EPA United States Environmental Protection Agency

High Throughput Transcriptomics (HTTr) Concentration-Response Screening in MCF7 Cells

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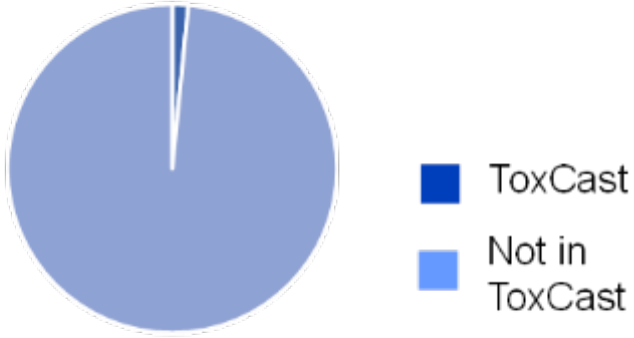
October 2, 2018

Outline

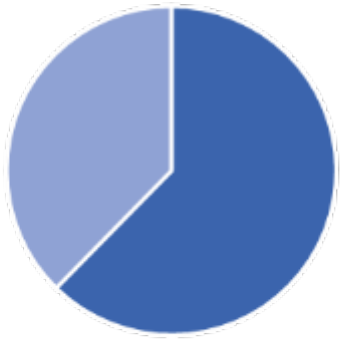
- Background & Objectives
- HTTr Pilot Experiment
    - Optimization Steps
    - Attenuation
    - Experimental Layout
- Results
    - Assay Performance Metrics
    - Concentration-Response Modeling
- Current Activities & Future Directions

Background

Gene Coverage



Pathway Coverage*

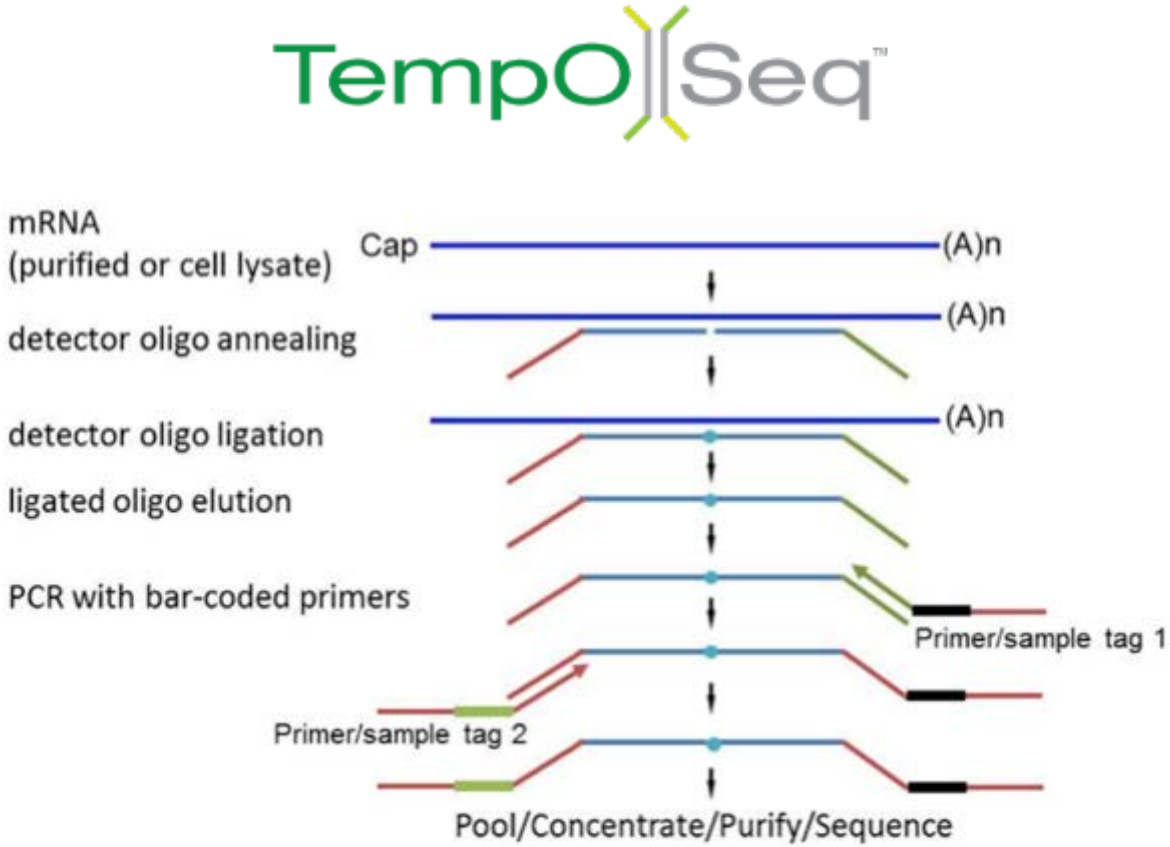


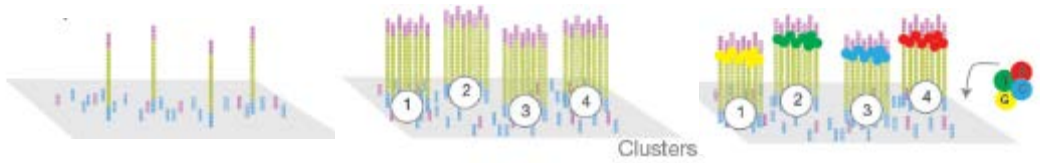
*At least one gene from pathway represented

- ToxCast assays cover about 320 genes.
- Pathway coverage is higher but still leaves large gaps
- Recent technological advances in transcriptomics are very promising for rapid and cost-effective whole transcriptome screening.
- Increase biological coverage by using high throughput transcriptomics (HTTr) as broad-based Tier 0 bioactivity screen.

BioSpyder TempO-Seq

- Targeted RNA-Seq technology
- Whole transcriptome assay provides output on > 20,000 transcripts.
- Requires very low input (< 10 pg total RNA).
- Performed on “standard” PCR and Next Gen Sequencers.
- Compatible with purified RNA or cell lysates.





Objectives

- Optimize culture and assay conditions for HTTr screening in MCF7 cells using the TempO-Seq human whole transcriptome assay.

- Perform a pilot experiment with a limited number of chemicals (n=44) in order to:

    1) Evaluate TempO-Seq assay performance.
    2) Determine the ability of the TempO-Seq assay to detect known biological signatures following chemical perbations
    3) Guide experimental design of larger screening studies.

HTTr Pilot: Experimental Design

Parameter	Multiplier	Notes
Cell Type(s)	1	MCF7
Culture Condition	2	DMEM + 10% HI-FBS PRF-DMEM + 10% CS-HI-FBS
Chemicals	44	see subsequent slides
Time Points:	3	6, 12, 24 hours
Assay Formats:	3	TempO-Seq HCI-Apoptosis HCI-Cytotoxicity
Concentrations:	8	3.5 $\log_{10}$ units; ½ $\log_{10}$ spacing
Biological Replicates:	4	3 TempO-Seq; 1 Reserve

[a] MCF7 cells cultured in DMEM + 10% HI-FBS was selected as the test system to facilitate comparability to the Broad Institute Connectivity Map (CMAP) database (http://portals.broadinstitute.org/cmap/).

[a] Dulbecoo's Modified Eagle's Media (MediaTech 10-013) + Heat-Inactivated FBS (Sigma-Aldrich F4135)
[B] Phenol Red Free Dulbecco's Modified Eagle's Media (MediaTech 17-205) + Charcoal-Stripped Heat-Inactivated FBS (Sigma-Aldrich 6765)

HTTr Pilot: Workflow

Generate Cryopreserved Cell Stocks

Cell Expansion

Cell Plating

BioTek MultiFlo FX

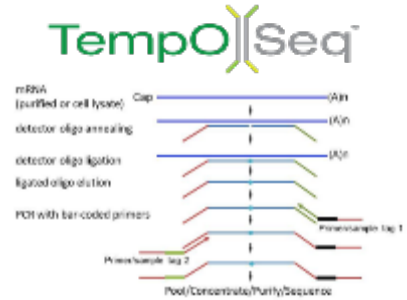
Cell Dosing

LabCyte Echo® 550 Liquid Handler

Track 1: Targented RNA-Seq

Generating Cell Lysates

TempO-Seq WT



Track 2: Cytotoxicity / Apoptosis

Cell Labeling

High Content Imaging

Thermo Cellomics ArrayScan® VTI HCS Reader

Assay Optimization

- **MCF7 Cell Culture**
  - Authentication
  - Expansion Protocol
  - Media Formulation
  - Seeding Density

- **TempO-Seq Assay**
  - Lysis Conditions
  - Attenuation of Highly Expressed Genes

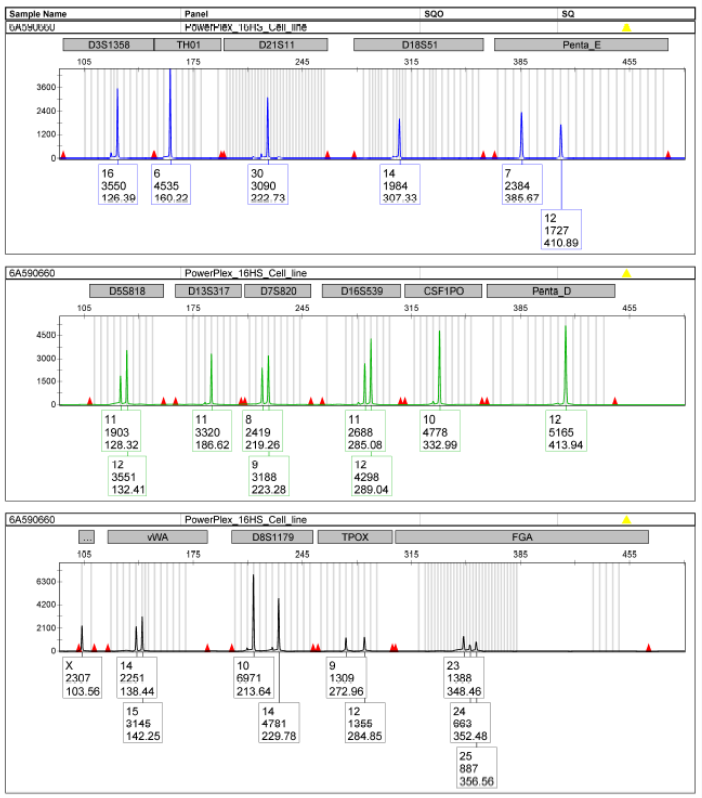
- **Chemical Treatments**
  - Concentration Range
  - Plate Map Design
  - Exposure Duration

MCF7 Cell Line Cryopreserved Stocks & Authentication

Cell Sourcing: ATCC® HTB-22™

Cryo Stock Expansion Strategy:
Procured 5 vials of cells
Expanded in parallel to internal Passage 3.
Pooled cells prior to cryopreservation (~120 vials @ $2x10^6$ cells / vial)

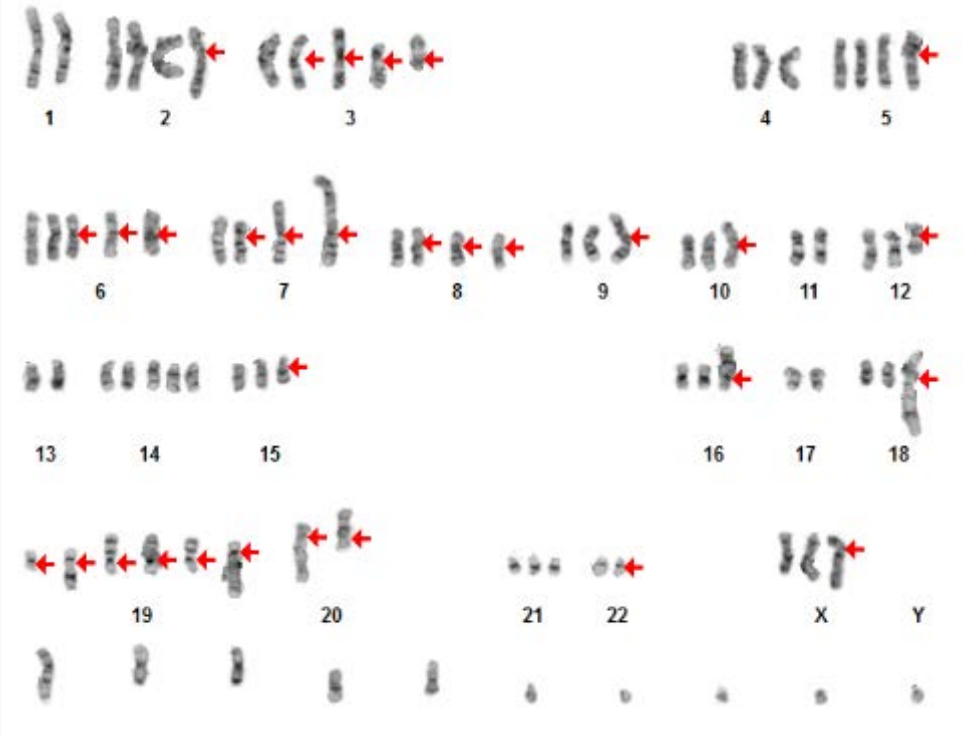
Authentication 1: STR Profiling



Loci	MCF7 Ref.	MCF7 NCCT
D5S818	11,12	11,12
D13S317	11	11
D7S820	8.9	8,9
D16S539	11,12	11,12
vWA	14,15	14,15
TH01	6	6
AMEL	X	X
TPOX	9.12	9,12
CSF1PO	10	10

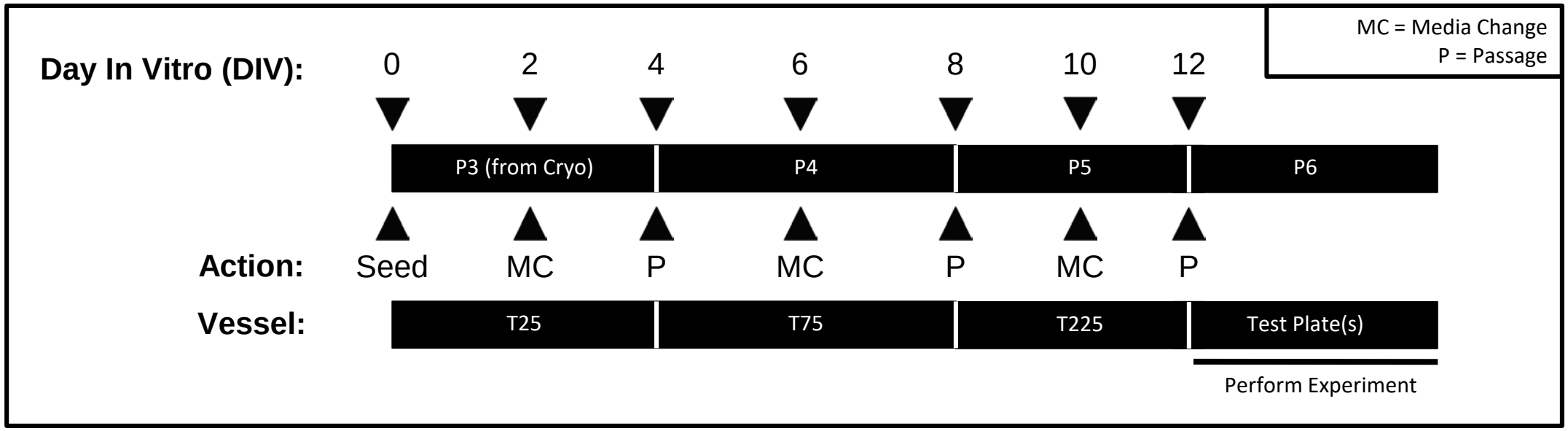
100% Concordance with Reference Profile

Authentication 2: Karyotyping



NCCT MCF7 Karyotype similar (but not identical) to reference profile.

MCF7 Expansion Protocol

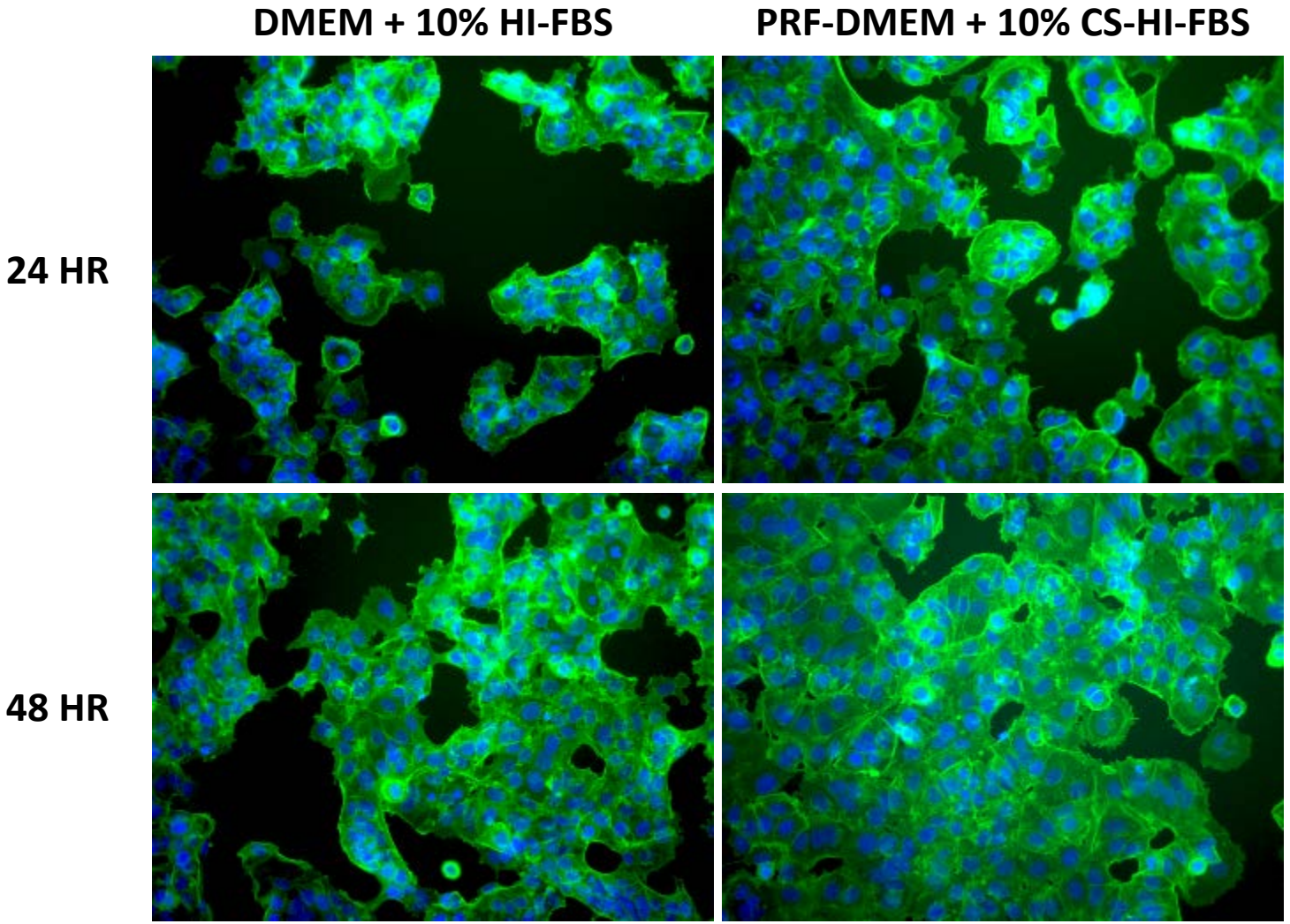


Stage	Culture Vessel	Average Cell Yield [a]	Number of Treatment Wells [b]	Number of Test Plates [c]
Initial Seeding	NA	$1.28x10^7$	182	0.47
P (3→4)	T25	$2.43x10^7$	346	0.90
P (4→5)	T75	$5.86x10^7$	837	2.18
P(5→6)	T225	$1.47x10^8$	2100	5.47

[a] Median values from c2017-08-14, c2017-08-15, c2017-08-19, c2017-08-20
[b] Assumes 384 well plate, 10,000 cells / well.
[c] For experimental needs > 5 plates / experiment, expand multiple cryopreserved MCF7 cell aliquots in parallel. Pool at each passaging stage.

