

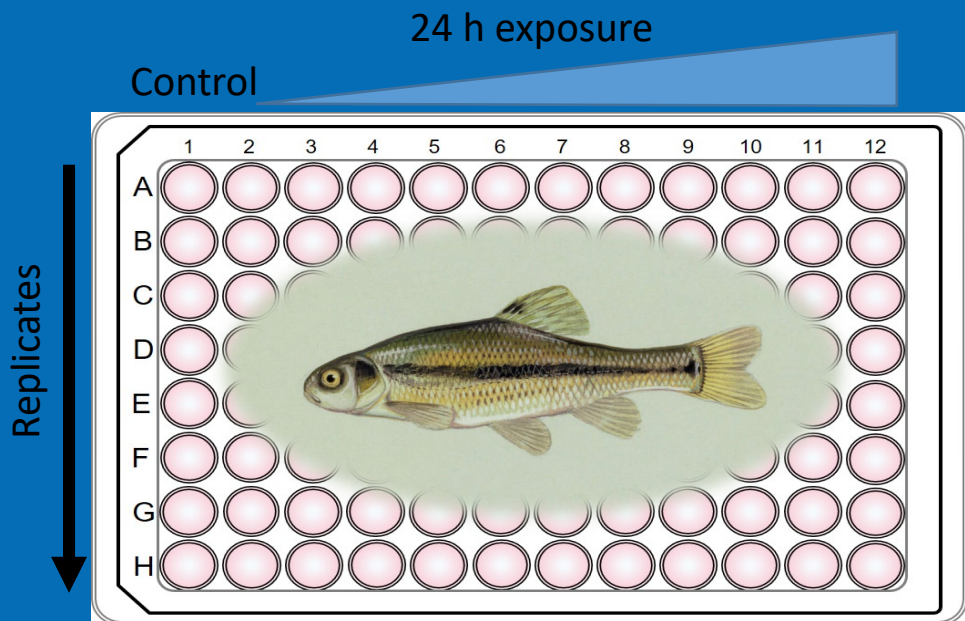
Transcriptomics-Based Points of Departure for Fish

Use of In Silico Sub-Sampling to Inform High Throughput Assay Design

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Michelle Le¹, Kendra Bush¹, Dan Villeneuve²**

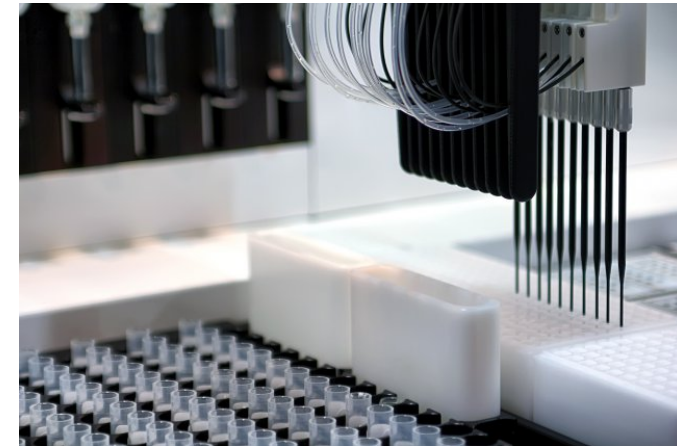
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Services at USEPA GLTED.

Summary: A minimum of 10,000 transcript features and 5 biological replicates in fathead minnow high throughput assays are needed to generate reliable transcriptomic-based points of departure for regulatory hazard characterization. Quantifying additional criteria is anticipated, pending more chemical data.



Background

- Traditional animal toxicity testing is time and resource intensive
- High-throughput transcriptomics as an alternative
 - Uses gene expression profiling as an endpoint for rapidly assessing the effects of chemicals
 - Can provide potency estimates for the concentrations of chemicals that produce perturbations




Objective

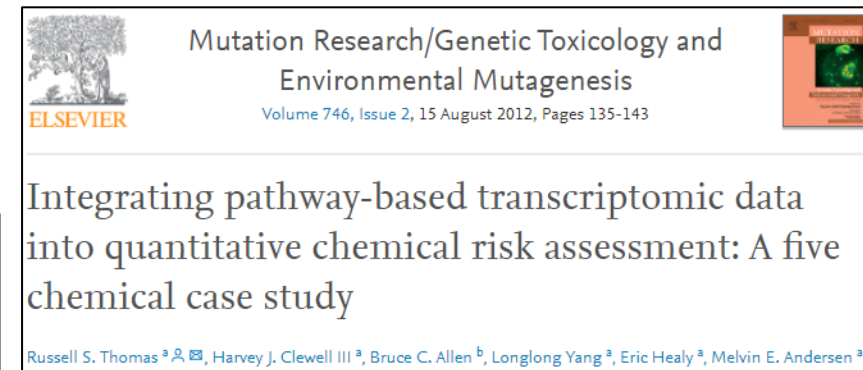
- Identify high-throughput transcriptomic assay parameters that can produce reliable points of departure estimates while minimizing time and resource use



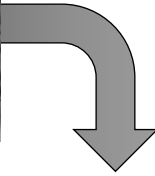
Temporal Concordance Between Apical and Transcriptional Points of Departure for Chemical Risk Assessment FREE

Russell S. Thomas , Scott C. Wesselkamper, Nina Ching Y. Wang, Q. Jay Zhao, Dan D. Petersen, Jason C. Lambert, Ila Cote, Longlong Yang, Eric Healy, Michael B. Black, Harvey J. Clewell, III, Bruce C. Allen, Melvin E. Andersen

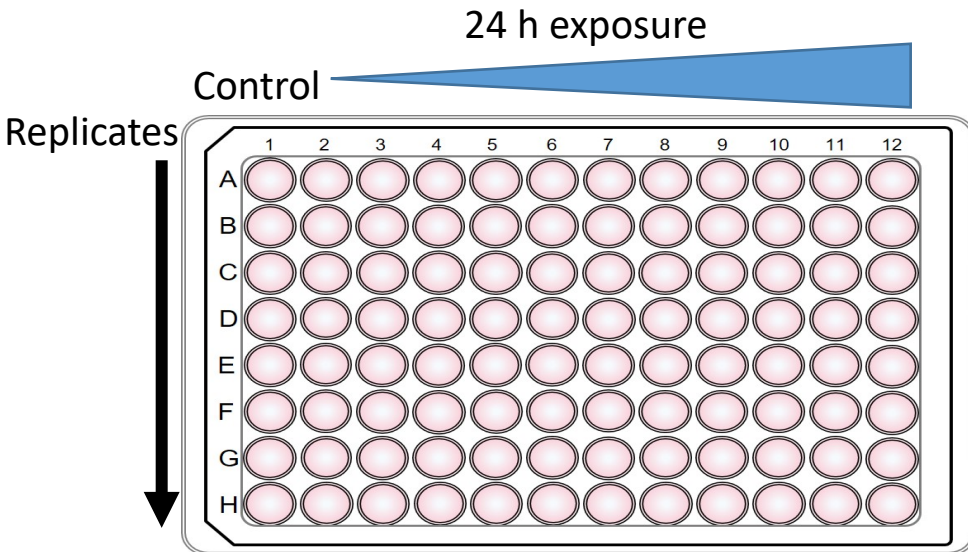
Toxicological Sciences, Volume 134, Issue 1, July 2013, Pages 180–194,
<https://doi.org/10.1093/toxsci/kft094>



Methods – Assay Design



Species	Age at Start	Temp	Time to Load Plate	Control 24-hr Survival	RNA Qty per Well
Pimephales promelas	24-hour	25° C	~30 minutes	24-hour	~1500 ng



Exposure Design

- 1 ml deep 96-well plates
- 12 concentrations – 8 replicates per concentration
- Half-log dilution series
- 1 individual per well
- 24-hour static exposures
- Phenotypic endpoints assessed
 - Survival and behavior
- After homogenization, RNA extracted for transcriptomics

