

Systematic Evaluation of Factors Affecting the Characterization of Wastewater Effluents Using Gene Expression

Adam Biales¹, David Bencic, Mary Jean See¹, Susan Glassmeyer¹, Dana Kolpin², Marc Mills¹, Edward Furlong², Robert Flick¹, Weichun Huang¹, S. Thomas Purucker¹

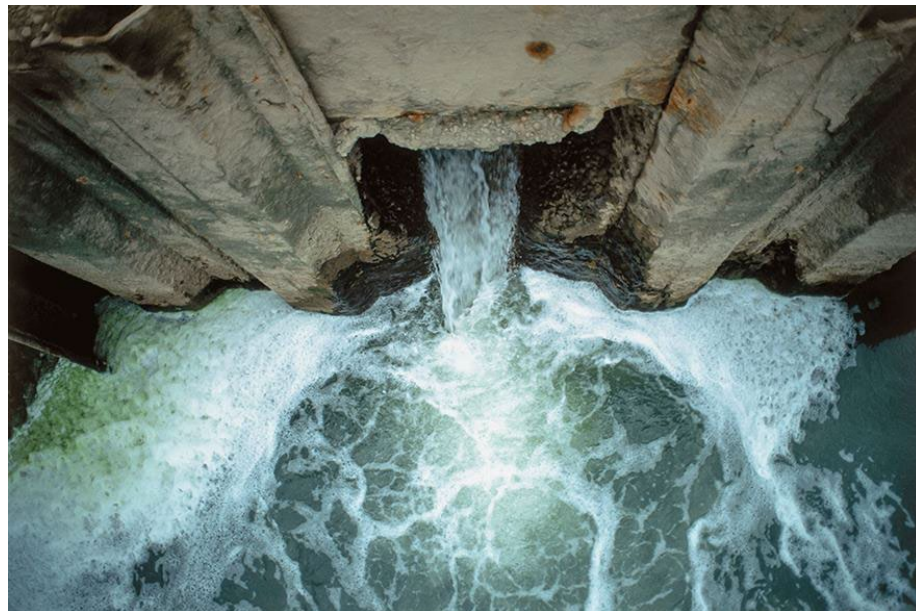
1. US Environmental Protection Agency

2. U.S. Geological Survey

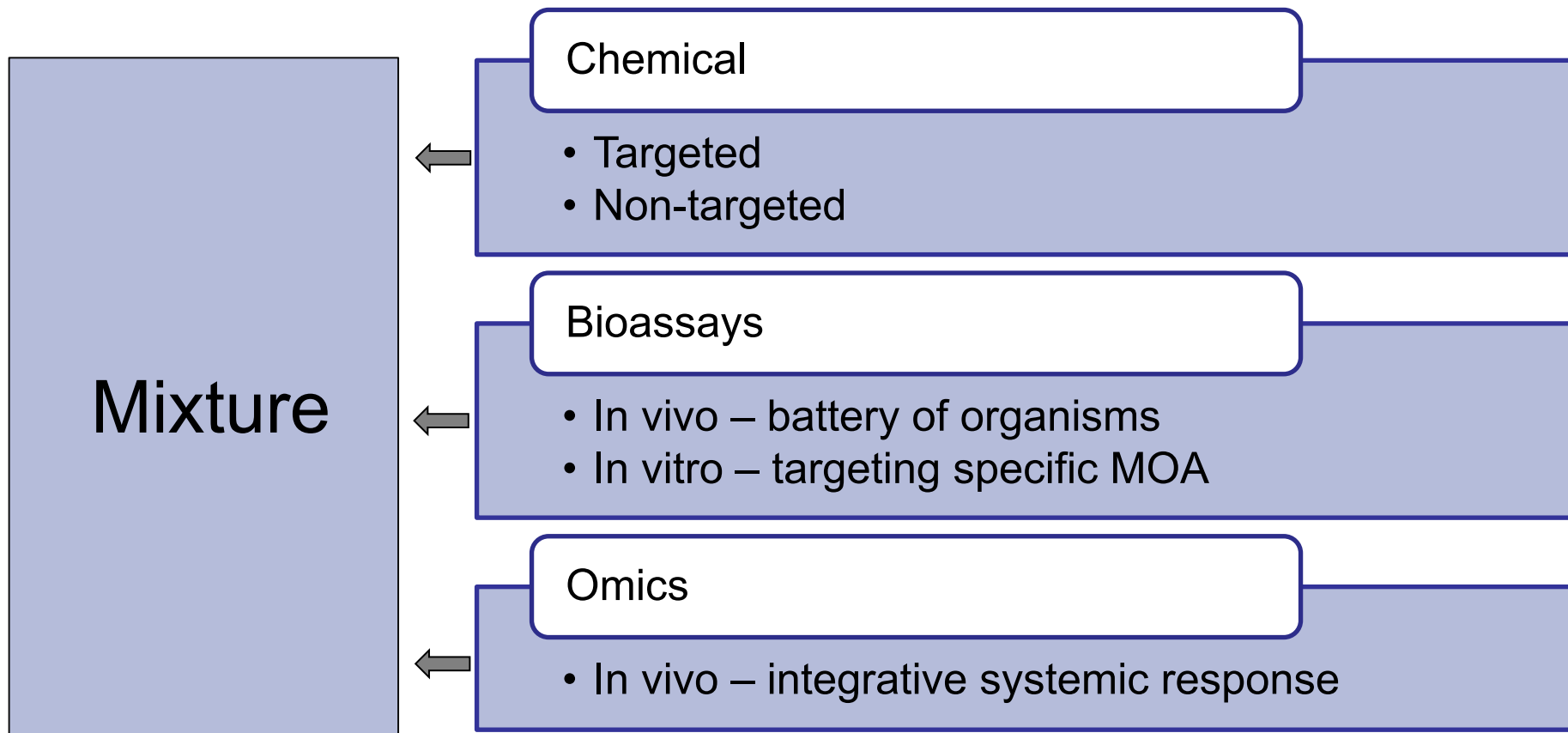
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Background