Media Effects on MCF7 Growth

- DMEM + 10% HI-FBS contains phenol red and an unknown compliment of serum factors which may stimulate ER activation.
- Phenol red-free media with charcoal-stripped FBS reduces endogenous estrogen receptor activation.



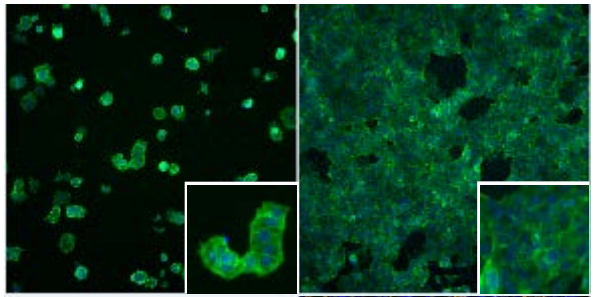
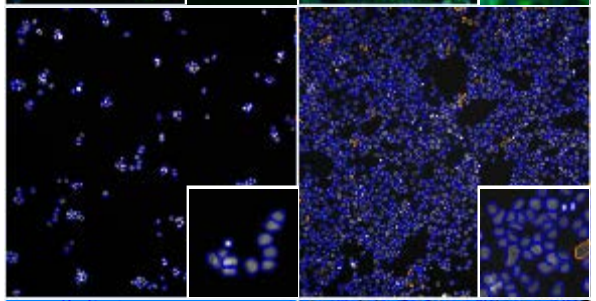
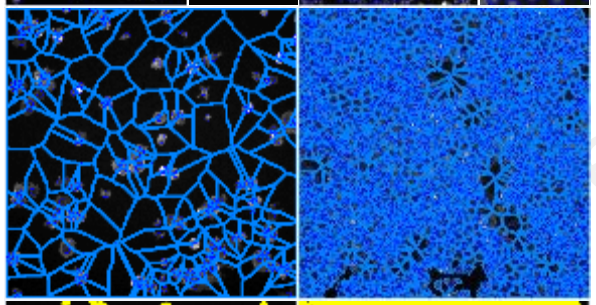
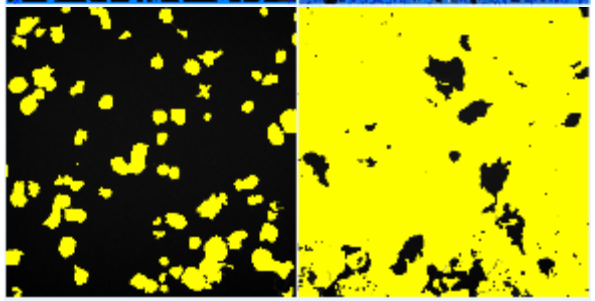
- Cells seeded at 5,000 cells / well

Qualitative Observations

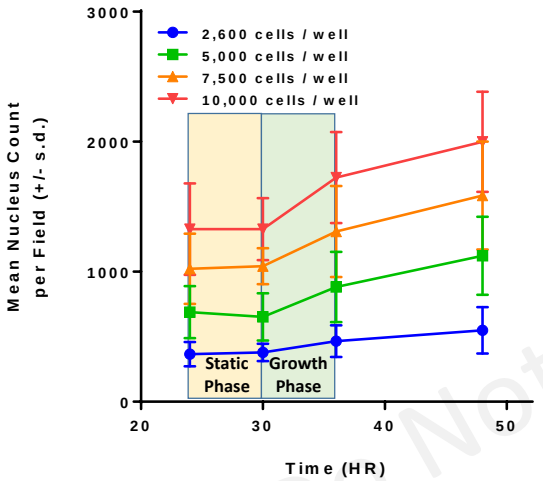
- More cell attachment and cell spreading with PRF-DMEM + 10% CS-HI-FBS.
- Greater increase in cell confluency over time in PRF-DMEM + 10% CS-HI-FBS.
- More proliferation over time in DMEM + 10% HI-FBS.

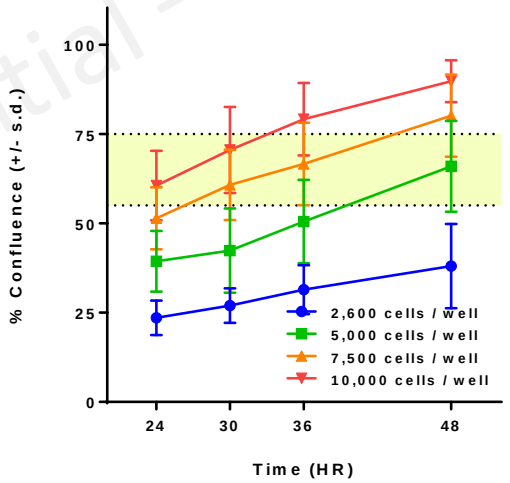
Quantification of Growth in NCCT MCF7 Cells & Selection of Seeding Densities

24 HR

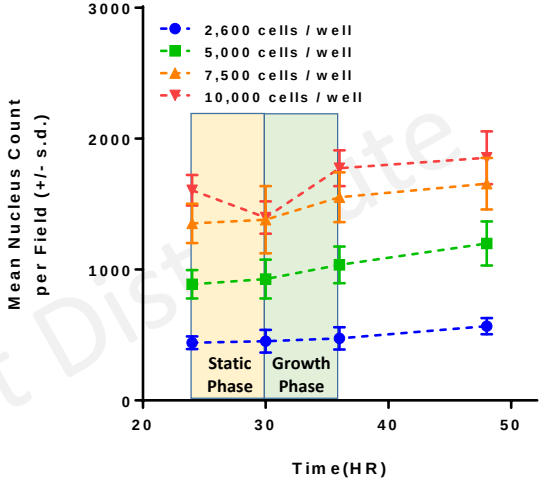
	2,600 cells / well	10,000 cells / well
Composite		
Nucleus Trace		
ZOI Masking		
Pixel Detection		

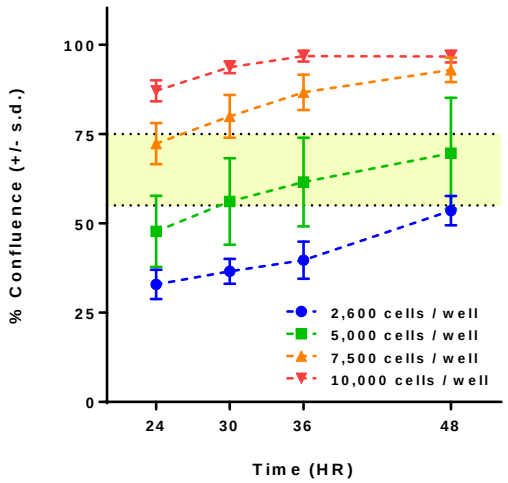
DMEM + 10% HI-FBS [a,c]





PRF-DMEM + 10% CS-HI-FBS [b,c]





- Increases in cell proliferation do not begin until 30 hours post-plating.
- Proliferation is greater in DMEM+10% HI-FBS versus PRF-DMEM + 10% CS-HI-FBS
- Cells seeded at concentrations yielding 55-75% confluence at time of sampling.
- **DMEM + 10% HI-FBS:**
  - 30 HR → 10,000 cells
  - 36 HR → 7,500 cells
  - 48 HR → 5,000 cells
- **PRF-DMEM + 10% CS-HI-FBS:**
  - All Times → 5,000 cells.

[a] Dulbecoo's Modified Eagle's Media (MediaTech 10-013) + Heat-Inactivated FBS (Sigma-Aldrich F4135)
[B] Phenol Red Free Dulbecco's Modified Eagle's Media (MediaTech 17-205) + Charcoal-Stripped Heat-Inactivated FBS (Sigma-Aldrich 6765)
[C] n = 72 replicate wells across two independent cultures.

Attenuation

- A method used with BioSpyder TempO-Seq assay to prevent highly expressed genes from occupying a disproportionate amount of available read space and increase the ability to quantify low abundance transcripts.

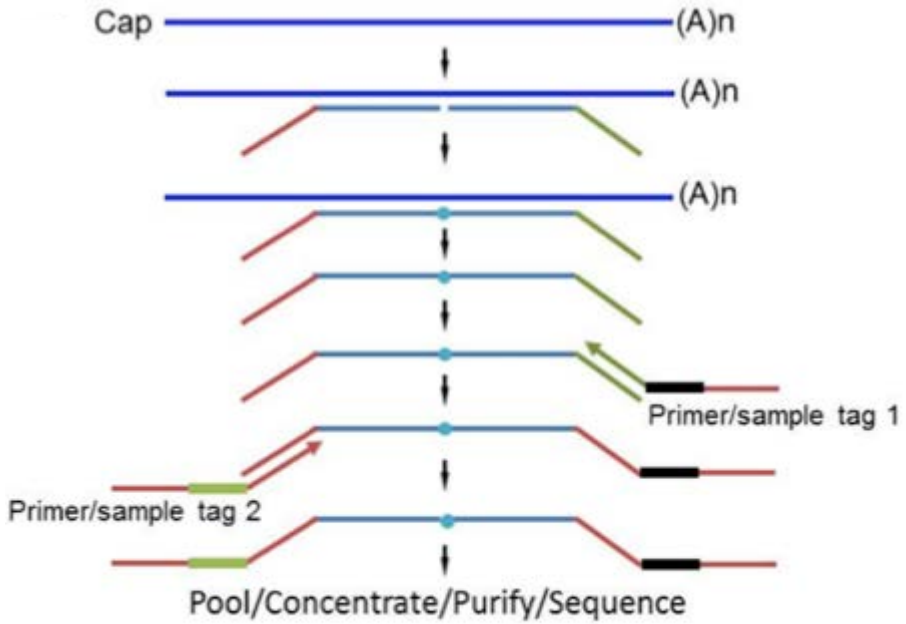
- Attenuation is accomplished by adding “cold probes” which do not have the PCR amplification tags at the 5’ and 3’ ends of the ligated detector oligos.

- The attenuation probe will bind to the same site as the detector oligos, thus decreasing the amount of the target RNA species available for PCR ampliciation.

- A “standard” attenuation for ribosomal RNAs is applied to TempO-Seq whole transcriptome assays.

- For additional attenuation, the end user must define:
  - The set of genes to be attenuated, and…
  - What degree of attenuation is appropriate

- **Question(s):**
  - Is additional attenuation needed in the MCF7 cell model?
  - If so, how is the attenuation set defined?



Study Design: TempO-Seq Attenuation in MCF7 Cells

Study Component 1:
6 HR Exposure, Static Phase

Cell Type:	MCF7
Media Type:	DMEM + 10% HI-FBS PRF-DMEM + 10% CS-HI-FBS
Treatments:	DMSO (0.5%) Trichostatin A (1 µM)
Replicates:	3
Dose Time:	24 hr post-plating
Sample Time:	30 hr post-plating
Total # of Samples:	12

Media Type, Exposure Window

Study Component 2:
6 HR Exposure, Growth Phase

Cell Type:	MCF7
Media Type:	DMEM + 10% HI-FBS PRF-DMEM + 10% CS-HI-FBS
Treatments:	DMSO (0.5%) Trichostatin A (1 µM)
Replicates:	3
Dose Time:	30 hr post-plating
Sample Time:	36 hr post-plating
Total # of Samples:	12

Media Type, Exposure Window

Study Component 3:
Time Course, Untreated Cells

Cell Type:	MCF7
Media Type:	DMEM + 10% HI-FBS PRF-DMEM + 10% CS-HI-FBS
Treatments:	None
Replicates:	3
Dose Time:	n/a
Sample Time:	30, 36, 48 hr post-plating
Total # of Samples:	18

Media Type, Time Course of Cell Growth

Lysis Option 1:
40 µL Media: 40 µL 2X Lysis Buffer

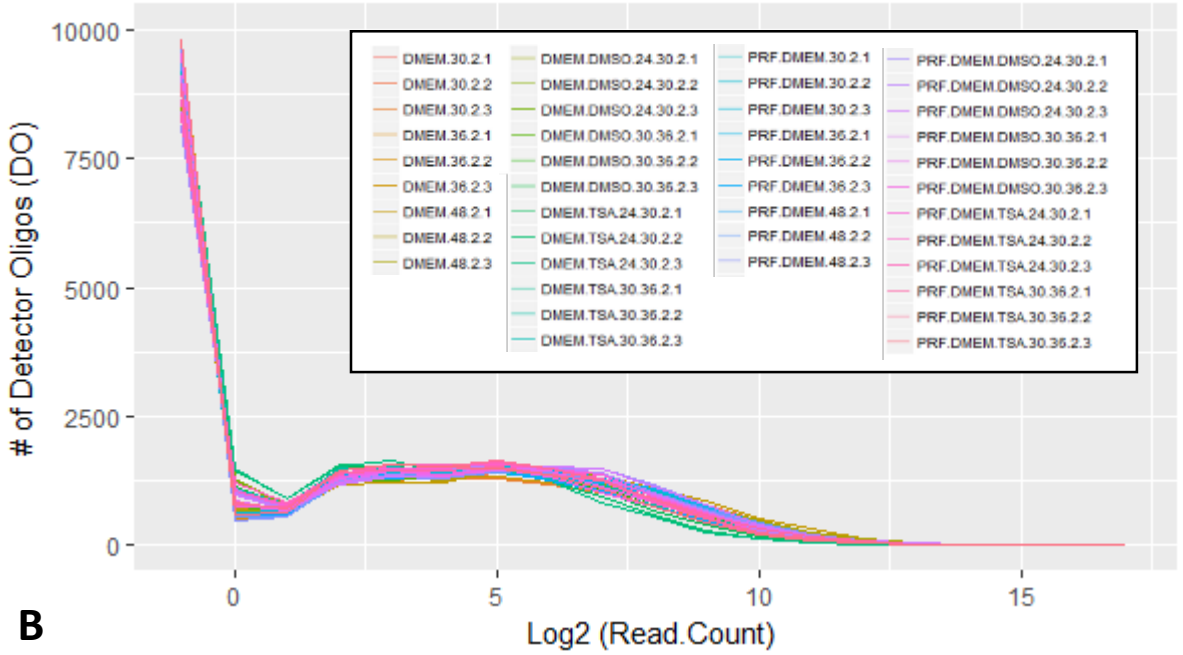
Lysis Option 2:
Drain to 10 µL Media: 10 µL 2X Lysis Buffer

Lysis Option 3:
Complete Drain → 10 µL 1X Lysis Buffer

- Each study component was performed using each lysis option.
- Samples from **Lysis Option 2** (n = 42) were used for identification of candidate Detector Oligos (DOs) for attenuation.

Distribution of Read Counts

A



B

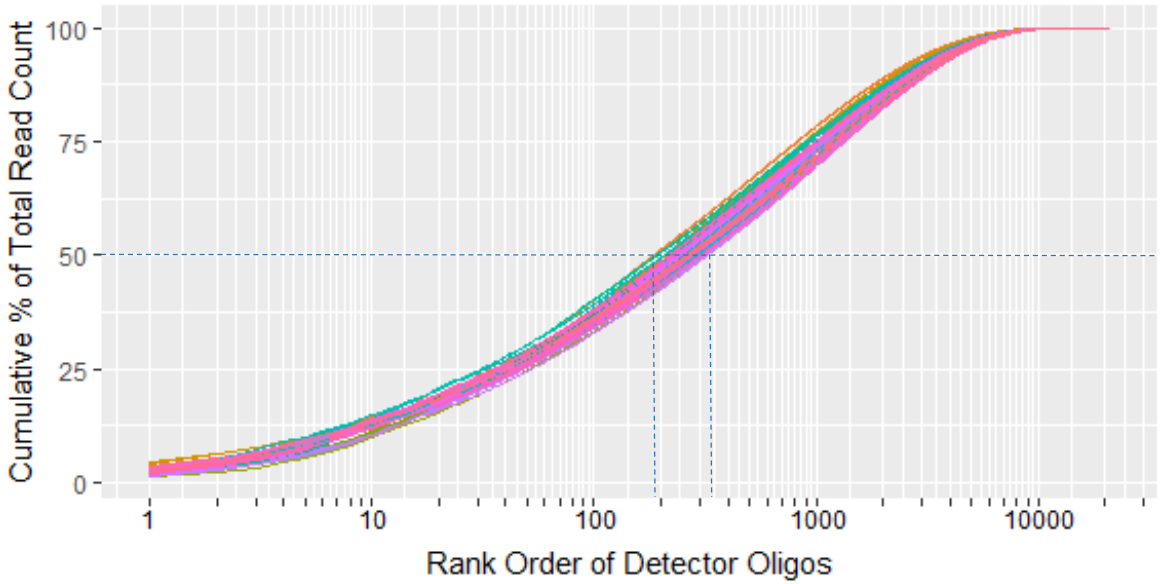


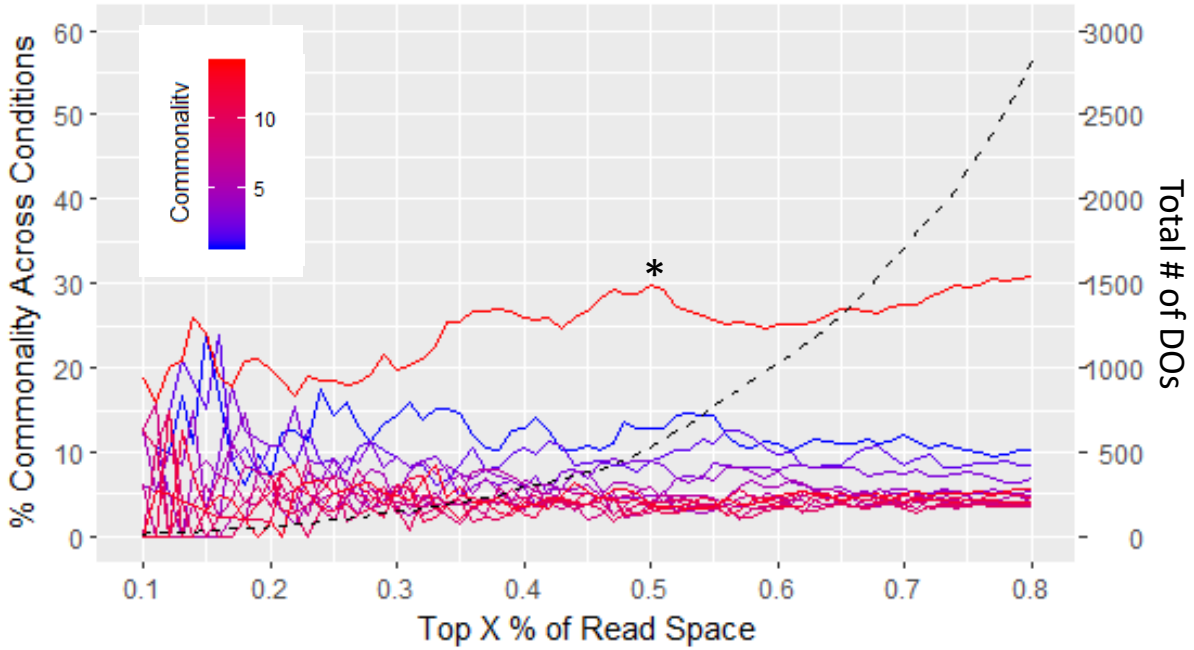
Table 1. Number of DOs Accounting for 50% of Total Read Space, Per Sample Basis