**See Michelle Le's poster for more details on assay design*

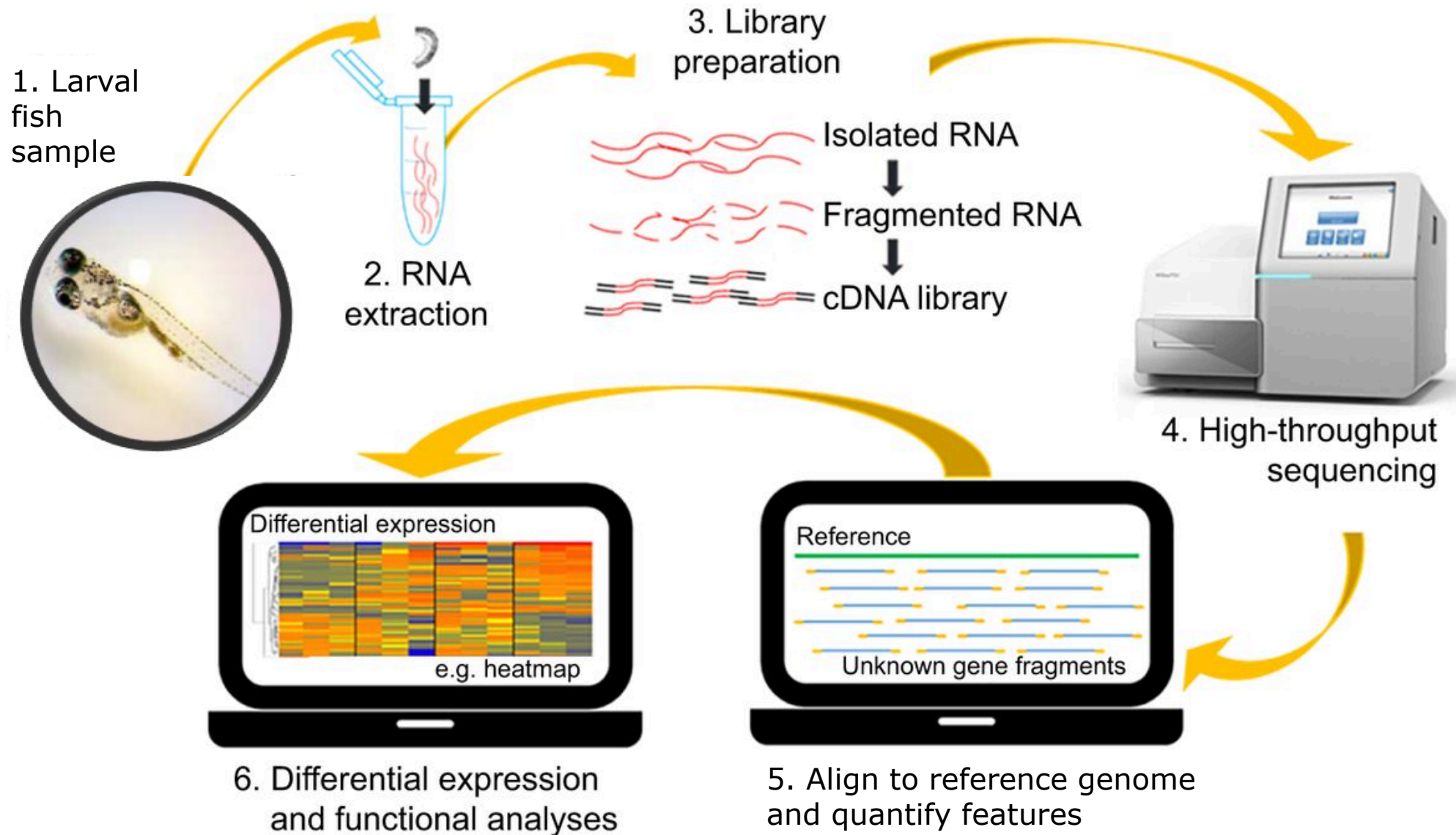
Chemicals

Metals	Neonicotinoids	Pharmaceuticals
CuSO ₄	Clothianidin	Fluoxetine
NiSO ₄	Flupyradifurone*	Paroxetine
ZnSO ₄	Imidacloprid	Sertraline
	Thiacloprid	

**Belongs to butenolide class of insecticides, but has similar mode of action to neonicotinoid insecticides*



Methods – Transcriptomic Analysis



Methods – *In silico* Subsampling

Sample ID

Transcript ID

Dose used in
that sample

	A	B	C	D	E	F	G	H	I
1		Cu_P1_A1	Cu_P1_A10	Cu_P1_A2	Cu_P1_A3	Cu_P1_A4	Cu_P1_A5	Cu_P1_A6	Cu_P1_A7
2	Dose	0	0.2	0.00002	0.00006	0.0002	0.0006	0.002	0.006
3	FMt000003	6.396698281	7.165080955	6.139417185	5.625463693	5.936491943	5.984797836	6.526667329	6.526667329
4	FMt000004	2.727597133	2.819530386	2.476866196	2.260722943	2.072535549	2.408035192	2.829599109	2.829599109
5	FMt000005	2.666574282	2.757823089	2.957500498	3.294229757	3.698358555	3.209724111	2.681578019	2.681578019
6	FMt000006	4.675333003	4.768559009	4.858302489	5.139450164	4.551132645	4.819989912	4.829061001	4.829061001
7	FMt000008	5.816765605	5.550398239	5.373006159	5.467031575	5.756057101	5.692205772	5.572099115	5.572099115
8	FMt000009	1.921778265	1.337509659	1.842399017	1.364723466	1.820179855	2.227968209	1.720804988	1.720804988
9	FMt000010	0.400578876	1.02468777	0.580341068	1.428654949	1.048759955	1.133331622	0.796197253	0.796197253
10	FMt000011	1.036715489	1.337509659	1.179840942	1.762168511	2.033423943	1.907113212	1.682372652	1.682372652
11	FMt000013	4.339705191	4.231422779	4.457041355	4.158758634	4.390170142	4.287434091	4.354221981	4.354221981
12	FMt000014	0.753428738	1.497088068	1.179840942	1.227677204	0.694880669	1.737858491	1.117537912	1.117537912
13	FMt000016	4.685621982	4.677065189	4.791080697	4.988545119	4.631905782	4.902412506	5.063939077	5.063939077
14	FMt000017	1.15990699	1.392687117	1.548825352	2.575114318	1.623516583	2.058579857	2.227306515	2.227306515
15	FMt000019	2.982521854	1.092914161	1.969452091	3.276966231	2.985273802	2.37954184	2.472248844	2.472248844
16	FMt000020	4.326615646	3.729709358	4.659064377	3.978274305	3.280771887	3.922966112	3.700540563	3.700540563
17	FMt000022	0.753428738	0.427434191	0.350624657	0.608894253	0.224966545	0.332838013	0.997448031	0.997448031
18	FMt000026	2.536192677	2.859249401	3.166436765	3.031435449	2.856737829	2.90442897	2.963836436	2.963836436
19	FMt000031	1.886534004	2.036875017	2.086216064	2.709867426	2.474112535	2.463387273	2.898278494	2.898278494
20	FMt000032	4.210281411	3.872414951	4.088442997	3.934904526	3.907492756	4.116679066	3.631612409	3.631612409
21	FMt000034	3.641040659	3.975408278	3.930816133	3.425369888	3.928963604	4.116679066	4.285372312	4.285372312
22	FMt000035	3.20831699	4.413005717	4.267645049	4.435879765	4.332726221	4.354221981	4.354221981	4.354221981
23	FMt000036	0.025579454	3.364405188	3.409614619	2.5591869	2.982290068	3.505201762	3.505201762	3.505201762
24	FMt000037	0.220389325	0.470050181	0.081807702	0.59096166	0.072994421	0.796197253	0.796197253	0.796197253

