AMR CASE STUDY OF QPCR VERSUS WGS METAGENOMICS IN WASTEWATER: A TIME AND PLACE

WILLIAM TAYLOR

SENIOR SCIENTIST, ESR

BACKGROUND

- Superbugs are coming

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- Superbugs are coming
- They will kill us all

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- Superbugs are coming
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- Farmer's fault

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BACKGROUND

- Superbugs are coming
- They will kill us all
- Dr's Fault
- Farmer's fault
- Our fault

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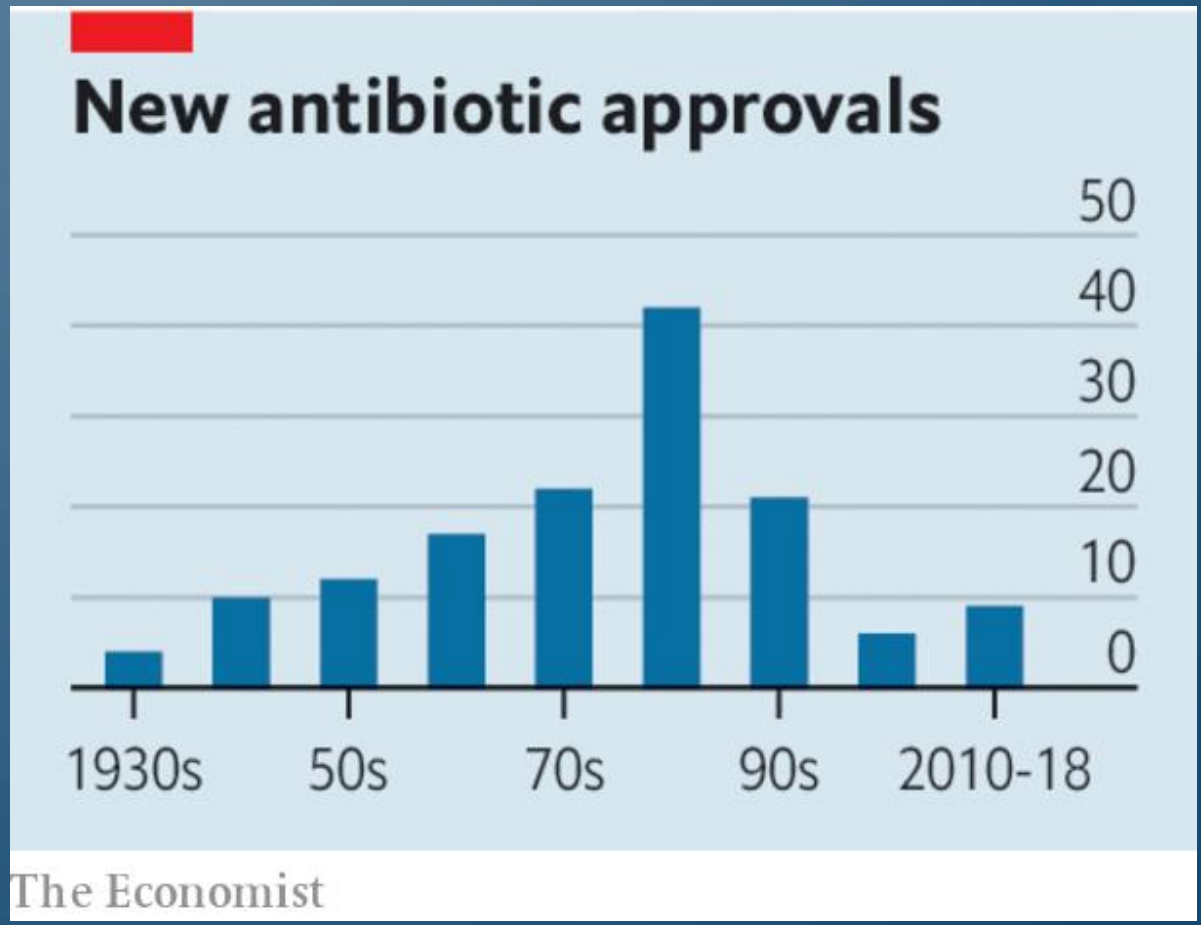
WHY NOT JUST MAKE NEW ONES?

- Costs on average $1 billion (USD) to bring a drug to market
- Huge investment for production
- Generic drugs are too cheap
  - Amoxicillin ~1$
  - Single course
- Ozempic $1000 a month
  - Ongoing consumption
- Kimmtrack $975,000 a year



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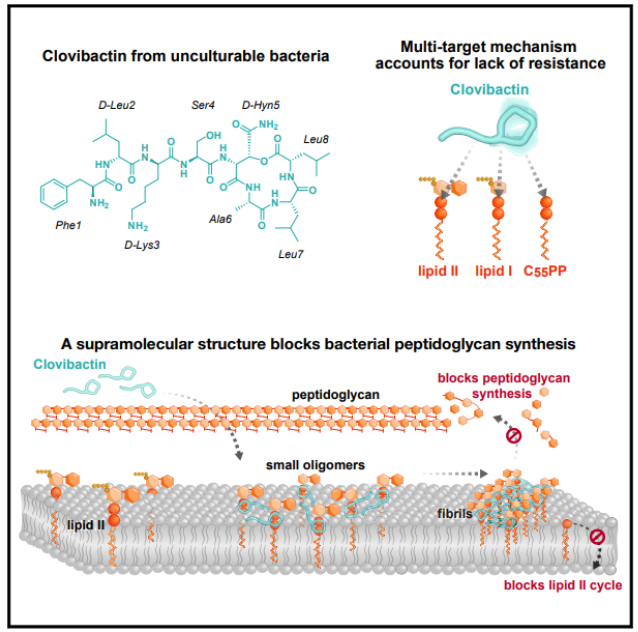
HOPE?

Cell

Article

An antibiotic from an uncultured bacterium binds to an immutable target

Graphical abstract



Authors

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In brief

Clovibactin, a new antibiotic isolated from soil bacteria, blocks bacterial cell wall synthesis by targeting essential peptidoglycan precursors, allowing it to kill drug-resistant bacterial pathogens without detectable resistance.

Journal of Medicinal Chemistry

Methylation of Daptomycin Leading to the Discovery of Kynomycin, a Cyclic Lipodepsipeptide Active against Resistant Pathogens