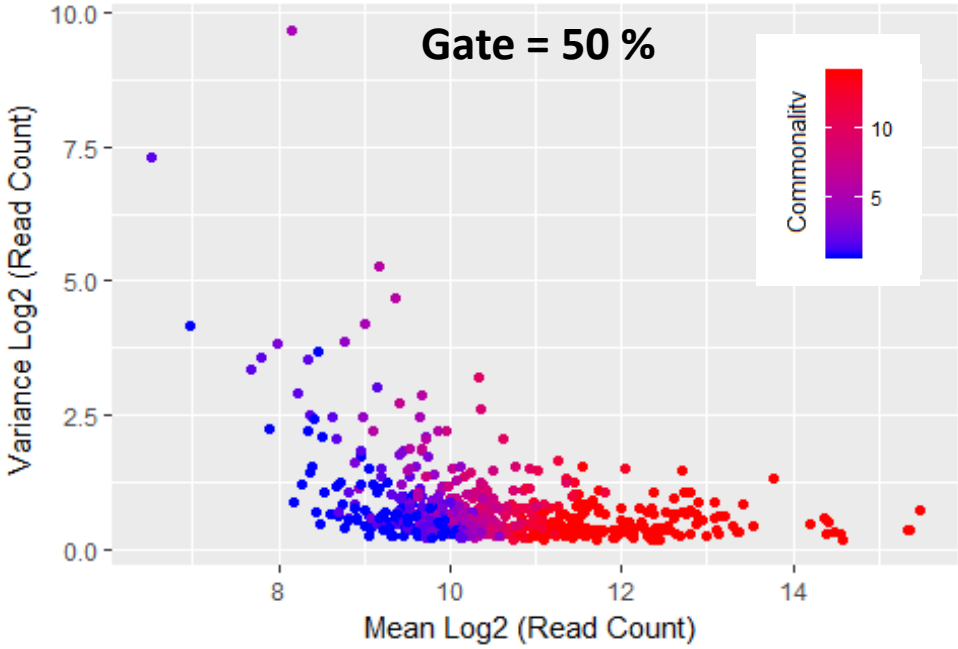
	Media Type	Treatment Type	Treatment Time, h	Sample Time, h	Replicate Number		
					1	2	3
Time Course	DMEM	--	--	30	242	246	186
Time Course	DMEM	--	--	36	273	220	208
Time Course	DMEM	--	--	48	238	249	239
Time Course	PRF.DMEM	--	--	30	276	288	289
Time Course	PRF.DMEM	--	--	36	268	248	244
Time Course	PRF.DMEM	--	--	48	240	240	262
C.Resp.1	DMEM	DMSO	24	30	308	259	269
C.Resp.1	DMEM	TSA, 1 µM	24	30	231	248	253
C.Resp.1	PRF.DMEM	DMSO	24	30	307	303	322
C.Resp.1	PRF.DMEM	TSA, 1 µM	24	30	273	278	303
C.Resp.2	DMEM	DMSO	30	36	242	233	249
C.Resp.2	DMEM	TSA, 1 µM	30	36	192	222	208
C.Resp.2	PRF.DMEM	DMSO	30	36	245	242	232
C.Resp.2	PRF.DMEM	TSA, 1 µM	30	36	220	273	263
				Range of DO Counts:	**186 - 322**		

Results

- Read count distributions similar across samples.
- Broad range of read counts within each sample (0 - ~32K).
- Within each sample, ~50-60% of DOs with non-zero read counts.
- Between 186 - 322 DOs account for 50% of the available read space (varies with sample).

Evaluating Commonality of Highly Expressed Genes Across Test Conditions





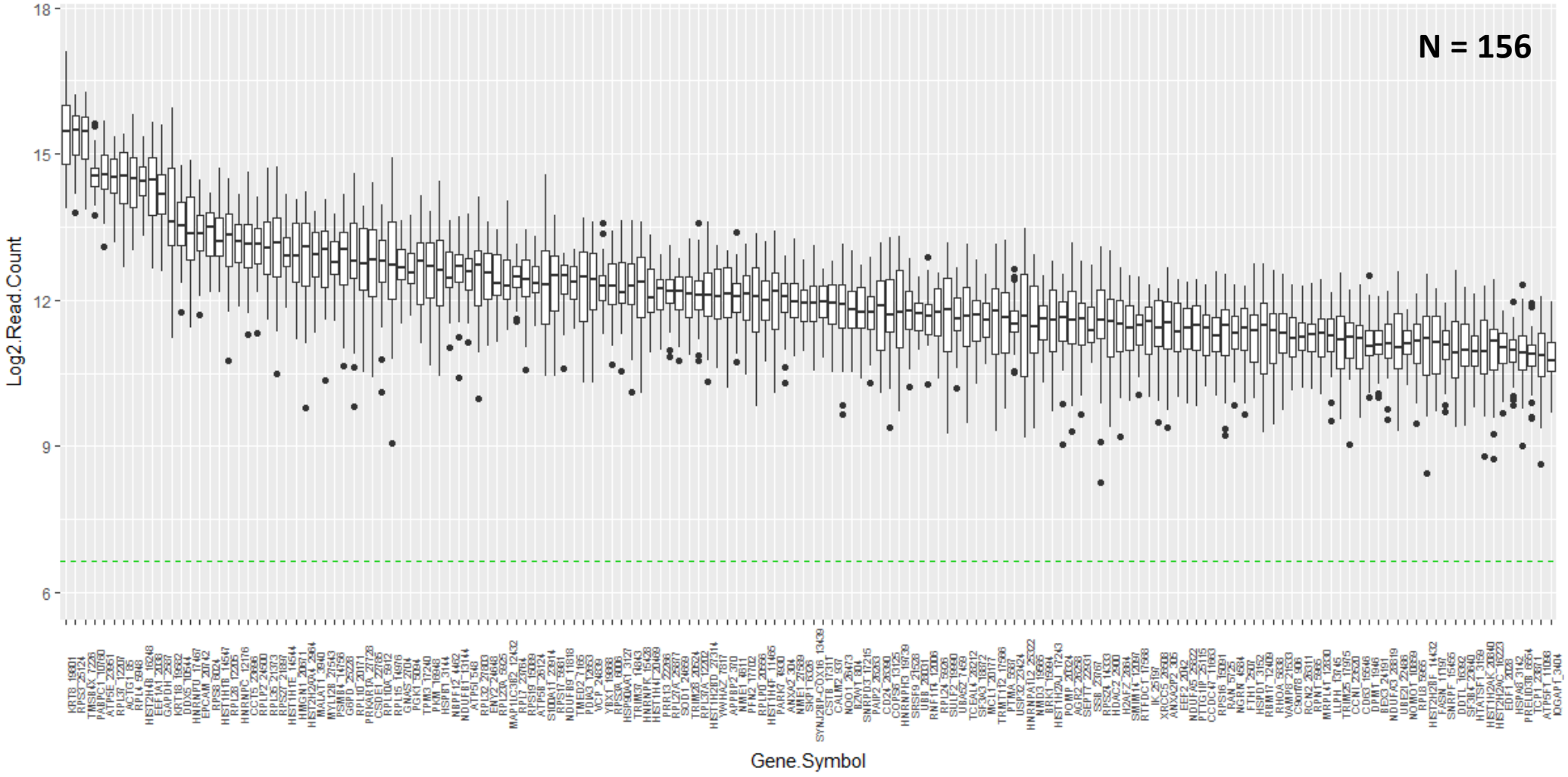
Using a Gate of 50 % of the total read space (*):

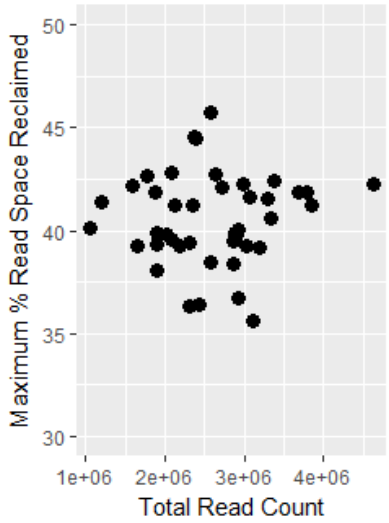
- ***Commonality Score = 14:*** ~ 30% of the DOs are identified as "highly-expressed" in all 14 test conditions (red).
- ***Commonality Score = 1:*** ~12.5% are identified as "highly-expressed" in only 1 test condition (blue).
- ***Commonality Score = 2 – 13:*** Varying number of DOs (< 10%) identified as "highly-expressed" in 2 to 13 test conditions.
- ***Variance:*** Tended to increase in DOs with lower commonality scores.

Conclusions:

- At Gate = 50 %, DOs with Commonality Scores of 14 are consistently identified as "highly-expressed" across all test conditions and have relatively lower variance and higher read counts across all test conditions.
- **N = 156** DOs identified as candidates for attenuation.

Candidate “Highly Expressed Genes” for Attenuation





- Rank ordered on x-axis by average read count across all test conditions.
- Green line → Raw read count = 100.

Top 12 Candidates for Attenuation

Gene Symbol	Official.Full.Name	Category	Description
TMSB4X	thymosin beta 4, X-linked	Cytoskeleton	This gene encodes an actin sequestering protein which plays a role in regulation of actin polymerization.
KRT8	keratin 8	Cytoskeleton	This gene is a member of the type II keratin family clustered on the long arm of chromosome 12. Type I and type II keratins heteropolymerize to form intermediate-sized filaments in the cytoplasm of epithelial cells.
ACTG1	actin gamma 1	Cytoskeleton	Actin, gamma 1, encoded by this gene, is a cytoplasmic actin found in non-muscle cells.
KRT18	keratin 18	Cytoskeleton	KRT18 encodes the type I intermediate filament chain keratin 18. Keratin 18, together with its filament partner keratin 8, are perhaps the most commonly found members of the intermediate filament gene family.
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Energy Metabolism	This gene encodes a member of the glyceraldehyde-3-phosphate dehydrogenase protein family. The product of this gene catalyzes an important energy-yielding step in carbohydrate metabolism
HIST2H4B	histone cluster 2 H4 family member b	Histone	This gene is intronless and encodes a replication-dependent histone that is a member of the histone H4 family
ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	Mitochondrial	This gene encodes a subunit of mitochondrial ATP synthase.
RPS3	ribosomal protein S3	Ribosomal	This gene encodes a ribosomal protein that is a component of the 40S subunit, where it forms part of the domain where translation is initiated.
RPL37	ribosomal protein L37	Ribosomal	This gene encodes a ribosomal protein that is a component of the 60S subunit.
RPL4	ribosomal protein L4	Ribosomal	This gene encodes a ribosomal protein that is a component of the 60S subunit.
PABPC1	poly(A) binding protein cytoplasmic 1	Ribosomal Transport	This gene encodes a poly(A) binding protein. The protein shuttles between the nucleus and cytoplasm and binds to the 3' poly(A) tail of eukaryotic messenger RNAs via RNA-recognition motifs.
EEF1A1	eukaryotic translation elongation factor 1 alpha 1	Ribosomal Transport	This gene encodes an isoform of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome.

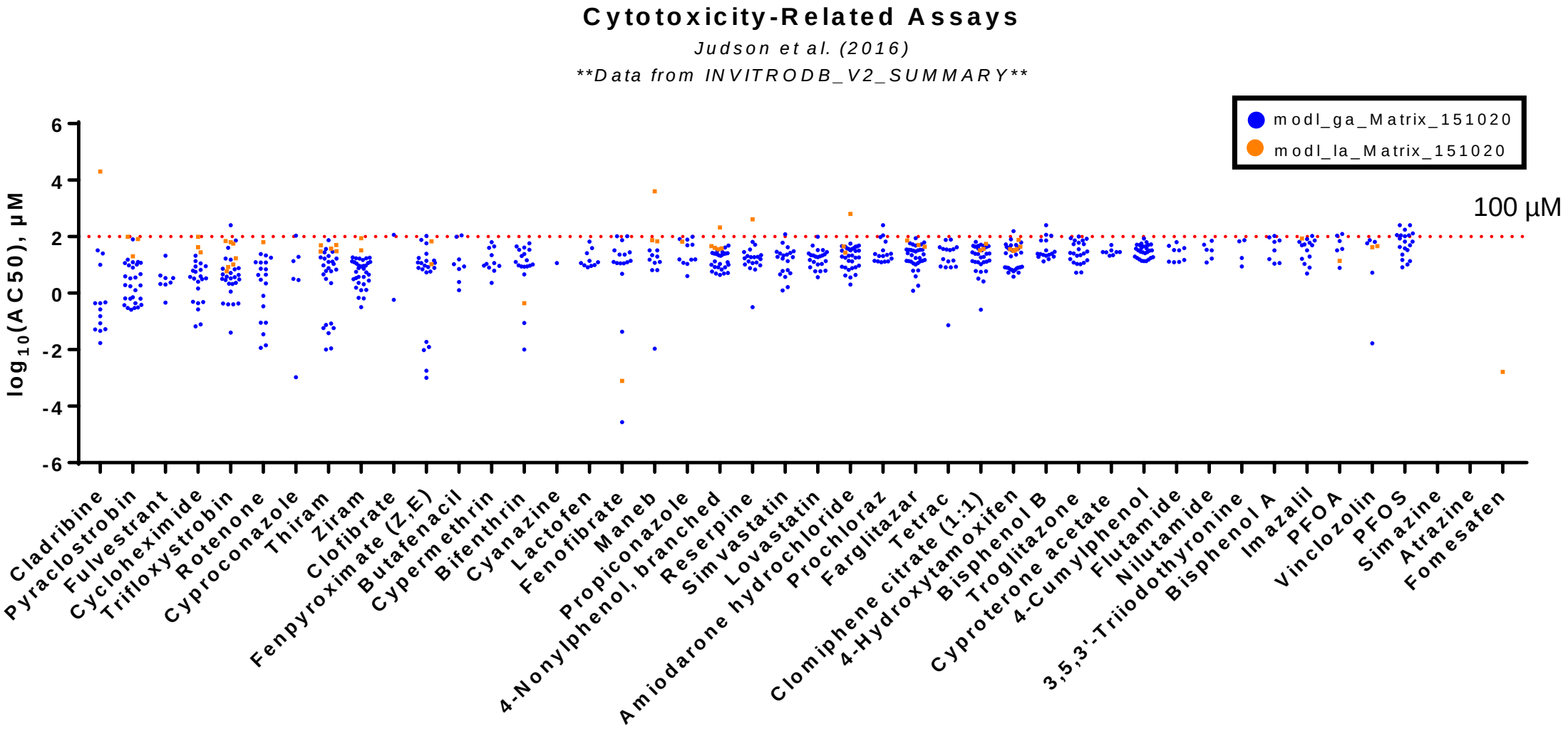
- The most highly expressed genes in the attenuation set are “housekeeping” genes.

HTTr Pilot: Chemical Test Set

Chemical Name	MIE Family	Chemical Name	MIE Family
Flutamide	ANTIANDROGEN	Rotenone	MITOCHONDRIA (COMPLEX I)
Nilutamide		Fenpyroximate (Z,E)	
Cyproterone acetate		Trifloxystrobin	MITOCHONDRIA (COMPLEX II)
Vinclozolin		Pyraclostrobin	
4-Hydroxytamoxifen	ANTIESTROGEN	PFOS	PPAR
Clomiphene citrate (1:1)		PFOA	
Fulvestrant		Troglitazone	
Atrazine	cAMP INDUCERS / PDE INHIBITORS	Farglitazar	
Cyanazine		Lactofen	PPO INHIBITOR / PPAR
Cladribine	CYTOTOXICANTS	Fomesafen	PPO INHIBITOR
Cycloheximide		Butafenacil	
Bisphenol A	ESTROGENS	Maneb	SH REACTIVE
4-Nonylphenol, branched		Thiram	
Bisphenol B		Ziram	
4-Cumylphenol		Imazalil	STEROIDOGENESIS
Clofibrate	FIBRATES	Prochloraz	
Fenofibrate		Cyproconazole	
Lovastatin	HMGCR	Propiconazole	
Simvastatin		Tetrac	THR
Bifenthrin	NA+ CHANNEL	3,5,3'-Triiodothyronine	
Cypermethrin		Reserpine	VMAT
Simazine	PHOTOSYSTEM II INHIBITOR	Amiodarone hydrochloride	

- Chemical set covers broad range of mechanistic diversity with redundancy within mechanistic class.

Dose Range Selection

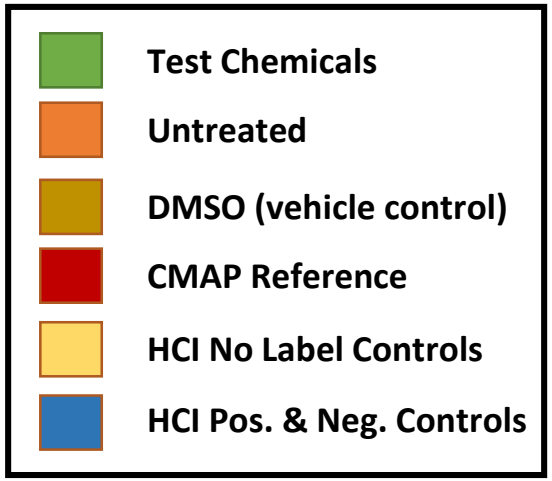


- Upper bound in testing range set at 100 µM based on upper limit of cytotoxicity range for most chemicals.
- **Final dose range:** 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 µM

Dosing Plate Layout

DOSING PLATE MAP																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	A	Ionomycin (30 µM)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	non-treated
2	B	Ionomycin (30 µM)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	non-treated
3	C	Ionomycin (30 µM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	non-treated
4	D	Staurosporine (1 µM)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	DMSO
5	E	Staurosporine (1 µM)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	DMSO
6	F	Staurosporine (1 µM)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	DMSO
7	G	Saccharin (100 µM)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	DMSO [No Label]
8	H	Saccharin (100 µM)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	Trichostatin (1 µM)
9	I	Saccharin (100 µM)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	Trichostatin (1 µM)
10	J	Sorbitol (100 µM)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	Trichostatin (1 µM)
11	K	Sorbitol (100 µM)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	Genistein (10 µM)
12	L	Sorbitol (100 µM)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Genistein (10 µM)
13	M	Ionomycin (30 µM) [No Label]	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Genistein (10 µM)
14	N	Staurosporine (1 µM) [No Label]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	Sirolimus (0.1 µM)
15	O	Saccharin (100 µM) [No Label]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Sirolimus (0.1 µM)
16	P	Sorbitol (100 µM) [No Label]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	Sirolimus (0.1 µM)

- 44 chemicals in 8-point concentration-response → all on one plate
- Non-treated (n=3) and DMSO (n=3) control wells.
- Three “CMAP” Reference Compounds, single point, in triplicate
- First column reserved for addition of RNA QC samples by NCCT (pre-shipment) and BioSpyder (post-shipment).

