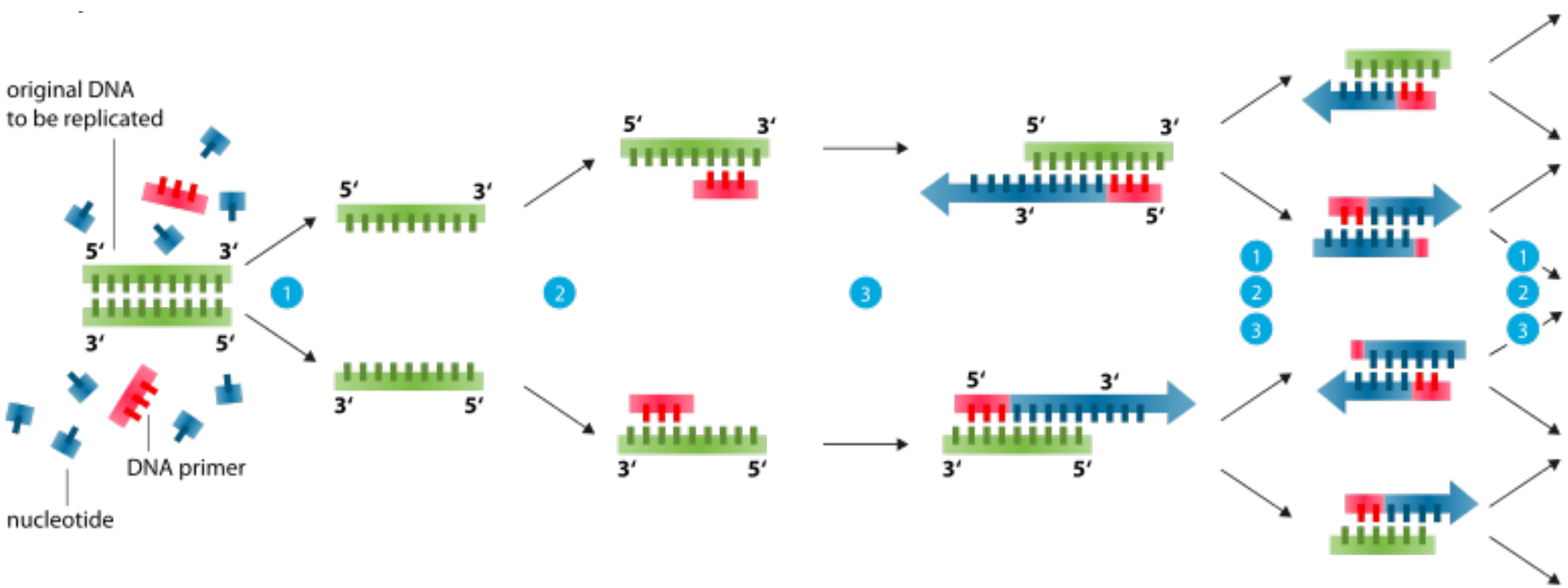
- DNA sequencing:
    - 16S rRNA gene sequencing
    - Metagenomics
    - Whole genome sequencing
    - Marker gene sequencing