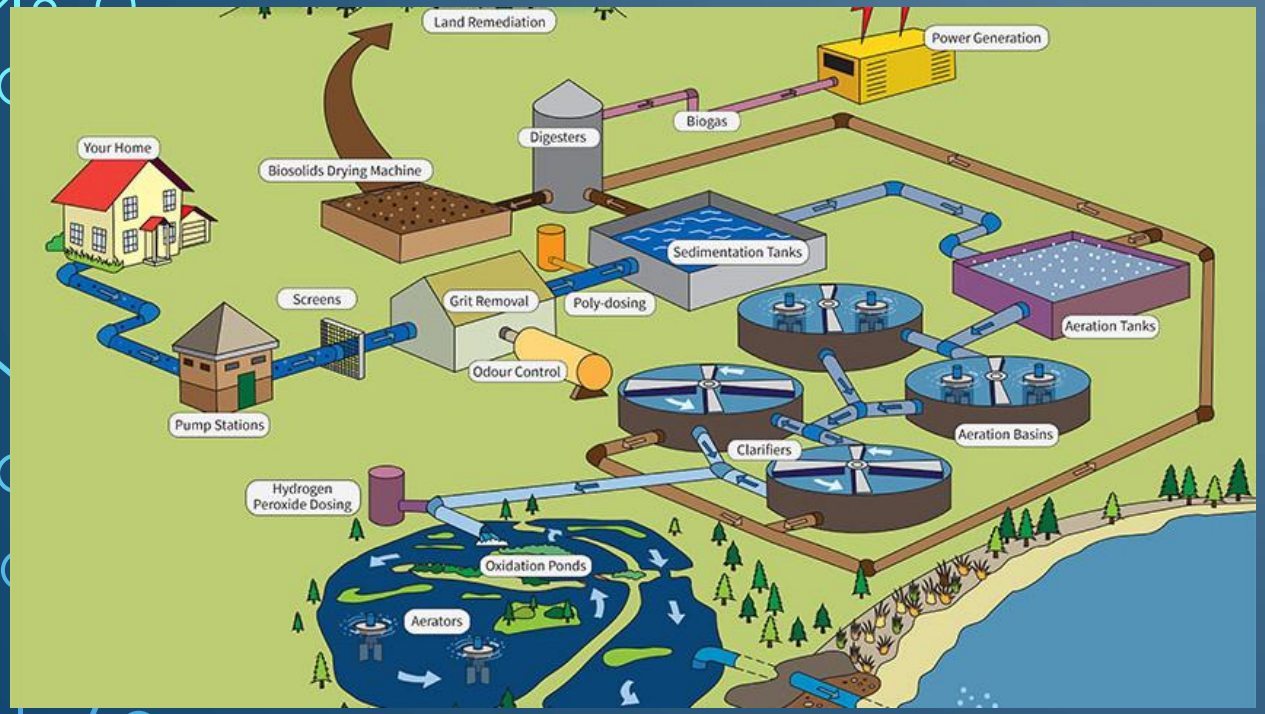
Hoi Yee Chow, Kathy Hiu Laam Po, Peng Gao, Pilar Blasco, Xiukun Wang, Congran Li, Lianwei Ye, Kang Jin, Kaichao Chen, Edward Wai Chi Chan, Xuefu You, Richard Yi Tsun Kao, Sheng Chen*, and Xuechen Li*

NMPA gives approval to test antibiotic drug in Mainland China

Kynomycin is designed to target complex skin and soft tissue infections caused by bacteria.

October 10, 2023

MONITORING COMMUNITY AMR



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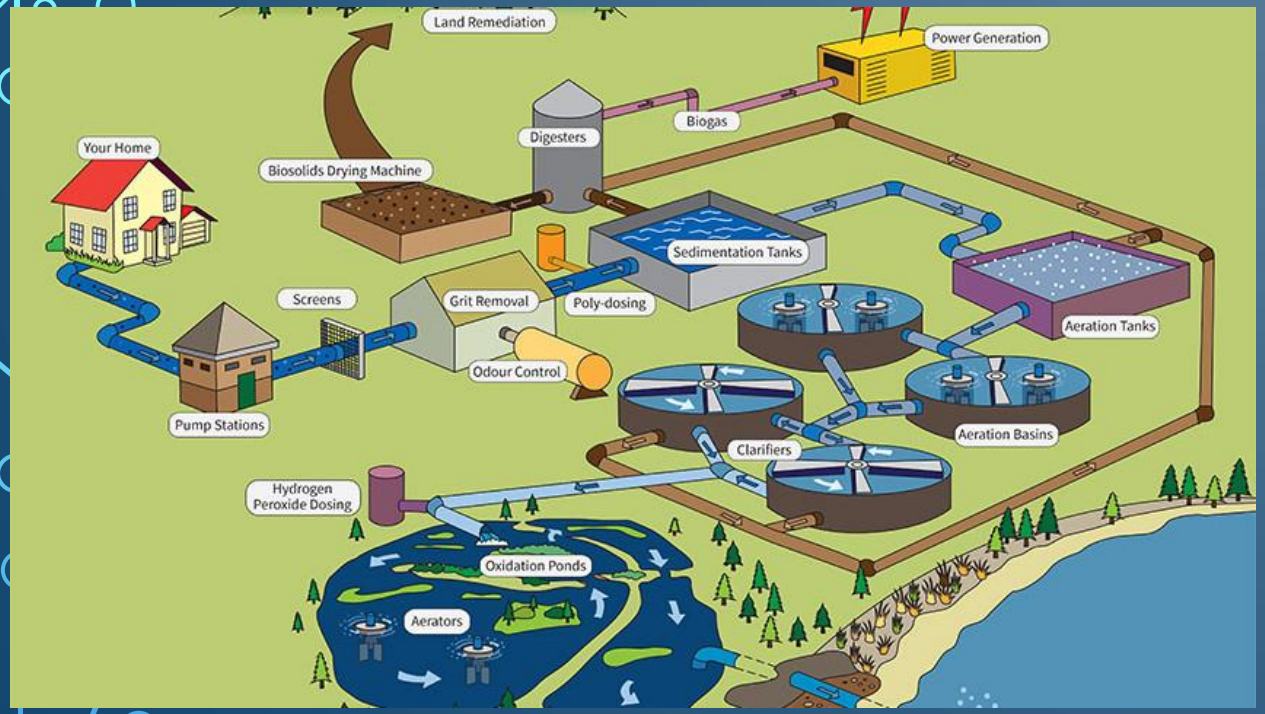
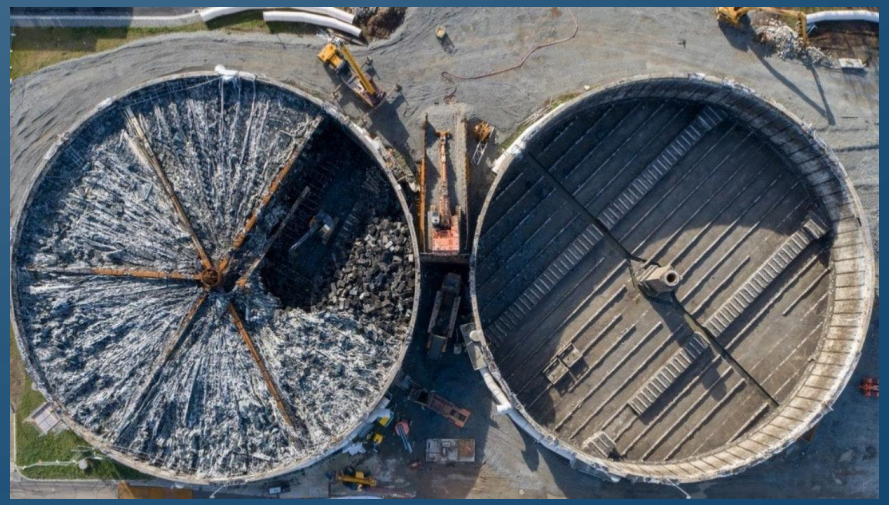
MONITORING COMMUNITY AMR