BMD
Express2.2

10th percentile(BMD) = tPOD



Methods – *In silico* Subsampling

LEGEND

----- Transition to next replicate set

Sampled data

Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04	B06	B07	B08	B09	B10	B11
Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015	0.0015	0.005	0.015	0.05	0.15	0.5
FMT000003	5.69	5.66	5.67	5.98	5.63	5.88	5.46	5.56	5.43	5.69	5.49	6.01	6.01	5.69	5.48	5.53	5.54	5.15	5.61	5.48	5.45
FMT000004	1.85	1.93	1.97	2.01	2.10	1.33	2.12	1.83	1.47	1.71	2.03	2.23	2.13	1.58	1.37	2.15	1.81	2.09	1.51	1.05	1.50
FMT000005	2.78	2.22	2.22	2.36	2.91	3.21	1.92	2.48	2.28	3.24	2.83	3.20	2.30	2.62	1.93	2.62	2.34	3.16	2.90	2.16	2.79
FMT000006	4.63	4.45	4.49	4.45	4.16	4.39	4.88	4.21	4.25	4.35	4.52	3.90	4.06	4.20	4.53	4.09	4.24	4.10	4.12	4.01	4.38
FMT000008	5.21	5.40	5.41	5.15	5.49	5.48	5.37	5.43	5.49	5.37	5.28	5.41	5.38	5.40	5.43	5.40	5.44	5.62	5.61	5.38	5.27
FMT000009	1.72	1.49	1.63	1.66	1.96	1.54	1.30	1.69	1.21	1.32	1.07	1.91	1.44	1.67	1.87	1.75	2.10	1.72	1.58	1.35	1.36
FMT000010	1.14	1.23	1.05	1.30	1.89	1.72	2.30	1.95	1.73	1.66	1.36	1.63	1.35	1.44	1.33	1.47	1.39	1.18	1.86	1.64	1.33
FMT000011	1.75	1.67	1.54	1.30	1.89	1.72	2.30	1.95	1.73	1.66	1.36	2.11	2.26	1.94	2.02	1.78	1.91	2.12	1.58	1.78	1.46
FMT000013	4.32	4.63	4.69	5.39	4.74	4.83	5.06	4.80	4.89	4.69	4.46	4.90	4.77	4.97	4.71	5.05	4.99	4.74	4.84	5.01	4.61
FMT000014	0.97	1.04	1.49	1.65	1.02	1.33	1.18	1.07	1.16	1.37	1.51	1.40	0.74	0.84	1.37	0.70	1.29	1.33	0.98	1.00	1.29
FMT000016	5.14	5.38	5.13	5.42	4.99	4.82	5.09	4.63	4.90	5.03	4.94	4.96	5.02	4.98	5.04	4.96	4.86	4.80	5.13	5.11	5.27
FMT000017	2.51	2.57	2.56	2.61	2.65	2.16	2.40	1.73	2.08	2.26	2.31	2.44	2.10	2.49	2.31	1.98	2.18	2.09	2.44	2.39	2.61
FMT000019	1.60	1.28	1.87	2.01	2.26	1.59	3.13	1.73	3.39	1.14	2.03	0.30	0.74	1.67	2.53	2.74	2.69	2.54	2.98	1.48	1.70
FMT000020	3.29	3.58	3.42	3.64	3.32	3.79	3.86	3.30	3.67	3.50	3.76	3.48	3.82	3.68	3.65	3.81	3.43	3.26	3.85	3.34	3.21

Original expression matrix

Full dataset:

- 31,158 transcripts
- 12 doses, 8 reps per dose
- 96 samples total

Transcript(m), m=100 - 30,000 at random intervals

"Transcript(100) example"

Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FMT000003	5.69	5.66	5.67	5.98	5.63	5.88	5.46	5.56	5.43	5.69	5.49	6.01	6.01	5.69	5.48
FMT000004	1.85	1.93	1.97	2.01	2.10	1.33	2.12	1.83	1.47	1.71	2.03	2.23	2.13	1.58	1.37
FMT000005	2.78	2.22	2.22	2.36	2.91	3.21	1.92	2.48	2.28	3.24	2.83	3.20	2.30	2.62	1.93
FMT000006	4.63	4.45	4.49	4.45	4.16	4.39	4.88	4.21	4.25	4.35	4.52	3.90	4.06	4.20	4.53
FMT000008	5.21	5.40	5.41	5.15	5.49	5.48	5.37	5.43	5.49	5.37	5.28	5.41	5.38	5.40	5.43
FMT000009	1.72	1.49	1.63	1.65	1.96	1.54	1.30	1.69	1.21	1.32	1.07	1.91	1.44	1.67	1.87
FMT000010	1.14	1.23	1.05	0.53	1.59	1.39	0.51	1.22	1.42	1.57	1.41	1.63	1.35	1.44	1.33
FMT000011	1.75	1.67	1.54	1.50	1.89	1.72	2.30	1.95	1.73	1.66	1.56	2.11	2.26	1.94	2.02
FMT000013	4.32	4.63	4.69	5.39	4.74	4.83	5.06	4.80	4.89	4.69	4.46	4.90	4.77	4.97	4.71
FMT000014	0.97	1.04	1.49	1.65	1.02	1.33	1.18	1.07	1.16	1.37	1.51	1.40	0.74	0.84	1.37
FMT000016	5.14	5.38	5.13	5.42	4.99	4.82	5.09	4.63	4.90	5.03	4.94	4.96	5.02	4.98	5.04
FMT000017	2.51	2.57	2.56	2.61	2.65	2.16	2.40	1.73	2.08	2.26	2.31	2.44	2.10	2.49	2.31
FMT000019	1.60	1.28	1.87	2.01	2.26	1.59	3.13	1.73	3.39	1.14	2.03	0.30	0.74	1.67	2.53
FMT000020	3.29	3.58	3.42	3.64	3.32	3.79	3.86	3.30	3.67	3.50	3.76	3.48	3.82	3.68	3.65

Transcript 100 dataset:

- 100 transcripts
- 12 doses, 8 reps per dose
- 96 samples total



- 31,158 transcripts
- 12 doses, **3 reps per dose**
- 36 samples total
- **12 iterations of each dataset**

LEGEND

----- Transition to next replicate set

 Sampled data

“Replicate(3,12x) example”

Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04	B05
Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015	0.0005
FM/FMD00000	5.69	5.66	5.67	5.65	5.63	5.88	5.46	5.56	5.43	5.69	5.49	6.01	6.01	6.01	5.69	5.48
Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04	B05
Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015	0.0005
FM/FMD00001	1.85	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00002	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00003	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00004	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00005	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00006	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00007	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00008	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00009	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00010	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00011	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00012	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00013	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00014	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00015	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00016	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00017	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00018	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00019	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00020	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00021	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00022	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00023	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00024	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00025	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00026	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00027	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00028	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00029	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00030	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00031	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00032	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00033	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00034	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00035	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00036	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00037	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00038	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00039	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00040	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00041	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00042	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00043	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00044	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00045	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00046	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00047	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00048	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00049	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00050	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00051	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00052	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00053	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00054	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00055	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00056	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00057	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00058	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00059	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00060	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00061	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00062	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00063	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00064	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00065	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00066	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00067	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00068	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00069	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00070	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00071	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00072	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00073	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00074	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00075	Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FM/FMD00076	Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
FM/FMD00077	Dose	0	1.5E-													

