Evaluating the Temporal Stability of Gene Expression Endpoints with Repeated Sampling Up and Down Stream of a Wastewater Treatment Plant

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Background

- Chemical input into ambient waters
  - Complex mixtures
    - Dynamic
  - Targeted chemical approaches
    - Lamplight effect
  - Toxicity endpoints
    - Uninformative -> MOA
  - Cell-based bioassays
    - Limited coverage
    - Matrix interference
  - Omics approaches
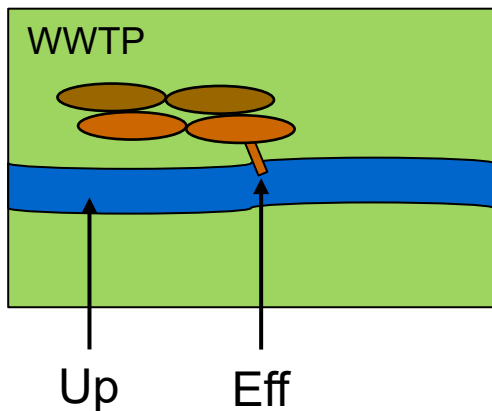   - False positives
    - Stability/Robustness
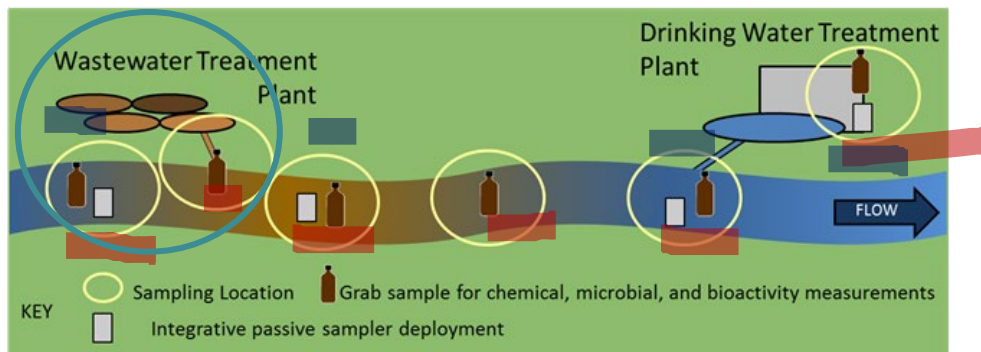
- Goal: Determine the consistency of gene expression in effluent exposed organisms over time and with different sampling approaches.
- Sample Collection:
  - Collected time matched samples (morning and afternoon) for two consecutive days up and down-stream (Eff) of WWTP.
  - Deploy fish up and down-stream of WWTP during collection period (Field)
- Gene Expression: RNA-seq - Compare different commonly used approaches.
  - Direct comparison of Up vs. Eff
  - Compare to clean laboratory control - Indirect comparison of Up vs. Eff

Wastewater Treatment Plant

- 1.2 MGD
- Serves ~1,700 people
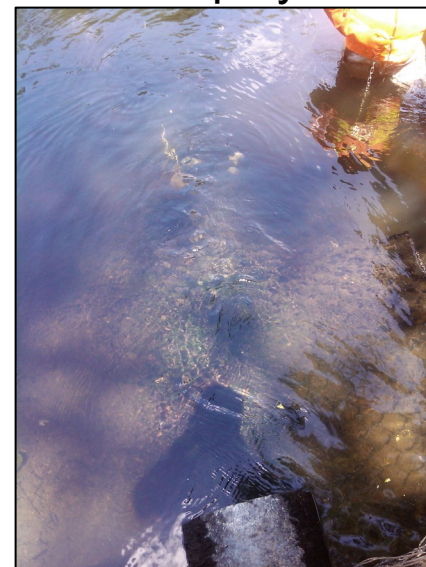- Advanced secondary treatment with UV disinfection