DNA sequencing: Sanger

- Uses two concepts you know about:
  - PCR
  - Gel electrophoresis

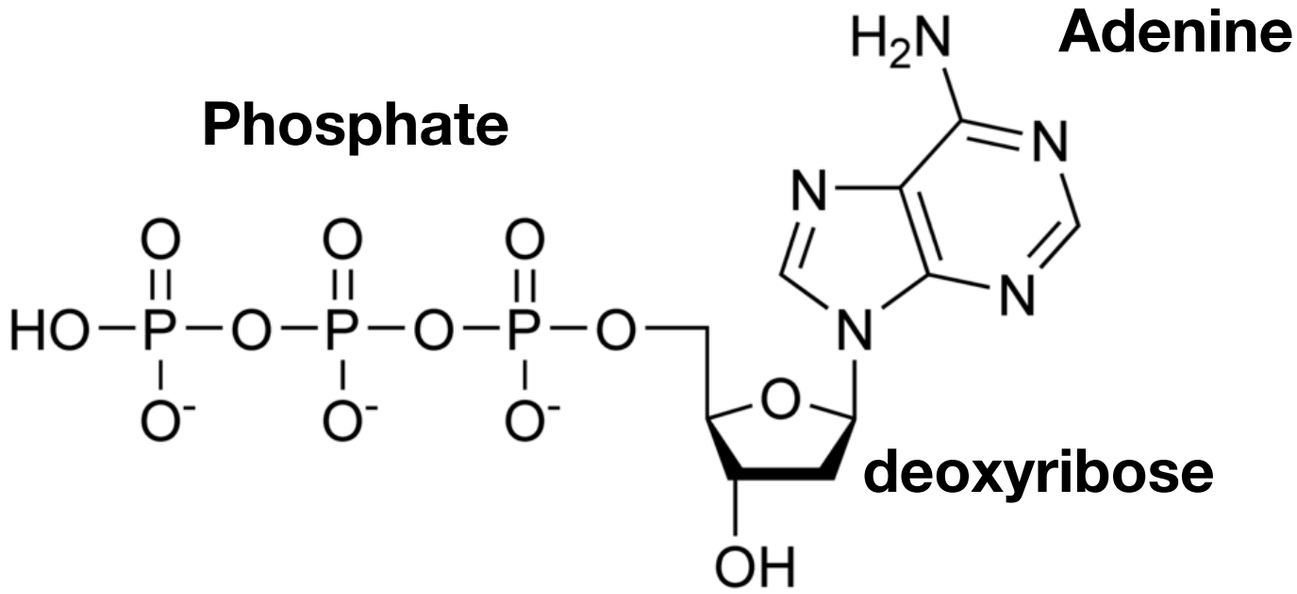


https://en.wikipedia.org/wiki/Gel_electrophoresis



https://en.wikipedia.org/wiki/Polymerase_chain_reaction

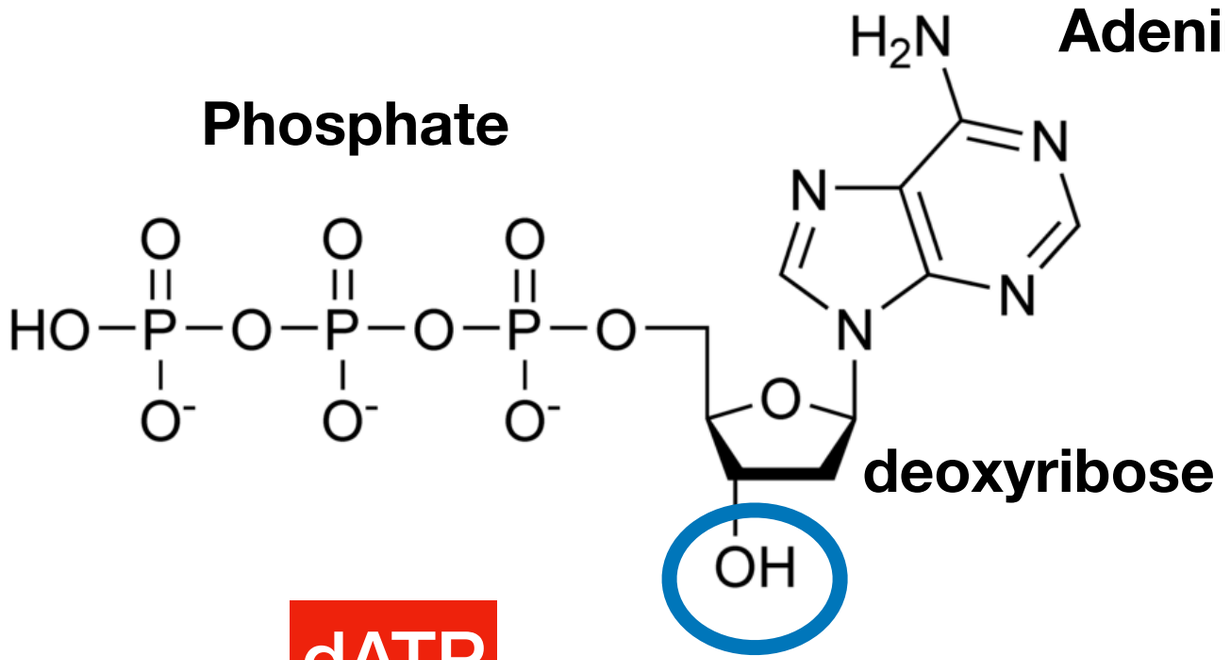
dNTPs vs. dye-ddNTP



dATP

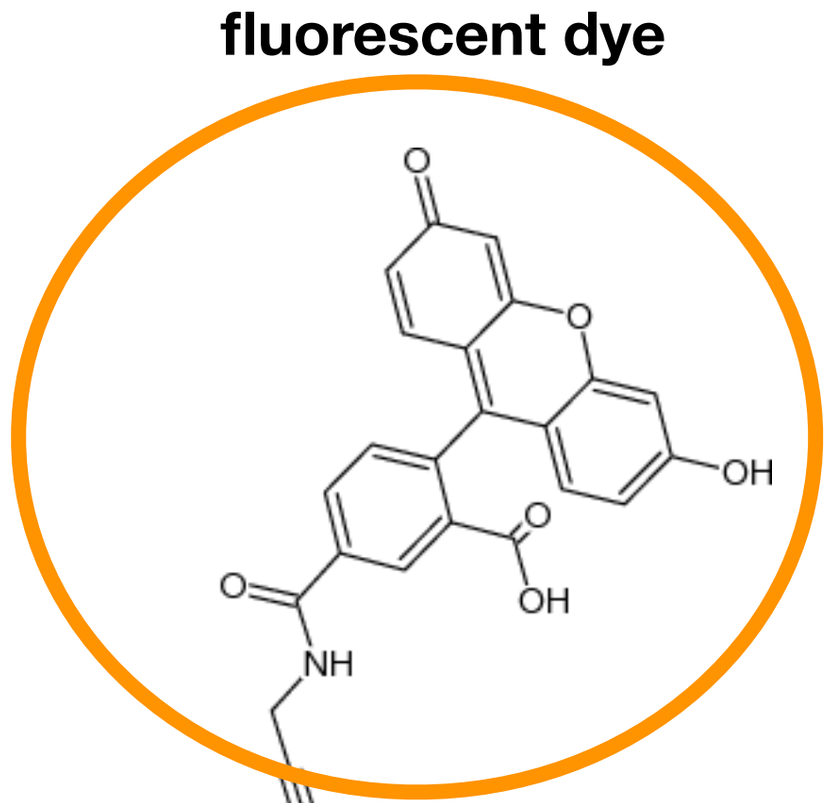
(dNTP = dATP, dCTP, dGTP, dTTP)

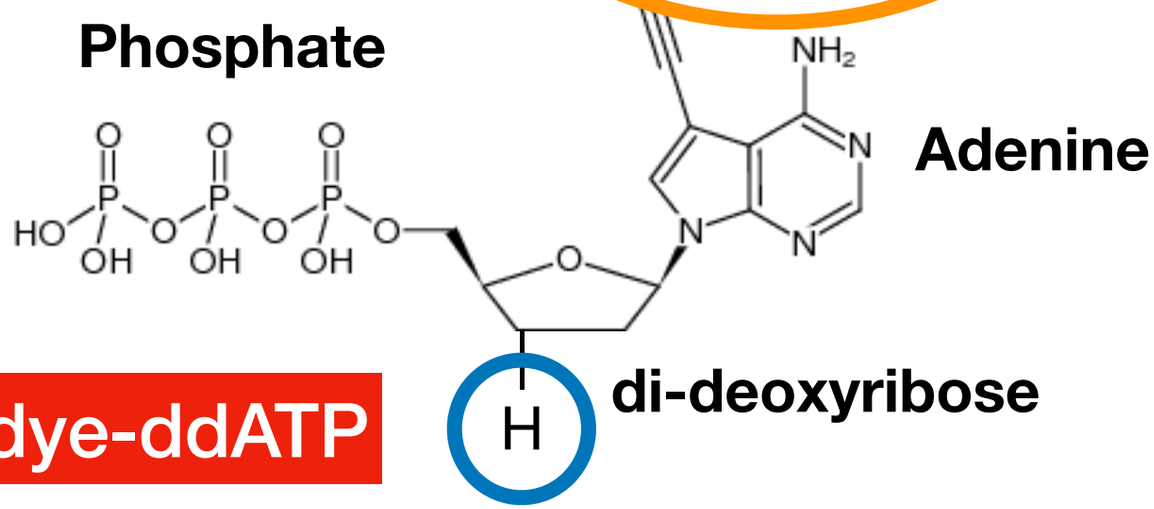
dNTPs vs. dye-ddNTP



dATP

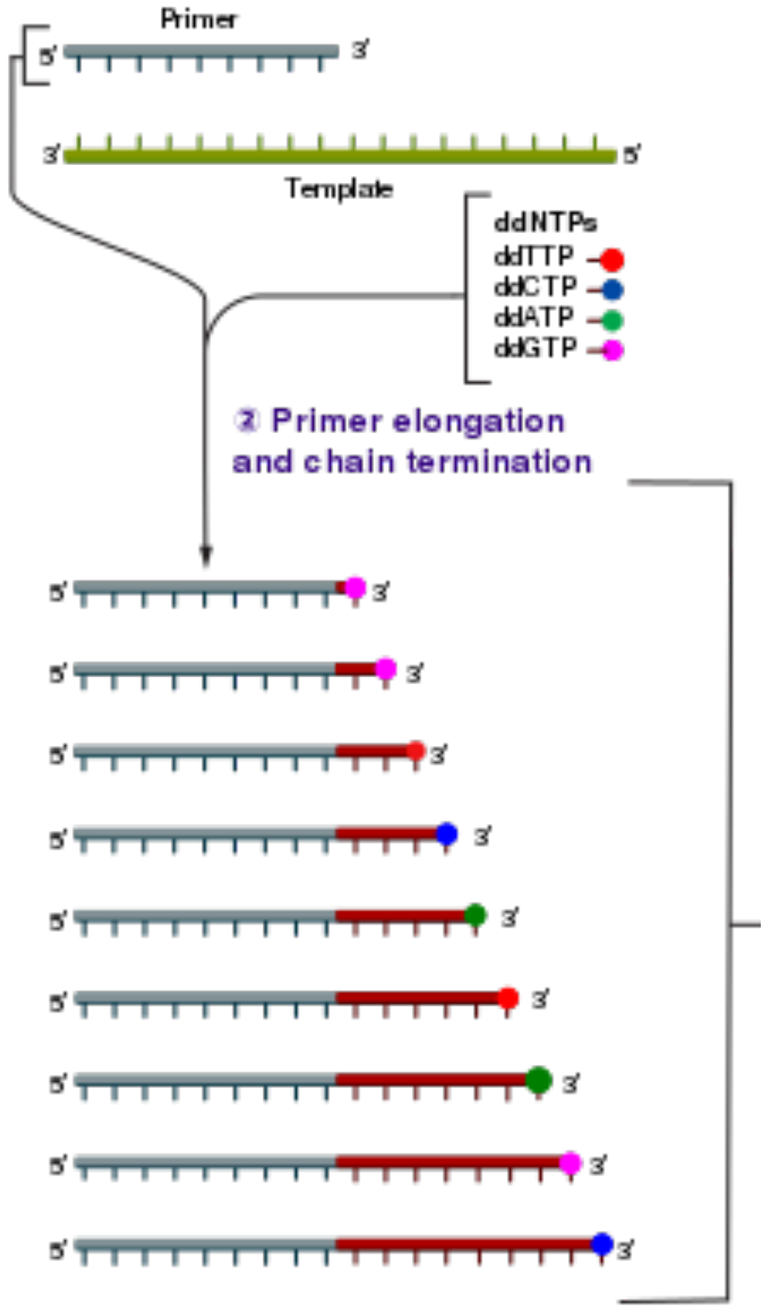
(dNTP = dATP, dCTP, dGTP, dTTP)





