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High Throughput Transcriptomics @ USEPA

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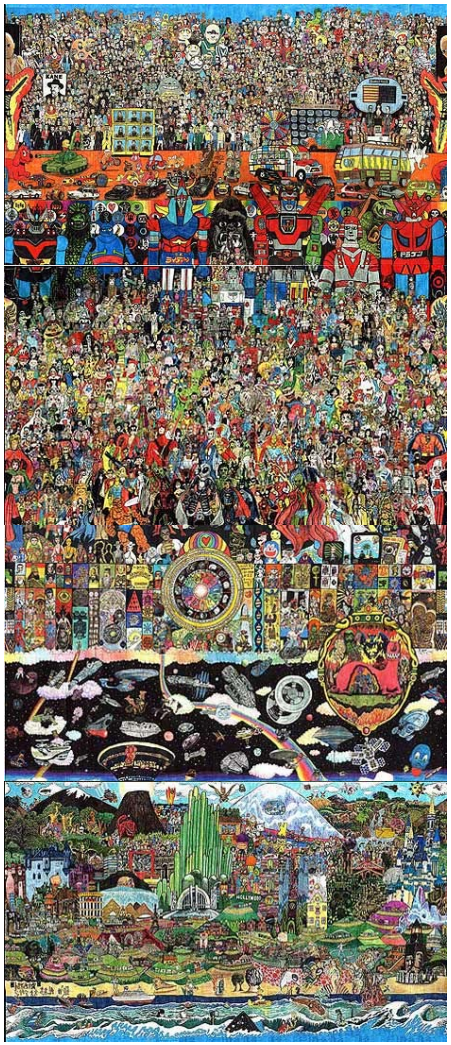
Toxicology is About Identifying What Can Go Wrong and Why

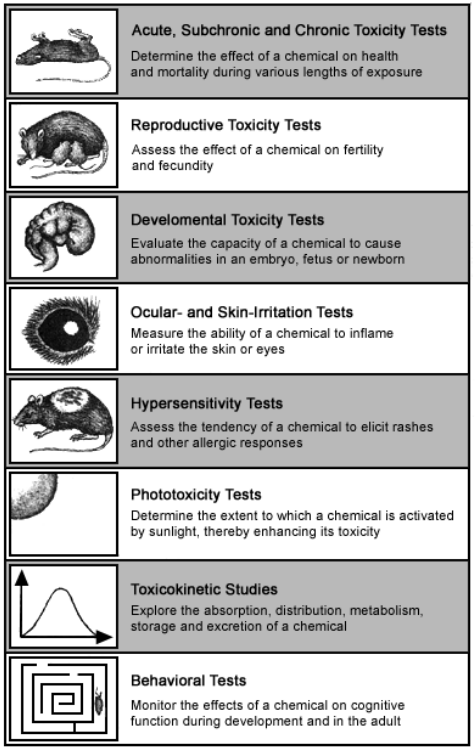


Picture of Everything - Howard Hallis

- The ideal chemical testing approach will provide complete coverage of all relevant toxicological responses
- It should be sensitive and specific
- It should identify the mechanism/mode-of-action (with dose-dependence)
- It should identify responses relevant to the species of interest
- Responses should ideally be translated into tissue-, organ-, and organism-level effects
- It must be economical and scalable

Traditional Animal Studies Are Less Than Ideal

Apical Responses With Limited Mechanistic Insight



Goldberg and Frazier (1989)

Variable Cross-Species Concordance

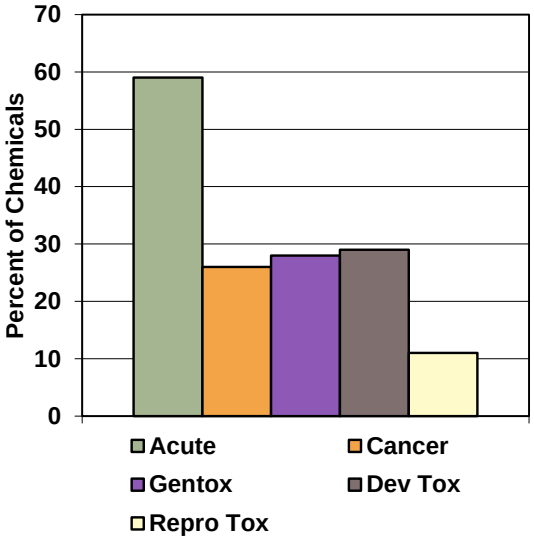
Table 3. Predictability of laboratory animal models for putative human teratogens.[a]

