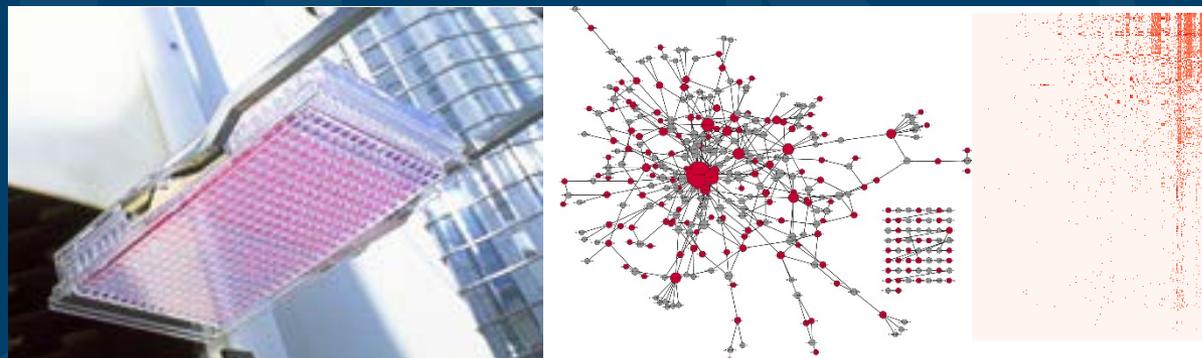


Strategies for Integrating Transcriptional Profiling into High-Throughput Toxicity Testing



Society of Toxicology Annual Meeting
March 25, 2015

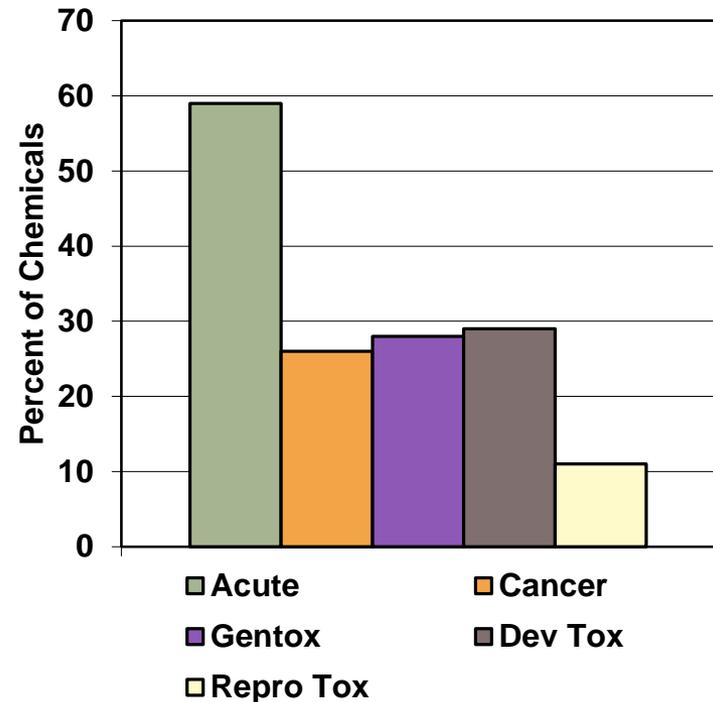
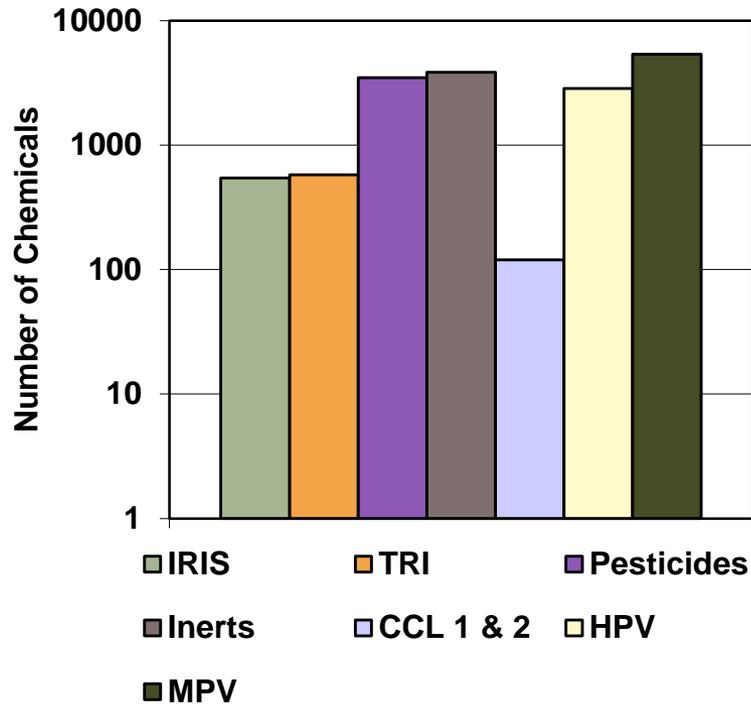
Rusty Thomas
Director
National Center for Computational Toxicology