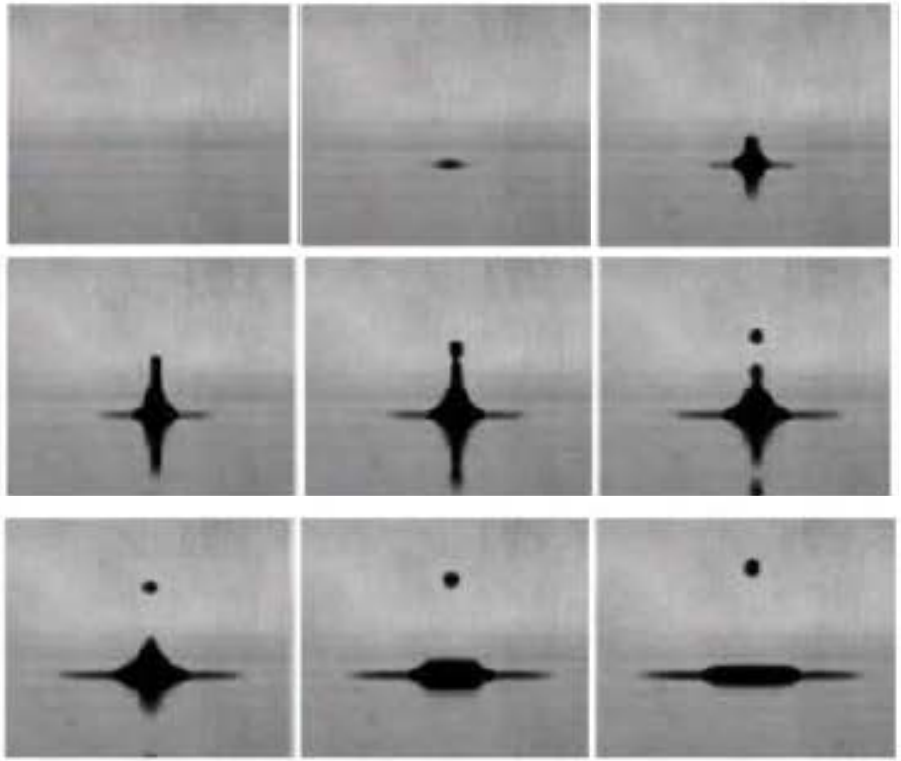


Dose Randomization using Echo 550

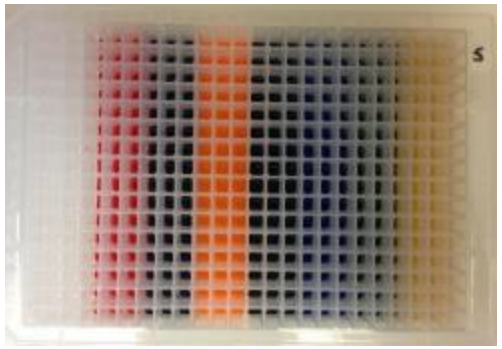
Acoustic dispensing technology:

- Uses soundwaves to precisely transfer small quantities of liquid (nL) from source plate to test plate.
- Allows for randomization of test wells → mitigate potential edge effects without "losing real estate."

LabCyte Echo® 550
Liquid Handler

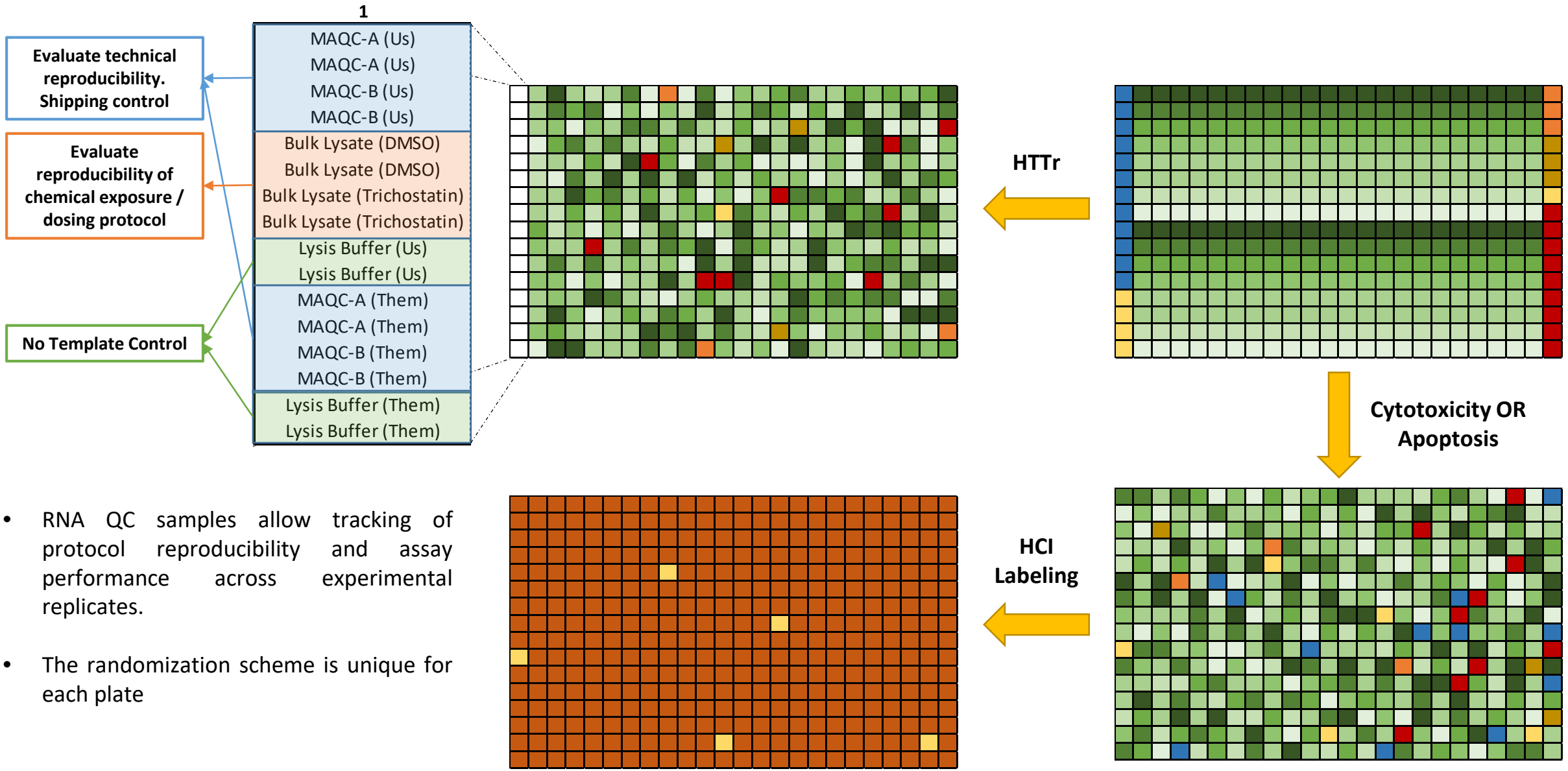


Source Plate



Test Plate

Echo Dispensing

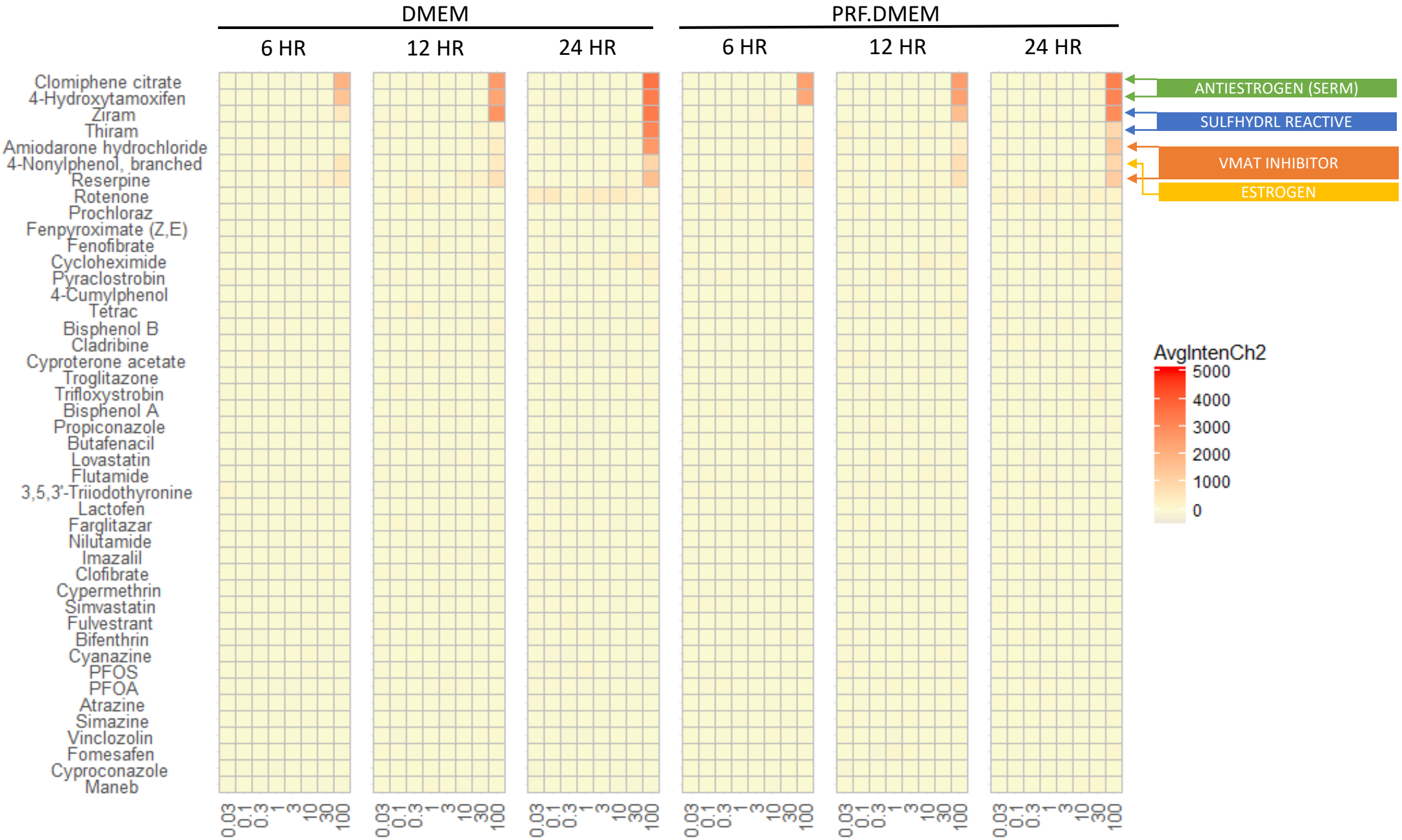


- RNA QC samples allow tracking of protocol reproducibility and assay performance across experimental replicates.
- The randomization scheme is unique for each plate

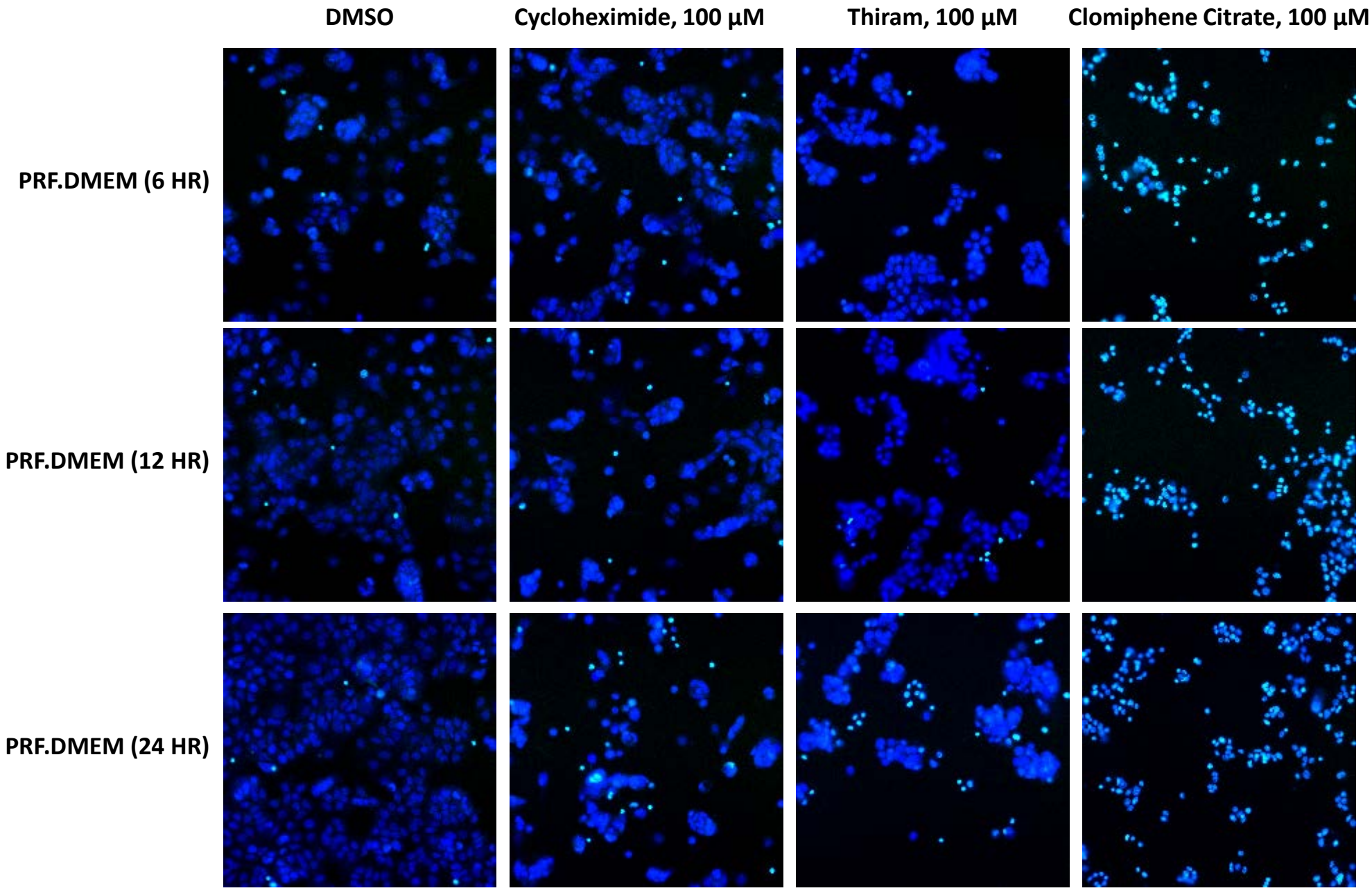
Results

- **Apoptosis & Cytotoxicity Assays**
- **Transcriptomics Data Analysis Pipeline**
- **HTTr Assay Performance Metrics**
- **Concentration Response Modeling**