Teratogen/group	Mouse	Rat	Rabbit	Hamster	Primate	Dog	Cat	Pig	Ferret	Guinea pig
Alcohol	+	+	−		+	+		+		+
Androgenic hormones[b]	+	+	+	+	+	+		+		+
Anticancer antimetabolites[c]	±	+	+	−	±	±	±	+		+
Anticancer alkylating agents[d]	+	+	+		+				+	
Anticonvulsants[e]	+	±	+	−	±	−	−			
Coumarin anticoagulants	−	−	−					−		
Antithyroid agents[b]	+	+	+							+
Progestrogenic hormones[b]	+	±	±		−	+				+
DES[b]	+	+	−	±	+				+	
Methylmercury	+	+	−	+	−	−	+	−		
Thalidomide	±	±	+	±	+	±	±	±	+	−
Lithium	+	−	−		−			−		
D-Penicillamine		+		+						
Streptomycin antibiotics[b]	−	+	−							−
Vitamin A analogs	+	+	+	+	+	+		+		+

[a] Legend: (+) teratogenic; (±) variably teratogenic; (−) not teratogenic.
[b] Defects related to functional activity.
[c] Includes azauridine, aminopterin, fluorouracil, methotrexate and cytarabine.
[d] Includes busulfan, chlorambucil, cyclophosphamide, and mechlorethamine.
[e] Includes hydantoin and dione groups, and valproate.

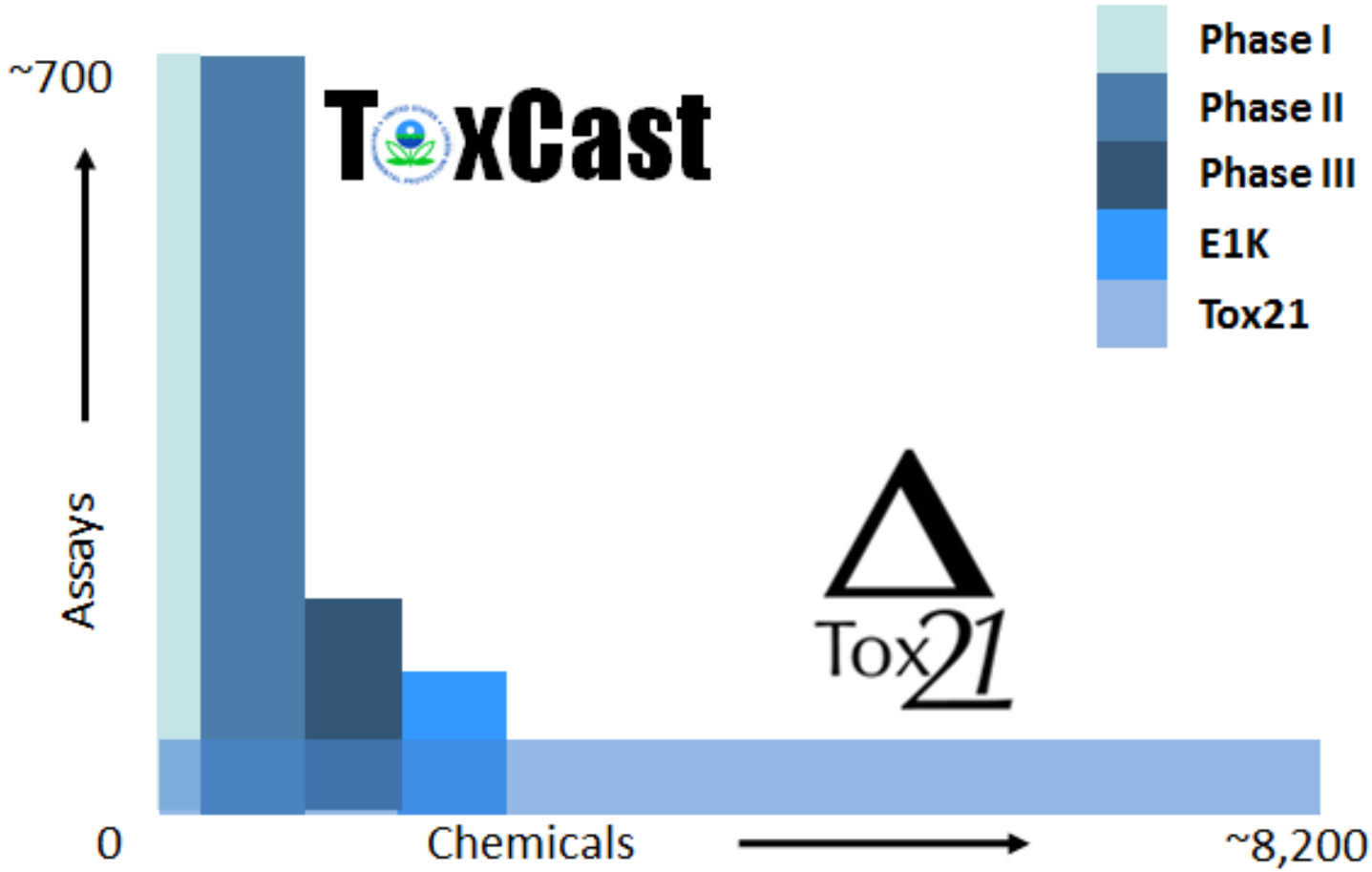
Schardein, et al *EHP* (1985)