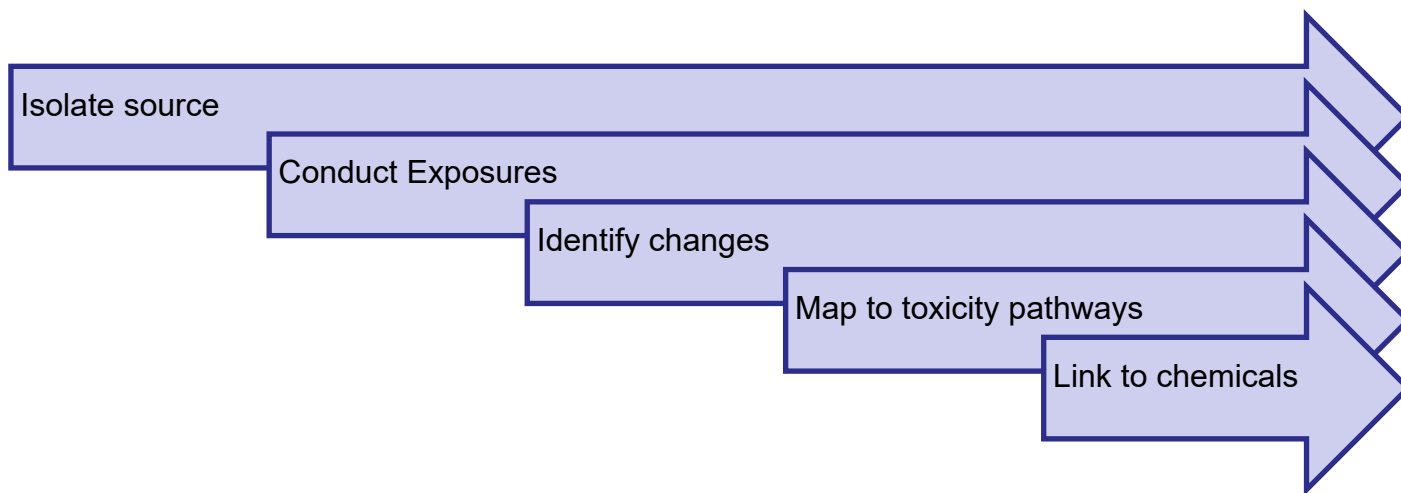
- Wastewater Treatment Effluent
 - Point sources of mixtures of chemical contamination
 - Variability in composition
 - Efficiency of removal
 - Temporal variability across scales
 - Variability in effects
 - Chemical and non-chemical factors
 - Diverse effects/MOA/target tissues