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Sampling

WWTP

Up   Eff

Effluent pipe sampling

Deployment

Effluent pipe

Day 1
- TS 1 – morning
- TS 2 - evening
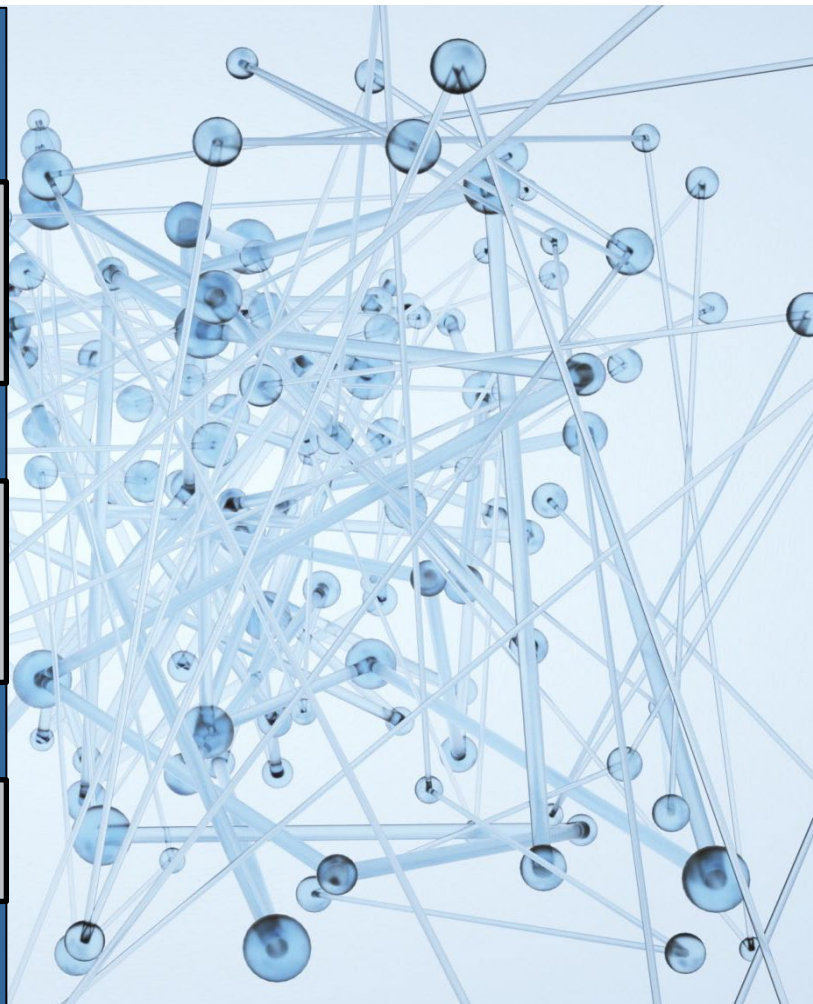
Time