Iterations 6 - 12

Iteration 5

Iteration 4

Iteration 3

Iteration 2

Iteration 1

Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04	B06	B07	B08	B09	B10	B11				
Dose	0	1.5E-05	0.000005	0.000015	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	1	0.15E-05	0.000005	0.000015	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5
FM1000003		5.69	5.66	5.67	5.98	5.63	5.88	5.46	5.56	5.43	5.69	5.49		6.01	6.01	5.69	5.48	5.53	5.54	5.15	5.61	5.48	5.45	5.78	
FM1000004		1.85	1.93	1.97	2.01	2.10	1.33	2.12	1.83	1.47	1.71	2.03		2.23	2.13	1.58	1.37	2.15	1.81	2.09	1.51	1.05	1.05	1.50	
FM1000005		2.78	2.22	2.22	2.36	2.91	3.21	1.92	2.48	2.28	3.24	2.83		2.30	2.30	2.62	1.93	2.62	2.34	3.16	2.90	2.16	2.78	4.35	
FM1000006		4.63	4.45	4.49	4.45	4.16	4.39	4.88	4.21	4.25	4.35	4.52		3.90	4.06	4.20	4.53	4.09	4.24	4.10	4.12	4.01	4.38	5.26	
FM1000008		5.21	5.40	5.41	5.15	5.49	5.48	5.37	5.43	5.49	5.37	5.28		5.41	5.38	5.40	5.43	5.40	5.44	5.62	5.61	5.38	5.27	5.36	
FM1000009		1.72	1.49	1.63	1.65	1.96	1.54	1.30	1.69	1.21	1.32	1.07		1.91	1.44	1.67	1.87	1.75	2.10	1.72	1.58	1.35	1.36	1.73	
FM1000010		1.14	1.23	1.05	1.05	1.14	1.14	1.14	1.14	1.14	1.14	1.14		1.63	1.35	1.44	1.33	1.47	1.39	1.18	1.86	1.64	1.33	1.46	
FM1000011		1.75	1.67	1.54	1.50	1.89	1.72	2.30	1.95	1.73	1.66	1.96		2.11	2.26	1.94	2.02	1.78	1.91	2.12	1.58	1.78	1.46	1.78	
FM1000013		4.32	4.63	4.69	5.39	4.74	4.83	5.06	4.80	4.89	4.69	4.46		4.90	4.77	4.97	4.71	5.05	4.99	4.74	4.84	5.01	4.61	4.61	
FM1000014		0.97	1.04	1.49	1.65	1.02	1.33	1.18	1.07	1.16	1.37	1.51		1.40	0.74	0.84	1.37	0.70	1.29	1.33	0.98	1.00	1.25	1.29	
FM1000016		5.14	5.38	5.13	5.42	4.99	4.82	5.09	4.63	4.90	5.03	4.94		4.96	5.02	4.98	5.04	4.96	4.86	4.80	5.13	5.11	5.27	5.27	
FM1000017		2.51	2.57	2.56	2.61	2.65	2.16	2.40	1.73	2.08	2.26	2.31		2.44	2.10	2.49	2.31	1.98	2.18	2.09	2.44	2.39	2.61	2.61	
FM1000019		1.60	1.28	1.87	2.01	2.26	1.59	3.13	1.73	3.39	1.14	2.03		0.30	0.74	1.67	2.53	2.74	2.69	2.54	2.98	1.48	1.70	1.70	
FM1000020		3.29	3.58	3.42	3.64	3.32	3.79	3.86	3.30	3.67	3.50	3.76		3.48	3.82	3.68	3.65	3.81	3.43	3.26	3.85	3.34	3.37	3.21	

Original expression matrix

Full dataset:

- 31,158 transcripts
- 12 doses, 8 reps per dose
- 96 samples total

Transcript(m), m=100 - 30,000 at random intervals

“Transcript(100) example”

Fluoxetine	A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	B01	B02	B03	B04
Dose	0	1.5E-05	0.00005	0.00015	0.0005	0.0015	0.005	0.015	0.05	0.15	0.5	0	1.5E-05	0.00005	0.00015
FMT000003	5.69	5.66	5.67	5.98	5.63	5.88	5.46	5.56	5.43	5.69	5.49	6.01	6.01	5.69	5.48
FMT000004	1.85	1.93	1.97	2.01	2.10	1.33	2.12	1.83	1.47	1.71	2.03	2.23	2.13	1.58	1.37
FMT000005	2.78	2.22	2.22	2.36	2.91	3.21	1.92	2.48	2.28	3.24	2.83	3.20	2.30	2.62	1.93
FMT000006	4.63	4.45	4.49	4.45	4.16	4.39	4.88	4.21	4.25	4.35	4.52	3.90	4.06	4.20	4.53
FMT000008	5.21	5.40	5.41	5.15	5.49	5.48	5.37	5.43	5.49	5.37	5.28	5.41	5.38	5.40	5.43
FMT000009	1.72	1.49	1.63	1.65	1.96	1.54	1.30	1.69	1.21	1.32	1.07	1.91	1.44	1.67	1.87
FMT000010	1.14	1.23	1.05	0.53	1.59	1.39	0.51	1.22	1.42	1.57	1.41	1.63	1.35	1.44	1.33
FMT000011	1.75	1.67	1.54	1.50	1.89	1.72	2.30	1.95	1.73	1.66	1.56	2.11	2.26	1.94	2.02
FMT000013	4.32	4.63	4.69	5.39	4.74	4.83	5.06	4.80	4.89	4.69	4.46	4.90	4.77	4.97	4.71
FMT000014	0.97	1.04	1.49	1.65	1.02	1.33	1.18	1.07	1.16	1.37	1.51	1.40	0.74	0.84	1.37
FMT000016	5.14	5.38	5.13	5.42	4.99	4.82	5.09	4.63	4.90	5.03	4.94	4.96	5.02	4.98	5.04
FMT000017	2.51	2.57	2.56	2.61	2.65	2.16	2.40	1.73	2.08	2.26	2.31	2.44	2.10	2.49	2.31
FMT000019	1.60	1.28	1.87	2.01	2.26	1.59	3.13	1.73	3.39	1.14	2.03	0.30	0.74	1.67	2.53
FMT000020	3.29	3.58	3.42	3.64	3.32	3.79	3.86	3.30	3.67	3.50	3.76	3.48	3.82	3.68	3.65

Transcript 100 dataset:

- **100 transcripts**
- 12 doses, 8 reps per dose
- 96 samples total

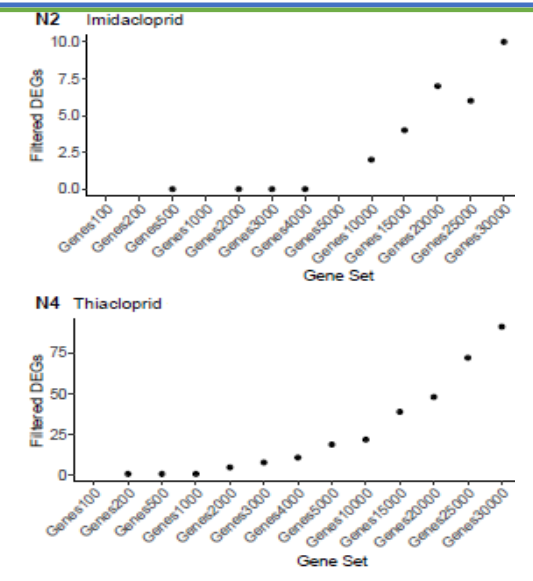
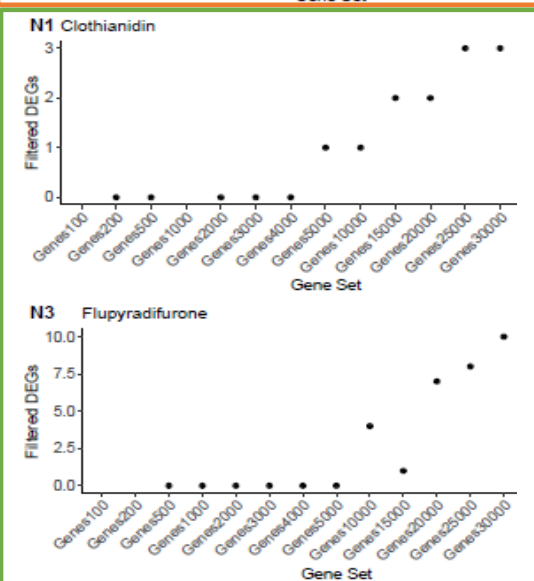
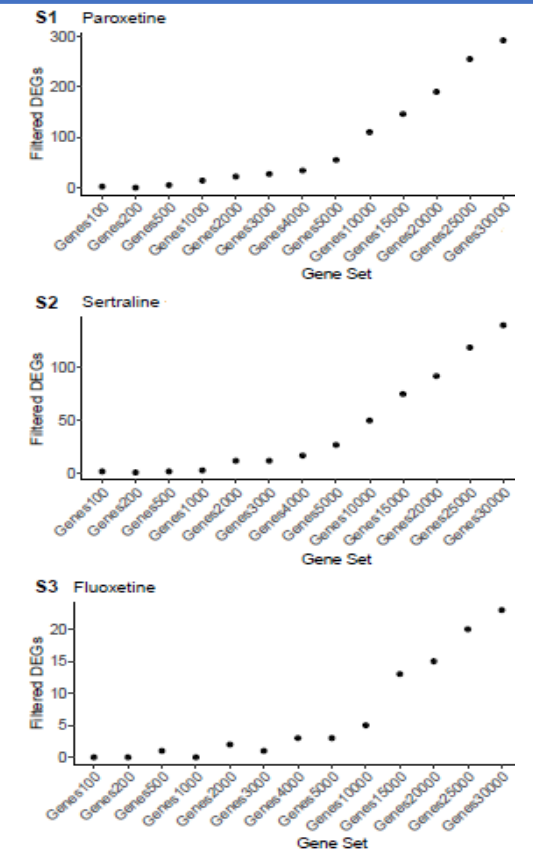
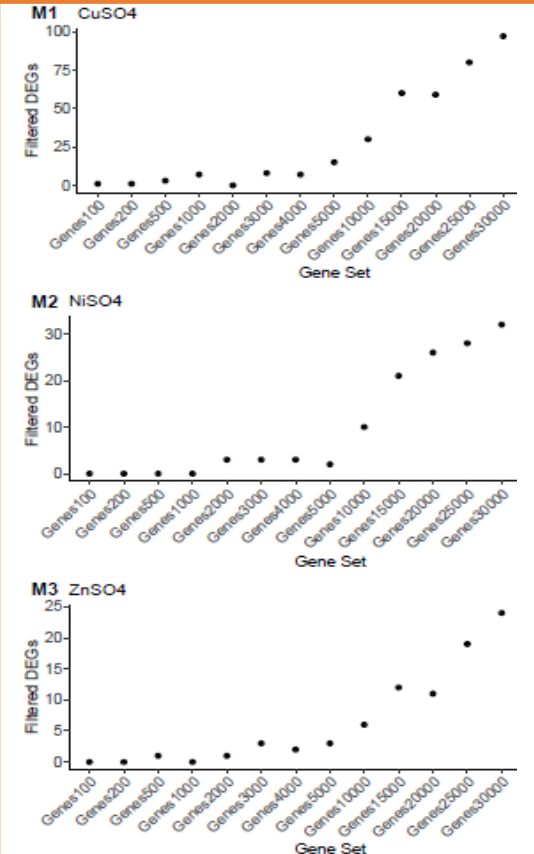