Traditional Studies Attempt to Cover Range of Potential Adverse Responses

	Acute, Subchronic and Chronic Toxicity Tests Determine the effect of a chemical on health and mortality during various lengths of exposure
	Reproductive Toxicity Tests Assess the effect of a chemical on fertility and fecundity
	Developmental Toxicity Tests Evaluate the capacity of a chemical to cause abnormalities in an embryo, fetus or newborn
	Ocular- and Skin-Irritation Tests Measure the ability of a chemical to inflame or irritate the skin or eyes
	Hypersensitivity Tests Assess the tendency of a chemical to elicit rashes and other allergic responses
	Phototoxicity Tests Determine the extent to which a chemical is activated by sunlight, thereby enhancing its toxicity
	Toxicokinetic Studies Explore the absorption, distribution, metabolism, storage and excretion of a chemical
	Behavioral Tests Monitor the effects of a chemical on cognitive function during development and in the adult

Goldberg and Frazier (1989)

Current System for Chemical Safety Testing Has Not Kept Pace

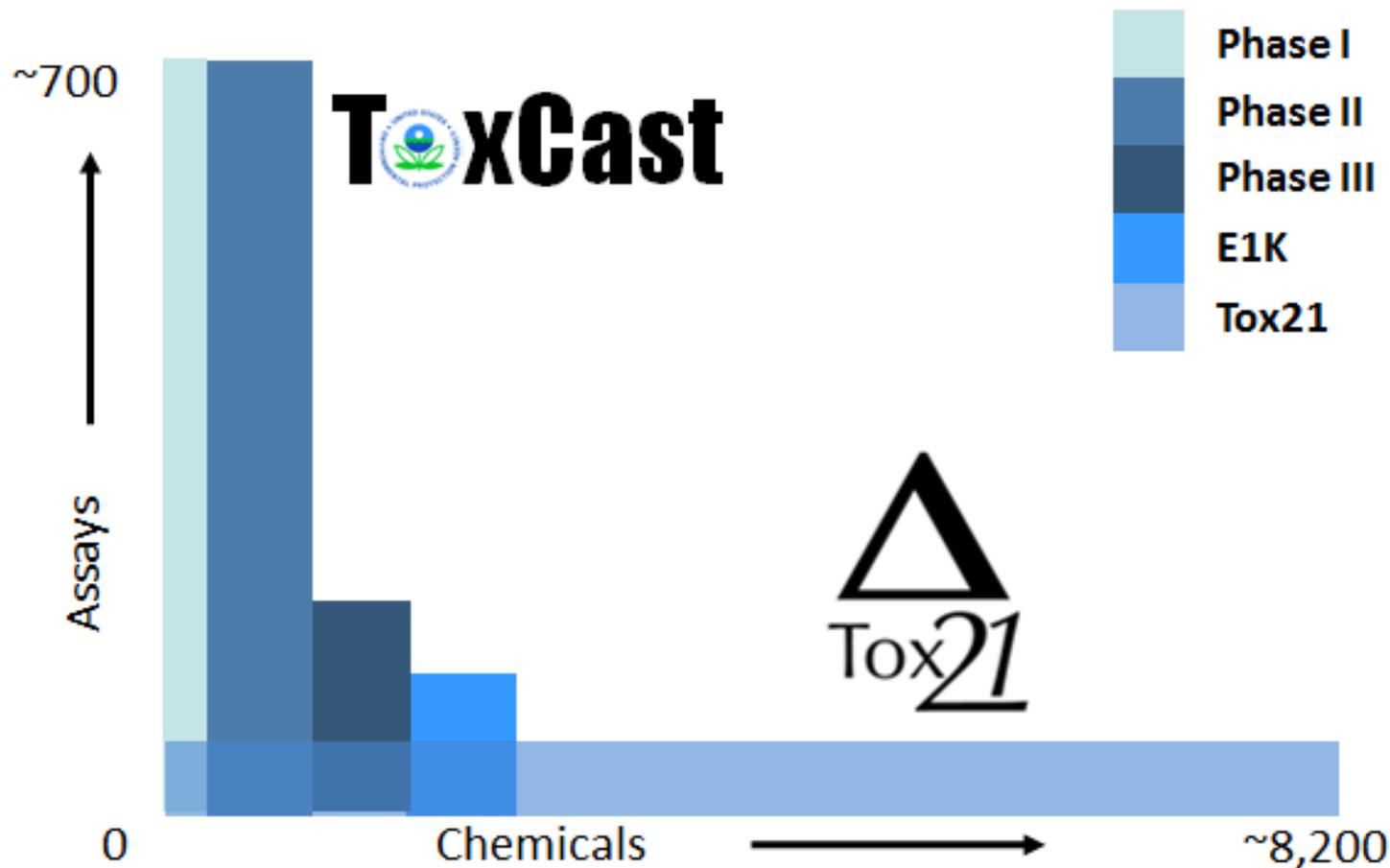


Significant Economic and Animal Costs Associated with Testing

Toxicity Study	Number of Animals	Approx. Cost
Skin sensitization (<i>in vivo</i>)	20	\$7,000.00
Acute toxicity by oral route	20	\$2,500.00
Repeated dose toxicity (one species, male and female (28 d), most appropriate route) (OECD407)	40	\$100,000.00
<i>In vivo</i> somatic cell genotoxicity study	80	\$35,000.00
Sub-chronic repeated dose toxicity, most appropriate route (90 d) (OECD 408)	80	\$220,000.00
Pre-natal developmental toxicity, one species, most appropriate route (OECD 414)	80	\$150,000.00
Chronic tox/Carcinogenicity study combined (> 12 month)	280	\$1,500,000.00
Two generation reproductive toxicity, one species, male, female (OECD 416)	360*	\$500,000.00
Developmental neurotoxicity (OECD 426)	80*	\$750,000.00