Day 2
- TS 3 – morning
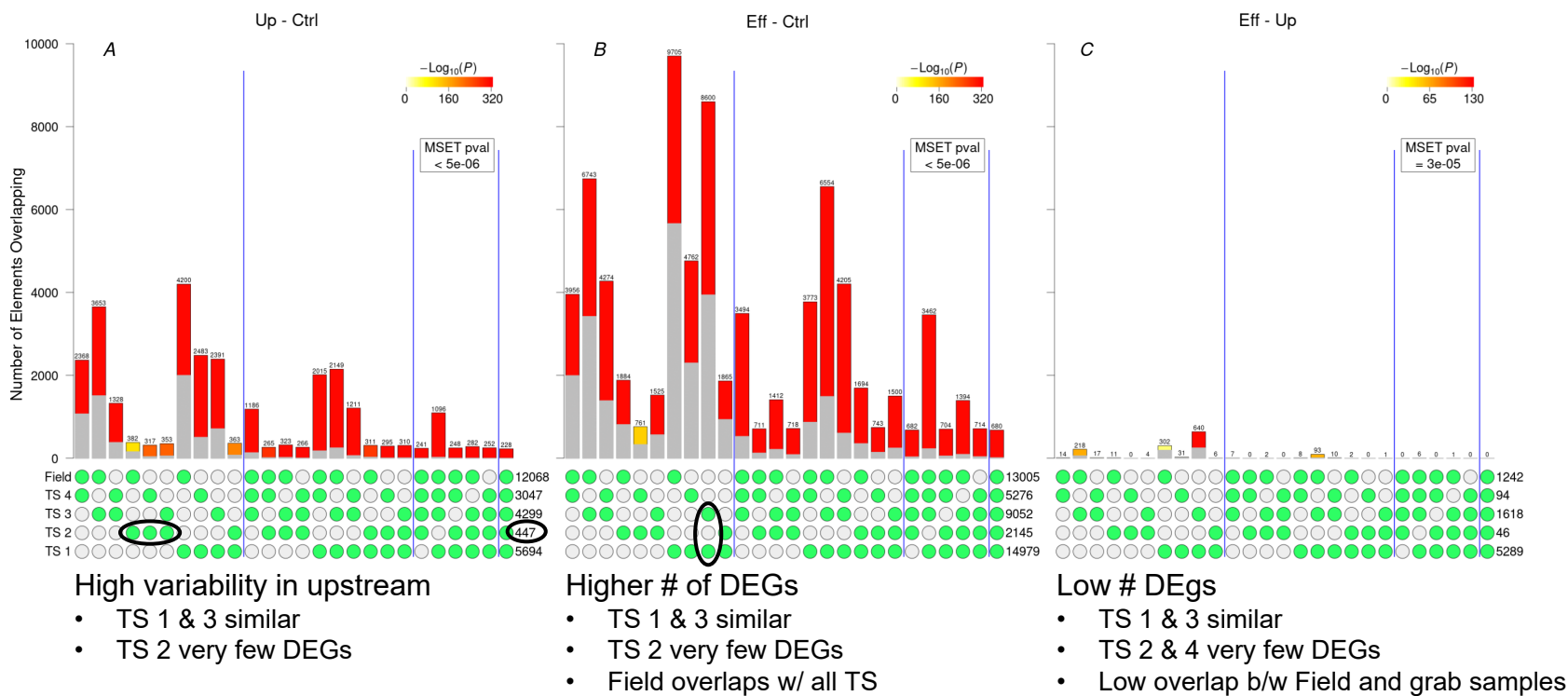- TS 4 - evening

Deployment

Analysis

DEGs – overlap
- Super Exact test

Functional Enrichment
- Semantic Analysis

Classification

Up - Ctrl

Eff - Ctrl

Eff - Up

High variability in upstream
- TS 1 & 3 similar
- TS 2 very few DEGs

Higher # of DEGs
- TS 1 & 3 similar
- TS 2 very few DEGs
- Field overlaps w/ all TS