Variable Transcript Set Sizes: DEGs

Metals

Pharms

Neonics

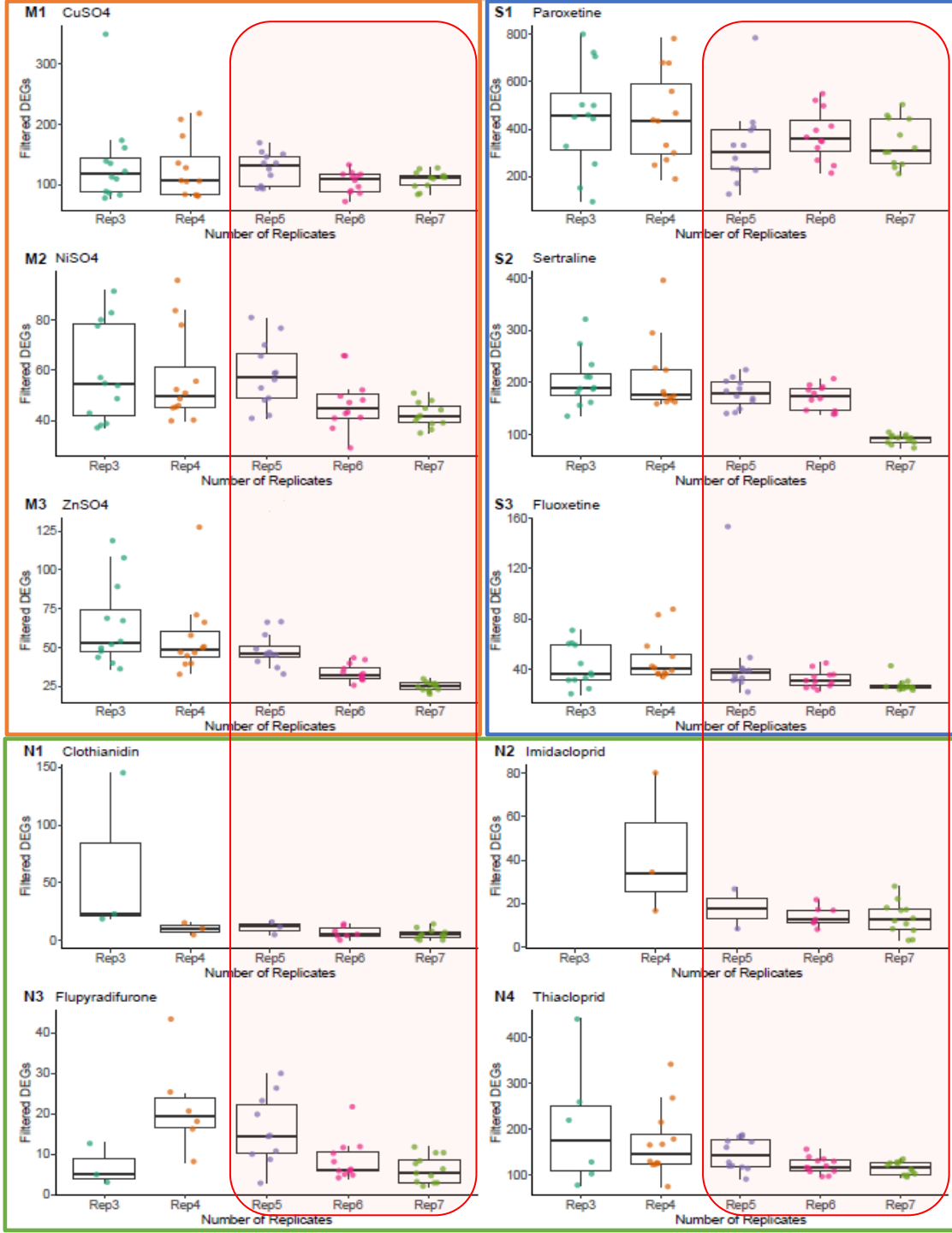


Variable Replicate (12x) Sizes: DEGs

Metals

Pharms

Neonics

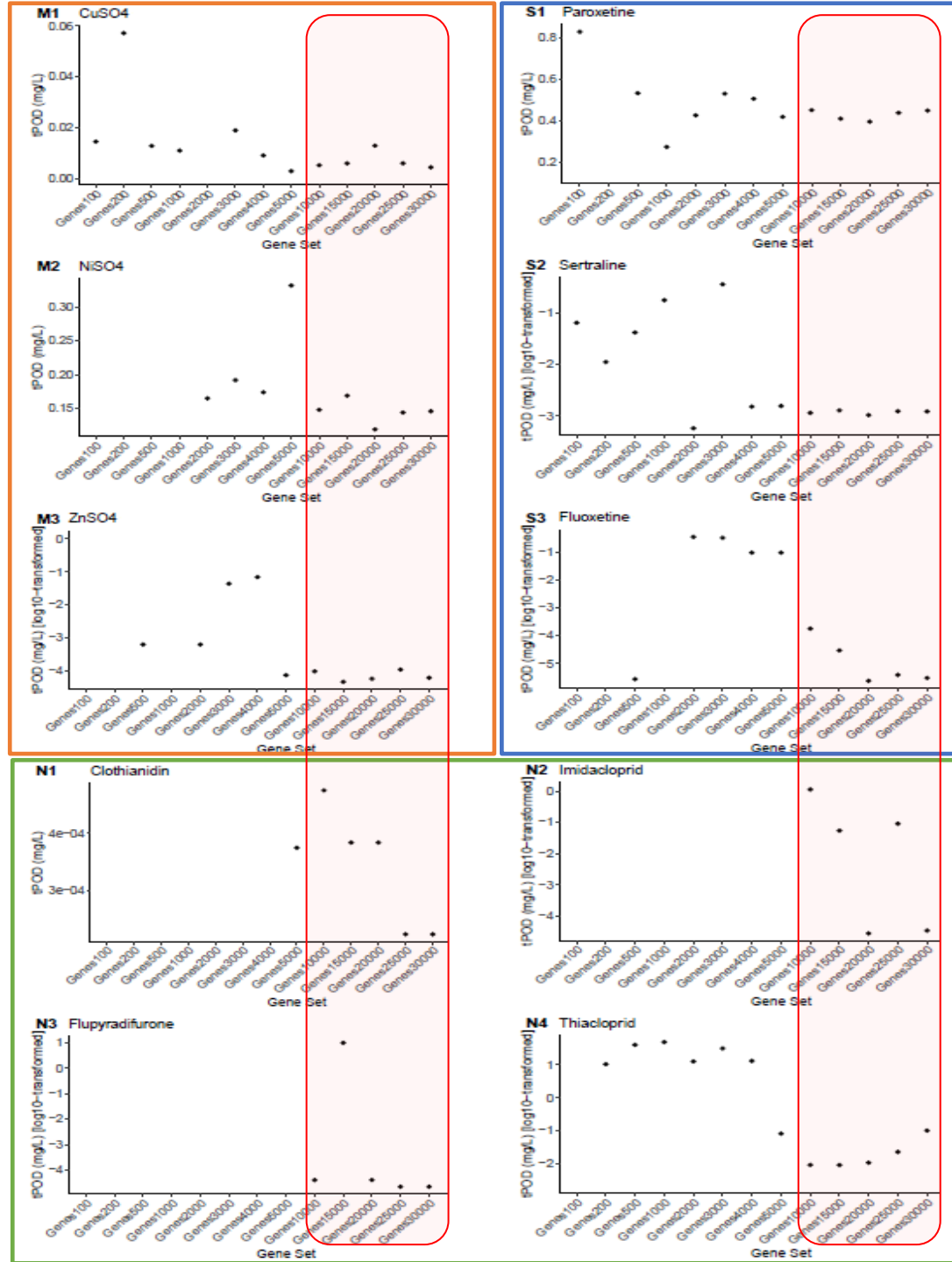


Variable Transcript Set Sizes: tPOD

Metals

Pharms

Neonics

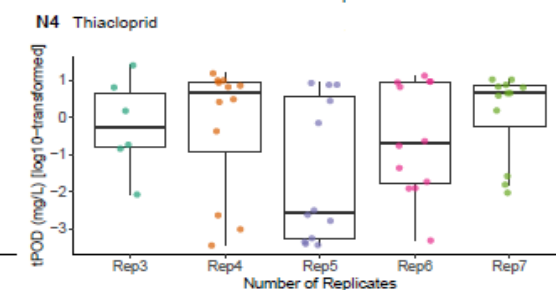
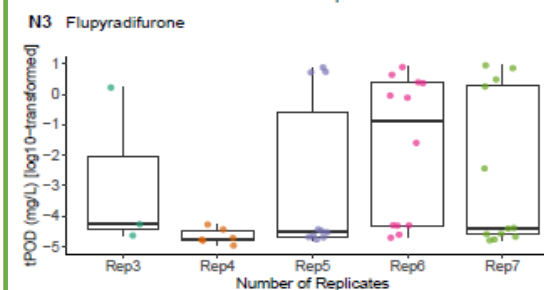