dye-ddATP

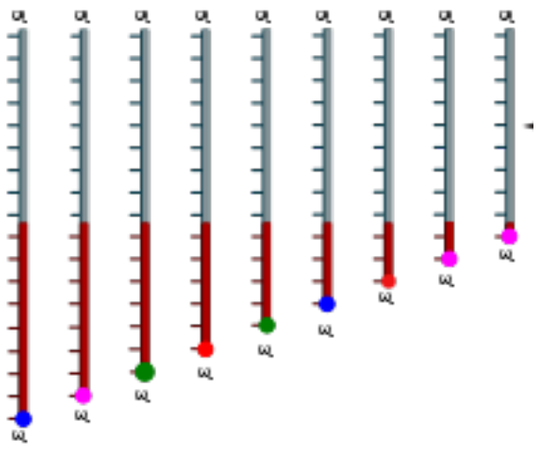
DNA sequencing: Sanger



- During PCR, labeled ddNTPs get incorporated randomly
- ddNTPs cause polymerase to STOP
- Result: copies of DNA with different lengths, last nucleotide is labeled

DNA sequencing: Sanger

DNA fragments from the PCR reaction are run through capillary electrophoresis



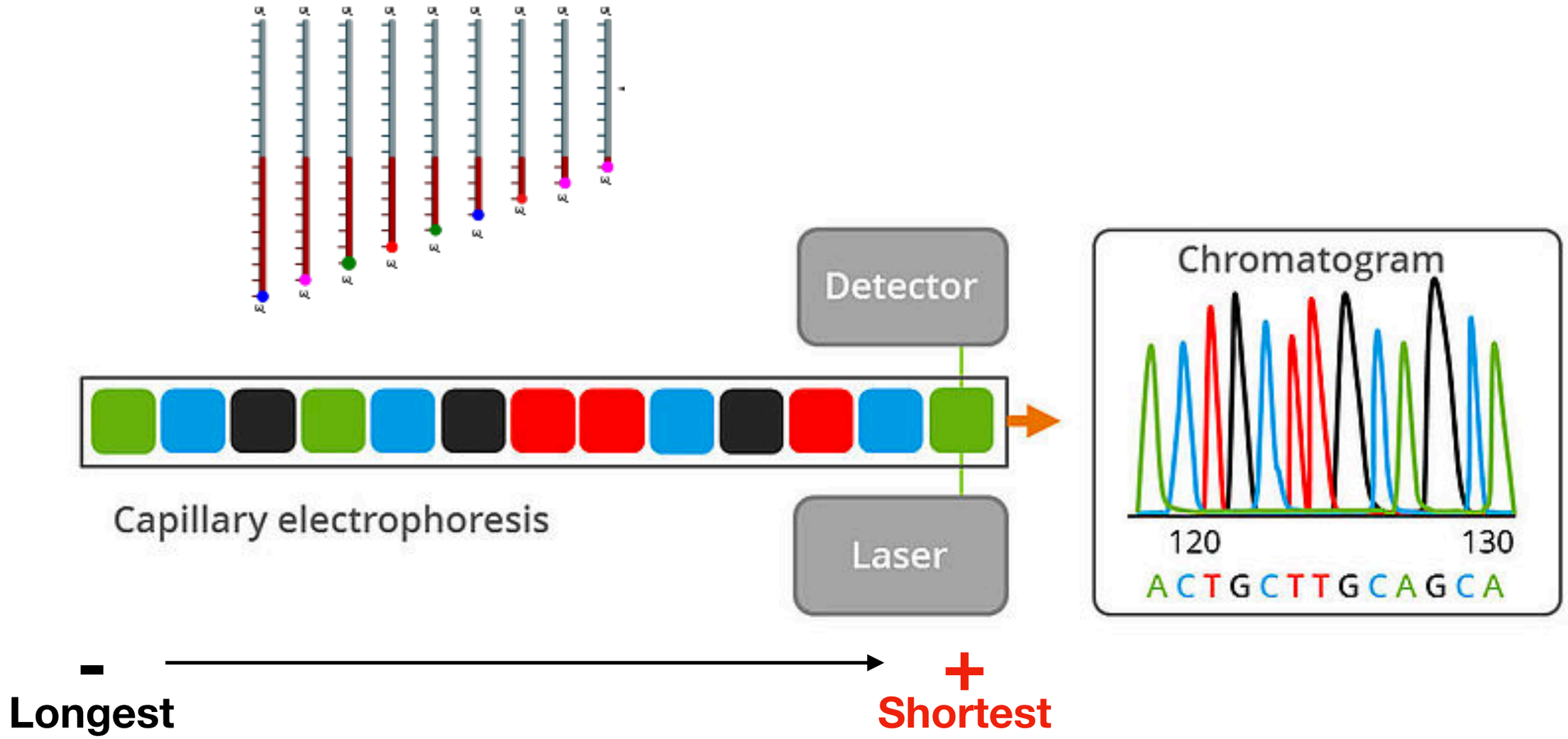
- **Longest**

+ **Shortest**

https://www.gatc-biotech.com/en/expertise/sanger-sequencing.html

DNA sequencing: Sanger

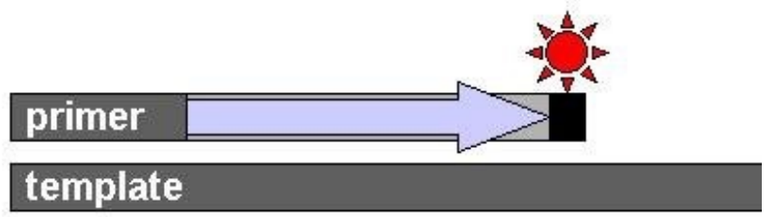
DNA fragments from the PCR reaction are run through capillary electrophoresis



https://www.gatc-biotech.com/en/expertise/sanger-sequencing.html

DNA sequencing: Sanger

- Need a known starting point



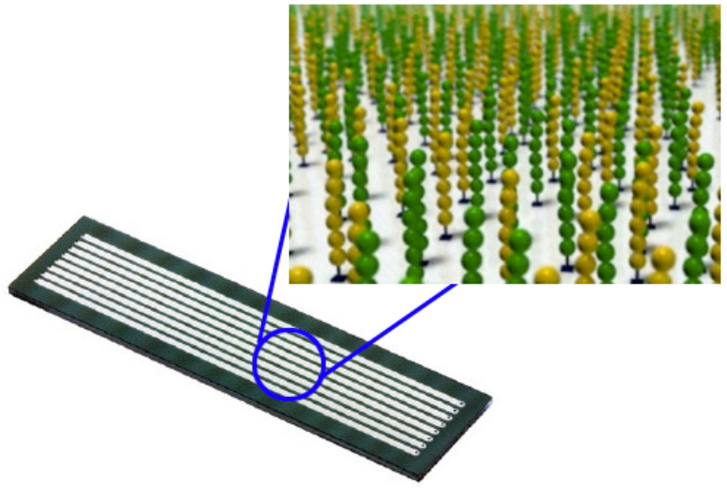
- Can sequence ONE template at a time (so you have to culture bacteria first)

- Long sequences: up to 1000 nucleotides at a time

- Video: (https://www.youtube.com/watch?v=ONGdehkB8jU)

https://commons.wikimedia.org/w/index.php?curid=3788287

DNA sequencing: high-throughput



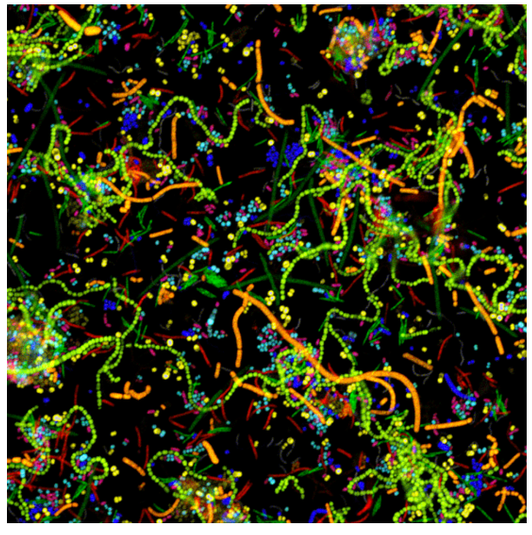
- Sequencing by synthesis (**Illumina**)