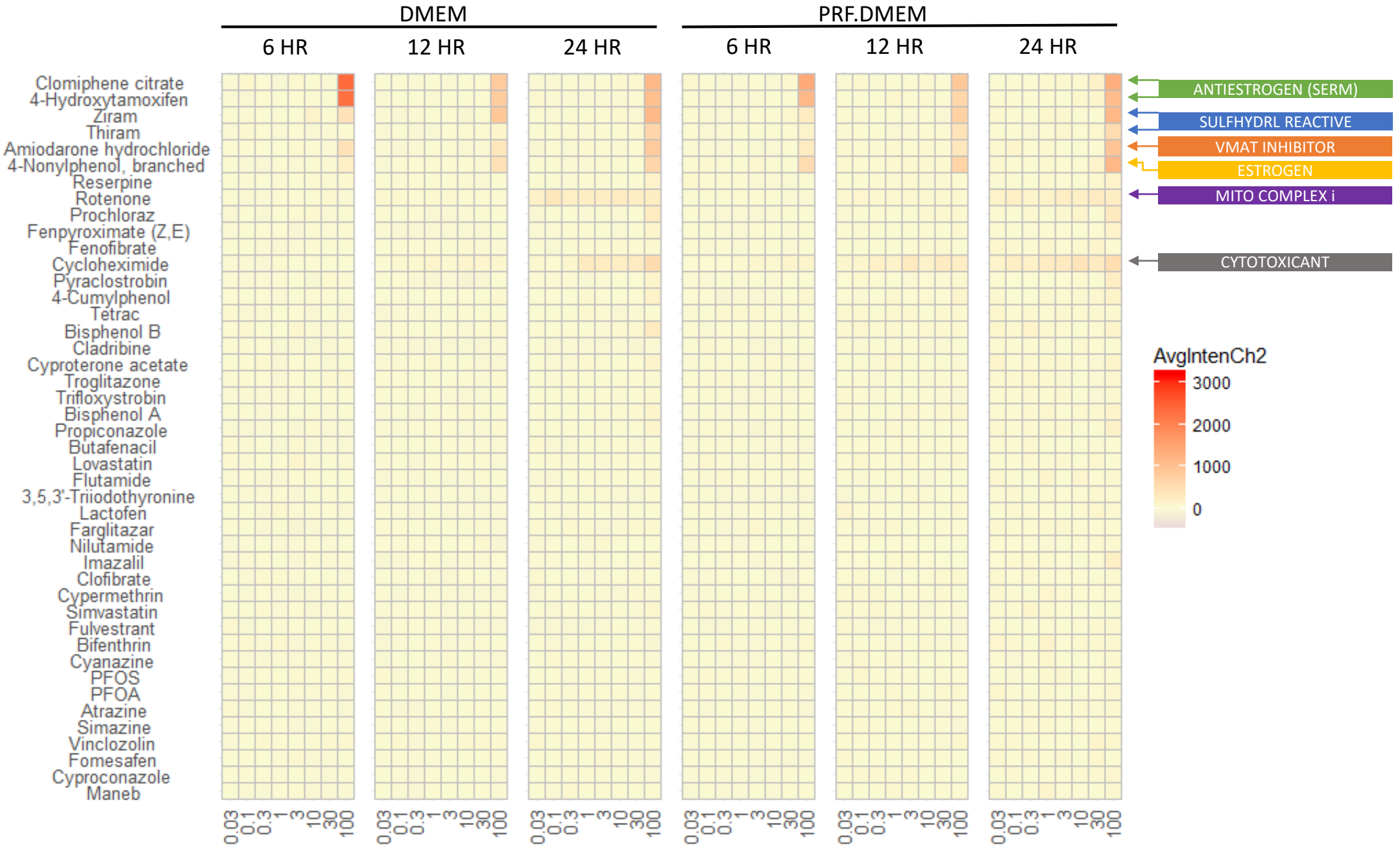
Apoptosis Assay Results



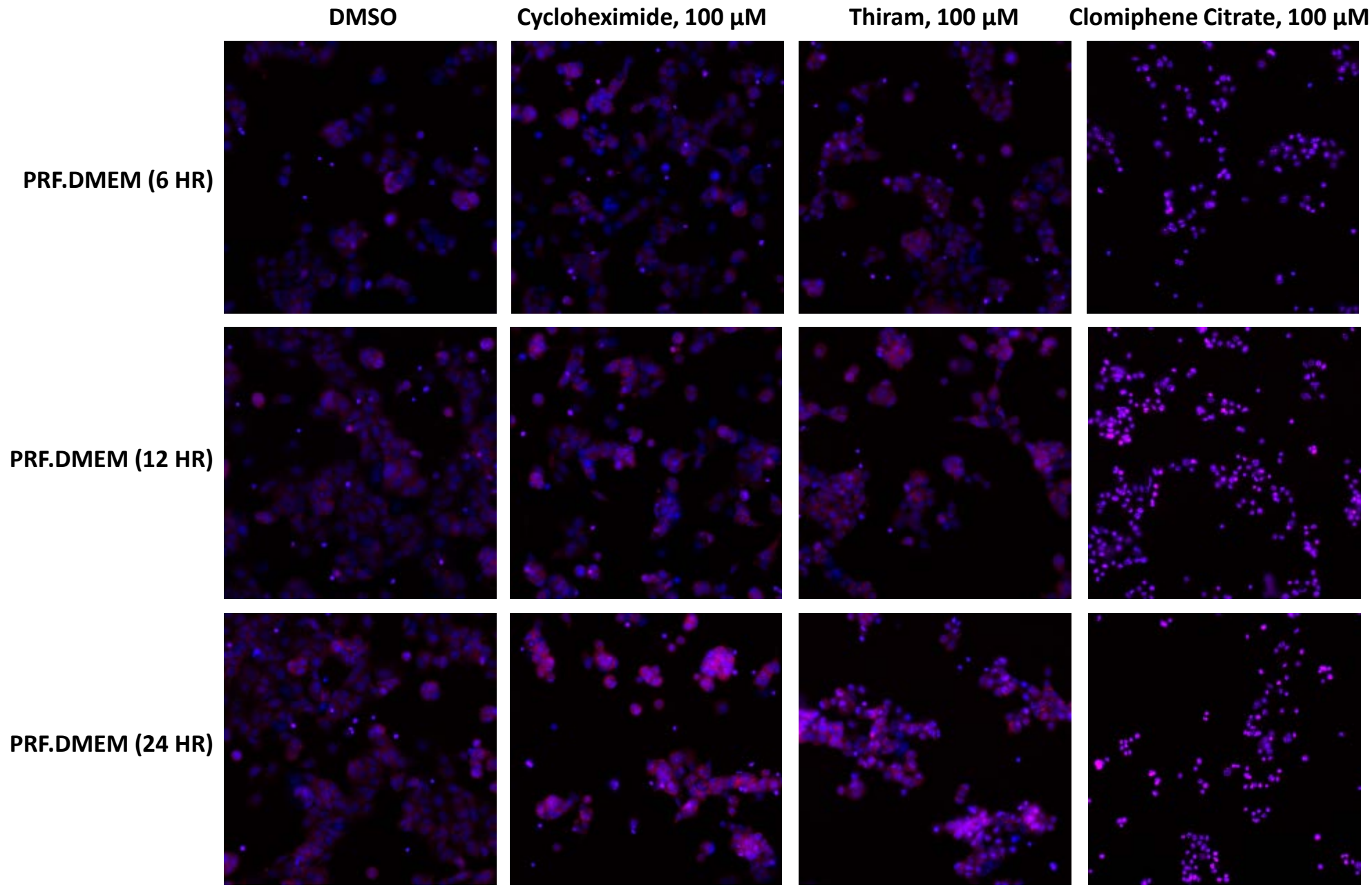
Apoptosis Assay, Ground Truth



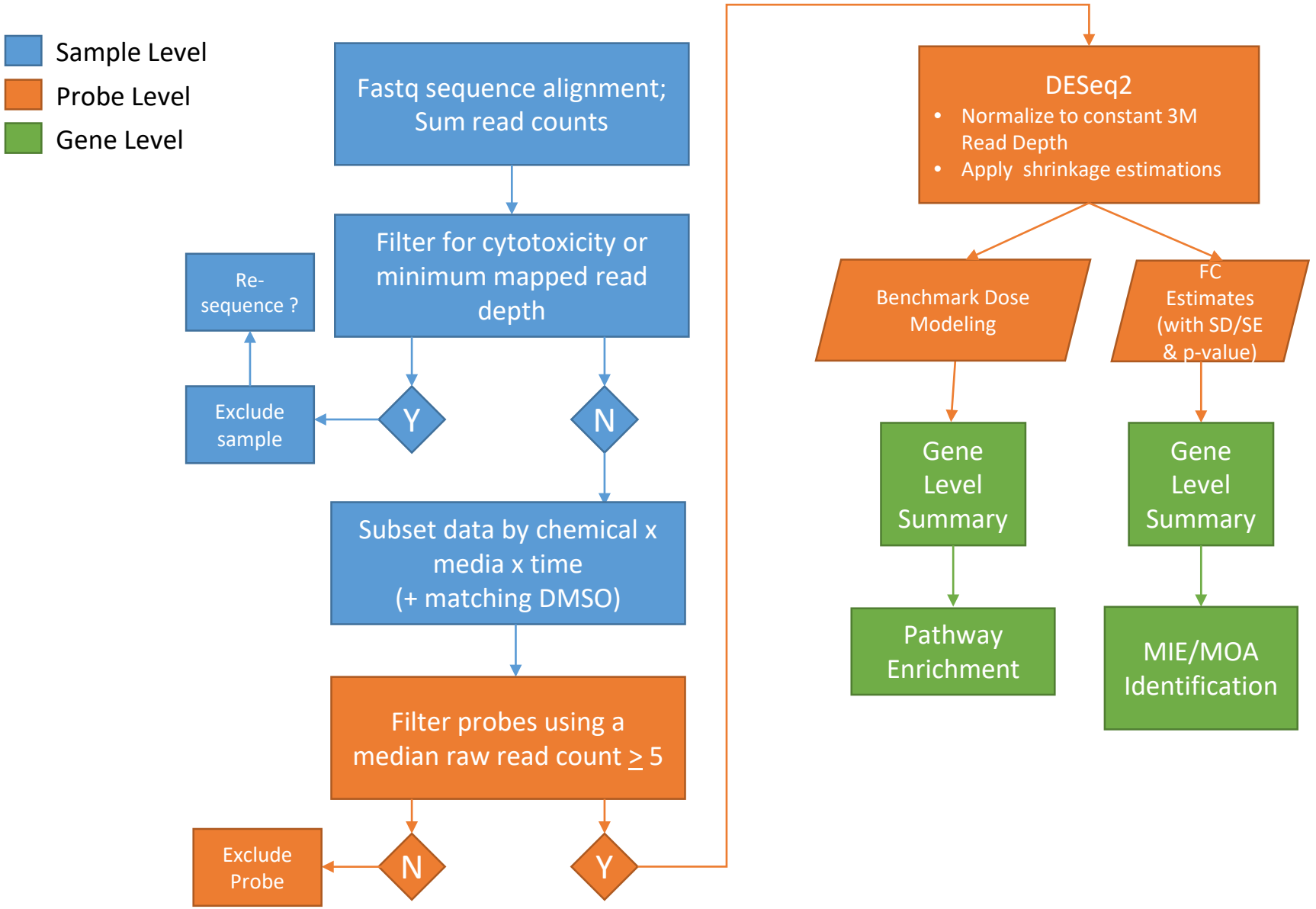
Cell Viability Assay Results



Cell Viability Assay, Ground Truth



Data Analysis Pipeline

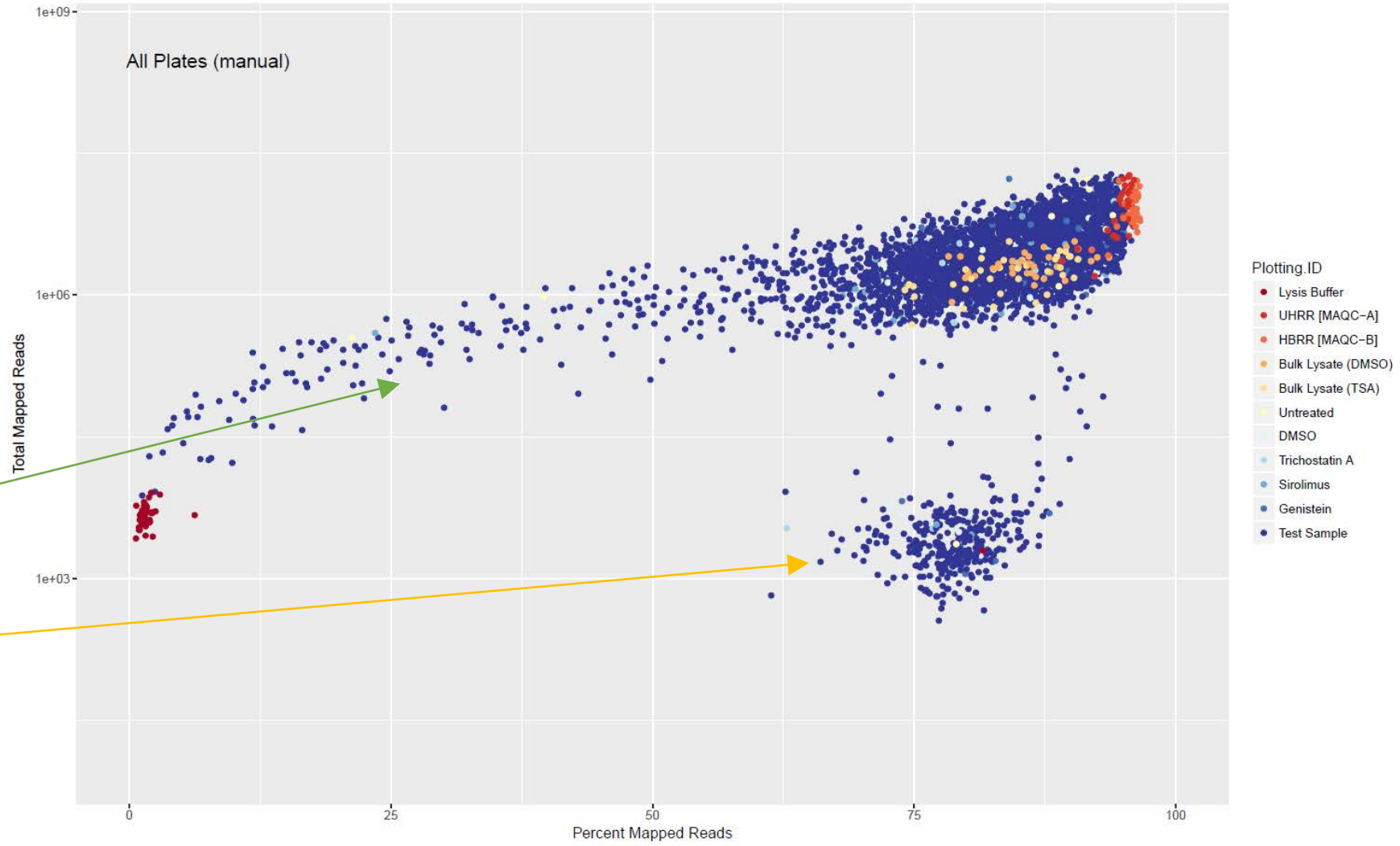


Assay Performance Metrics

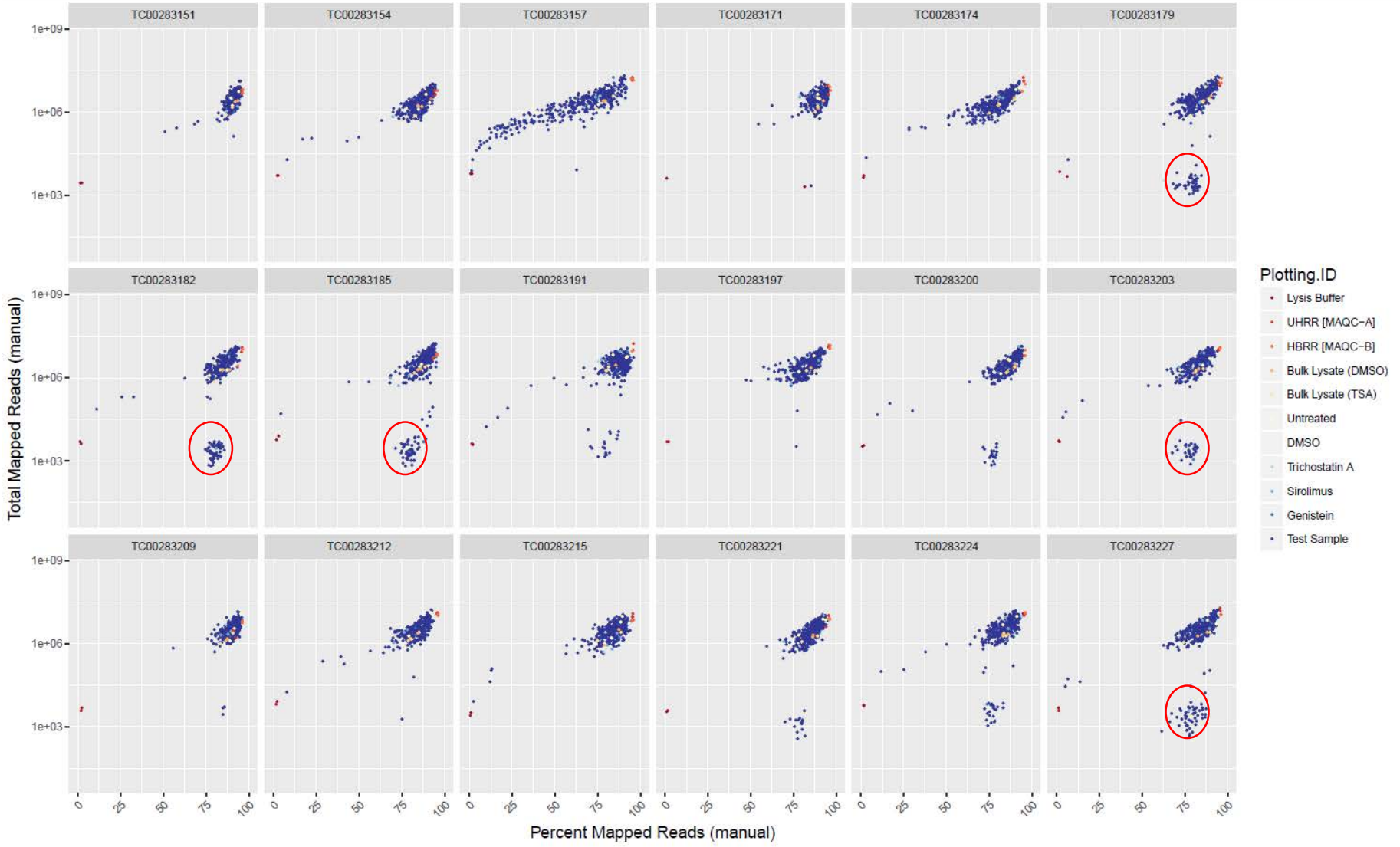
- **Total Mapped Reads vs. Percent Mapped Reads**
- **Correlation and Variation in Technical Replicates [within plate]**
- **Correlation and Variation in Biological Replicates [across plates]**
- **Detection of Biological Signal**
  - Transcriptional Biomarkers
  - Connectivity Mapping

Total Mapped Reads vs. Percent Mapped Reads [All Plates]

- Average total mapped reads of test samples ~ $3.0x10^6$
- Percent mapped reads > 75%
- Lysis Buffer blanks have low total reads, but not zero.
- Purified RNAs clustered at upper left.
- Comet tail ?
- Off-set cluster ?

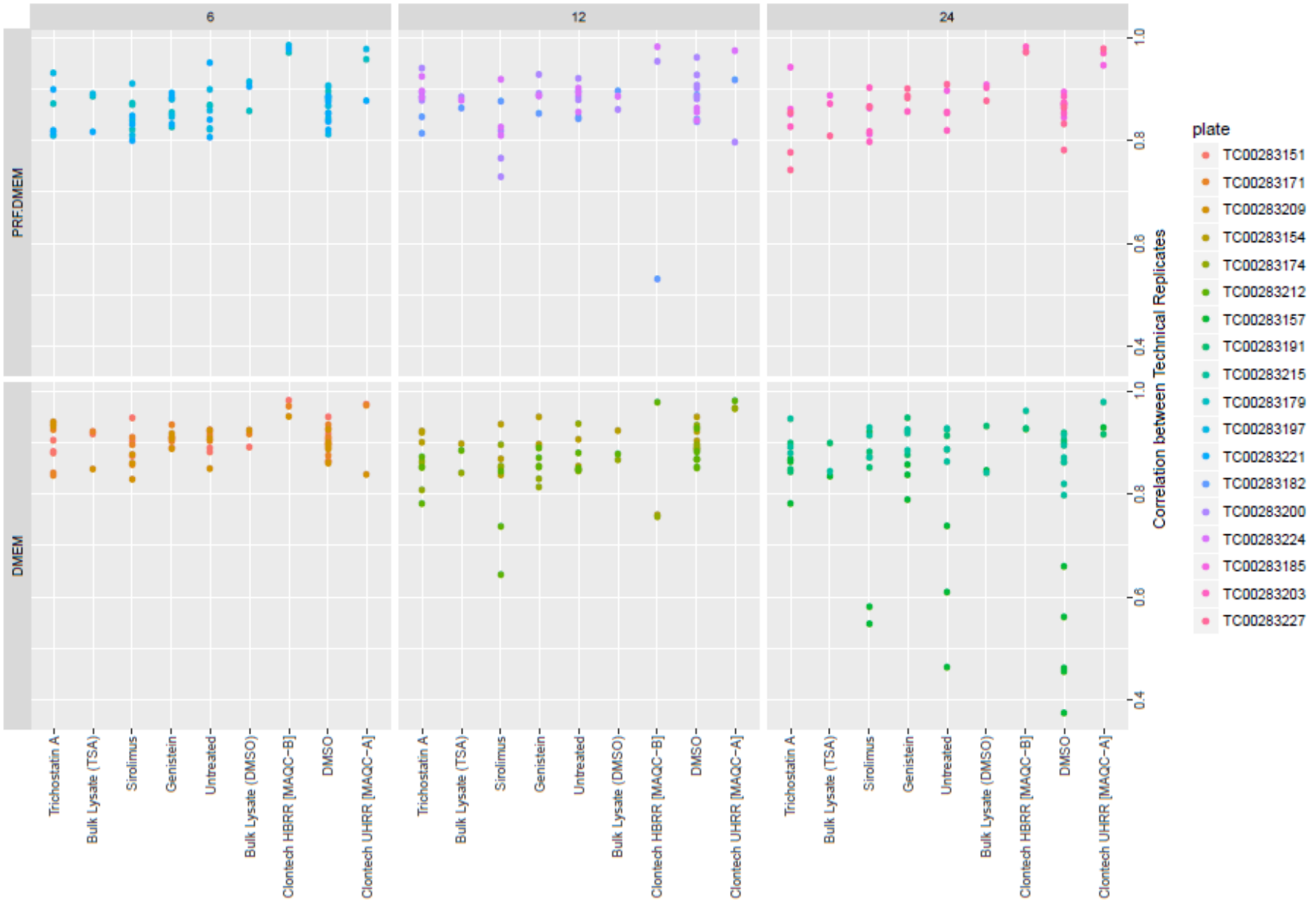


Total Mapped Reads vs. Percent Mapped Reads [By Plates]



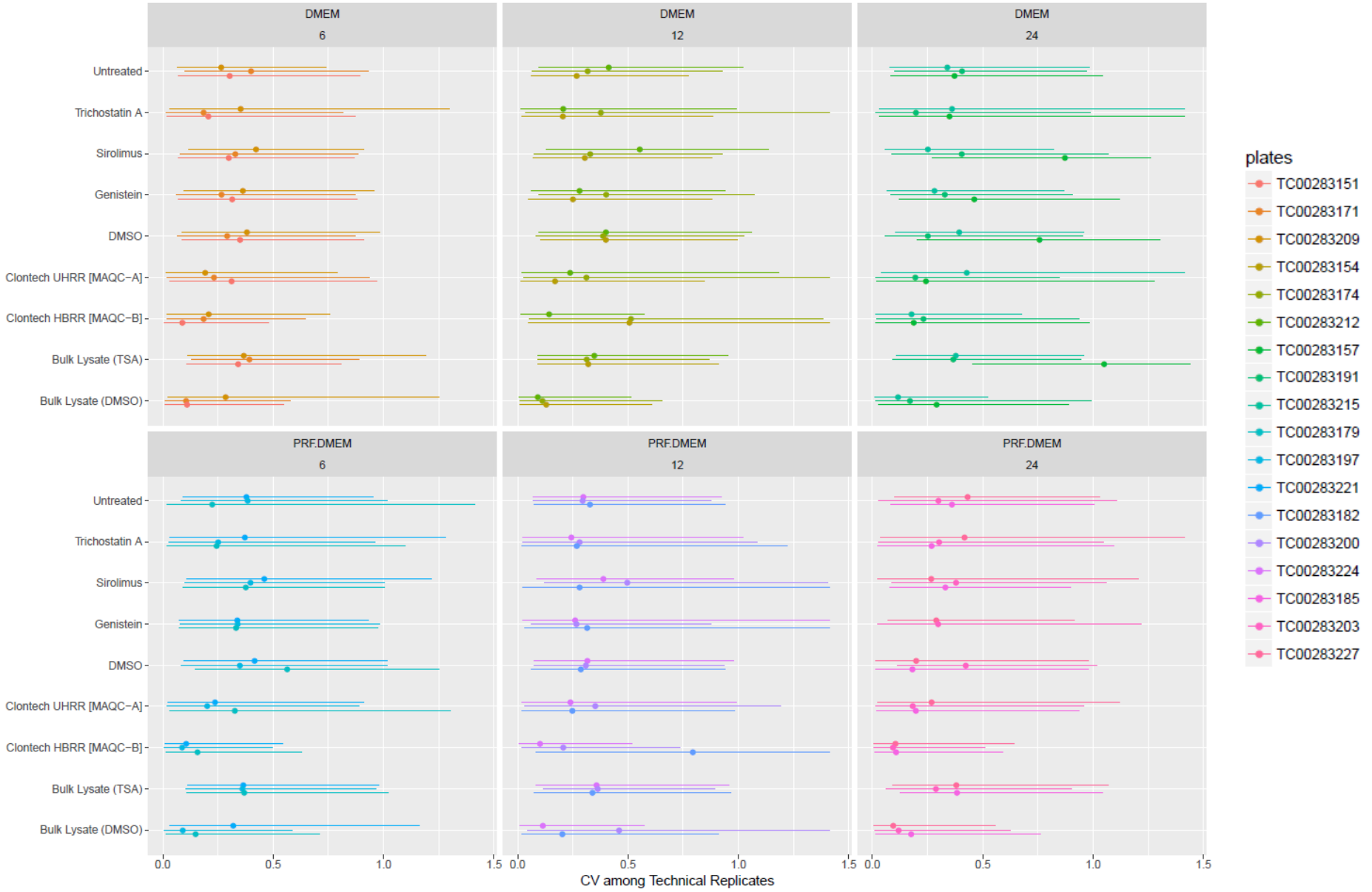
- Comet tail → Due to one “poor performing” plate
- Offset cluster → Low read count samples across many plates (red circles) → Candidates for resequencing.