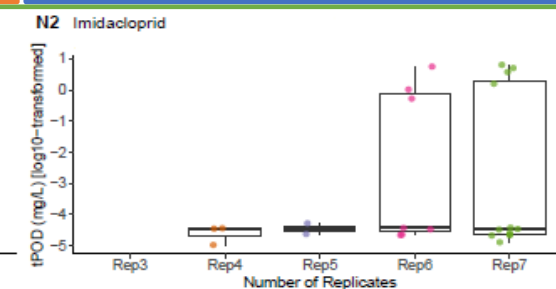
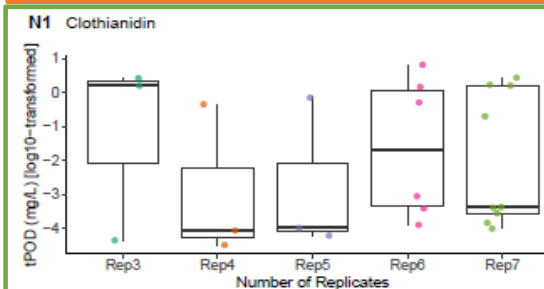
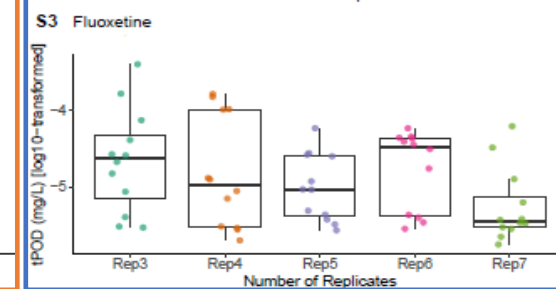
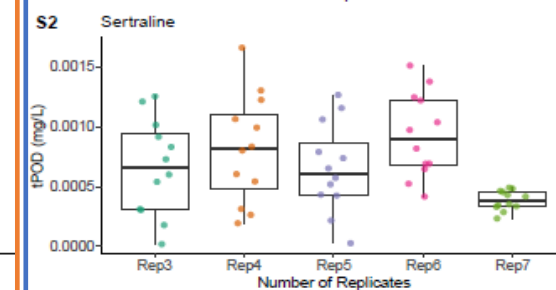
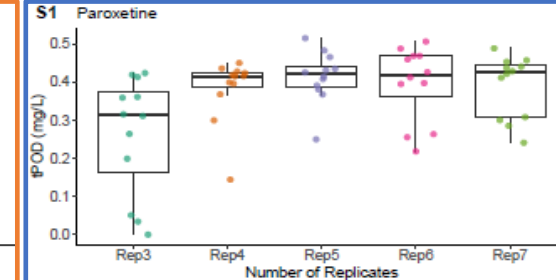
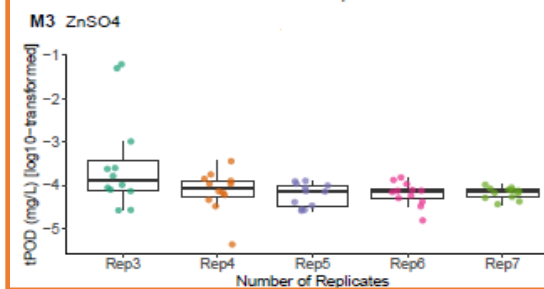
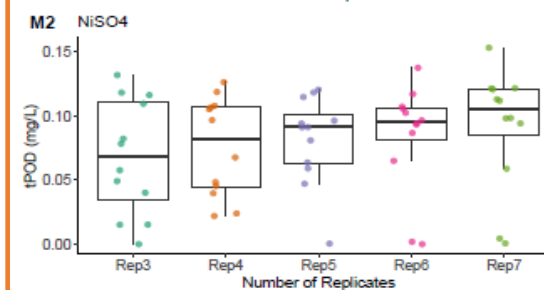
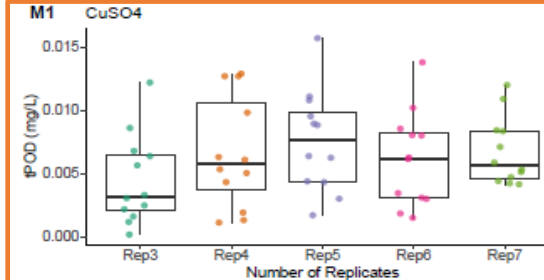