Christchurch City Council

Chris Brent



Christchurch City Council

E/S/R
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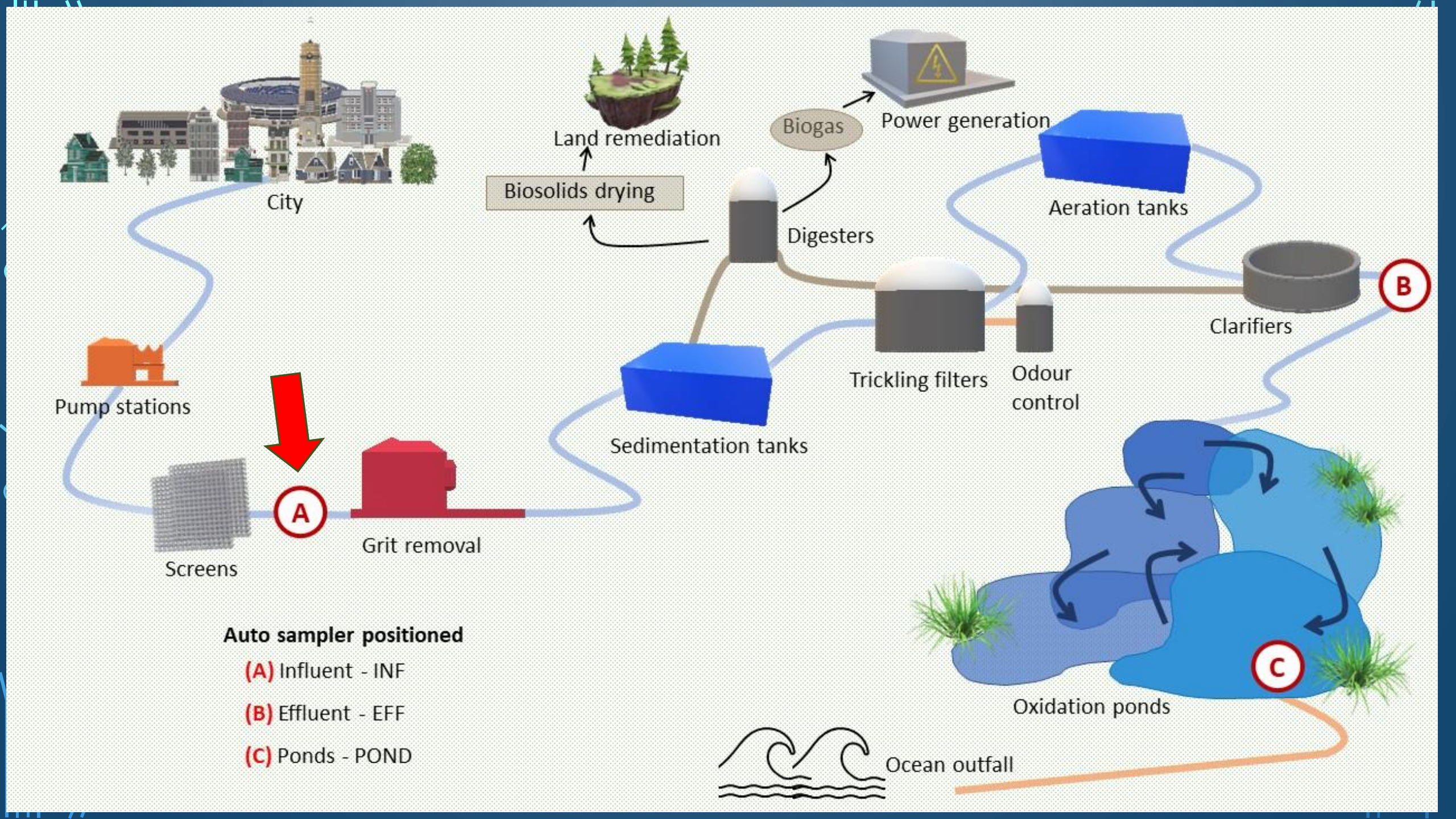
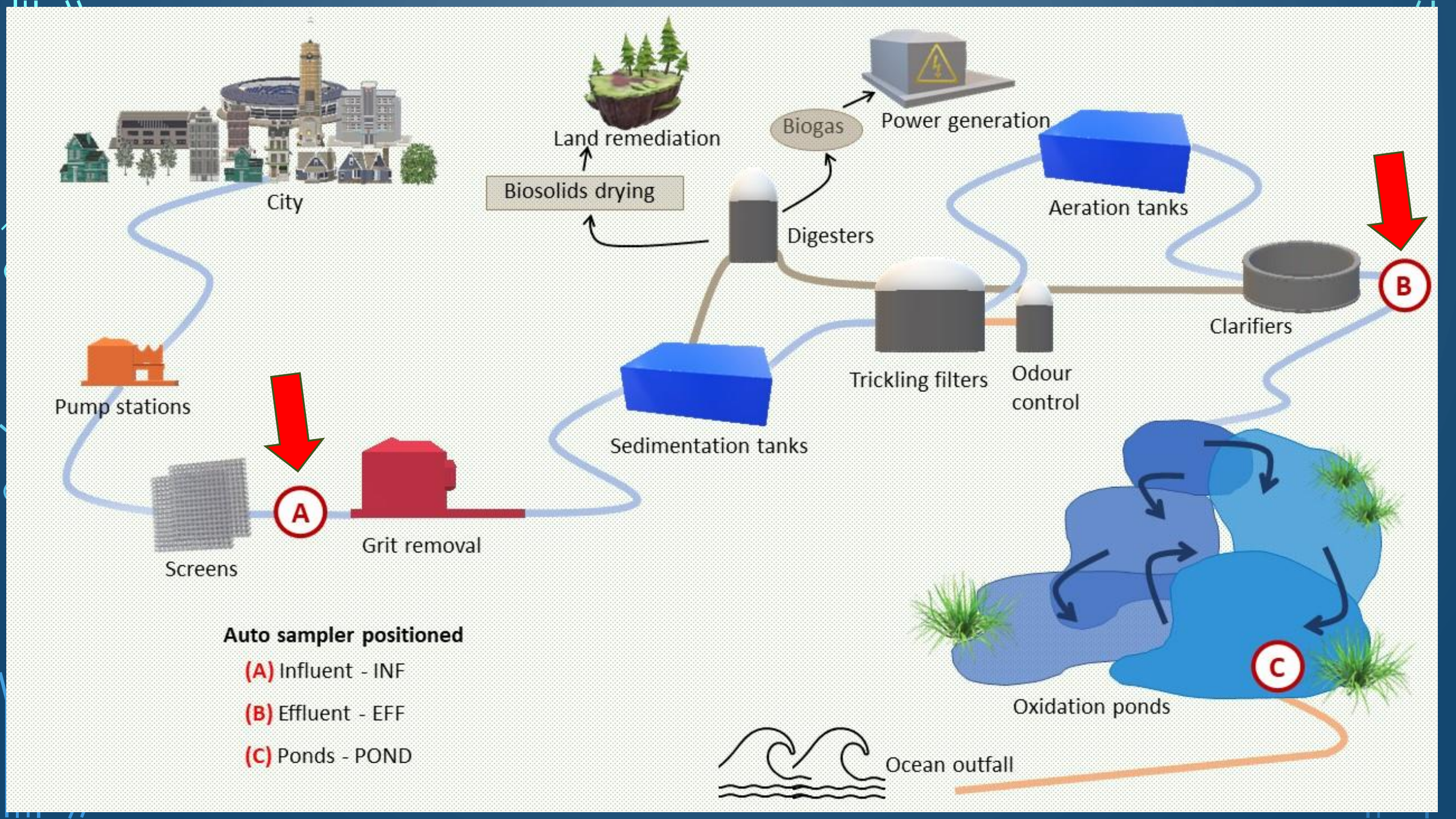