Low # DEgs
- TS 1 & 3 similar
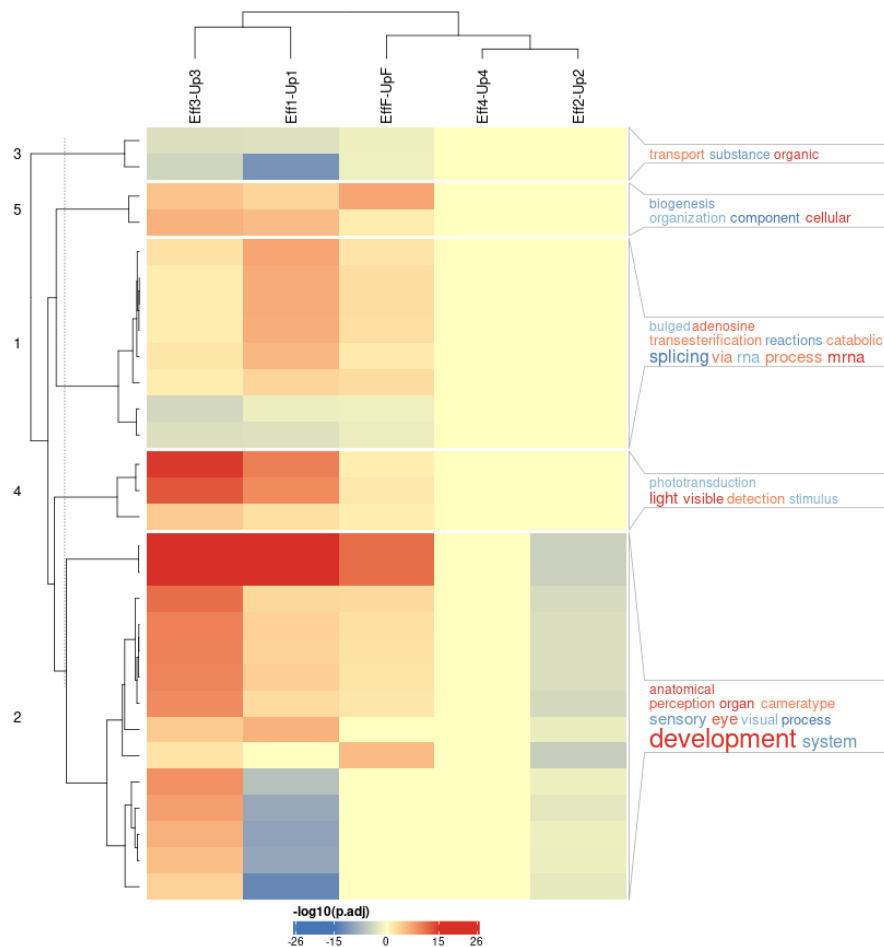- TS 2 & 4 very few DEGs
- Low overlap b/w Field and grab samples

# Cluster Analysis of Enriched Categories – Up vs. Eff

- Overall transcriptional levels were low
  - Upstream – highly variable across time
- Little to none in TS 2 & TS 4
- TS 1 & 3 cluster

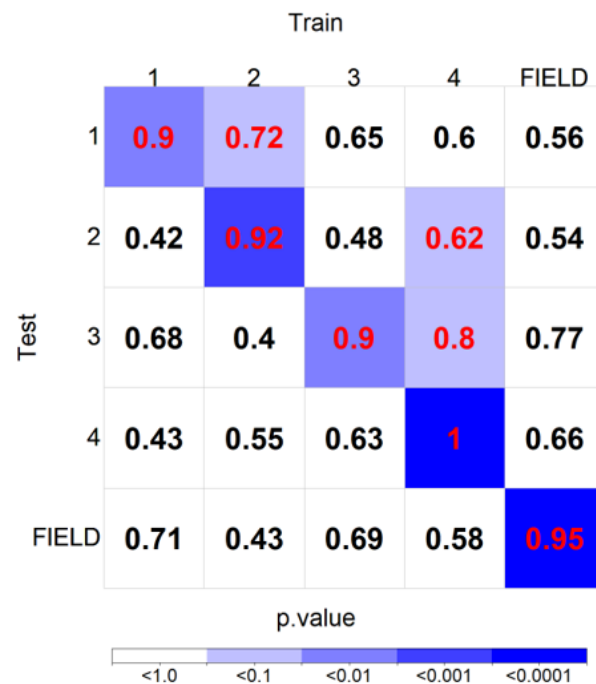# Cluster Analysis of Enriched Categories – Up and Eff vs. Control