High Cost and Limited Scalability



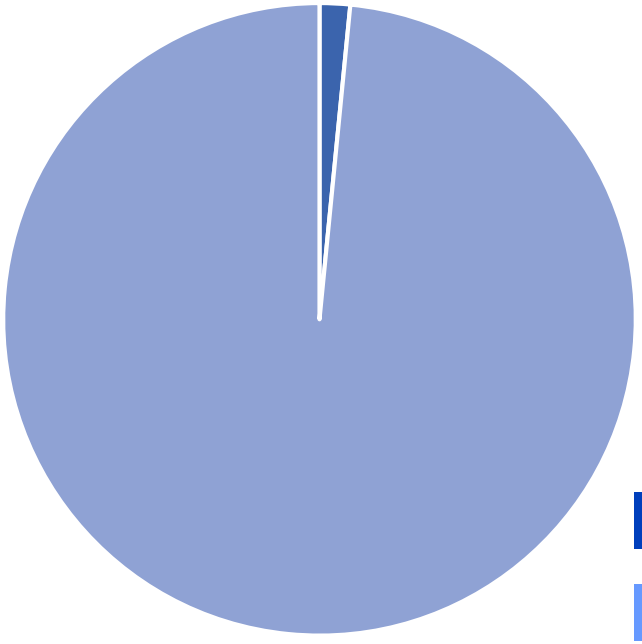
Judson, et al *EHP* (2010)