City
Land remediation
Biosolids drying
Biogas
Power generation
Digesters
Aeration tanks
Clarifiers
B
Pump stations
Sedimentation tanks
Trickling filters
Odour control
A
Grit removal
Screens
C
Oxidation ponds
Ocean outfall
Auto sampler positioned
(A) Influent - INF
(B) Effluent - EFF
(C) Ponds - POND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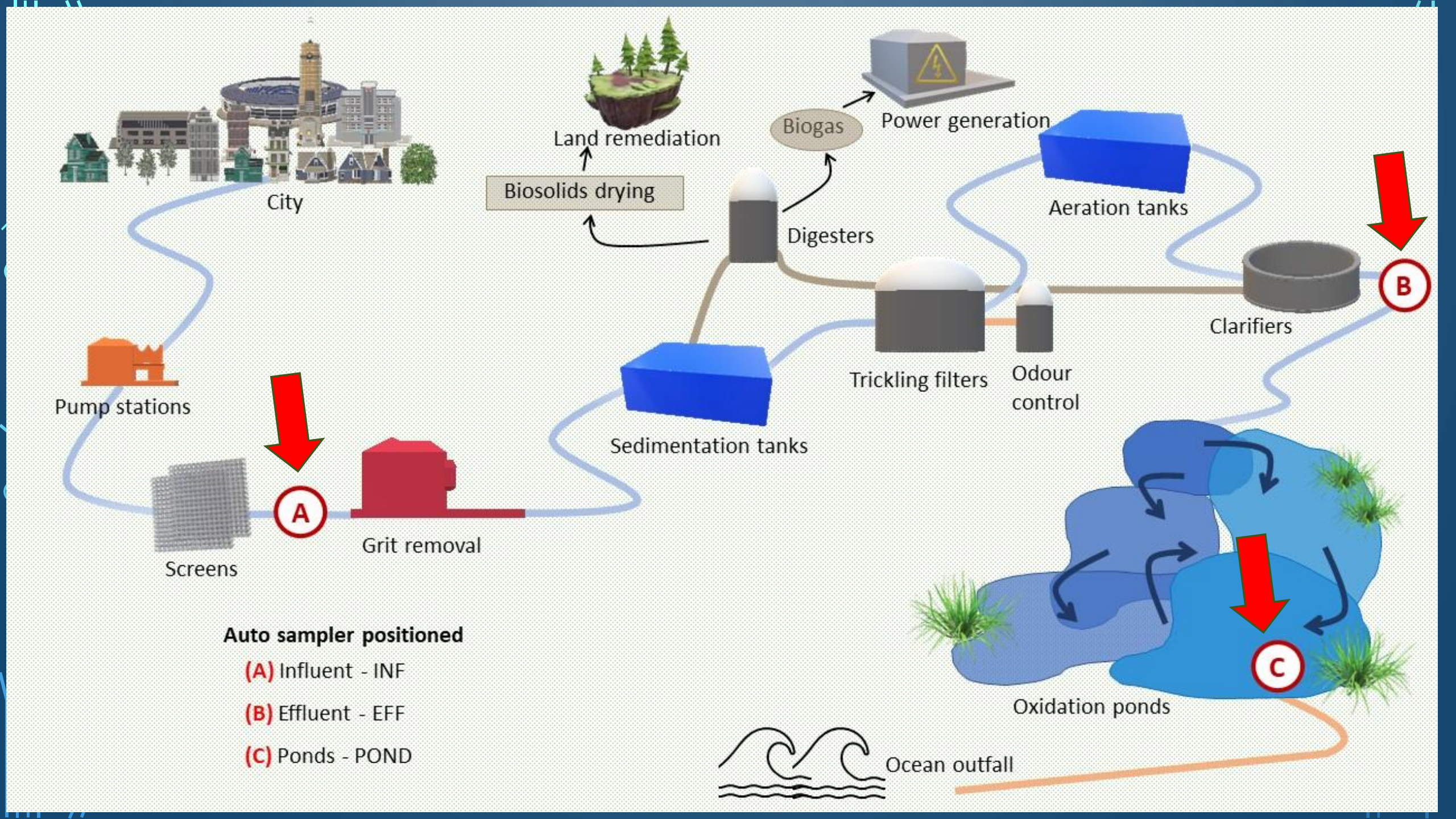
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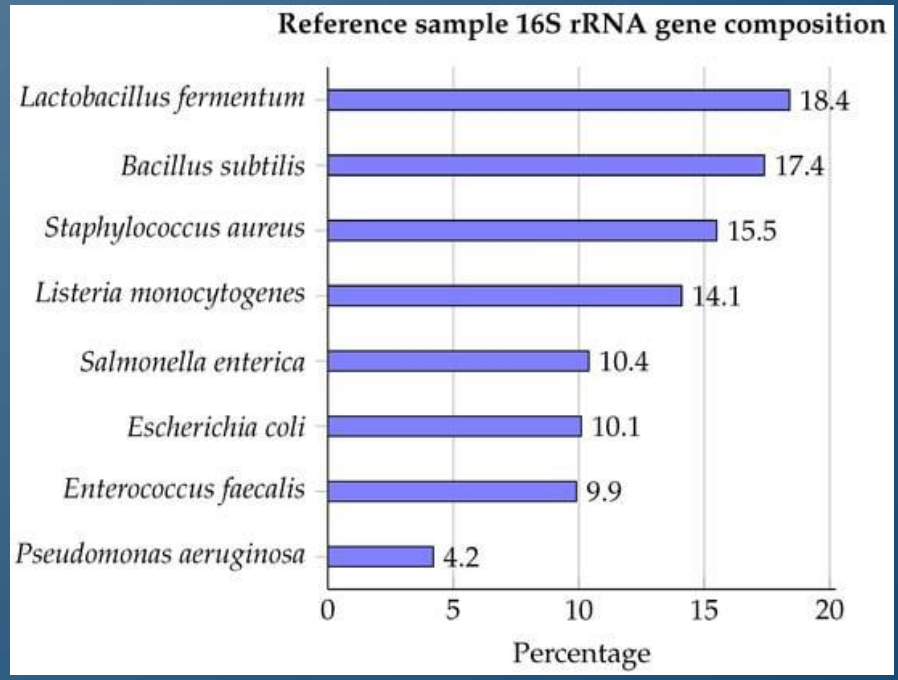
METHODOLOGY: qPCR AND MGS

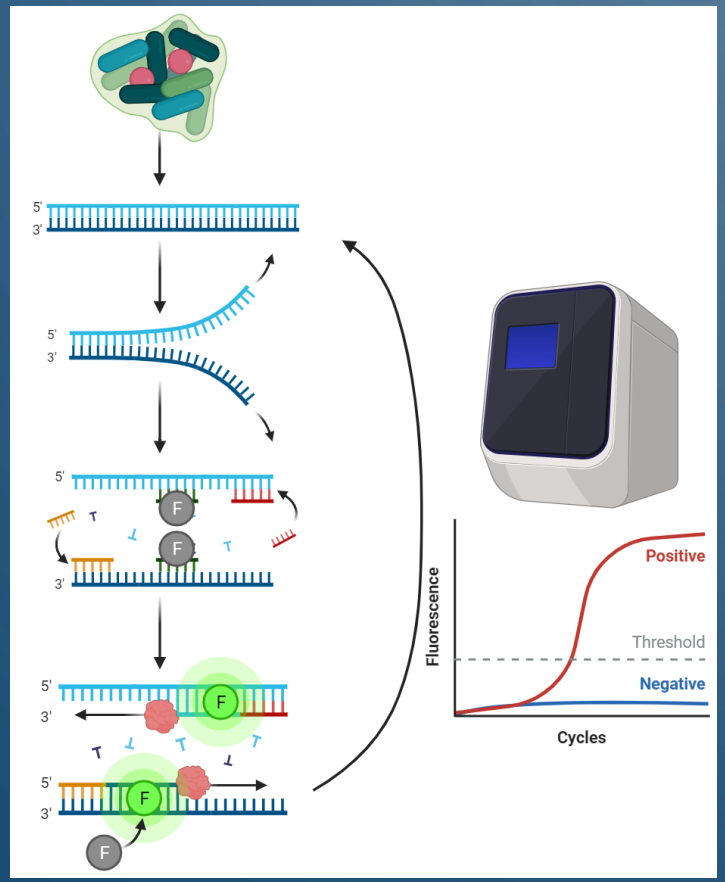
- 4 sample types × 3 replicates × 3 Days
  - INF, EFF, POND, SED (2 days)
  - 24-hour composite samples, *n* = 33
- qPCR 5 ARGs + 16S rRNA
  - *ermB*, *sul1*, *tetA*, *tetQ*, *tetW*
- Whole genome/metagenomic sequencing (MGS)
  - ResFinder DB and SILVA DB
- 16S rRNA adjusted 'ARG per bacteria'