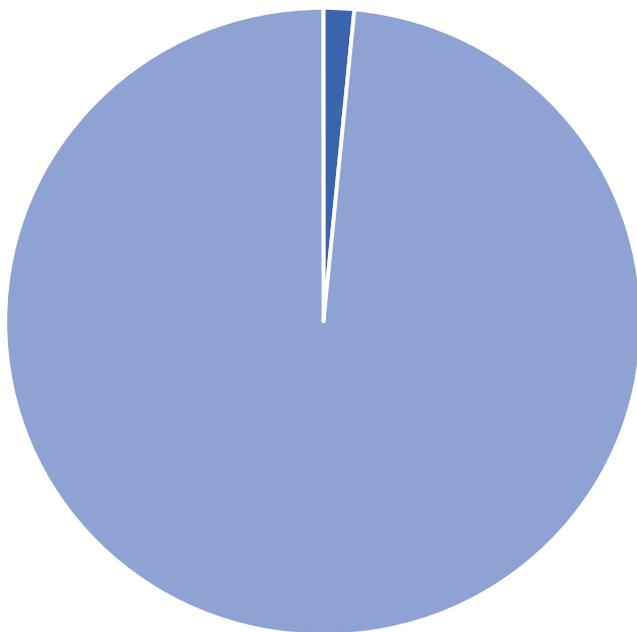
*Offspring not counted

Multiple Federal Efforts Have Begun to Address the Data Gap

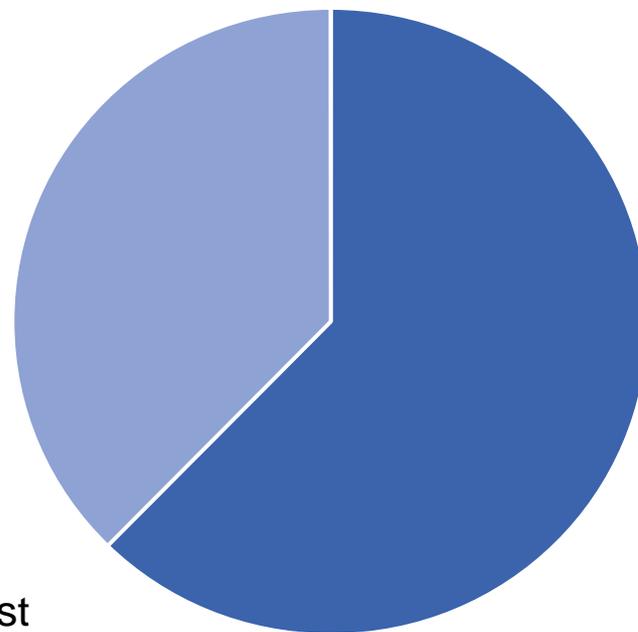


Current Coverage of Biological Space is Less Than Optimal

ToxCast Gene Coverage



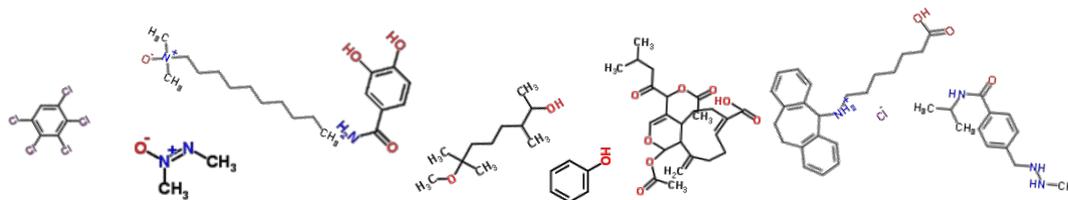
ToxCast Pathway Coverage*



 ToxCast
 Not in ToxCast

*At least one gene from pathway represented

Incorporating a Broad Biological Screening Platform



Broad Primary Screen for Bioactivity/MOA

Secondary Confirmation
Screen

Tertiary Screen to Discriminate
Perturbation from Adverse
Effect

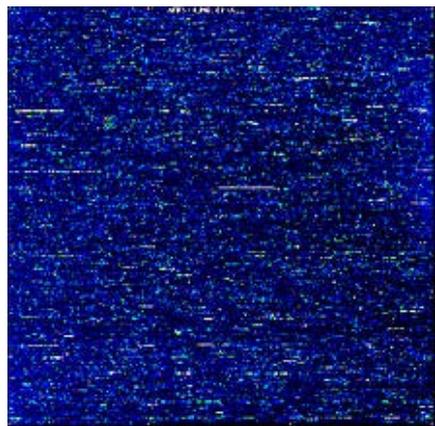
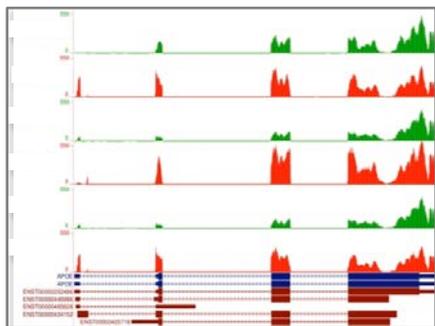
Requirements and Potential Platforms for HT Transcriptomics