High-Throughput Screening Efforts Have Attempted to Fill Gaps

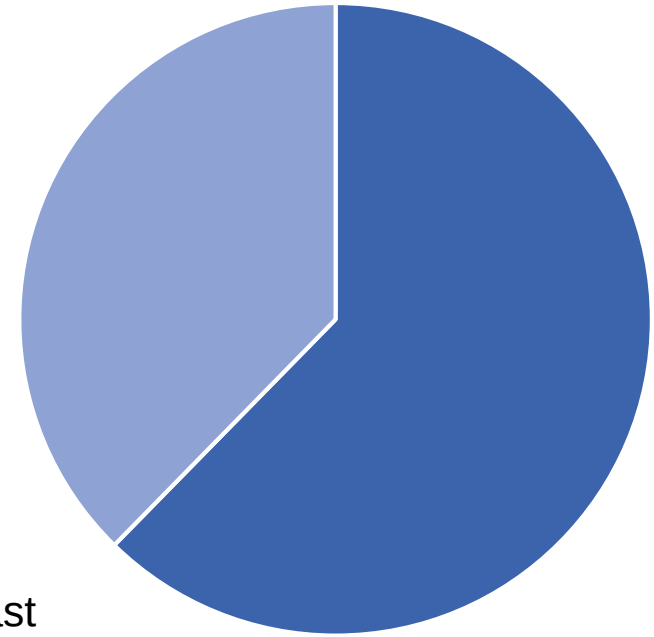


But, Current Coverage of Biological Space is Less Than Optimal

ToxCast Gene Coverage



ToxCast Pathway Coverage*

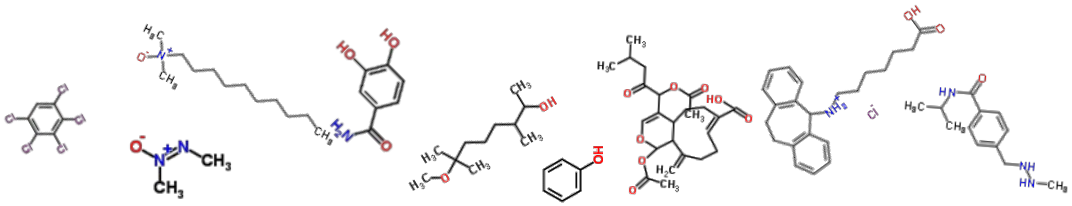


ToxCast

Not in ToxCast

*At least one gene from pathway represented

Incorporating a Comprehensive Biological Screening Platform



Broad Primary Screen for Bioactivity/MOA

HTTr: High Throughput Transcriptomics

Secondary Confirmation Screen

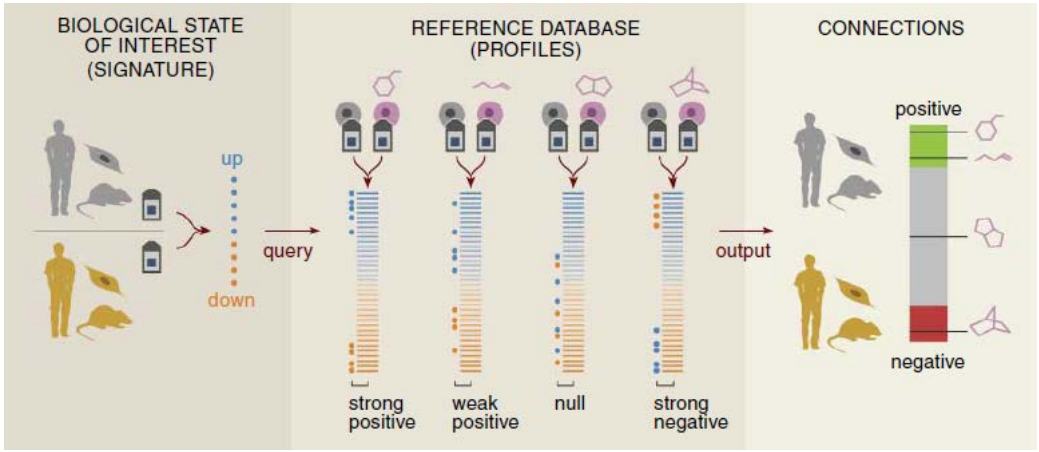
Tertiary Screen to Identify Likely Tissue/Organ Effects

Operationalizing & Deploying

HTTr Assay

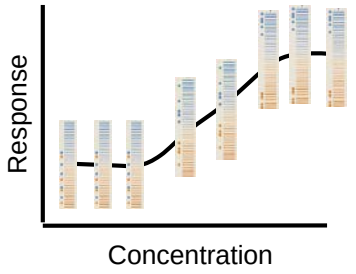
Tier 0

- Identify predominant mechanisms as a function of concentration
- Group chemicals by similar mechanism/bioactivity
- Identify a concentration that results in no transcriptional effects

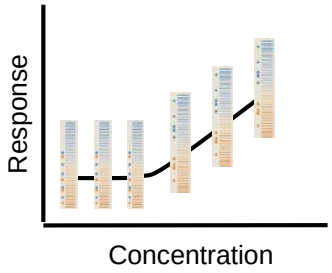


Lamb et al. *Science* (2006)

Broad CMAPdb: 7,000 profiles; 1,309 compounds
NIH LINCs CMAPdb: 9,000 shRNAs, 3,000 over expression ORFs, and 4,000 compounds in 20 cell types/lines (cell lines and primary cells)