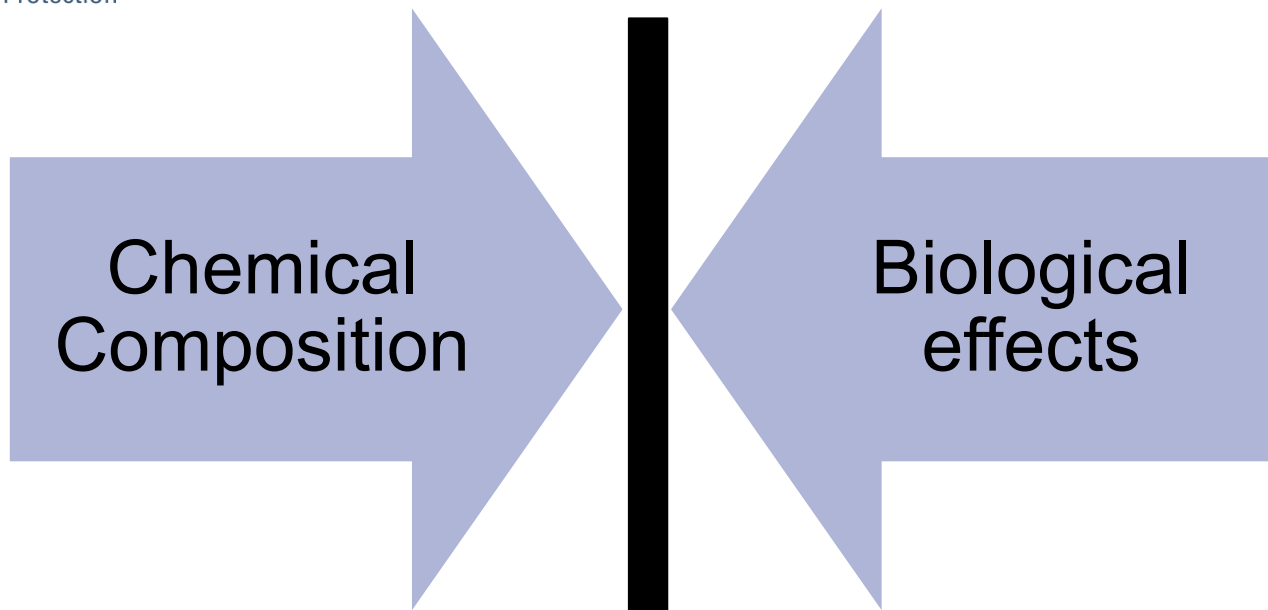
Characterization



Omics-based Assays



Characterization

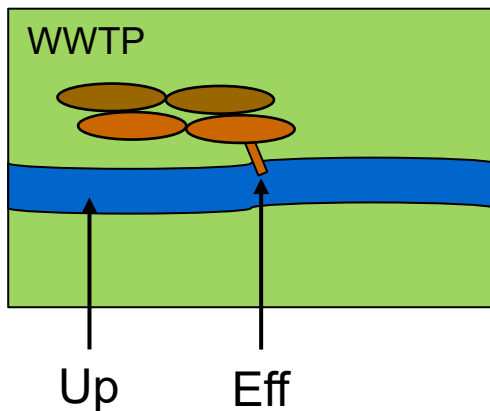


- Interpretation
 - Temporal effects
 - Sampling
 - Influence of reference

Objectives

- Use of gene expression to characterize the bioactivity of a complex, real-world mixture
 - Is gene expression in organisms exposed to a wastewater treatment effluent stable within and across days?
 - How do experimental factors, such as the selection of reference conditions, affect the interpretation of expression results?