- A PCR-like process, uses fluorescently labeled nucleotides, laser, imager

- Short reads: 100-250 nucleotides

- Video: https://www.youtube.com/watch?v=fCd6B5HRaZ8

DNA sequencing: high-throughput



- Doesn’t need known starting point
- Can sequence *multiple* different templates simultaneously
- Start with environmental sample without culturing
- Generates a LOT of data

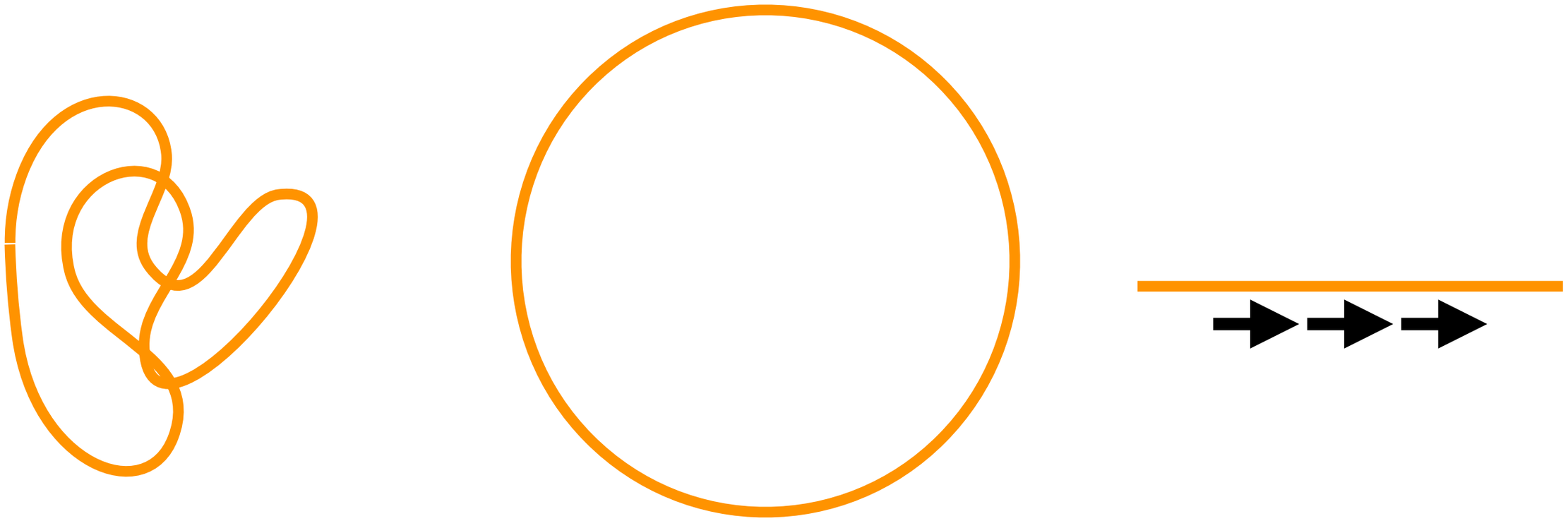
High-throughput sequencing: steps

- Filter water to collect biomass
- Isolate DNA
- Prepare a sequencing library or libraries
- Run sequencing (complex protocol/machine)

Uses of DNA sequencing

- Whole genome sequencing
- 16S rRNA gene sequencing
- Metagenomics
- Marker gene sequencing

Whole genome sequencing



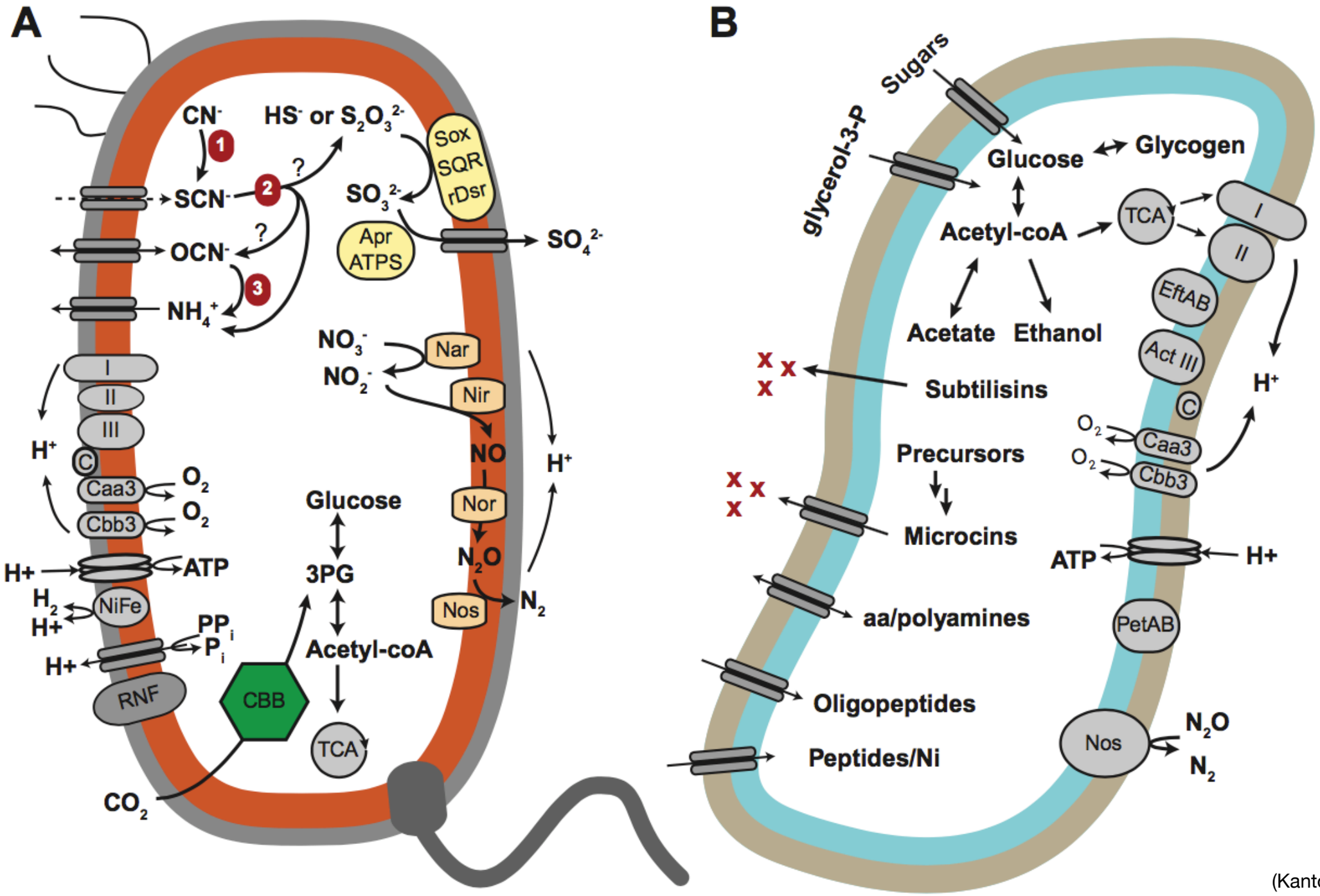
Bacterial chromosomes are circular- most bacteria have 1 chromosome

Genomes contain genes, which are the blueprint for how to make a cell

Question 4:

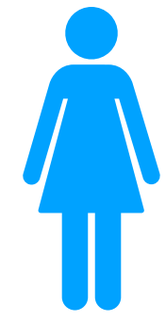
Name one trait or characteristic of bacteria that is determined by their genes.