Cell Type #1



Cell Type #2

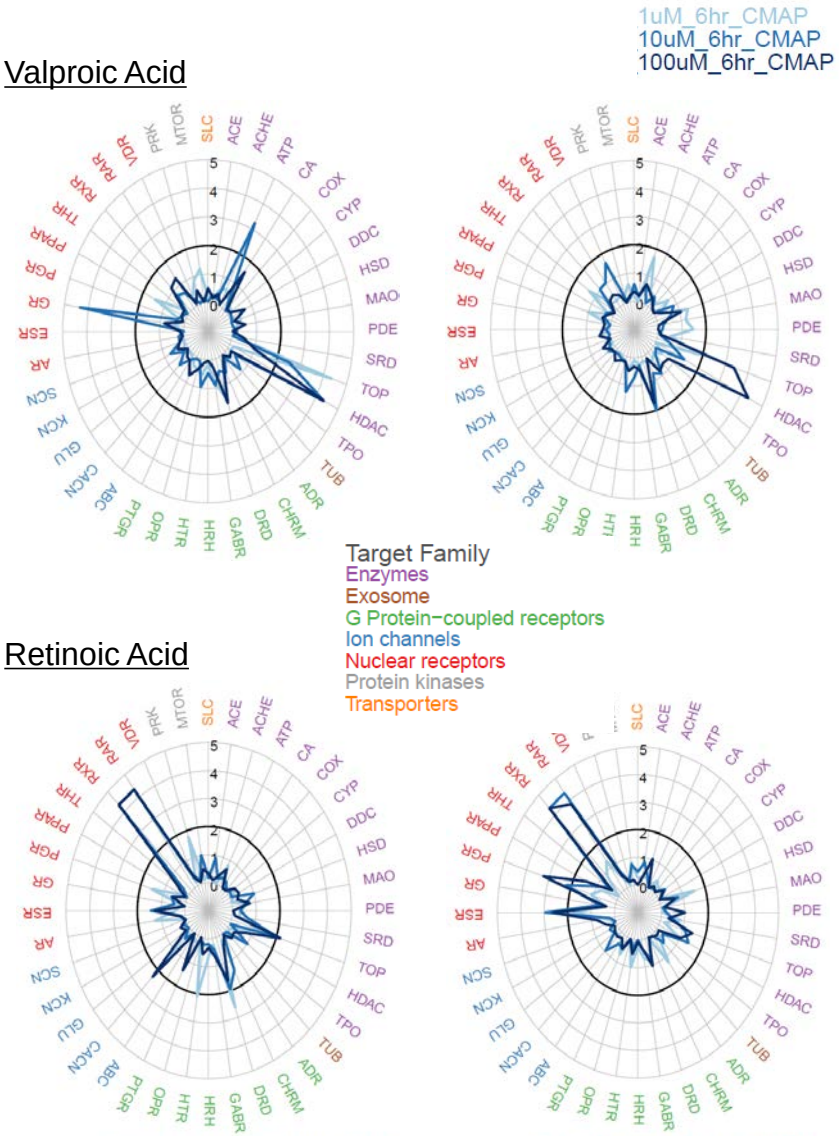
Cell Type #3

…

Identify Mode-of-Action

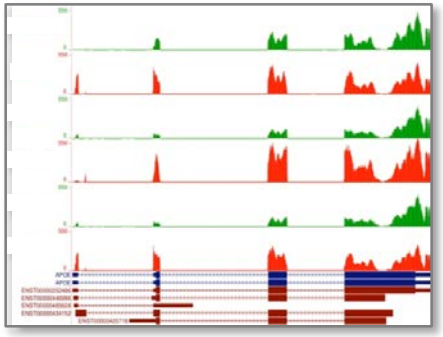
Target Family	Total Profiles	Target Genes	Chemicals	Cell Lines
Cytokine receptors	3	1	1	3
Enzymes	336	40	112	5
Exosome	14	1	4	4
G protein-coupled receptors	585	16	192	4
Ion channels	194	8	65	3
Nuclear receptors	227	10	71	5
Protein kinases	19	8	6	4
Transporters	102	2	35	3

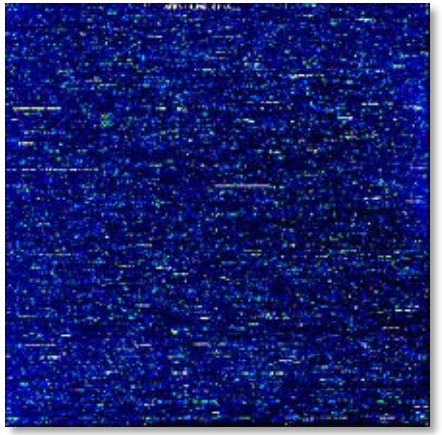
- Developed local database of Broad's CMAP data (~3,000 profiles)
- Annotated targets using KEGG (1,571 profiles)
- Significant genes identified using a z-score cutoff of 2
- Incorporated "JG" scoring method (Jiang and Gentleman 2007)
- Determine significance using a permuted rank approach across target family