Sampling



Effluent pipe sampling

Deployment



Effluent pipe

Day 1

- TS 1 – morning
- TS 2 – evening

Time



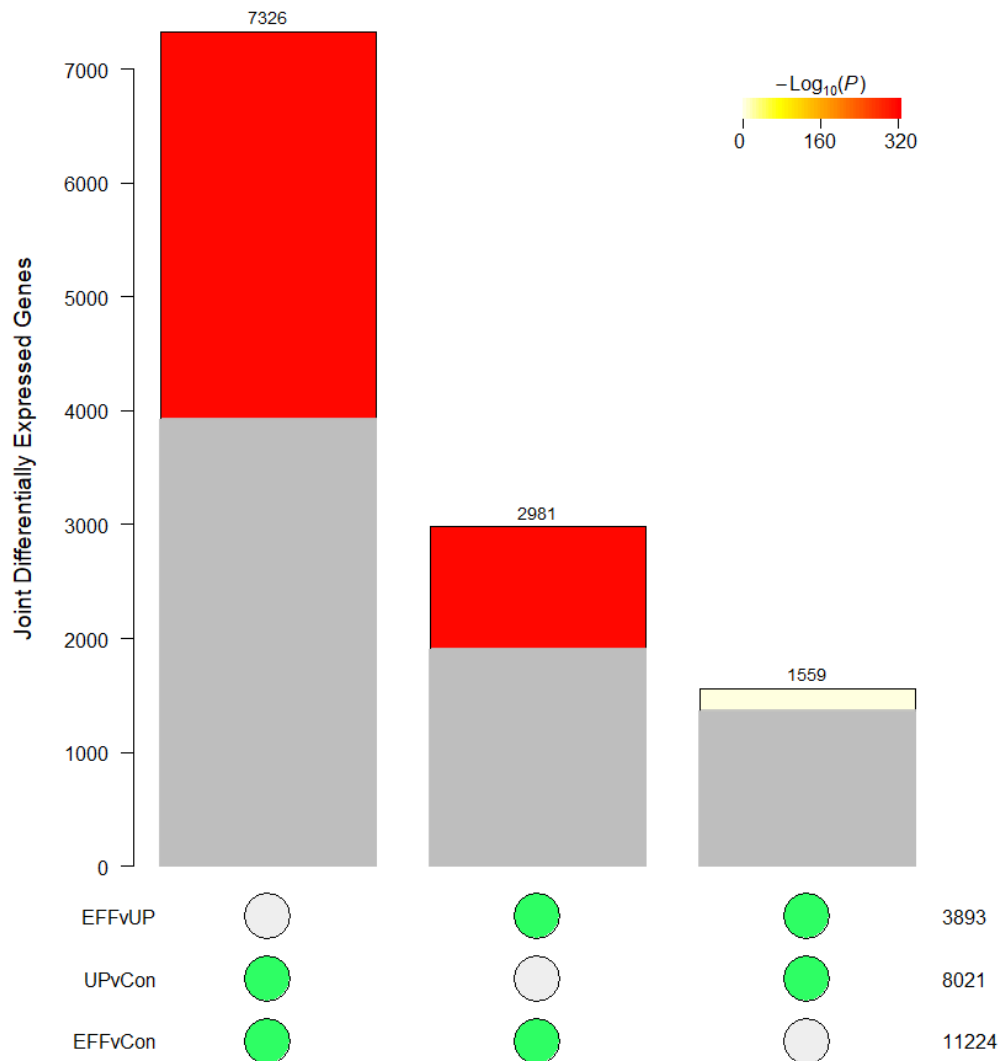
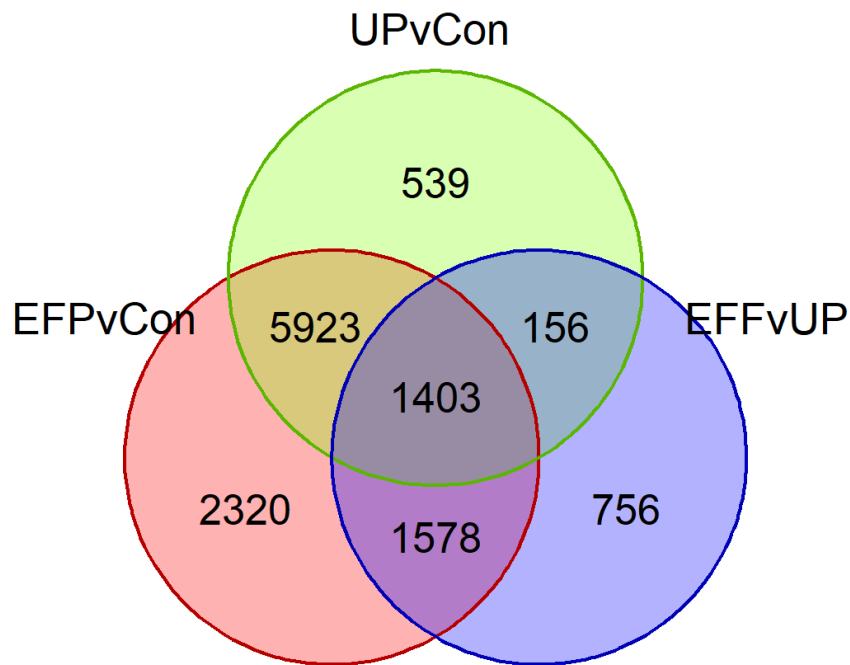
Day 2