Requirements

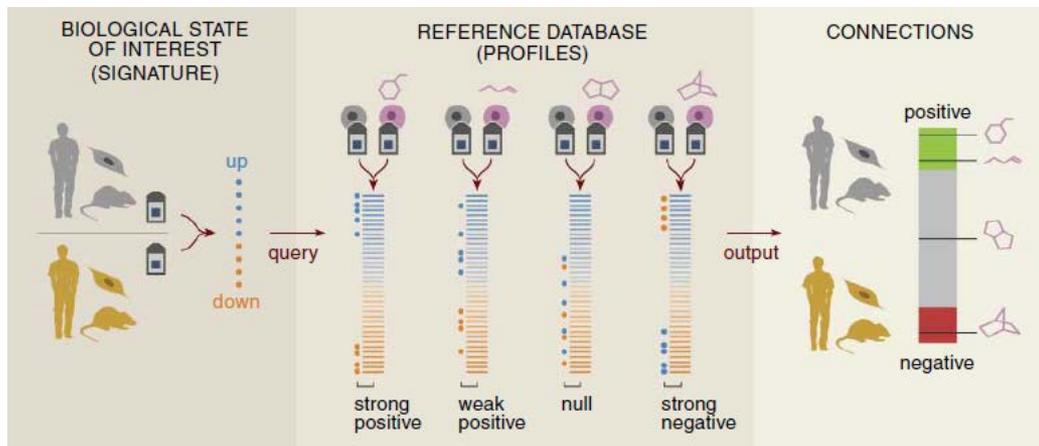
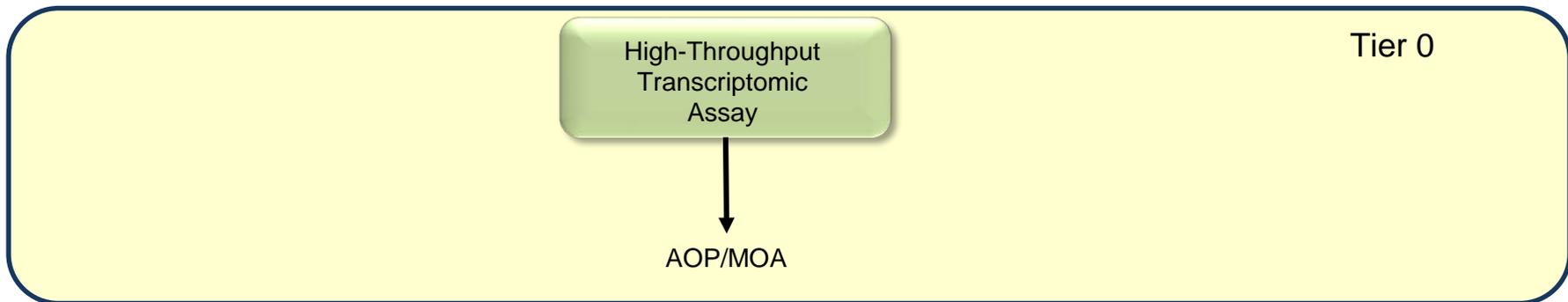
- Measure or infer transcriptional changes across the whole genome (or very close to it)
- Compatible with 96- and 384-well plate formats (maybe 1536?) and laboratory automation
- Work directly with cell lysates (no separate RNA purification)
- Compatible with multiple cell types and culture conditions
- Low levels of technical variance and robust correlation with orthogonal measures of gene expression changes
- Low cost (\$20 - \$40 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)

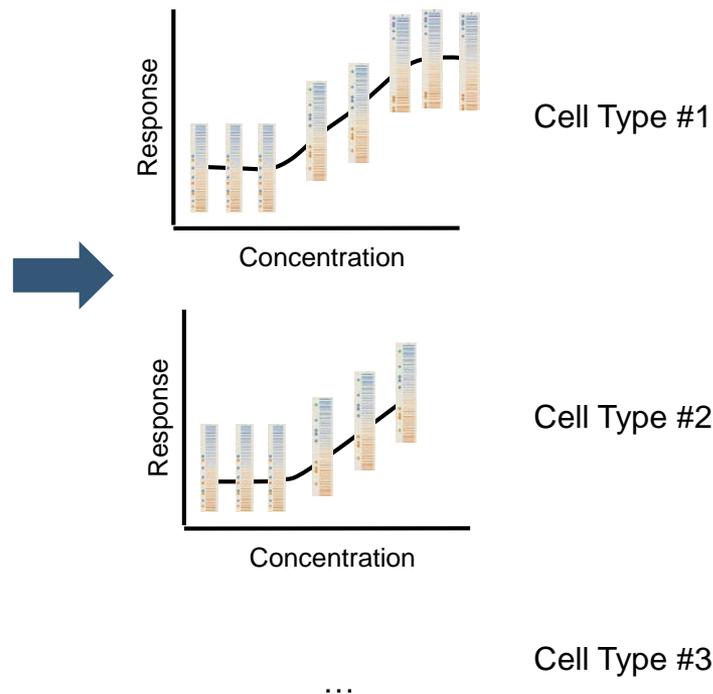


How Would a HT Transcriptomic Platform be Deployed?