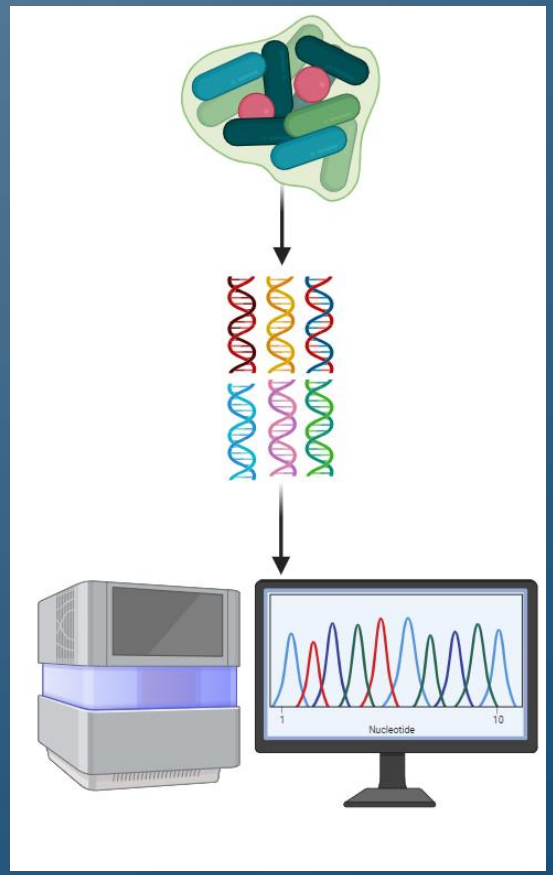
Winand et al. 2019.Targeting the 16S rRNA Gene for Bacterial Identification in Complex Mixed Samples: Comparative Evaluation of Second (Illumina) and Third (Oxford Nanopore Technologies) Generation Sequencing Technologies.

METHODOLOGY: qPCR AND MGS

qPCR



MGS



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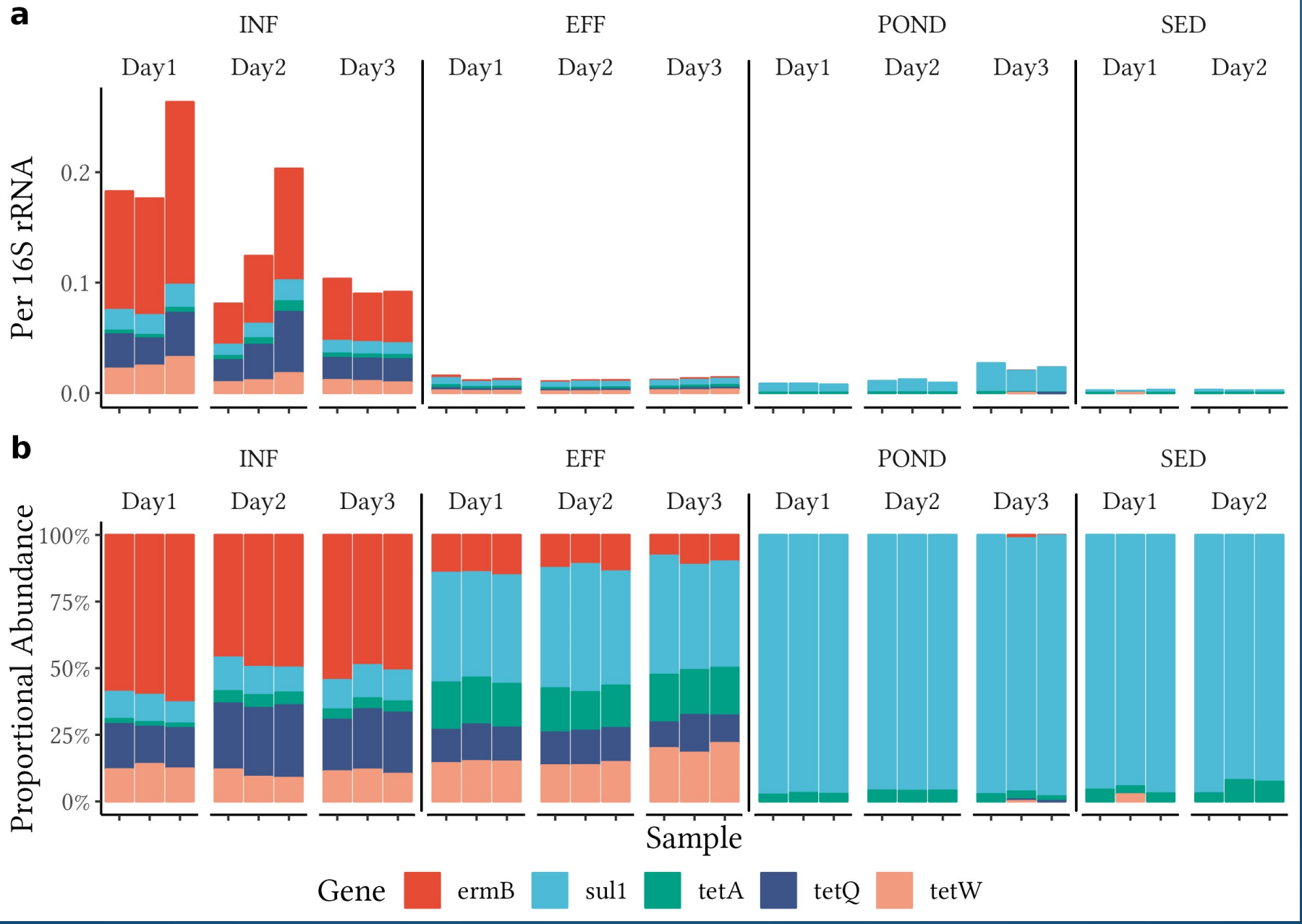
qPCR