- TS 3 – morning
- TS 4 – evening



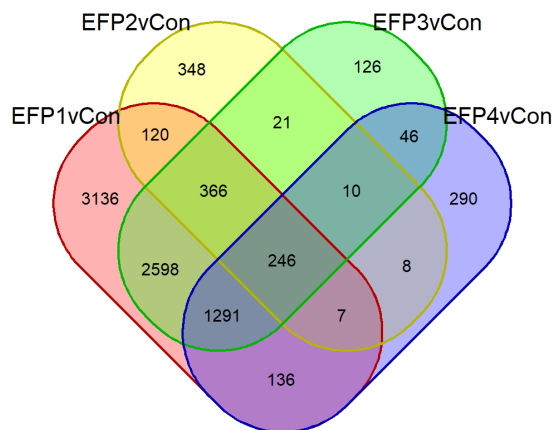
Deployment

Cumulative Gene Expression

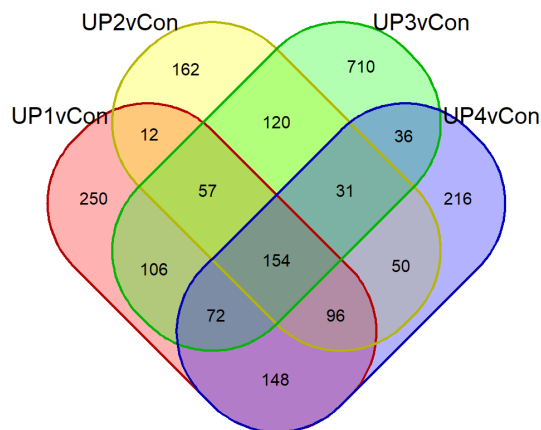


Temporal Stability

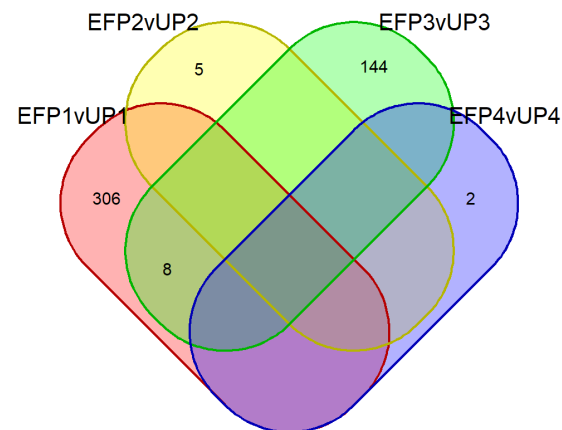
Effluent vs. Control



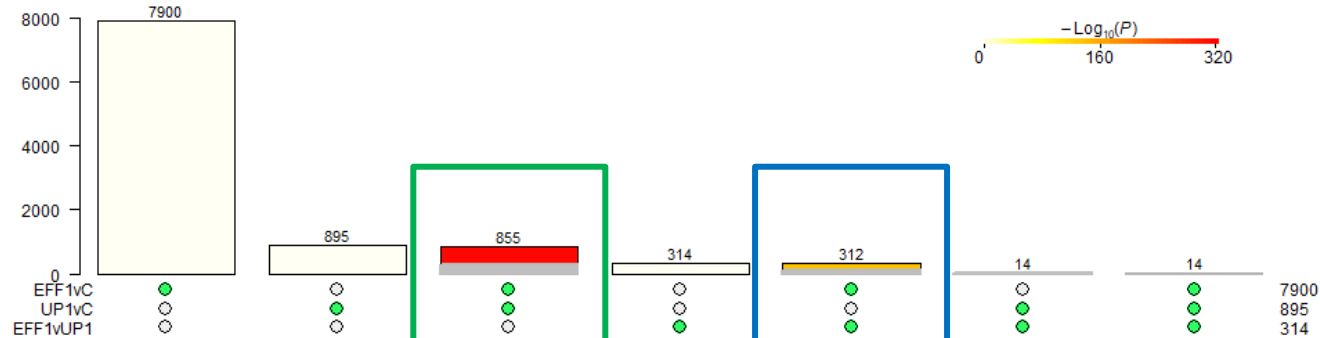
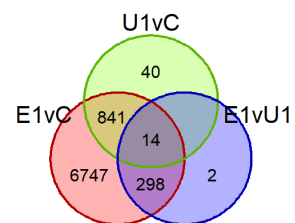
Upstream vs. Control