- ❑ GO Biological Function Enrichment analyses
- ❑ Filtered
    - ❑ terms present in at least 9 treatment groups
- ❑ Semantic analysis of clusters
- ❑ Eff grab samples clustered
- ❑ Up samples did not cluster
    - ❑ Changing background
- ❑ Field samples clustered
    - ❑ Suggested deployment is a complicating factor
        - ❑ Stress
- ❑ Up 2 few DEGs -> few GO terms
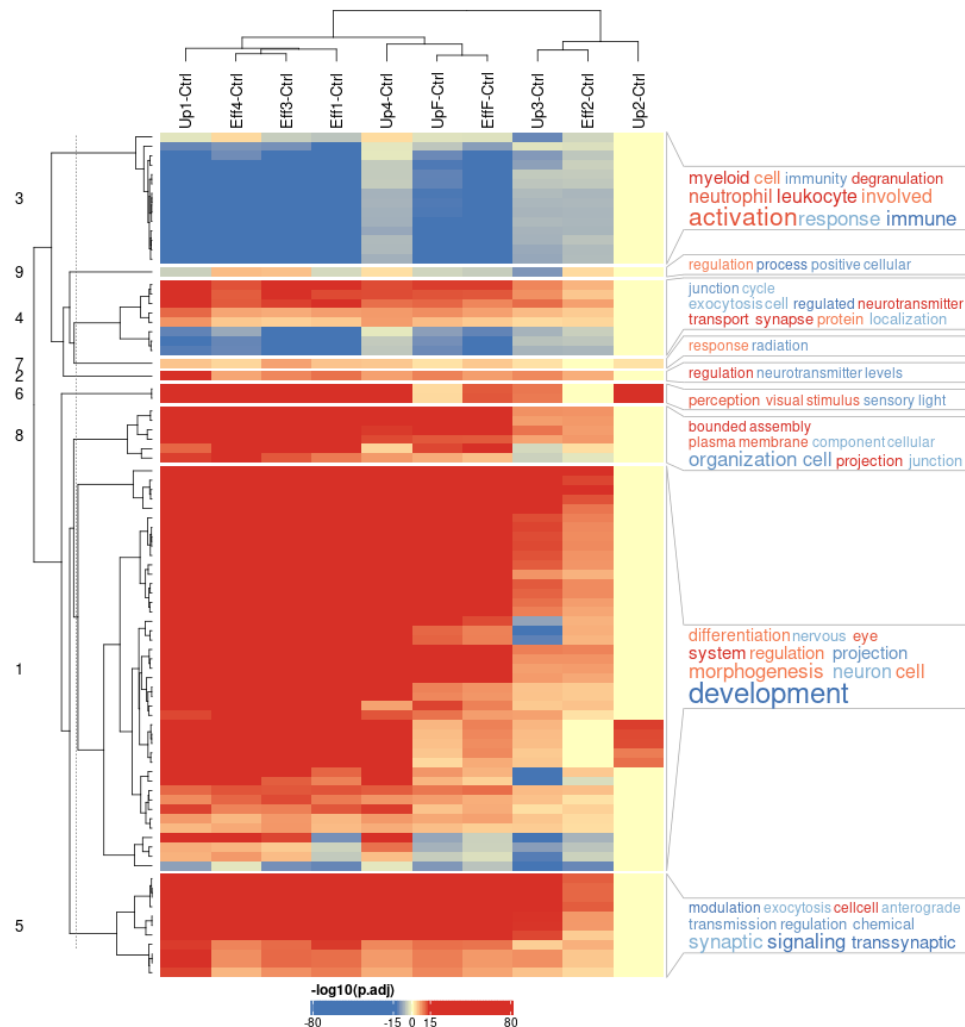
Classification

- Alternative approach to similarity
  - Not based on DEGs
  - Negative Binomial Linear Discriminant Analysis
- Effluent -> DEGs -> classification
- Permutations to determine p value
  - Only TS 4 able to classify other TS
- TS 1 and 3
  - Reciprocal -> higher AUC
  - High AUC w/ Field
    - Reciprocal (train on Field)
      - TS 3

Cross-timestep AUCs, Perm. Test pvals

Test \ Train	1	2	3	4	FIELD
1	0.9	0.72	0.65	0.6	0.56
2	0.42	0.92	0.48	0.62	0.54
3	0.68	0.4	0.9	0.8	0.77
4	0.43	0.55	0.63	1	0.66
FIELD	0.71	0.43	0.69	0.58	0.95

p.value: <1.0 <0.1 <0.01 <0.001 <0.0001

Conclusions

- Reference comparison
  - Up vs. Eff
    - High variability in Up – changing background
      - Independent of WWTP
      - Reference changes at each TP and field vs. grab
- Field vs. Grab Samples
  - Grab samples
    - Random noise – TS 2
    - Intra-daily differences (TS 1 & TS 3)
    - Can approximate field
      - Time dependent -> Composite sampling

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