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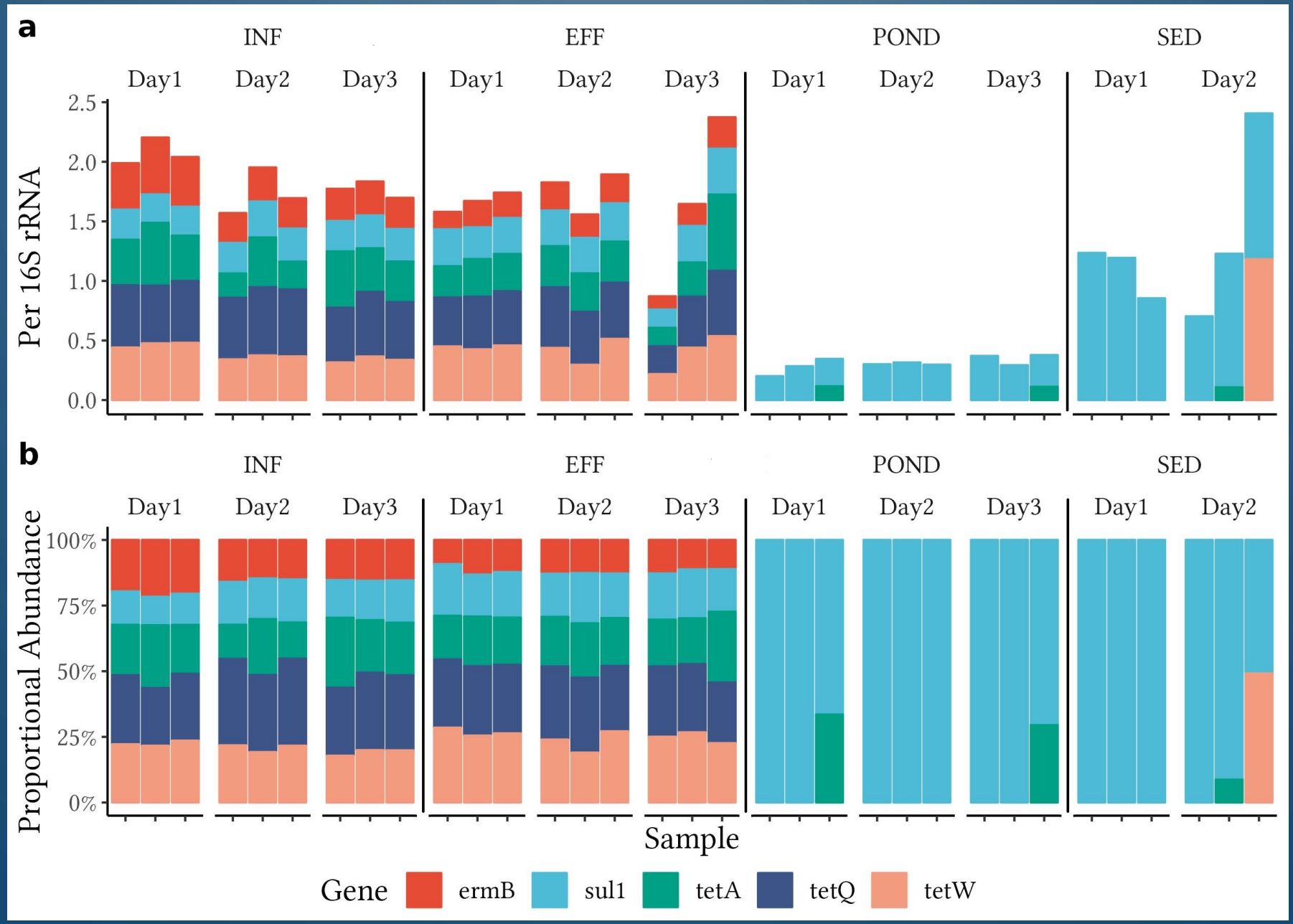
MGS



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DISCREPANCIES? BIAS?

- Gene length & Sequencing Depth
  - *ermB*: ~750bp
  - *tetQ* & *tetW*: ~1920bp
  - 16S rRNA ~1500bp
- Primer issues
  - Off target binding
  - Inhibitors interfere primer binding
    - Less impactful for MGS?
- Sample dilution

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PRESENCE ABSENCE

Percent of samples ARGs were detected.

Sample Type	Method	*ermB*	*sul1*	*tetA*	*tetQ*	*tetW*
INF (*n* = 9)	qPCR	100%	100%	100%	100%	100%
	MGS	100%	100%	100%	100%	100%
EFF (*n* = 9)	qPCR	100%	100%	100%	100%	100%
	MGS	100%	100%	100%	100%	100%
POND (*n* = 9)	qPCR	22.22%	100%	100%	22.22%	11.11%
	MGS	0%	100%	77.77%	0%	0%
SED (*n* = 6)	qPCR	0%	100%	100%	0%	16.6%
	MGS	0%	100%	66.6%	0%	16.6%

ESR
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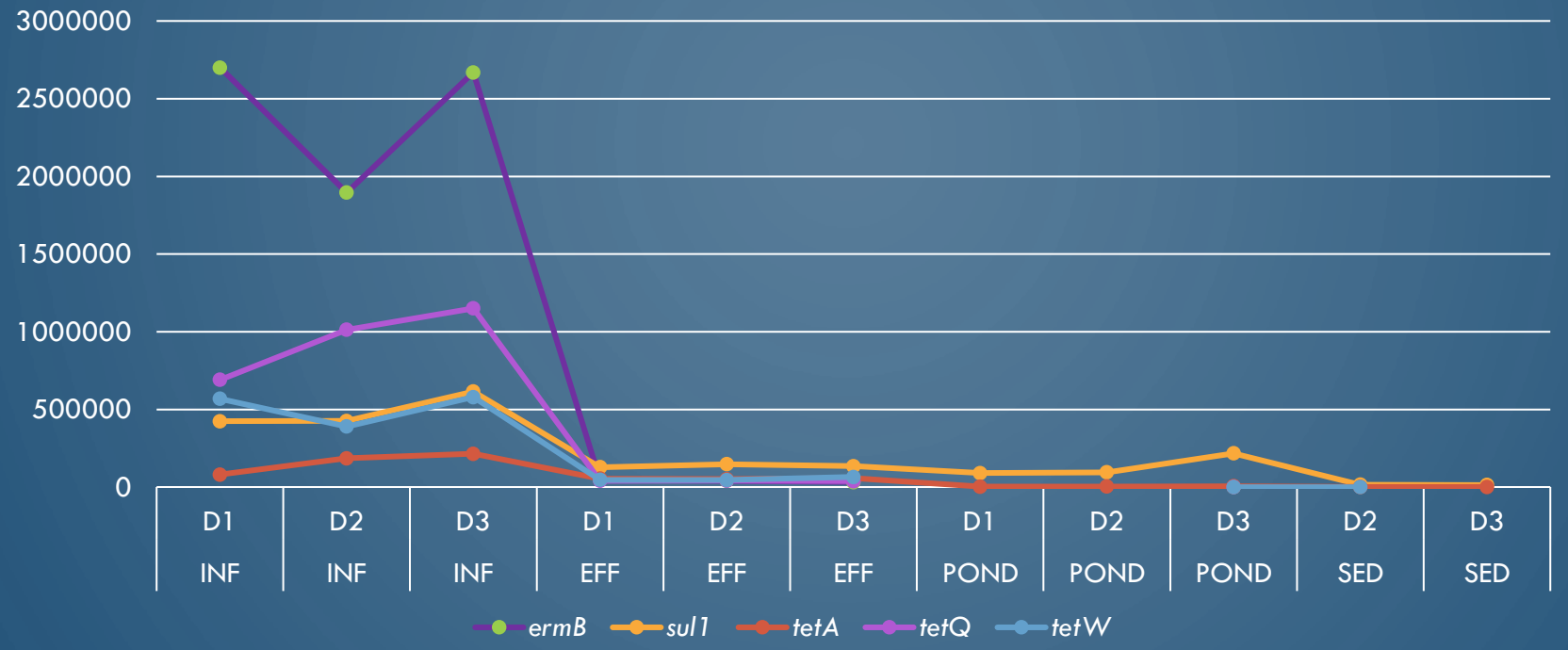
ARG throughout Treatment Process: qPCR



	INF	INF	INF	EFF	EFF	EFF	POND	POND	POND	SED	SED
gene	D1	D2	D3	D1	D2	D3	D1	D2	D3	D2	D3
ermB	2700636	1895615	2669494	45654	40384	31623	0	0	990	0	0
sul1	423532	425576	614870	127493	147182	135177	89977	94782	216803	14978	13501
tetA	80374	185020	213917	54195	50971	57182	2611	3985	5392	533	865
tetQ	690747	1012524	1150965	40802	41242	37512	0	0	521	0	0
tetW	568664	390419	579583	46496	45507	64931	0	0	74	103	0

Raw values summed for each day.