Correlation Among Technical Replicates



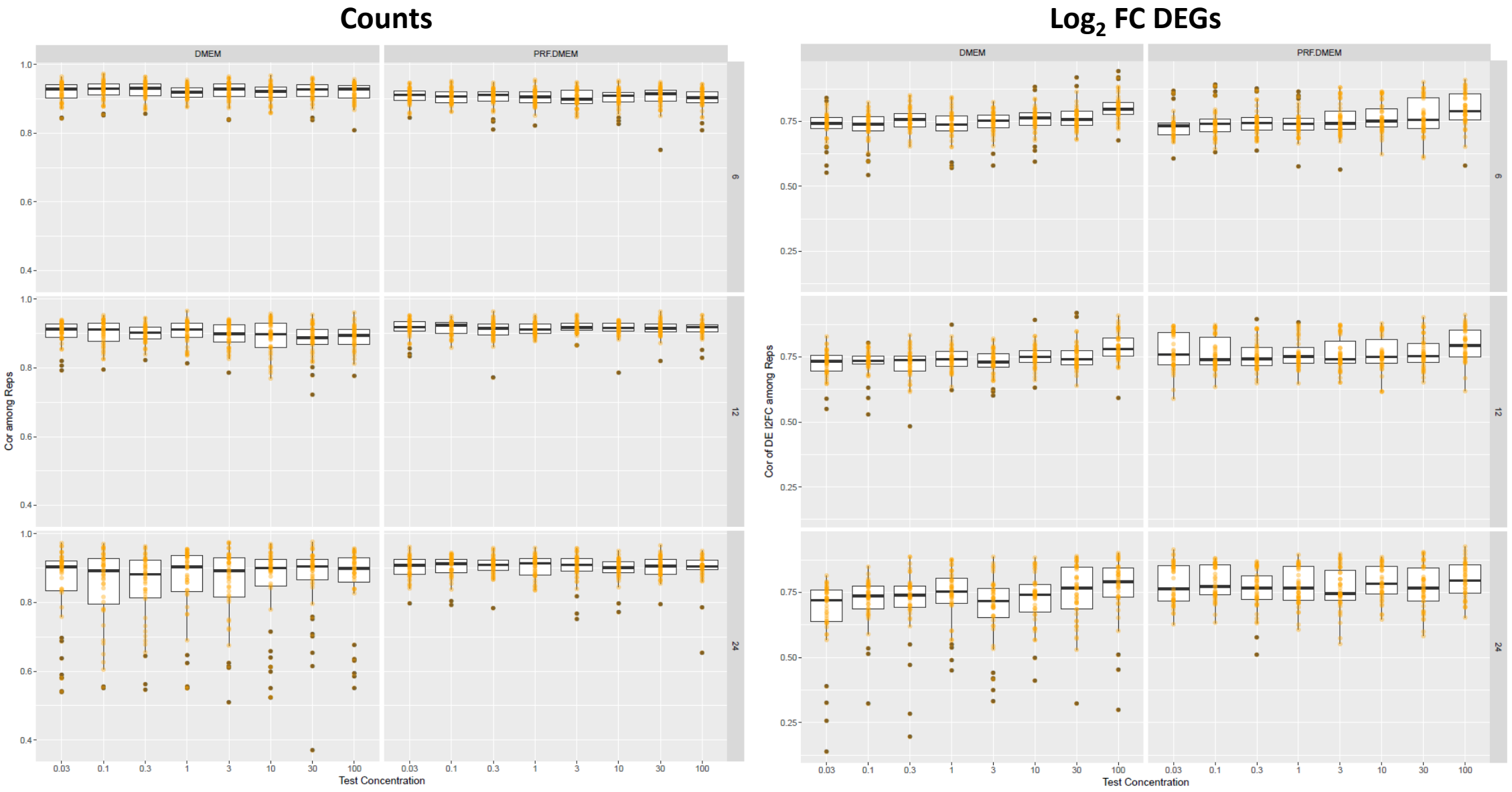
- Correlation among technical replicates is high (> 0.85 %).

Coefficient of Variation (CV) Among Technical Replicates



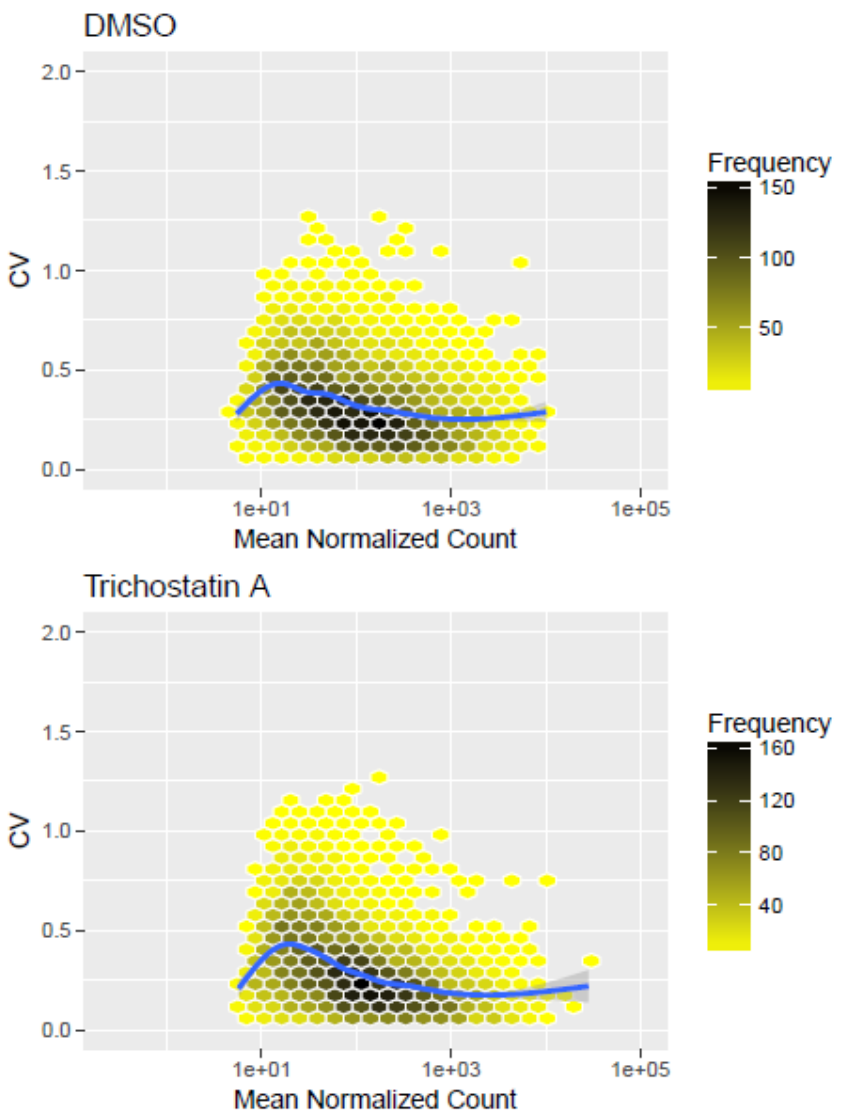
- Coefficient of variation in gene expression values is low (median ~30 %).

Correlations in Biological Replicates, Stratified by Expression Level



- Correlations of raw counts and $\log_2$ FC of DEGs is high ($\geq$ 0.85) for most conditions.

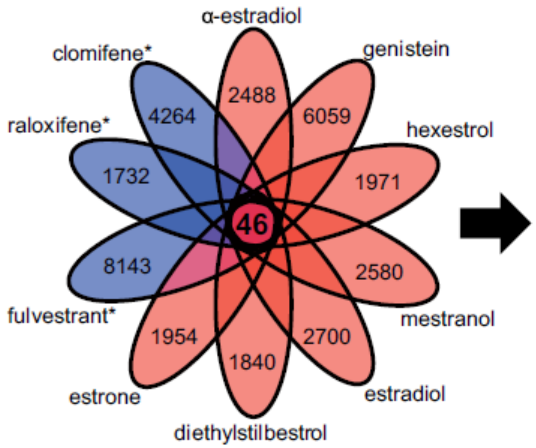
Coefficient of Variation (CV) Stratified by Expression Level

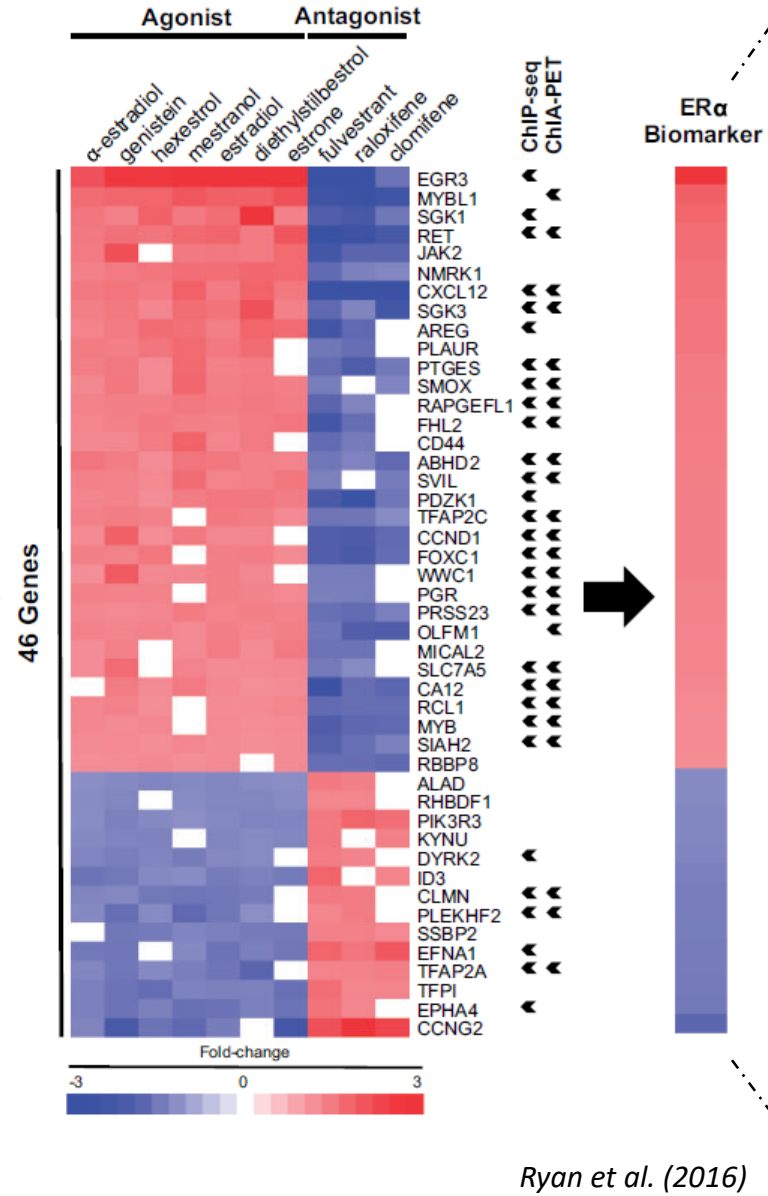


- CVs decrease as a function of mean expression level.

ERα Biomarker Signature

- Biomarker signature determined by treating MCF7 cells with various ERα agonists and antagonists.
- Can we use this to detect biologically meaningful signal in the BioSpyder data?

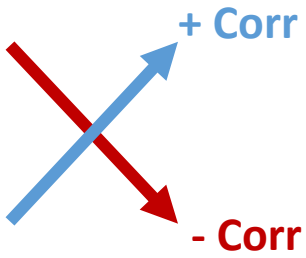


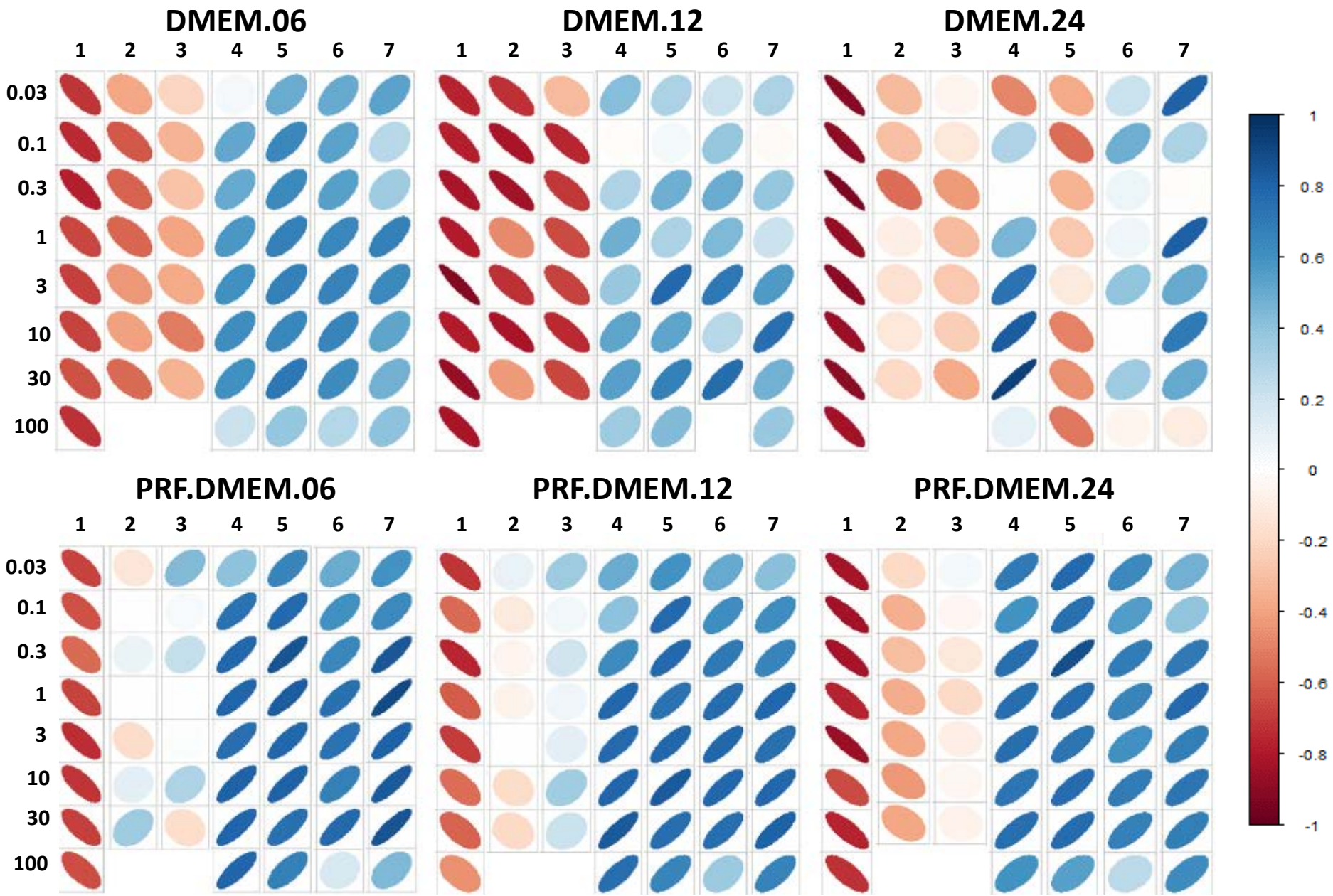


Ryan et al. (2016)