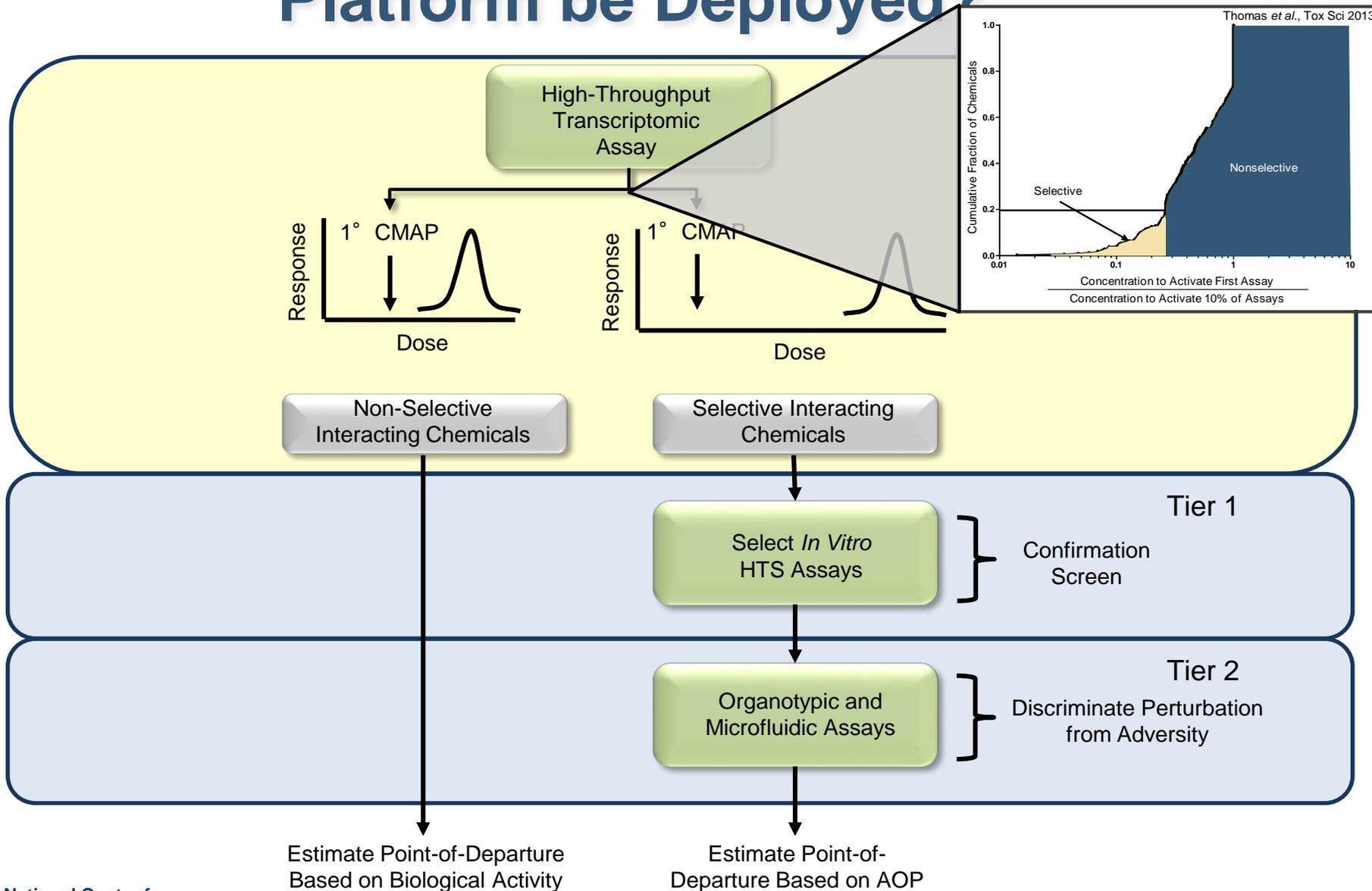


Lamb et al. *Science* (2006)

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)



How Would a HT Transcriptomic Platform be Deployed?