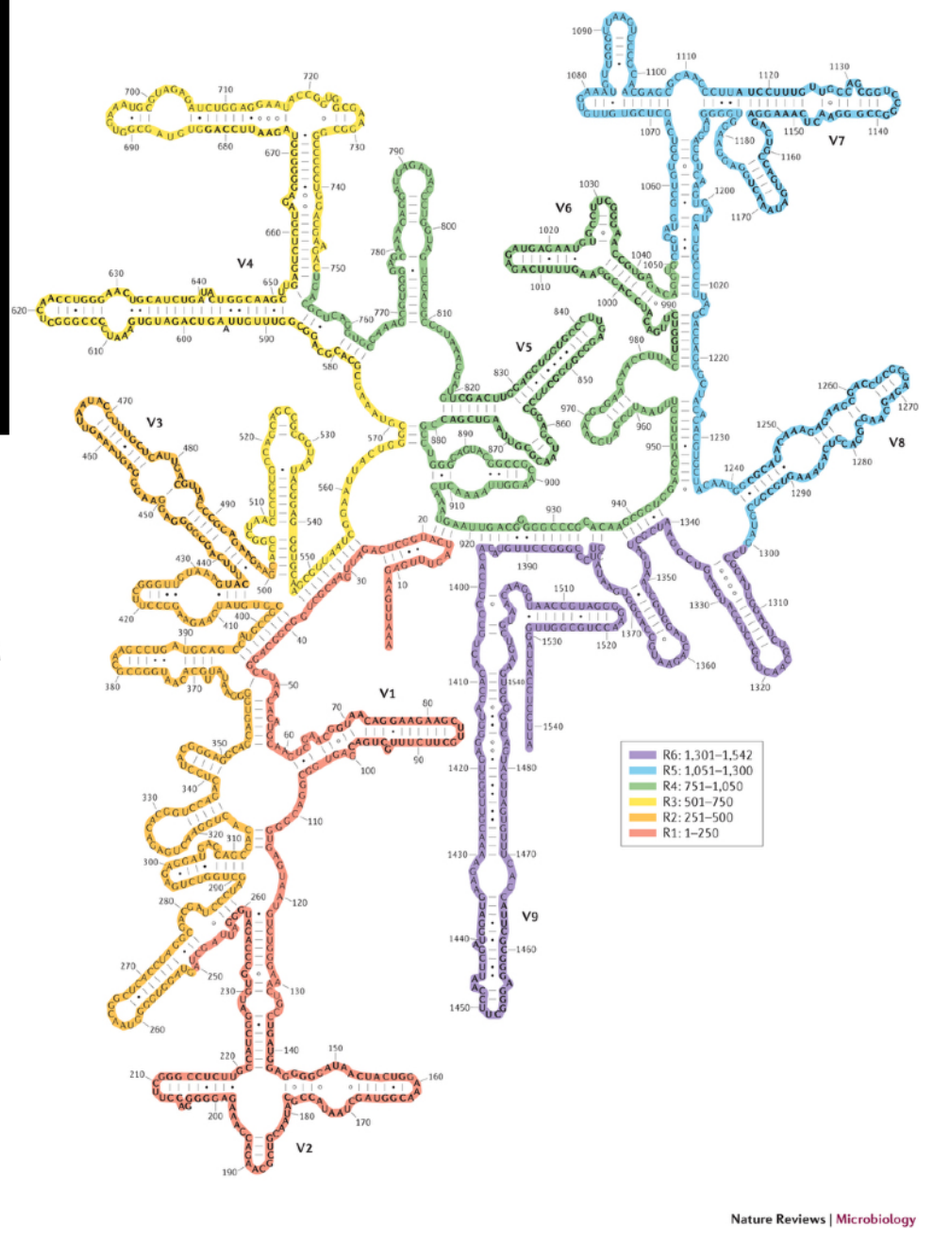
Genomes are blueprints



(Kantor et al. 2015)