Correlation with ERα Transcriptional Biomarker

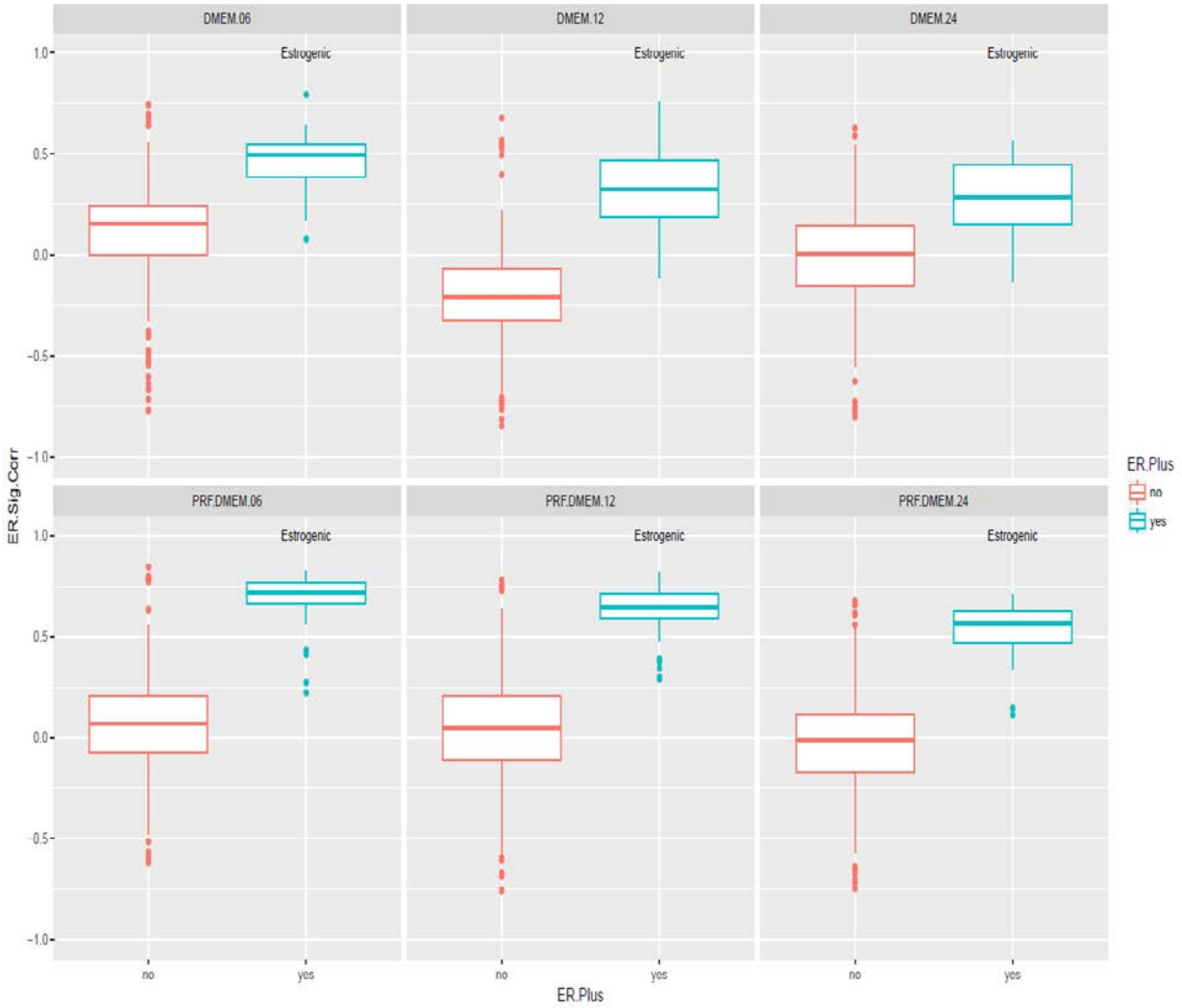
	Chemical	MOA
1	Fulvestrant	Antiestrogen (SERD)
2	4-Hydroxytamoxifen	Antiestrogen (SERM)
3	Clomiphene Citrate	
4	Bisphenol A	Estrogenic
5	Bisphenol B	
6	4-Nonylphenol, branched	
7	4-Cumylphenol	



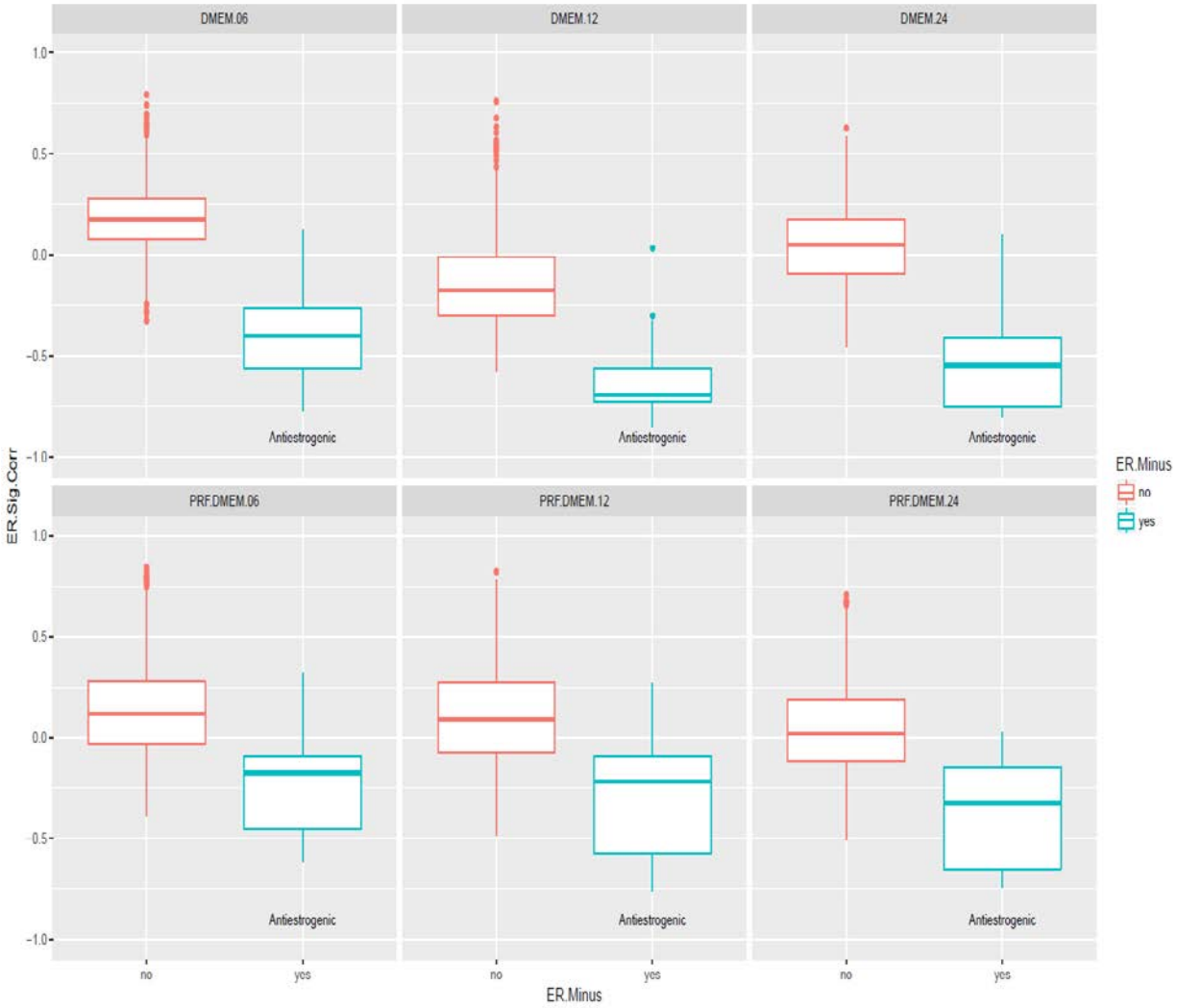


Correlation with ERα Transcriptional Biomarker - Antagonists

Agonists

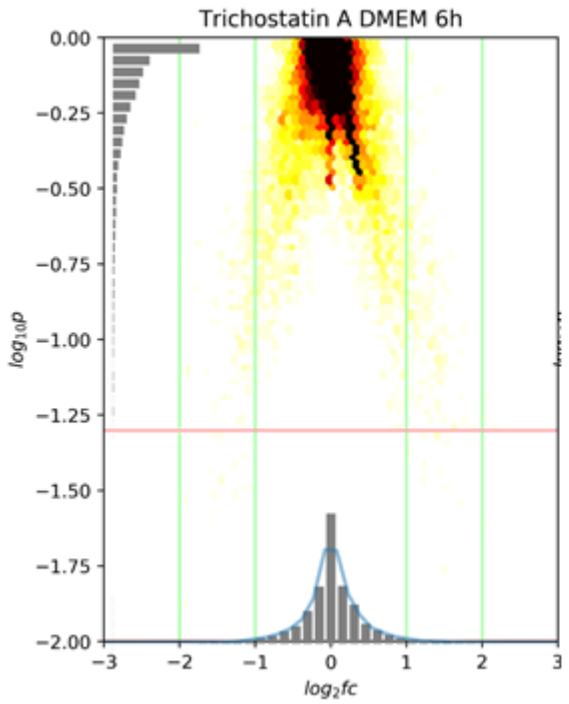


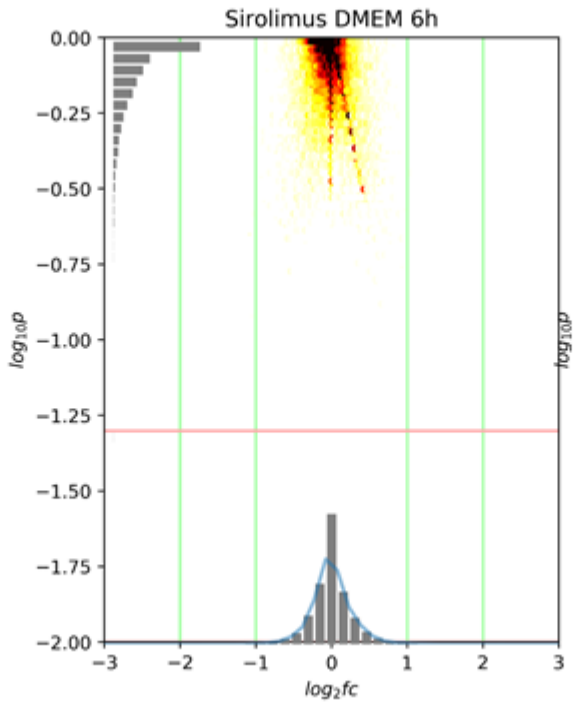
Antagonists

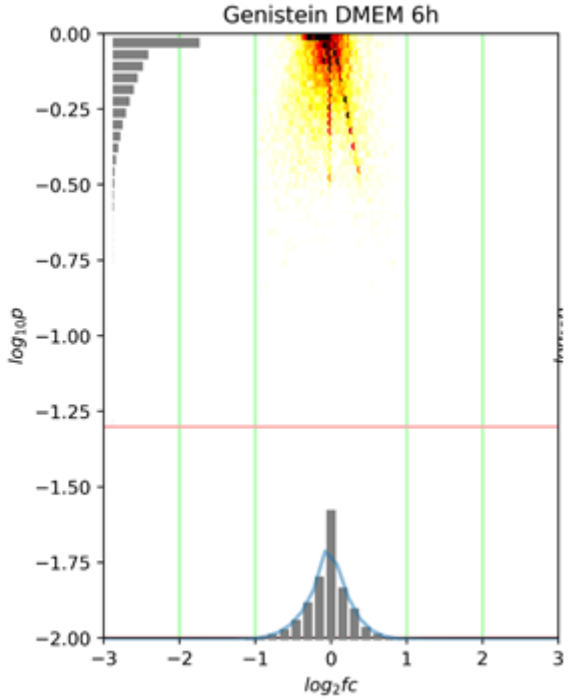


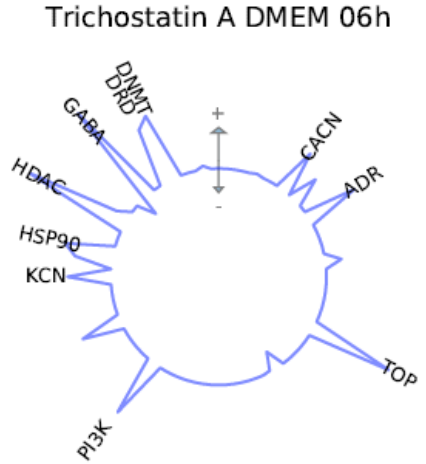
- The ability to detect ERa antagonists (particularly SERMs) was decreased by use of charcoal stripped serum.

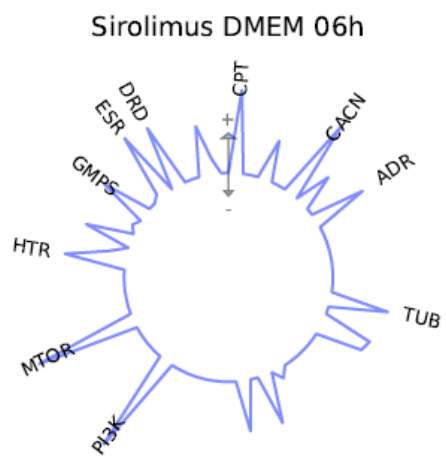
Connectivity Mapping

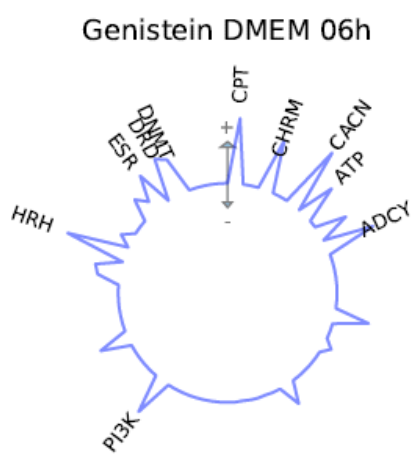






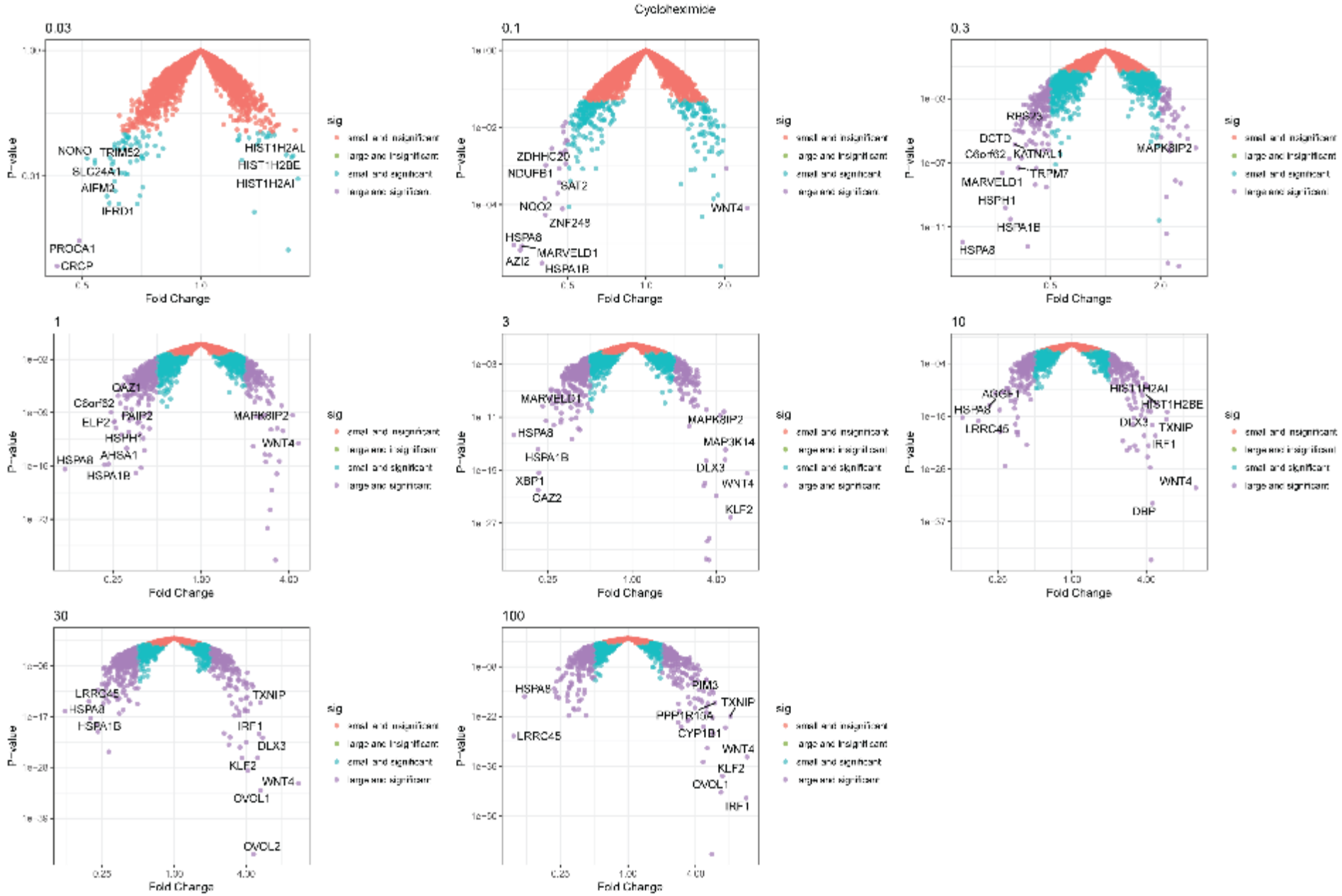






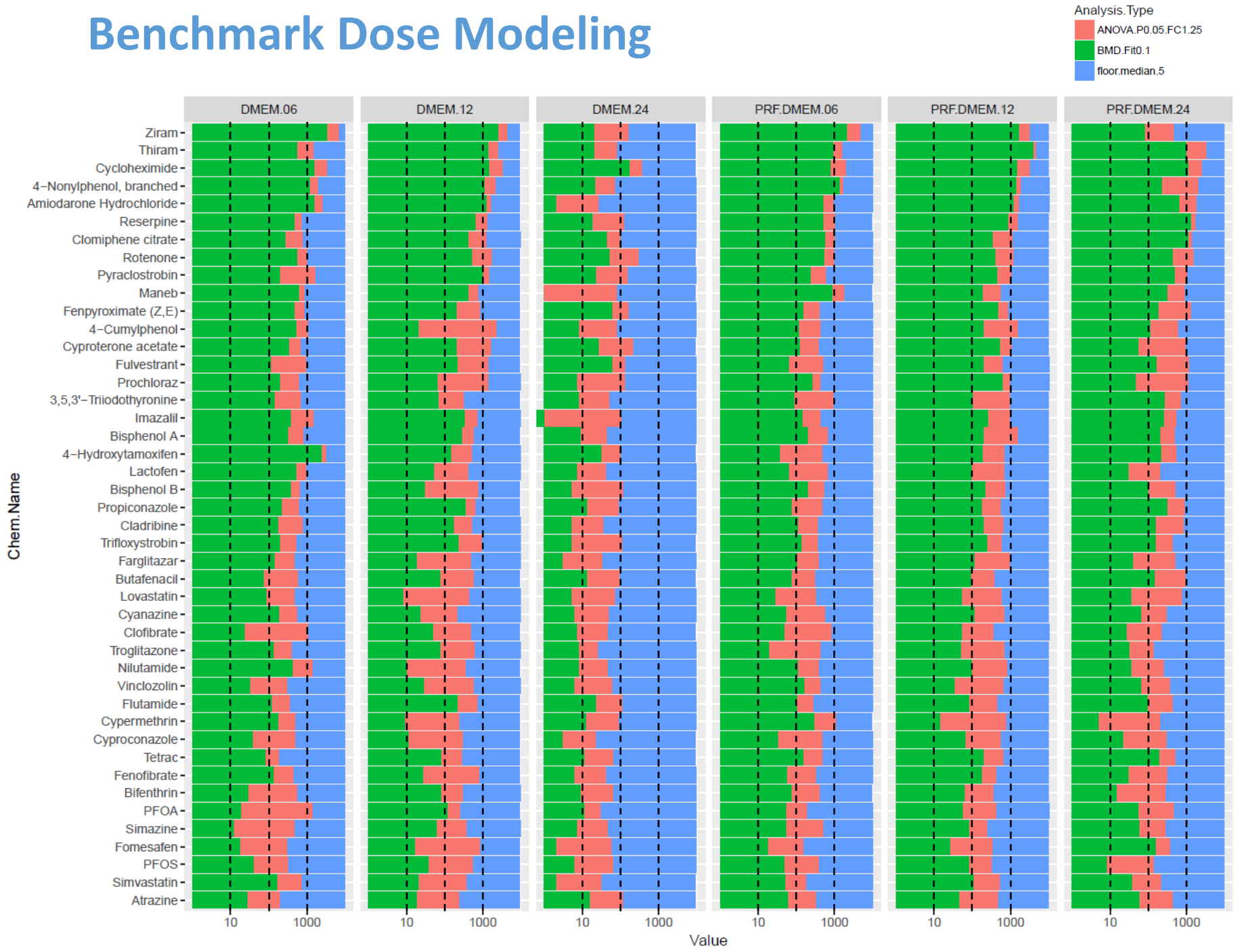
- Differential gene expression observed with reference chemicals.
- Putative targets identified using Connectivity Mapping
- Large degree of promiscuity of predicted targets observed.
- Currently evaluating additional methods for MIE prediction