Approaches for Estimating a Transcriptomic Point of Departure

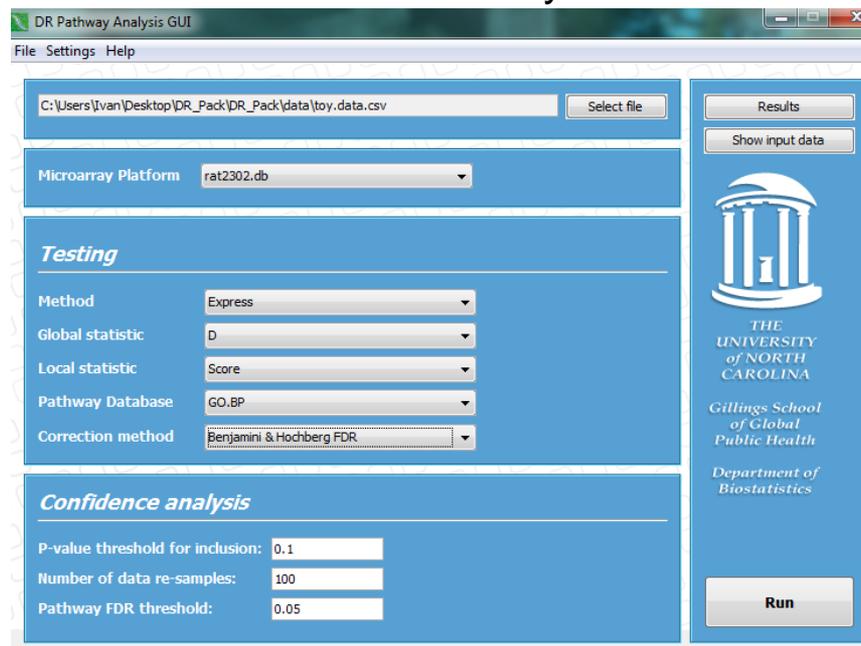
BMDEExpress



<http://sourceforge.net/projects/bmdexpress/>

Yang *et al.*, *BMC Genomics*, 2007
Thomas *et al.*, *Toxicol Sci.*, 2007

DR Pathway



DR Pathway Analysis GUI

File Settings Help

C:\Users\Ivan\Desktop\DR_Pack\DR_Pack\data\toy_data.csv

Microarray Platform: rat2302.db

Testing

Method: Express
Global statistic: D
Local statistic: Score
Pathway Database: GO_BP
Correction method: Benjamini & Hochberg FDR