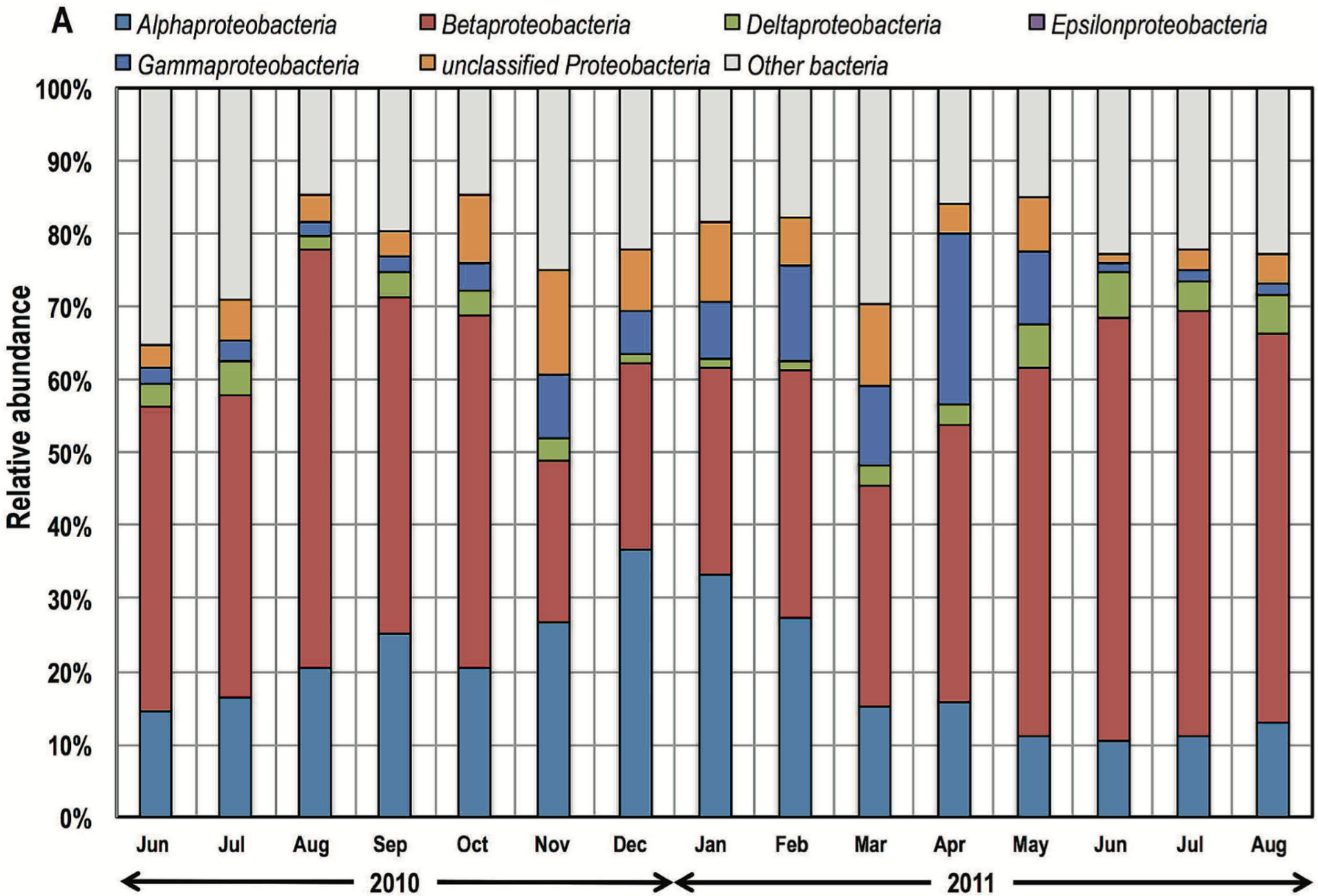
16S rRNA gene sequencing



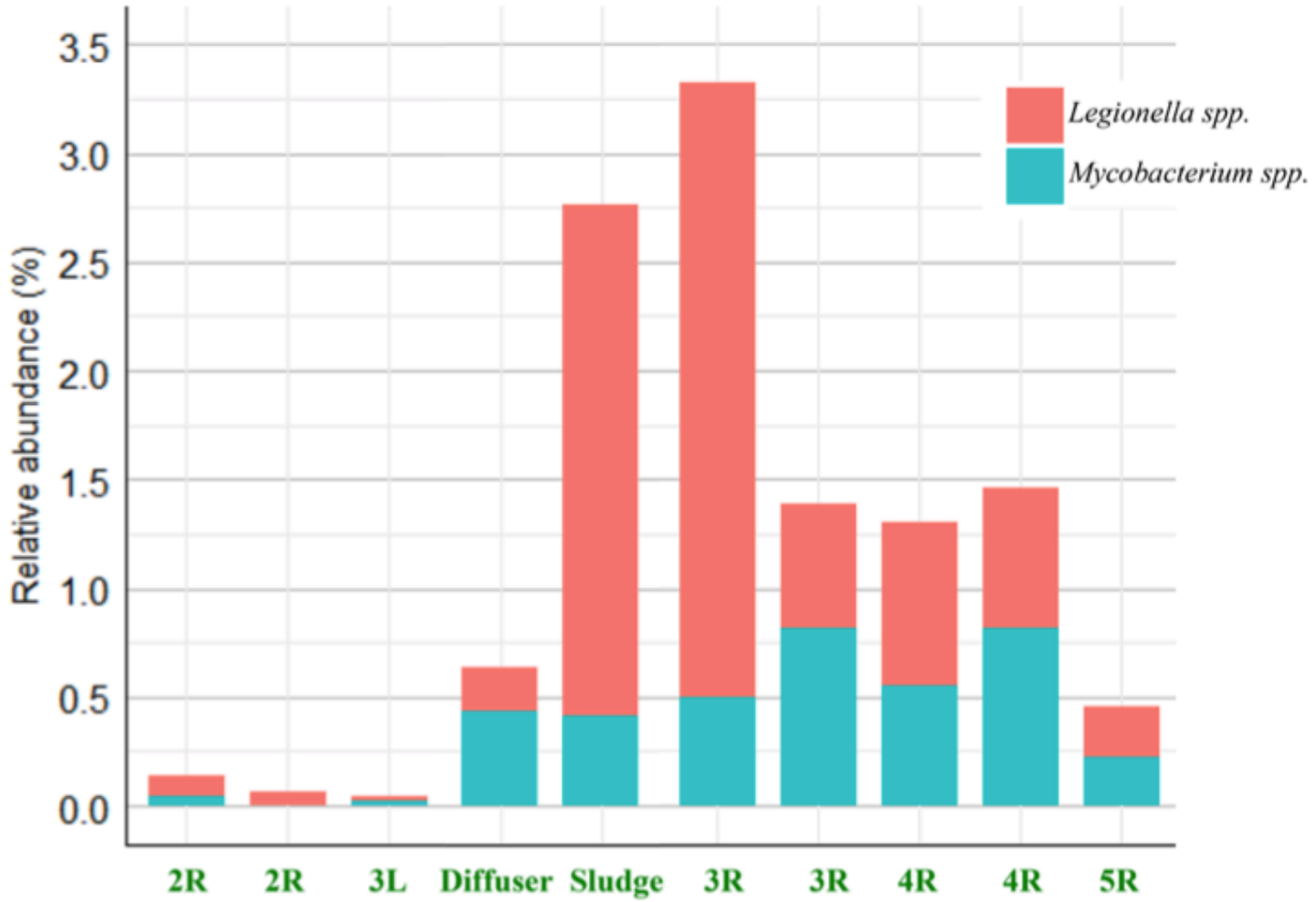


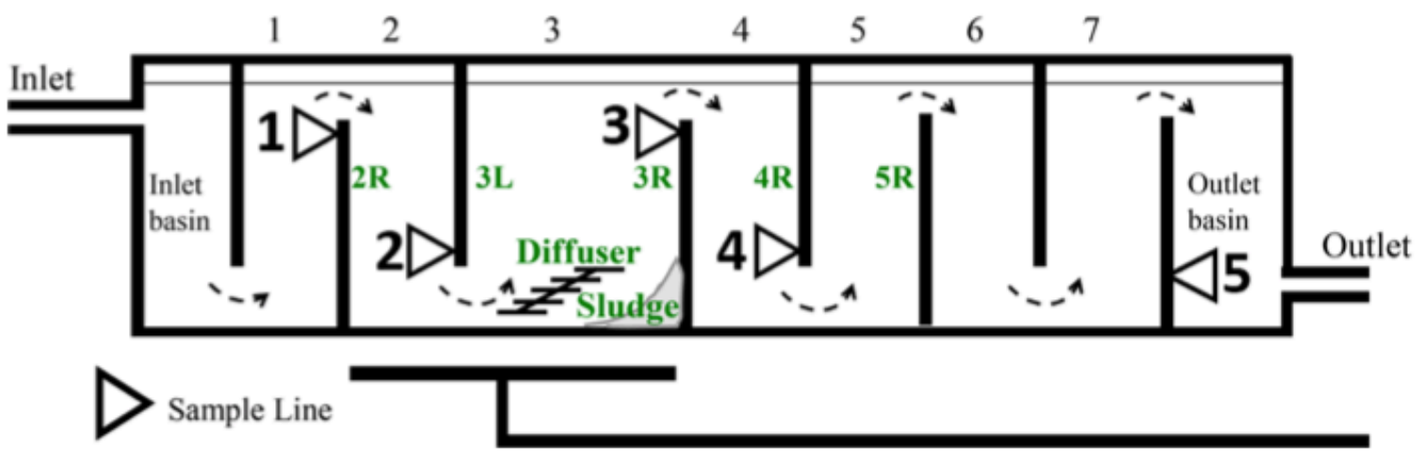
Yarza *et al.* (2014) Nat. Rev. Micro.

16S: seasonal patterns in a distribution system microbiome



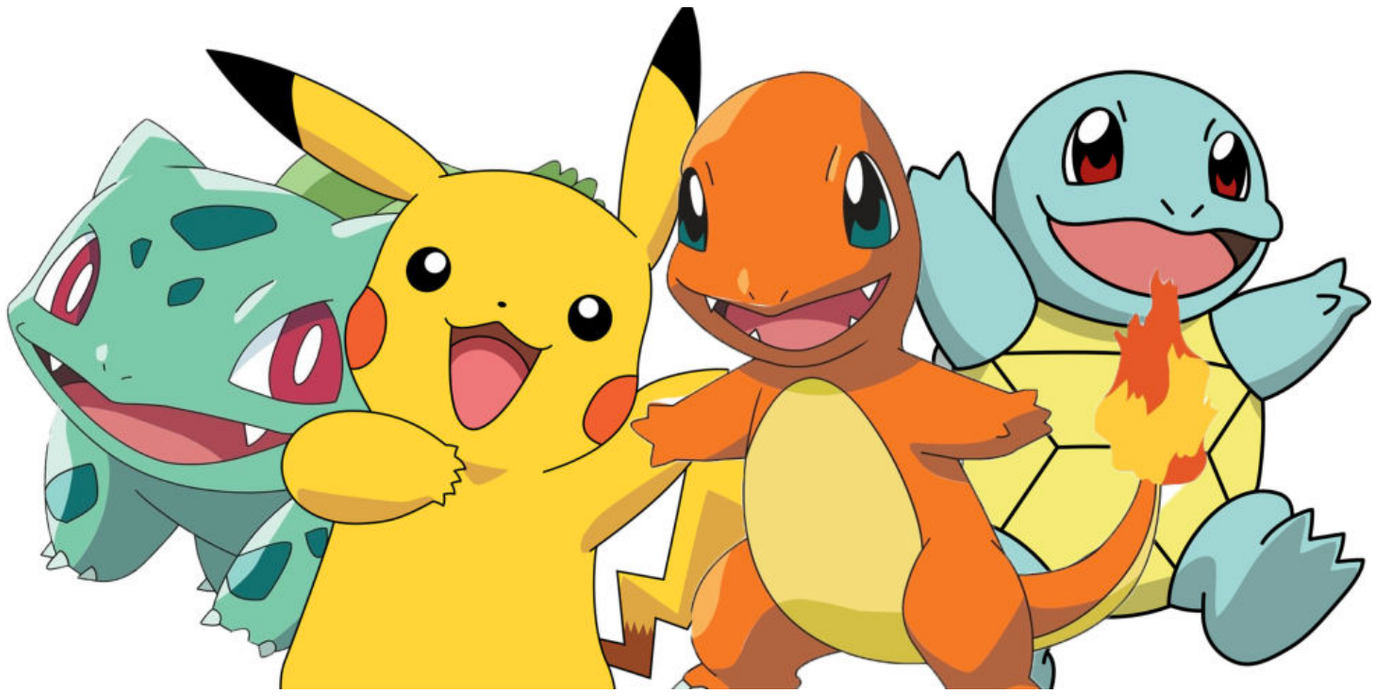
16S sequencing: opportunistic pathogens in ozone contactor at DWTP