Concentration Dependent Increases in Transcriptional Response

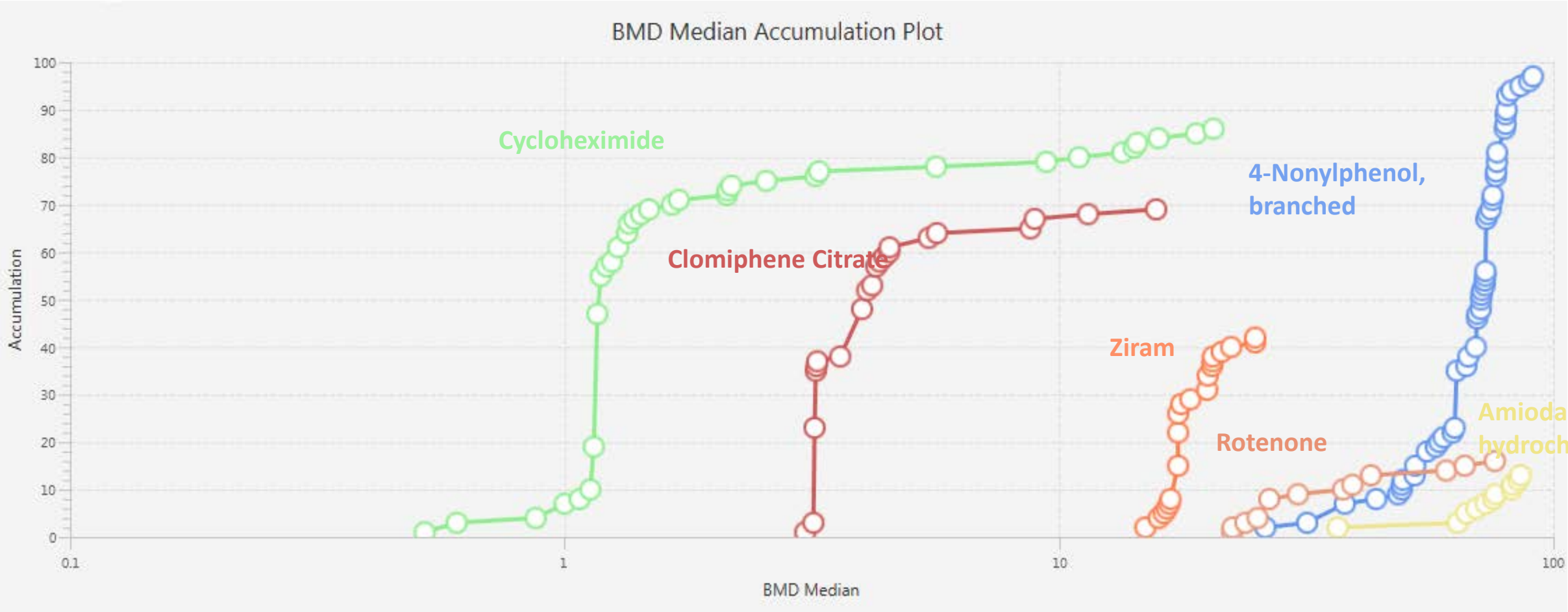


Benchmark Dose Modeling

Parameter	Criteria
Pre-filter	ANOVA ($p_{raw} < 0.05$ & $\lvert FC \rvert \geq 1.25$)
Models	Hill, power, linear, poly 2, exponential 2
BMR Factor:	1.349 (10 %)
Best Model Selection:	Lowest AIC
Hill Model Flagging:	'k' < 1/3 Lowest Positive Dose Select next best model with p > 0.05

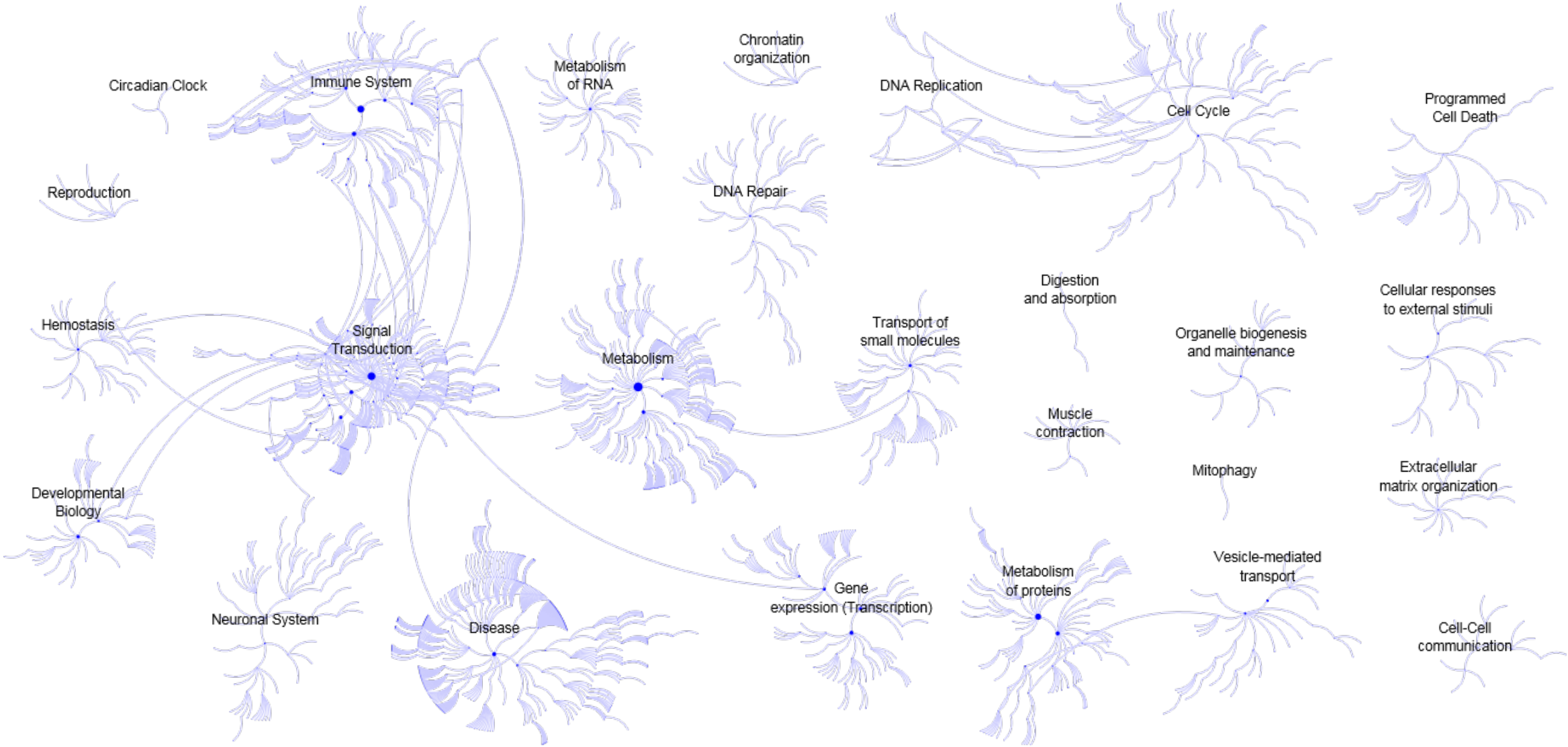


Benchmark Dose Modeling



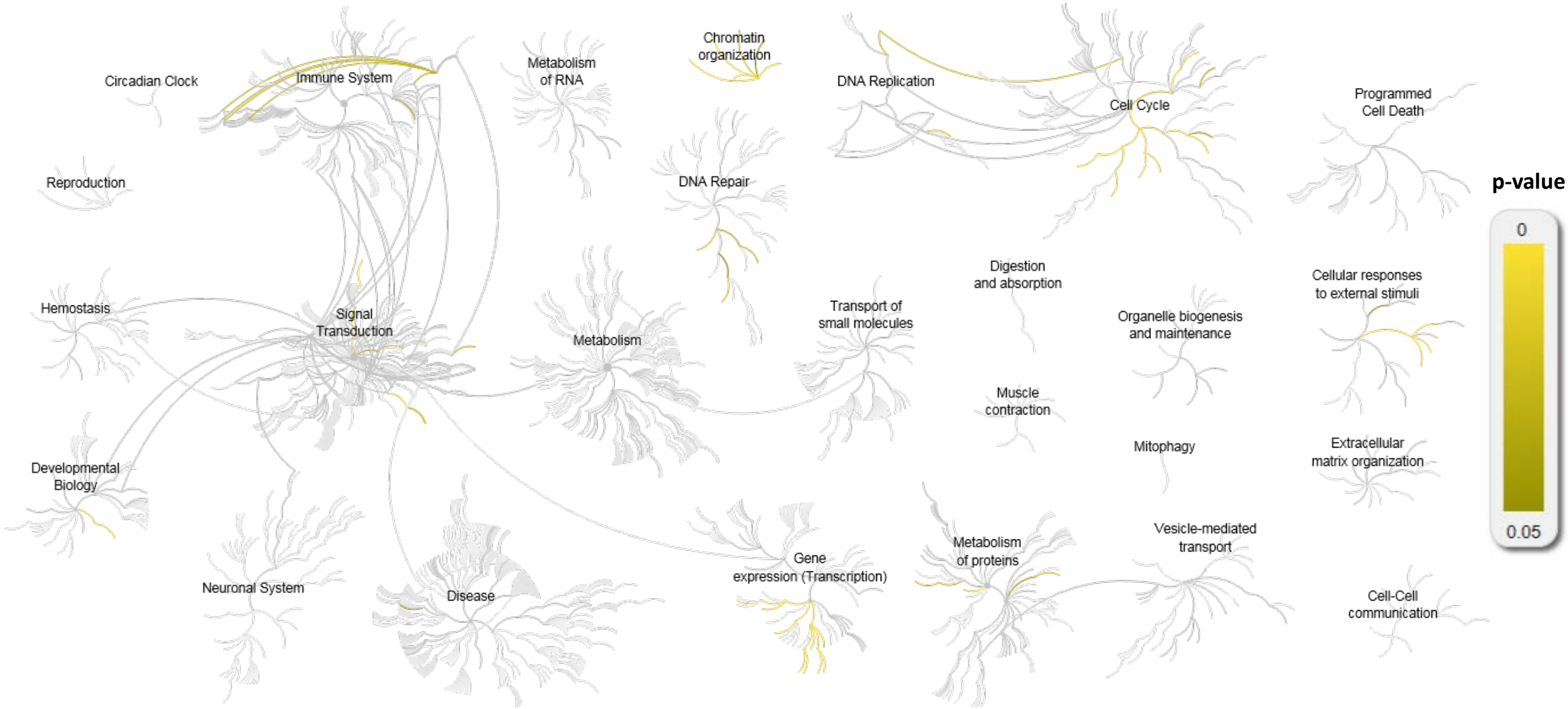
- Enrichment using Reactome Pathway Database
- Observed broad range of thresholds for chemical bioactivity.

Network Mapping



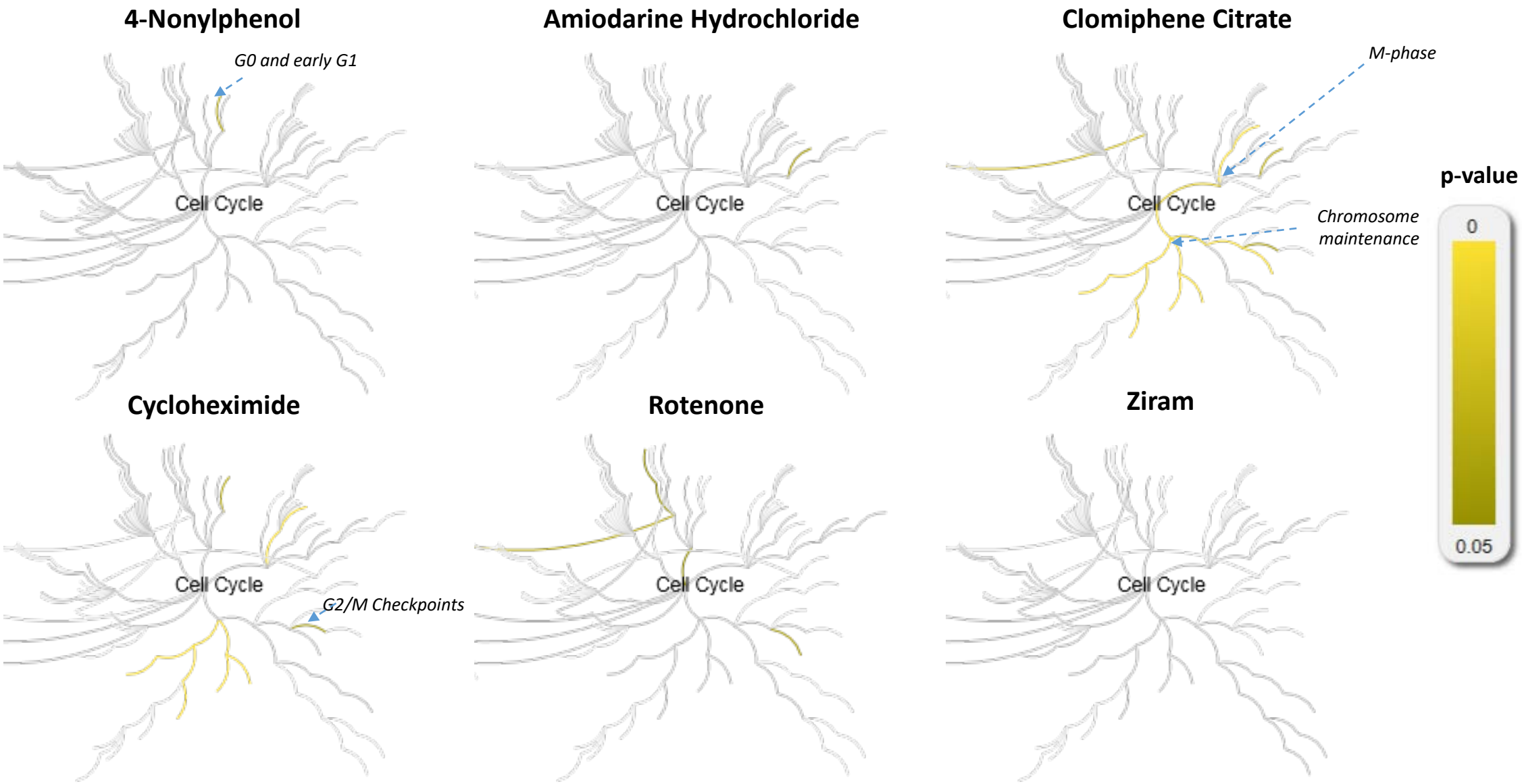
- Reactome (v60) Pathway Hierarchy

Network Mapping [Clomiphene Citrate]

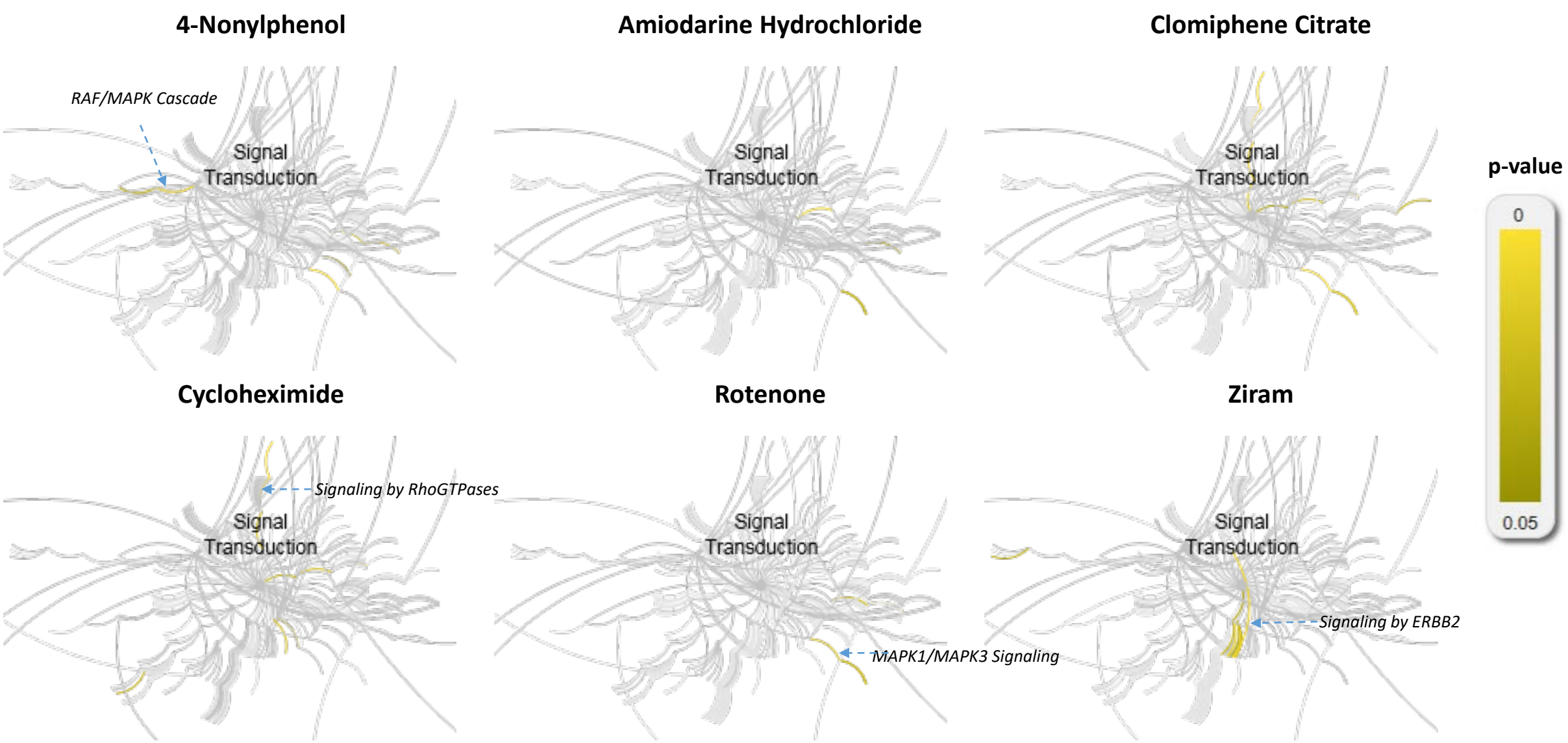


- Reactome (v60) Pathway Hierarchy → Overlaid with enrichment scores based on probes with acceptable BMD model fit
- Highlights different areas of biology affected by a chemical

Diversity in Response of Cell Cycle Networks



Diversity in Response of Signal Transduction Networks



Current Activities & Future Directions

- **Fall 2017:**
  - Refining data anlysis pipeline.
  - Exploring methods for MIE prediction & characterization of biological responses.
  - Prepping initial publication.
  - Conducting concentration-response screening of 2,200 chemicals in MCF7 cell model (8 conc., 6 HR exposure).

- **Beyond 2017:**
  - Tox21 reference chemical partner project
  - Screening in additional cell lines.
  - Coupling with image-based phenotypic screening assay.