Martin and Karmaus, Unpublished

Requirements and Potential Platforms for HT Transcriptomics





Requirements

- Measure or infer transcriptional changes across the whole genome (or very close to it)
- Compatible with 96- and 384-well plate formats (maybe 1536?) and laboratory automation
- Work directly with cell lysates (no separate RNA purification)
- Compatible with multiple cell types and culture conditions
- Low levels of technical variance and robust correlation with orthogonal measures of gene expression changes
- Low cost ($30 - $45 per sample or less)

Potential Platforms

- Low coverage whole transcriptome RNA-seq (3 – 5 million mapped reads)
- Targeted RNA-seq (e.g., TempO-seq, TruSeq, SureSelect)
- Microarrays (e.g., Genechip HT)
- Bead-based (e.g., L1000)

Proposed Plate Map

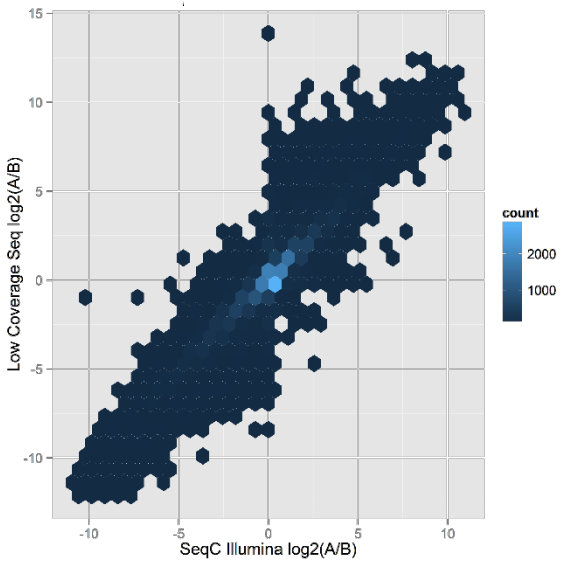
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	cndx
A	MAQC-A (Us)	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	DMSO	8
B	MAQC-A (Us)	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	DMSO	7
C	MAQC-B (Us)	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	DMSO	6
D	MAQC-B (Us)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	DMSO	5
E	Bulk Lysate (DMSO)	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Trichostatin	4
F	Bulk Lysate (DMSO)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Trichostatin	3
G	Bulk Lysate (DMSO)	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Trichostatin	2
H	Bulk Lysate (DMSO)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Trichostatin	1
I	Bulk Lysate (Trichostatin)	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	Genistein	8
J	Bulk Lysate (Trichostatin)	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	Genistein	7
K	Bulk Lysate (Trichostatin)	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	Genistein	6
L	Bulk Lysate (Trichostatin)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	Genistein	5
M	Lysis Buffer Only	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	Sirolimus	4
N	Lysis Buffer Only	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Sirolimus	3
O	MAQC-A (Them)	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Sirolimus	2
P	MAQC-B (Them)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Sirolimus	1