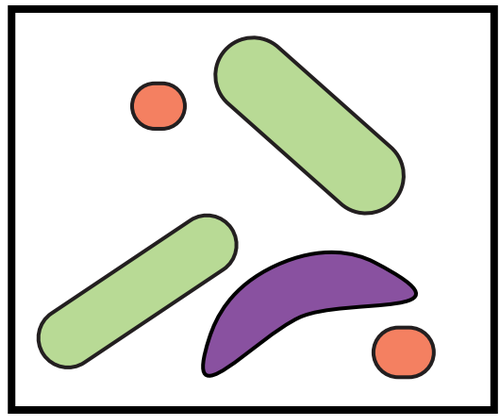
Metagenomics

What’s better than one genome? Lots of genomes!



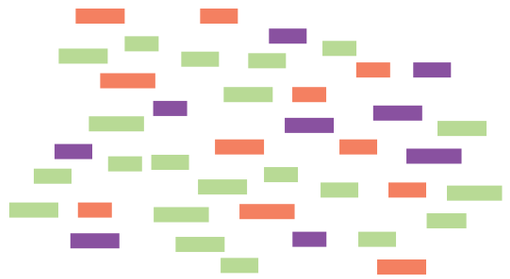
Metagenomics

1. Sampling



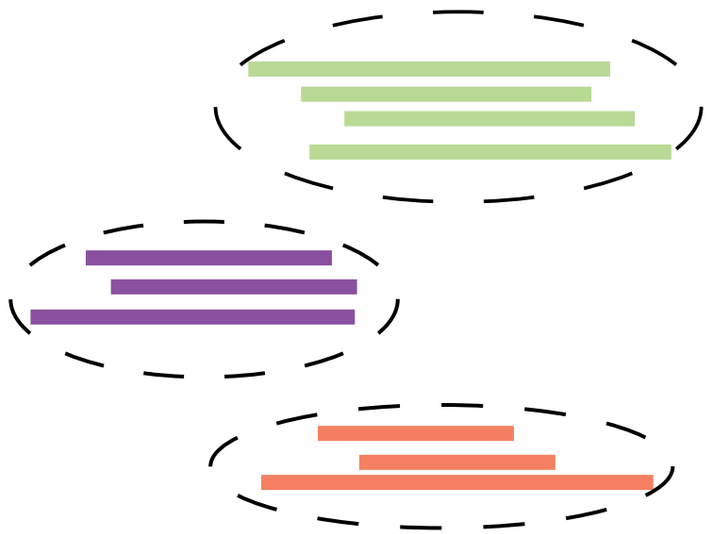
2. DNA extraction

3. Illumina sequencing



4. Reassembly

5. Binning



6. Genome curation

Marker gene sequencing for specific pathogenic groups

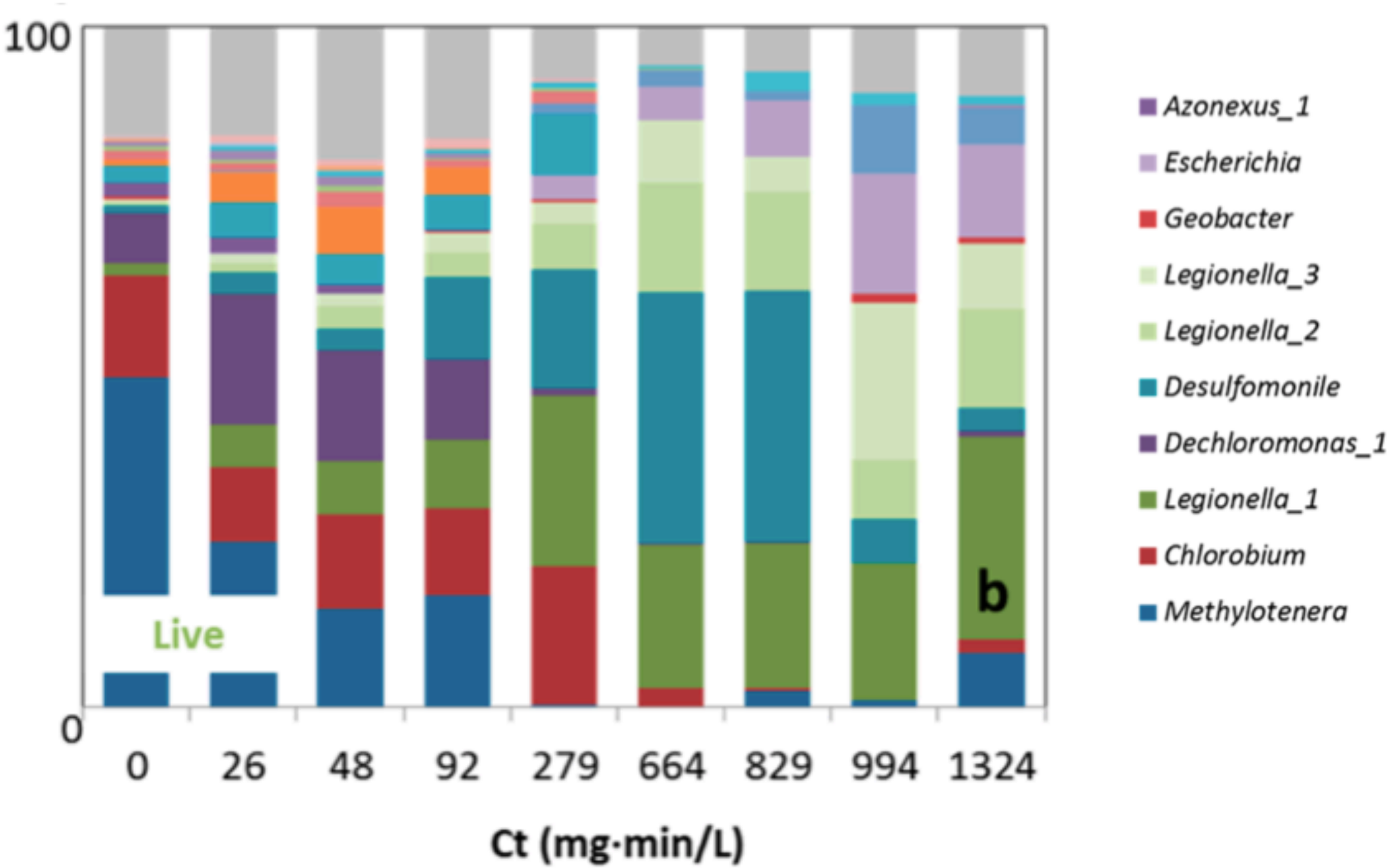
- “A **High-Throughput Approach** for Identification of **Nontuberculous Mycobacteria** in Drinking Water Reveals Relationship between Water Age and Mycobacterium avium”

- “Development of a genus-specific **next generation sequencing approach** for sensitive and quantitative determination of the **Legionella** microbiome in freshwater systems”