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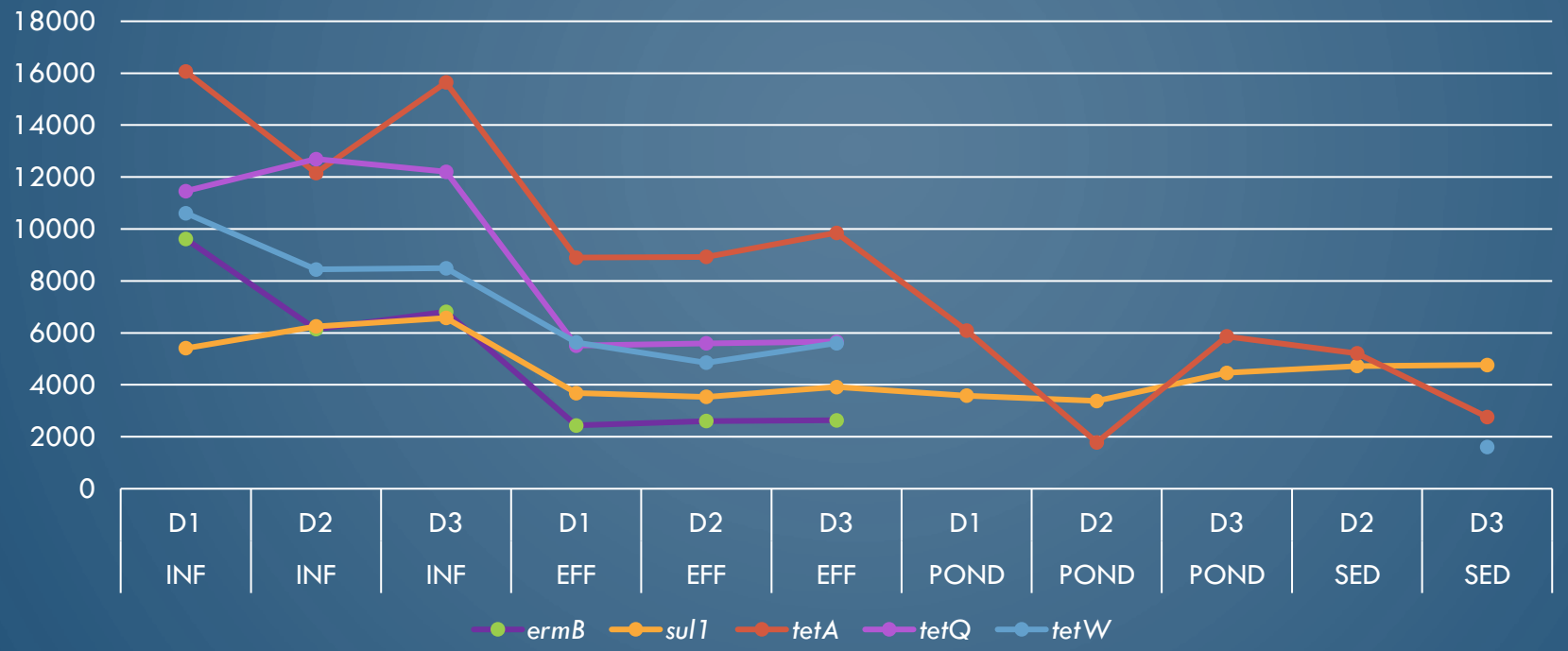
ARG throughout Treatment Process: MGS reads



	INF	INF	INF	EFF	EFF	EFF	POND	POND	POND	SED	SED
gene	D1	D2	D3	D1	D2	D3	D1	D2	D3	D2	D3
ermB	9613	6144	6811	2440	2606	2638	0	0	0	0	0
sul1	5409	6251	6578	3683	3539	3914	3586	3382	4464	4722	4763
tetA	16063	12158	15641	8895	8924	9850	6092	1787	5865	5214	2763
tetQ	11456	12689	12202	5511	5599	5662	0	0	0	0	0
tetW	10607	8442	8490	5630	4854	5603	0	0	0	0	1607

Raw values summed for each day.

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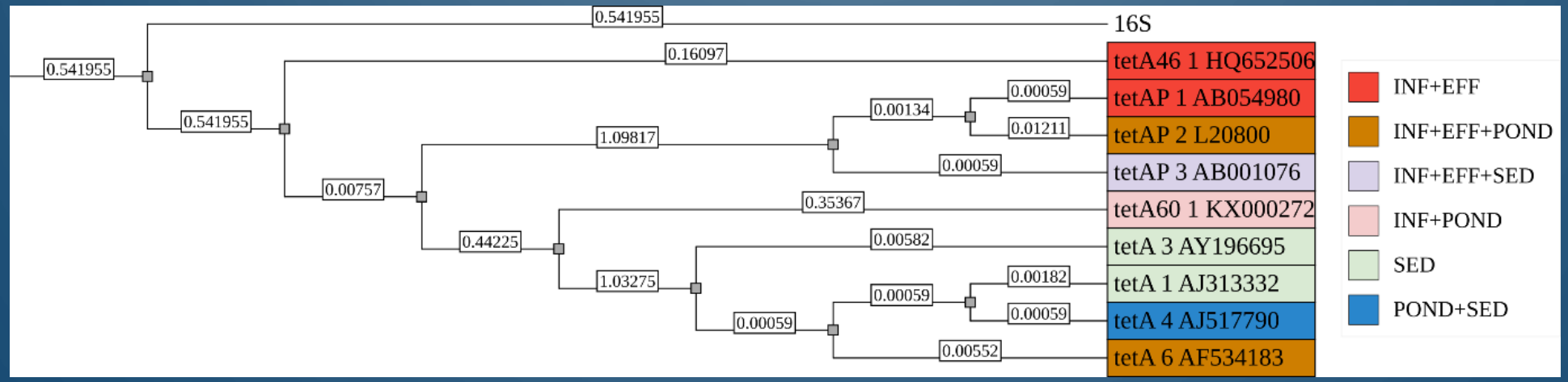
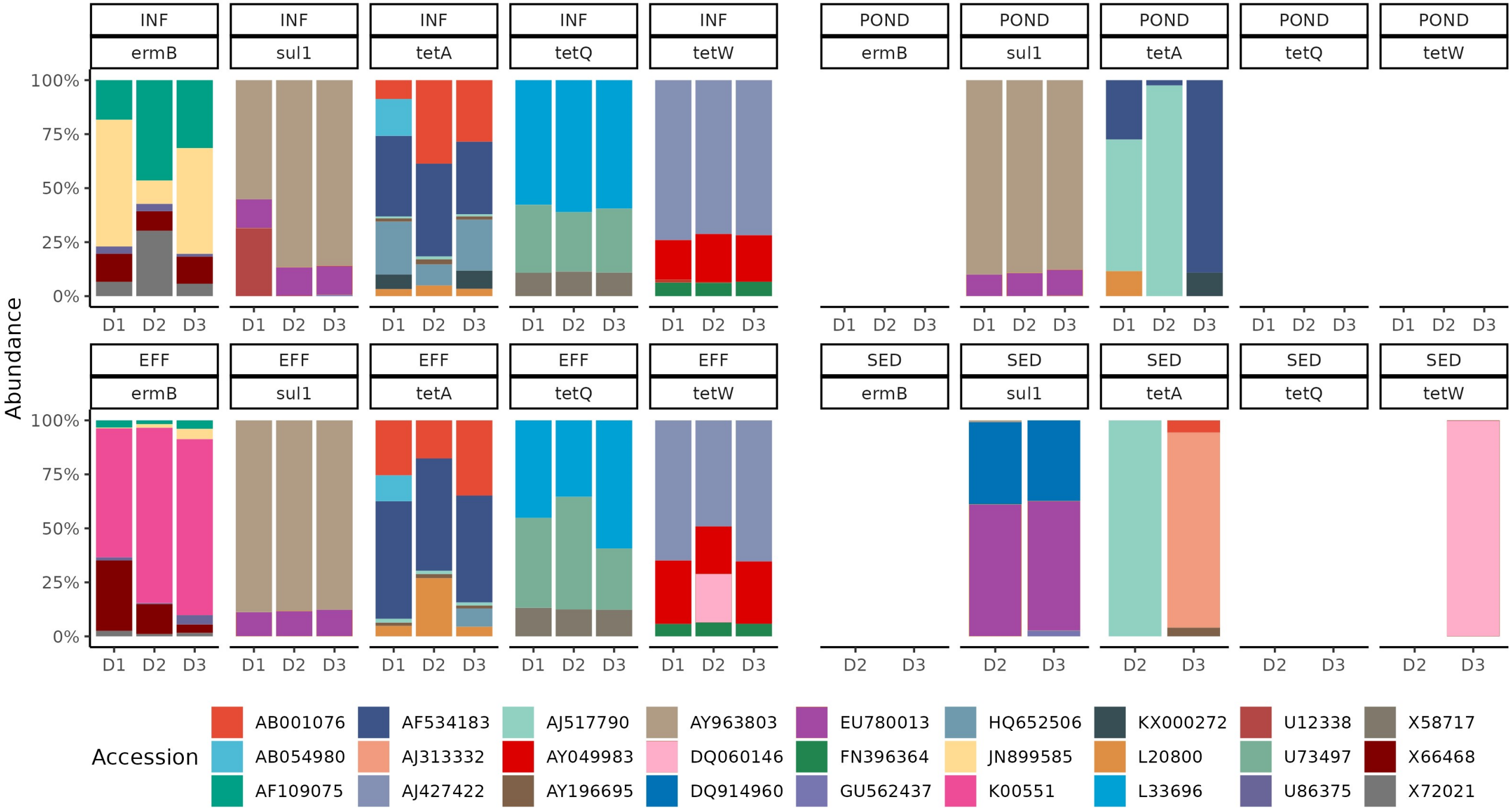
tetA REFERENCE MULTIPLE SEQUENCE ALIGNMENT