Replicates (n=3) from separate freeze/thaw

Technical Performance of the Three Sequencing Platforms

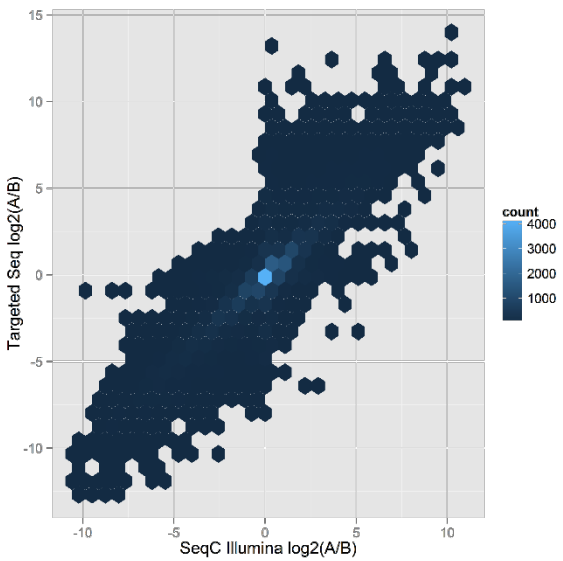
Low Coverage

r^2 0.83



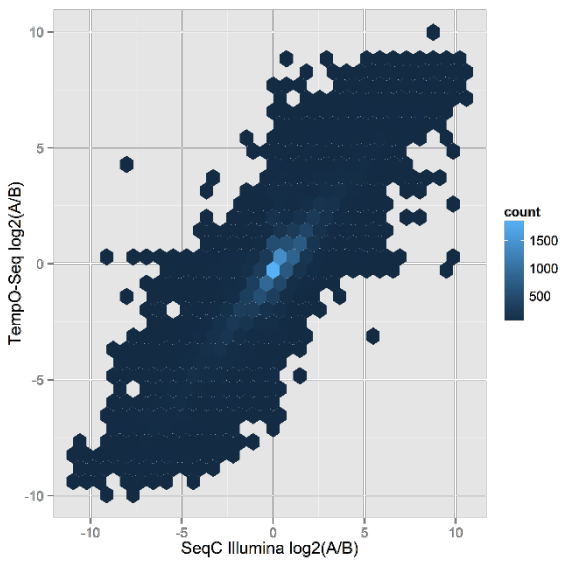
TruSeq

r^2 0.74



TempO-Seq

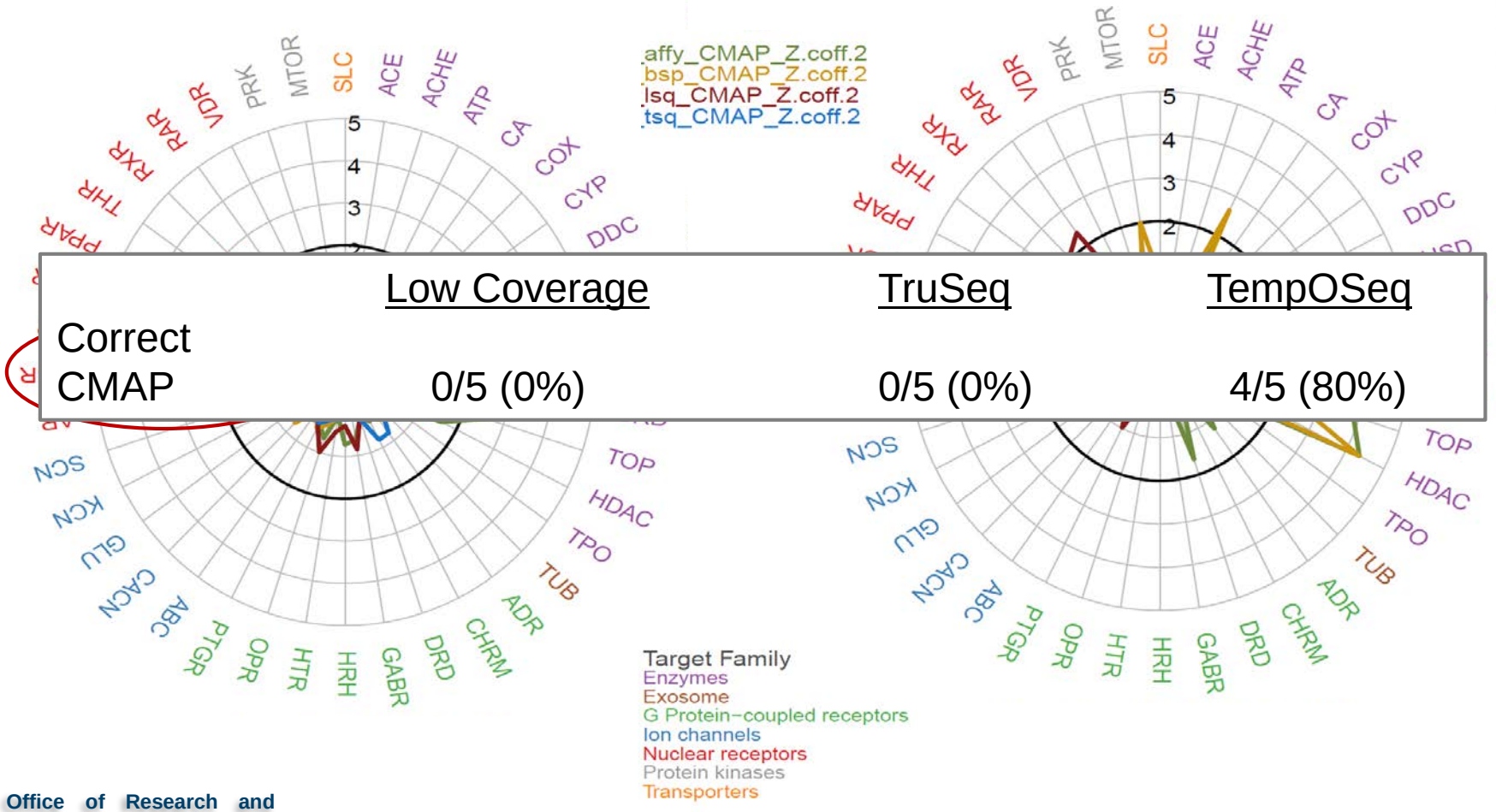
r^2 0.75



Functional Performance of the Three Sequencing Platforms

Genistein

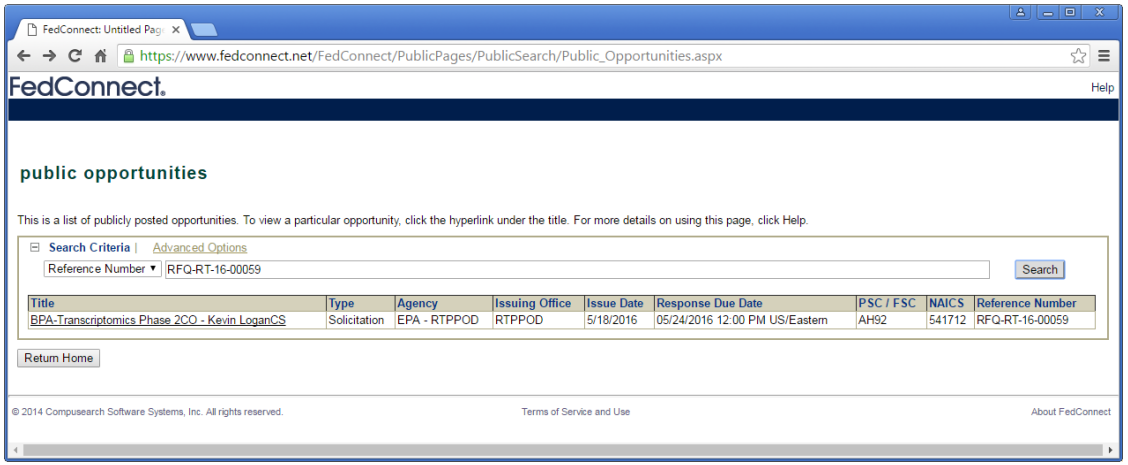
Trichostatin



	Low Coverage	TruSeq	TempOSeq
Correct CMAP	0/5 (0%)	0/5 (0%)	4/5 (80%)

Current Status

- Procurement underway for high-throughput transcriptomic services



- Strategy is to obtain services starting with submission of cell-lysates and delivery of raw and normalized transcriptomic data
    - This will allow multiple collaborative partners to use the same platform (i.e., harmonized) and contribute data from different cell types/models and chemicals

Anticipated Next Steps

- Perform pilot study (Summer) to validate workflow and refine experimental design
- Initiate large scale screen (Fall/Winter)
    - Cell type: MCF7
    - Compounds: 1,000+ (ToxCast Phase I/II + Reference Chemicals)
    - Time Point: Single
    - Concentration Response: 8 (Starting @ 300 uM)
- Perform secondary pilot study looking at cell type selection/ pooling strategies (Fall/Winter)
- Integrate HT transcriptomic platform with metabolic retrofit solution to allow screening +/- metabolism (FY17)
- Explore partnerships to build community database of common chemical set across multiple cell types/lines

EPA United States Environmental Protection Agency

Summary

- High-throughput transcriptomics will fundamentally change the way we evaluate chemicals for safety
  - Greater coverage of biological space
  - Reduced cost
  - Ability to leverage large existing databases of gene expression data
  - Fits logically in a tiered testing approach
  - Allows dose response characterization for both selective and non-selective chemicals
- Platform procurement underway
- Cell type/line selection challenges remain

Acknowledgements

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EPA's National Center for Computational Toxicology