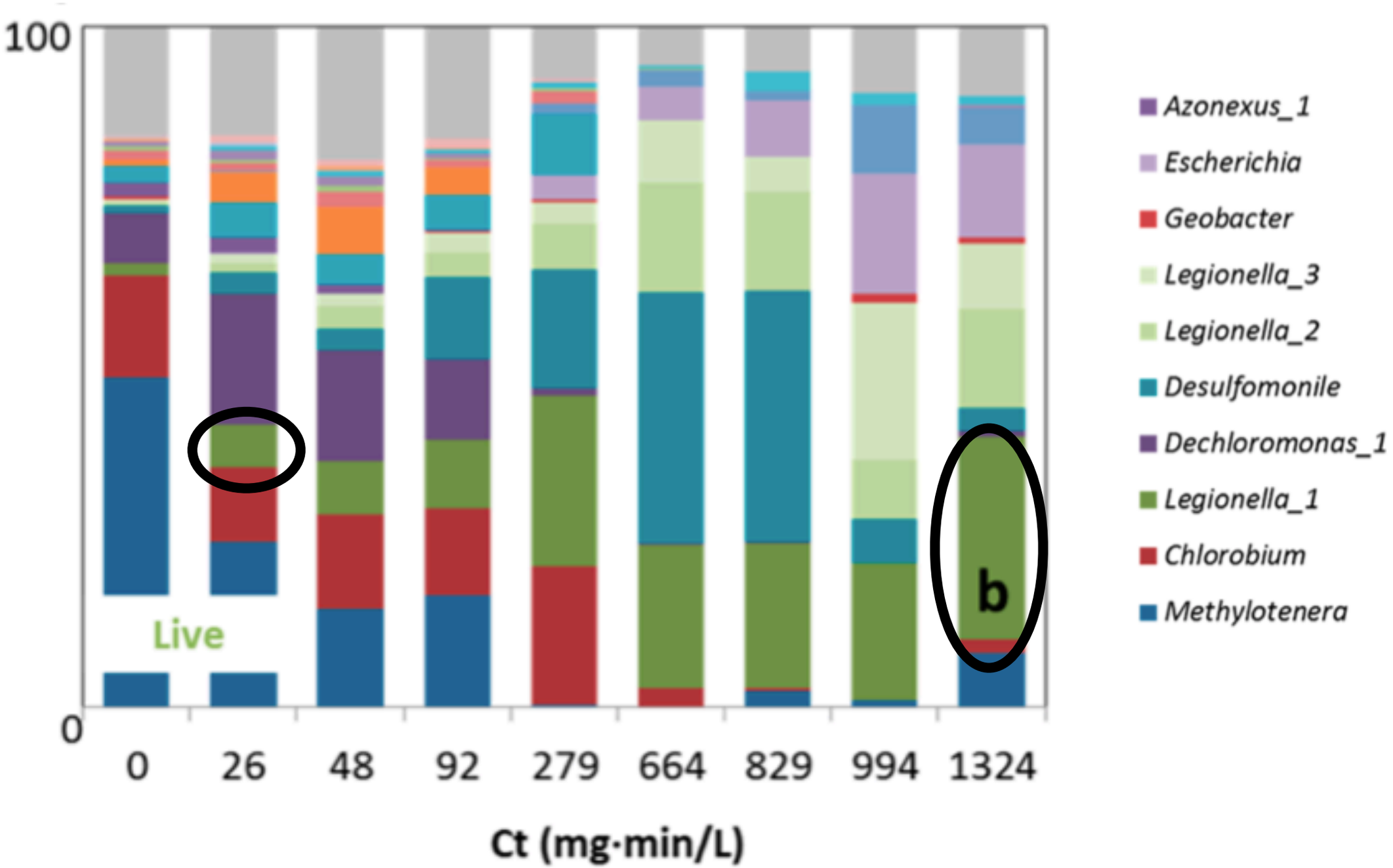
Question 5:

What factors could affect the **quantity** and **types** of bacteria in drinking water?

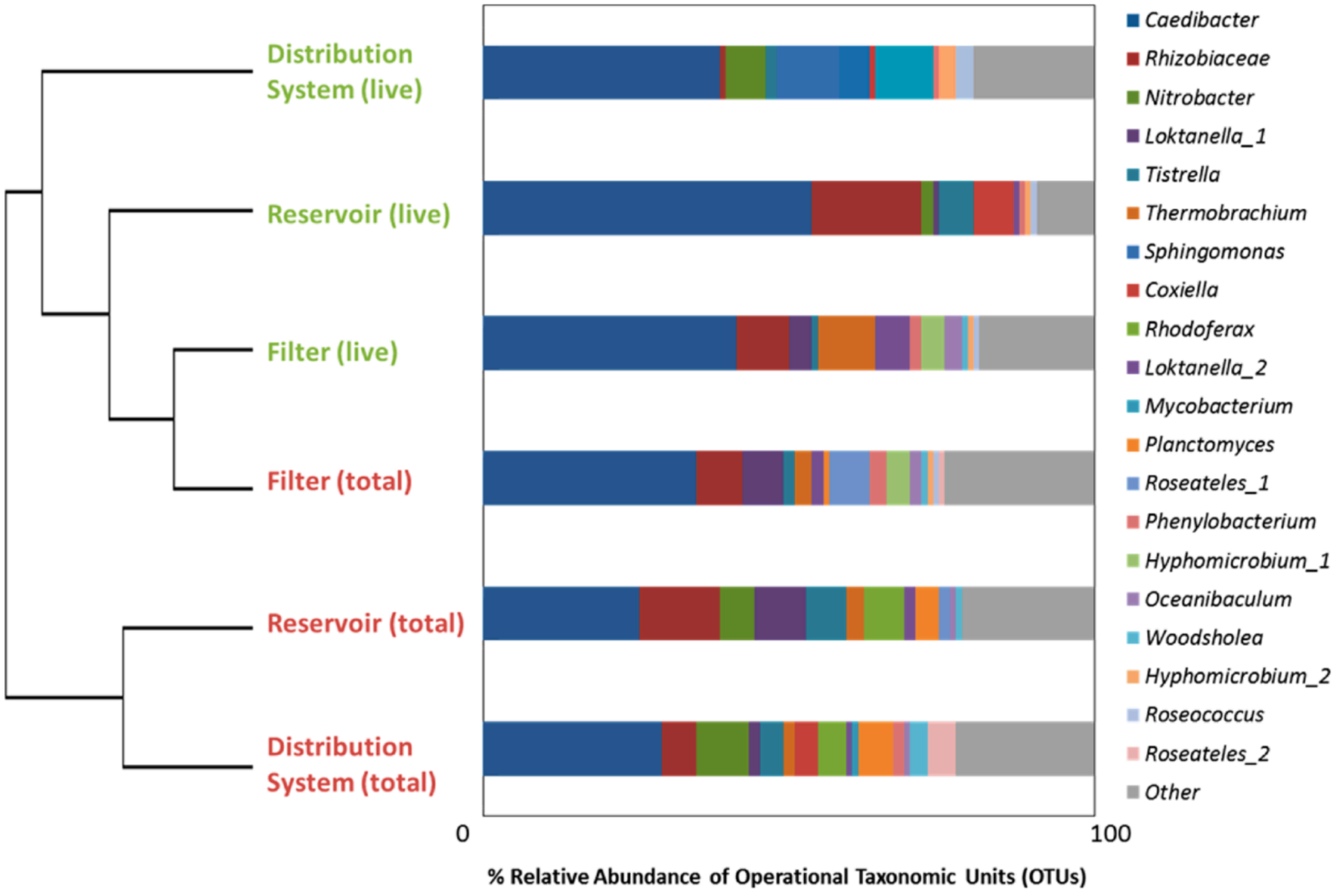
Effect of disinfection on microbiome



Relative abundance can be misleading



Treatment vs. distribution



Recap

- The water microbiome concept
- Methods for microbial quantification: flow cytometry, qPCR
- Methods for identification/classification: DNA sequencing
- Factors that affect the water microbiome