INF ermB
INF sul1
INF tetA
INF tetQ
INF tetW
POND ermB
POND sul1
POND tetA
POND tetQ
POND tetW
EFF ermB
EFF sul1
EFF tetA
EFF tetQ
EFF tetW
SED ermB
SED sul1
SED tetA
SED tetQ
SED tetW
Abundance
100%
75%
50%
25%
0%
D1 D2 D3
Accession
AB001076
AB054980
AF109075
AF534183
AJ313332
AJ427422
AJ517790
AY049983
AY196695
AY963803
DQ060146
DQ914960
EU780013
FN396364
GU562437
HQ652506
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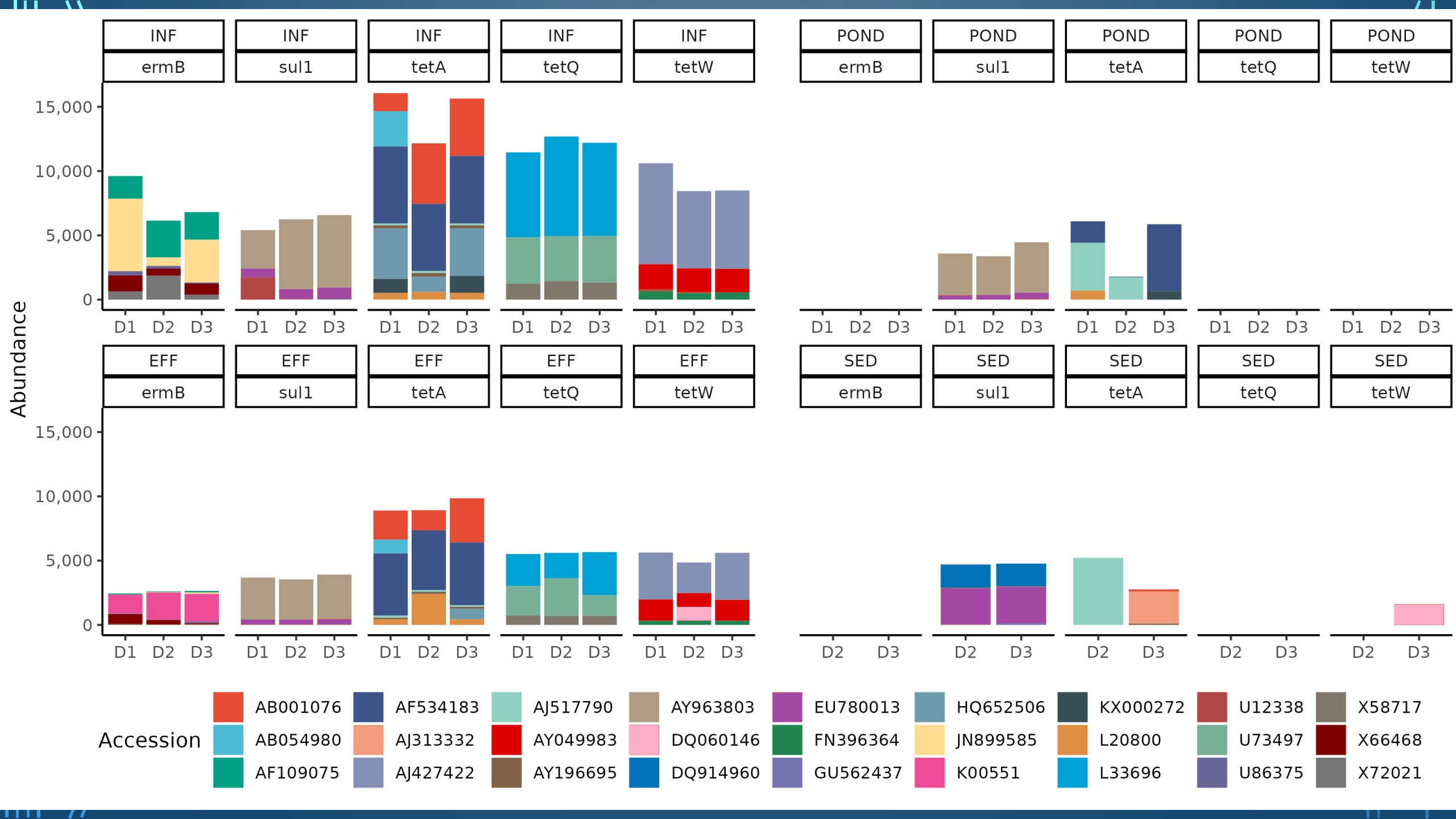
INF
ermB
INF
sul1
INF
tetA
INF
tetQ
INF
tetW
POND
ermB
POND
sul1
POND
tetA
POND
tetQ
POND
tetW
EFF
ermB
EFF
sul1
EFF
tetA
EFF
tetQ
EFF
tetW
SED
ermB
SED
sul1
SED
tetA
SED
tetQ
SED
tetW
Abundance
15,000
10,000
5,000
0
D1
D2
D3
Accession
AB001076
AB054980
AF109075
AF534183
AJ313332
AJ427422
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K00551
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L20800
L33696
U12338
U73497
U86375
X58717
X66468
X72021

PROS AND CONS: qPCR & MGS

- qPCR
  - Fast and effective
  - Ubiquitous and cheap
  - (more) Quantitative
  - Need a priori knowledge
- Metagenomic Sequencing (MGS)
  - Time consuming and expensive
  - Specialised knowledge, multiple methods
  - Limited quantification
  - Large amount of redundant information
  - Catch-all (with enough depth)
  - Broad with option of further analysis

| Applied and Industrial Microbiology | Research Article | 24 May 2023

Nonbacterial Microflora in Wastewater Treatment Plants: an Underappreciated Potential Source of Pathogens

Authors: Sujani Ariyadasa, William Taylor, Louise Weaver, Erin McGill, Craig Billington, Isabelle Pattis | AUTHORS INFO & AFFILIATIONS

DOI: https://doi.org/10.1128/spectrum.00481-23 • Check for updates

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