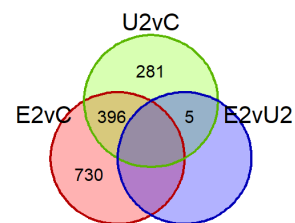
Effluent vs. Upstream



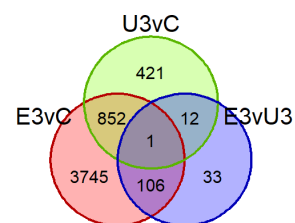
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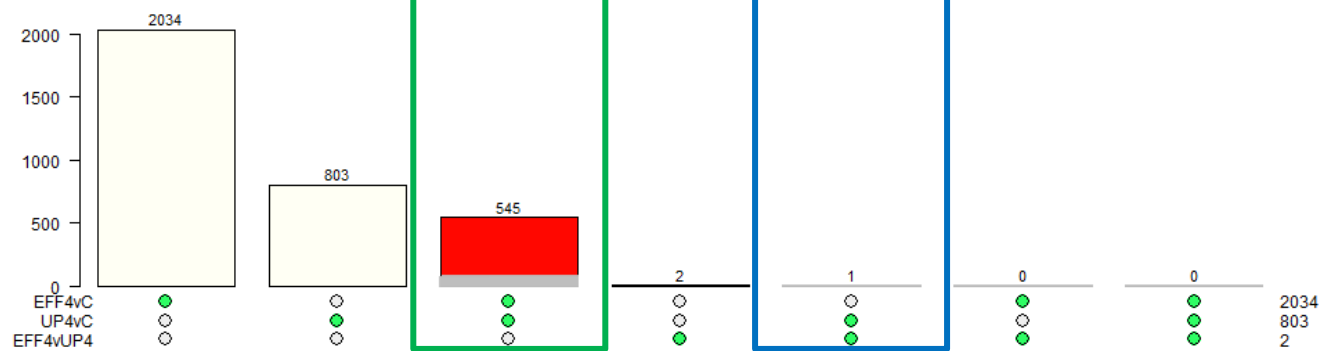
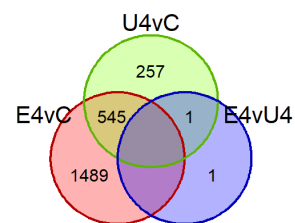
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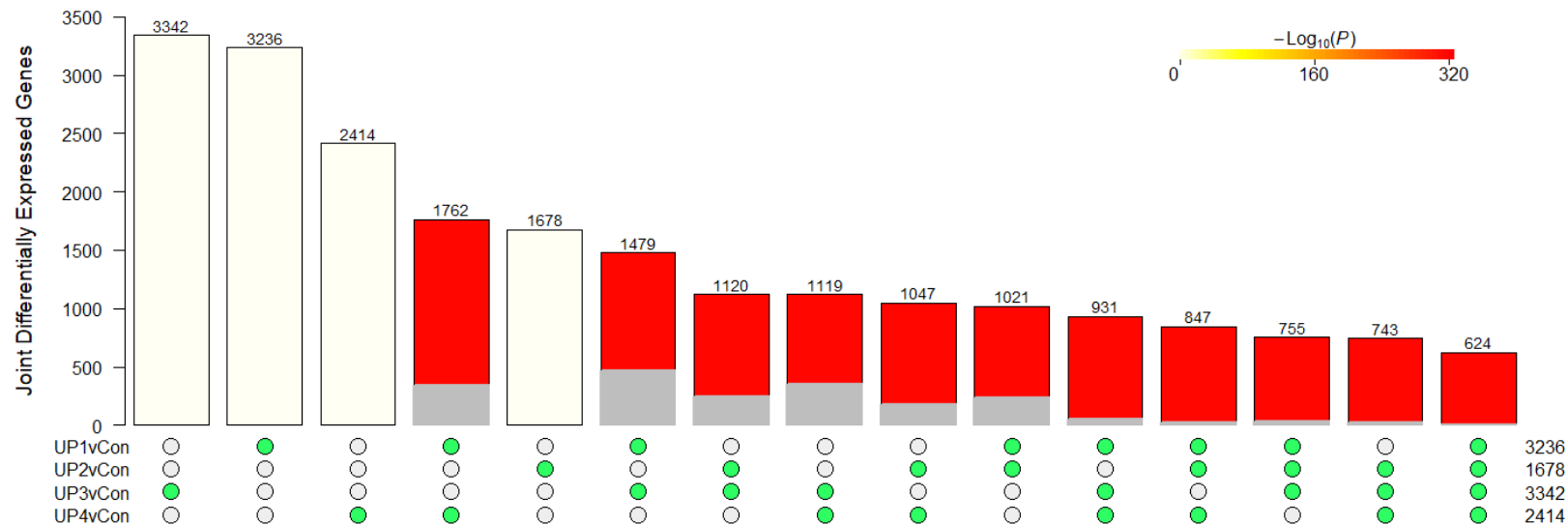
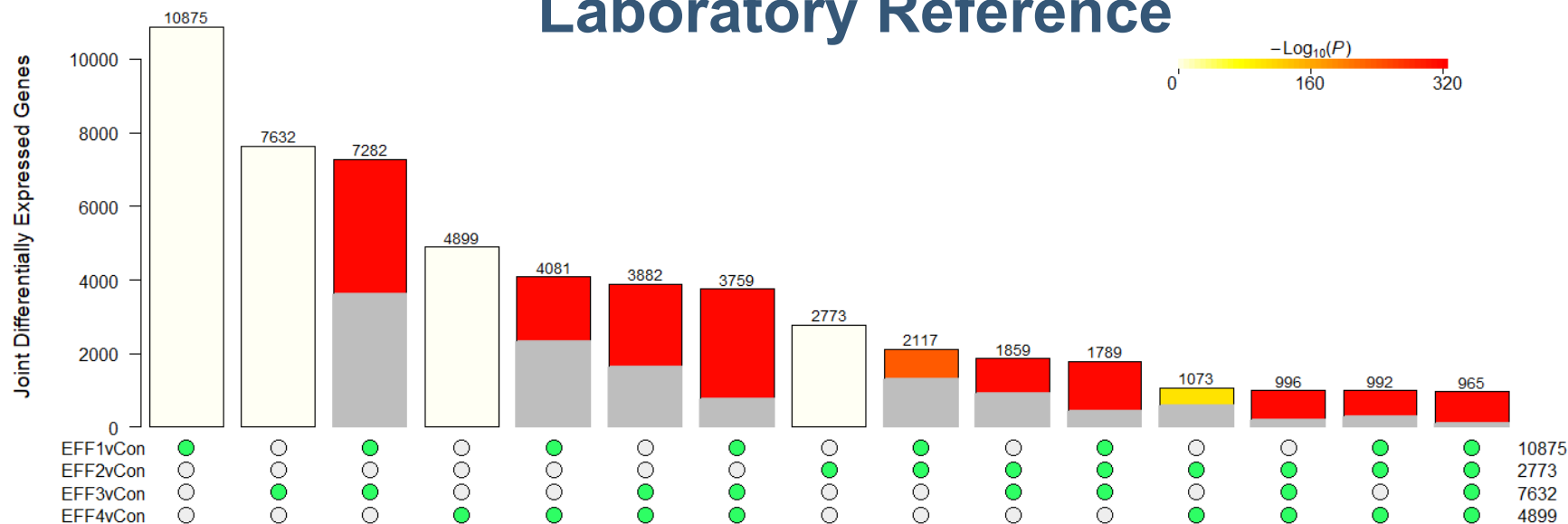