Variable Replicate (12x) Sizes: tPOD

Metals

Pharms

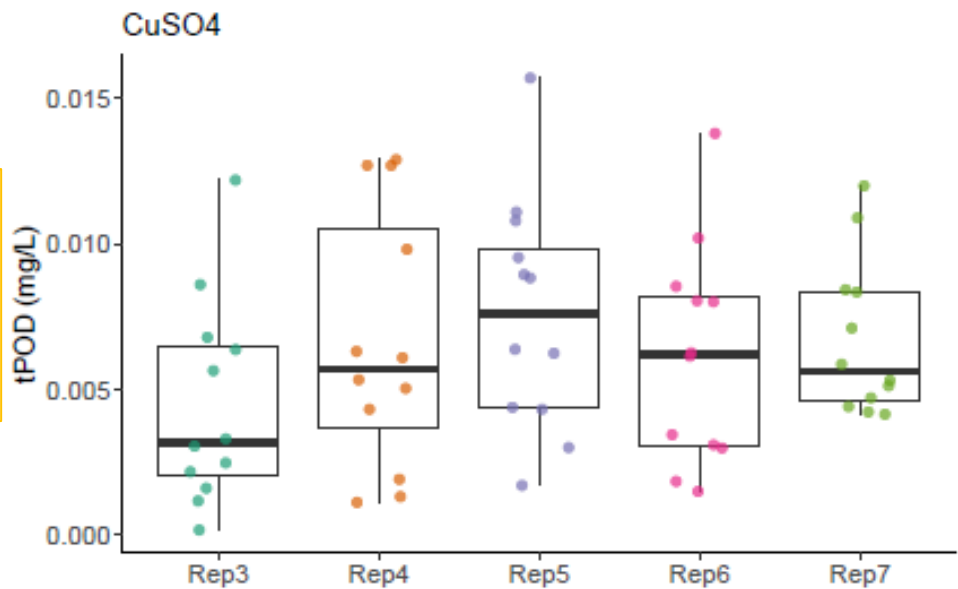
Neonics



tPOD Variability

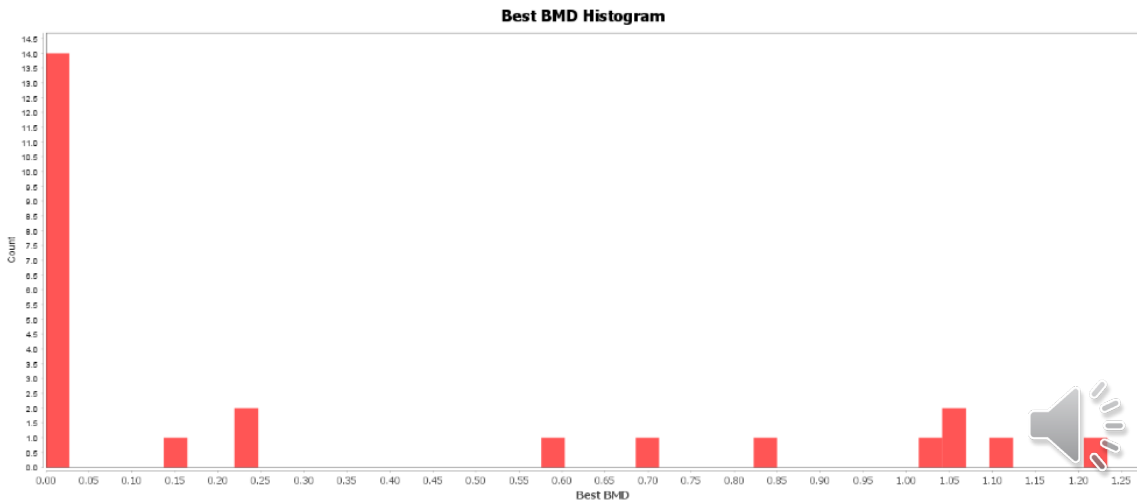
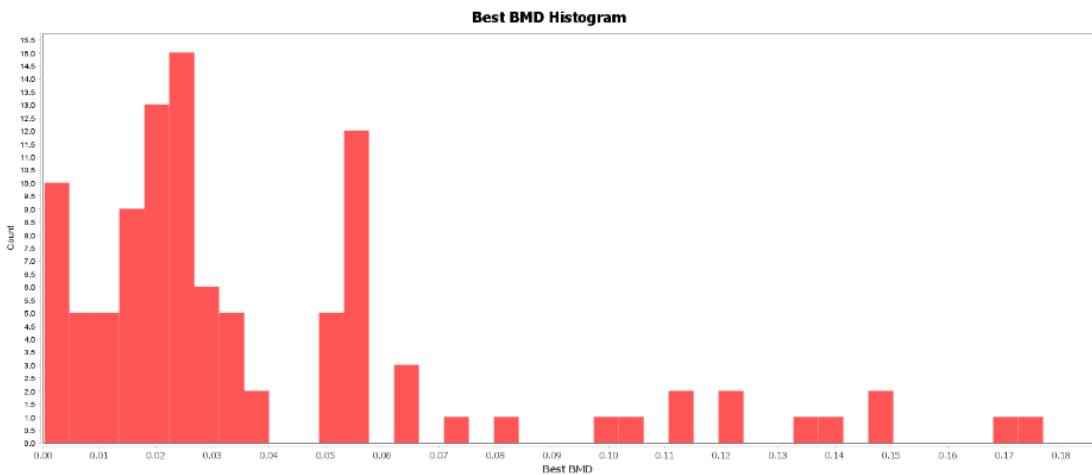
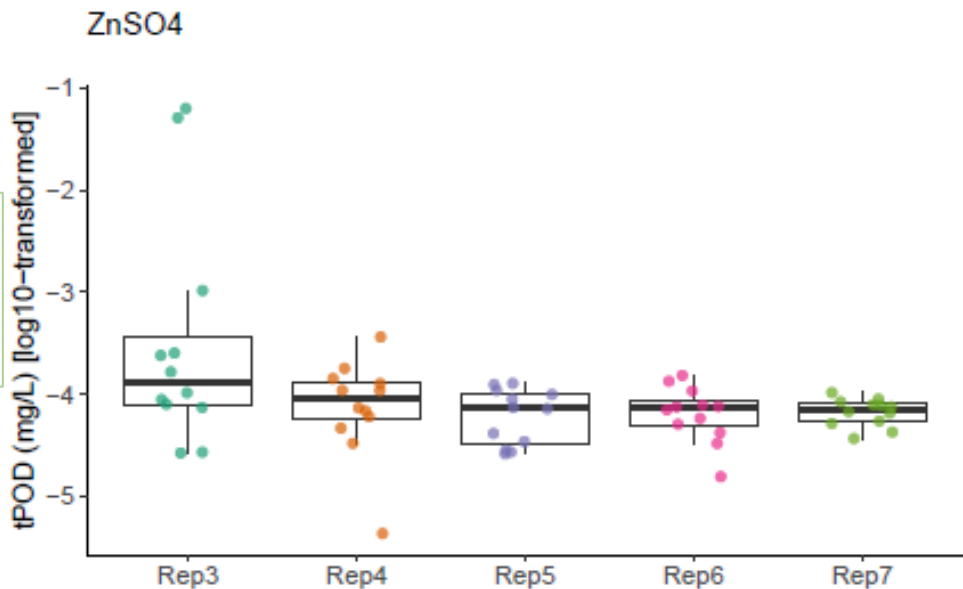
Copper sulfate (100-150 DEGs)

Linear
scale, 3-4
fold
variability



Log 10 scale,
≈ 1 log
variability

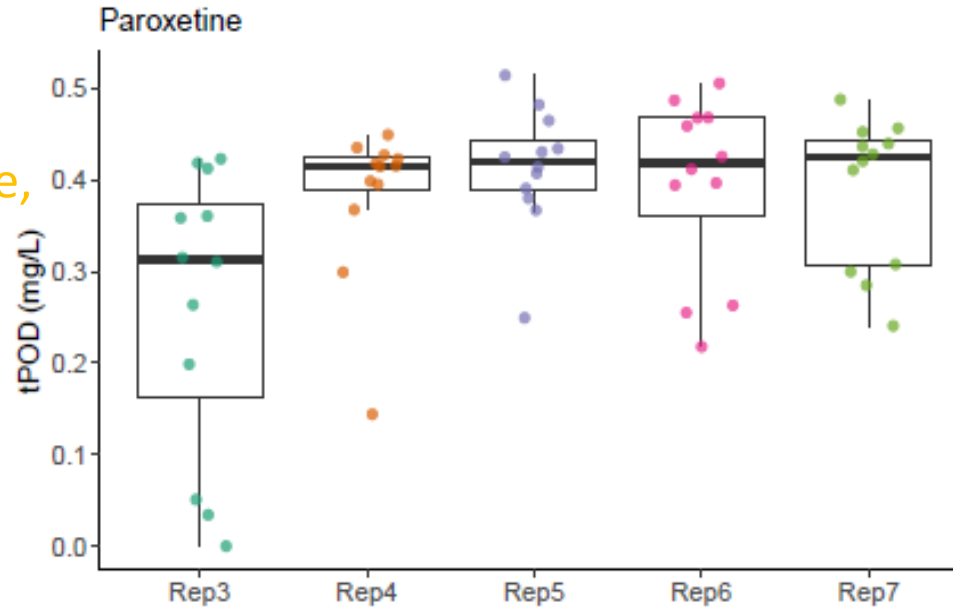
Zinc sulfate (20-60 DEGs)



tPOD Variability

Paroxetine (500-600 DEGs)