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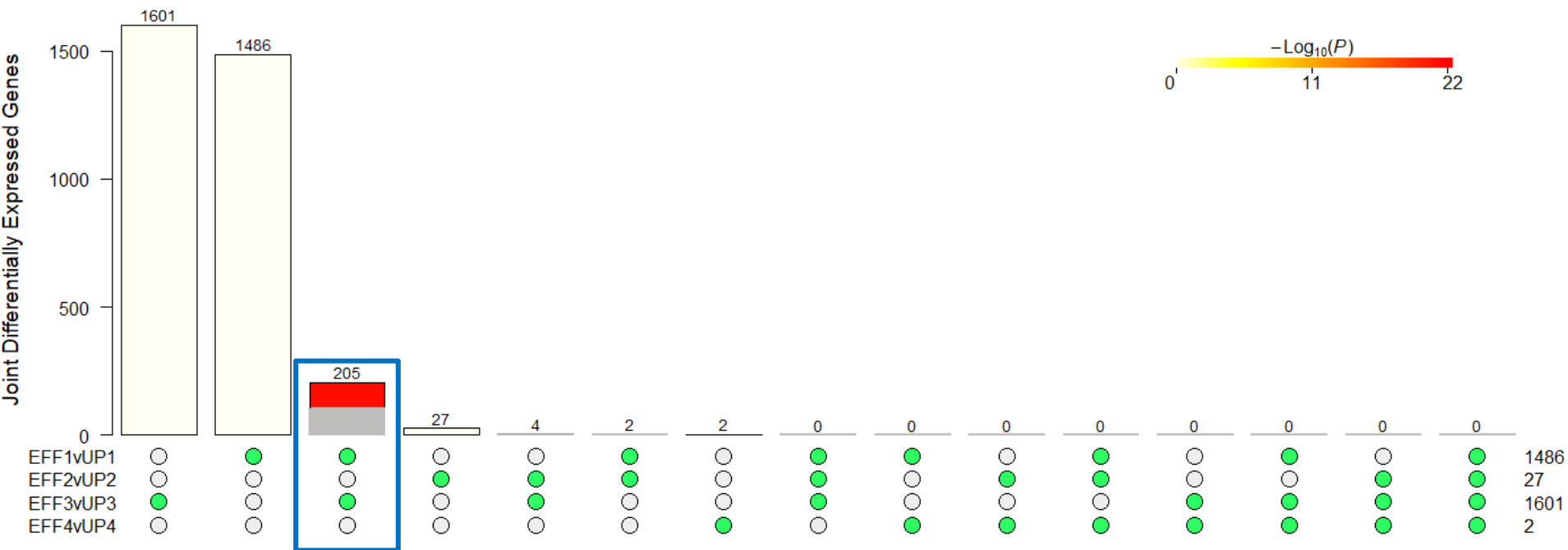
D



Laboratory Reference



Upstream Reference



Conclusions

- Stability
 - Cumulative gene expression reflects individual time points
 - Minimal day-to-day variability
 - Within day variability
- Reference selection
 - Laboratory reference
 - Relatively limited variability across time matched samples
 - Background water effects – non-WWTP dependent effects
 - Up vs. downstream
 - Reflects laboratory reference results
 - Relatively muted gene expression response
 - Suggests increased variability
 - Differences in background b/w up and downstream

USGS

Ed Furlong
Dana Kolpin
 Dave Alvarez
 Michelle Hladik
 Mike Meyer
 Jason Sorenson
 Marc Zimmerman



USEPA

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