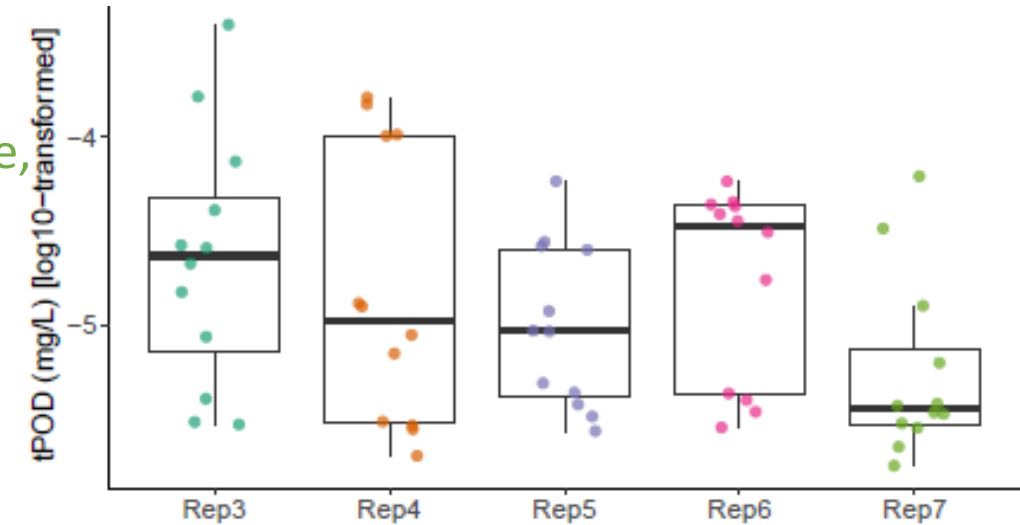
Linear scale,
2-3 fold
variability



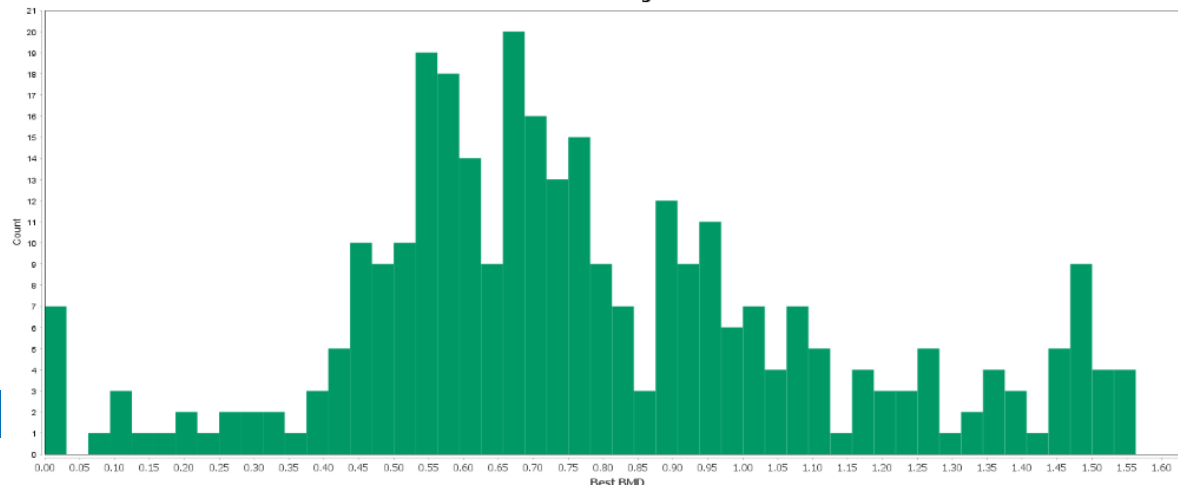
Log 10 scale,
1-1.5 log
variability

Fluoxetine (25-60 DEGs)

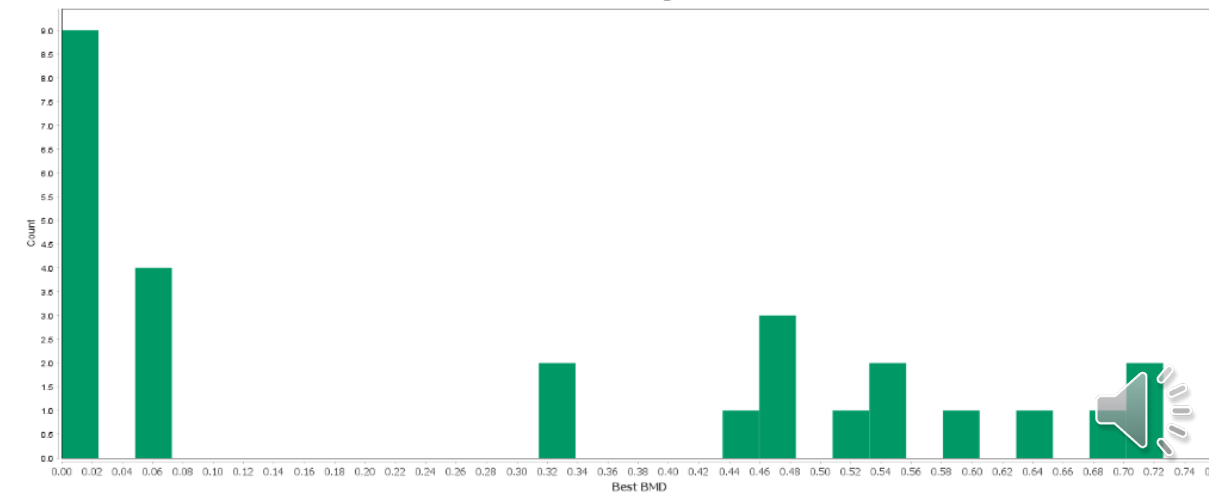
Fluoxetine



Best BMD Histogram



Best BMD Histogram



Conclusions

Minimum Assay Acceptance Criteria

- 10,000 transcript features
- 5 biological replicates
- Minimum number of differentially expressed genes **[TBD]**
- BMD distribution constraint **[TBD]**

tPODs that have reasonable, and quantifiable, levels of uncertainty is expected to aid the adoption of high-throughput transcriptomics in regulatory hazard characterization



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The authors have no conflicts of interest to declare.

The research presented here neither constitutes nor necessarily reflects official U.S. EPA policy.



References

- Harrill, J., Shah, I., Setzer, R. W., Haggard, D., Auerbach, S., Judson, R., & Thomas, R. S. (2019). Considerations for strategic use of high-throughput transcriptomics chemical screening data in regulatory decisions. *Current opinion in toxicology*, 15, 64-75.
- Richard E. Connon, Ken M. Jeffries, Lisa M. Komoroske, Anne E. Todgham, Nann A. Fangue; The utility of transcriptomics in fish conservation. *J Exp Biol* 15 January 2018; 221 (2): jeb148833. doi: <https://doi.org/10.1242/jeb.148833>
- Phillips JR, Svoboda DL, Tandon A, et al. BMDExpress 2: enhanced transcriptomic dose-response analysis workflow. *Bioinformatics*. 2019;35(10):1780-1782. doi:10.1093/bioinformatics/bty878
- Pagé-Larivière, F., Crump, D., & O'Brien, J. M. (2019). Transcriptomic points-of-departure from short-term exposure studies are protective of chronic effects for fish exposed to estrogenic chemicals. *Toxicology and applied pharmacology*, 378, 114634.
- Thomas, R. S., Wesselkamper, S. C., Wang, N. C. Y., Zhao, Q. J., Petersen, D. D., Lambert, J. C., ... & Andersen, M. E. (2013). Temporal concordance between apical and transcriptional points of departure for chemical risk assessment. *Toxicological sciences*, 134(1), 180-194.
- Thomas, R. S., Clewell III, H. J., Allen, B. C., Yang, L., Healy, E., & Andersen, M. E. (2012). Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: a five chemical case study. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 746(2), 135-143.