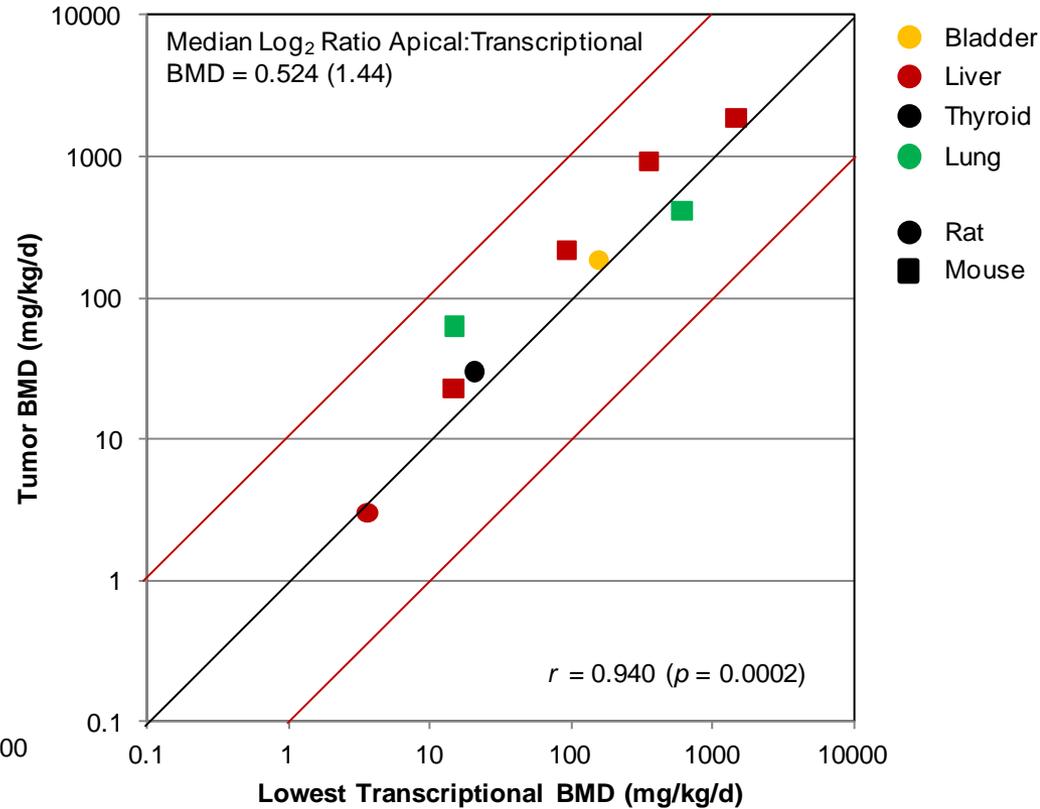
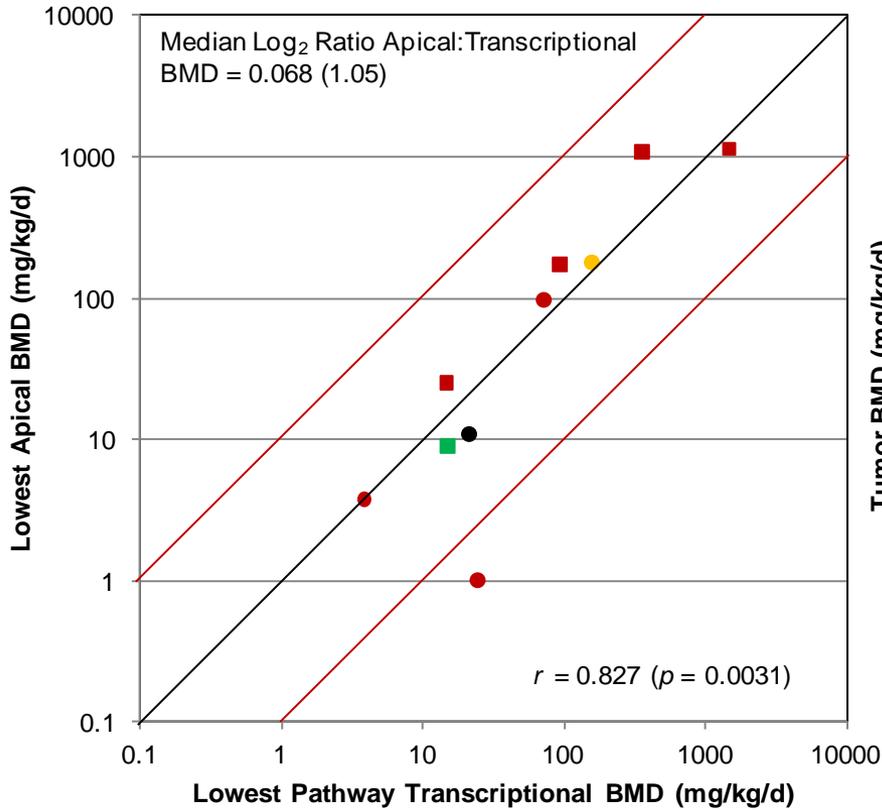
Confidence analysis

P-value threshold for inclusion: 0.1
Number of data re-samples: 100
Pathway FDR threshold: 0.05

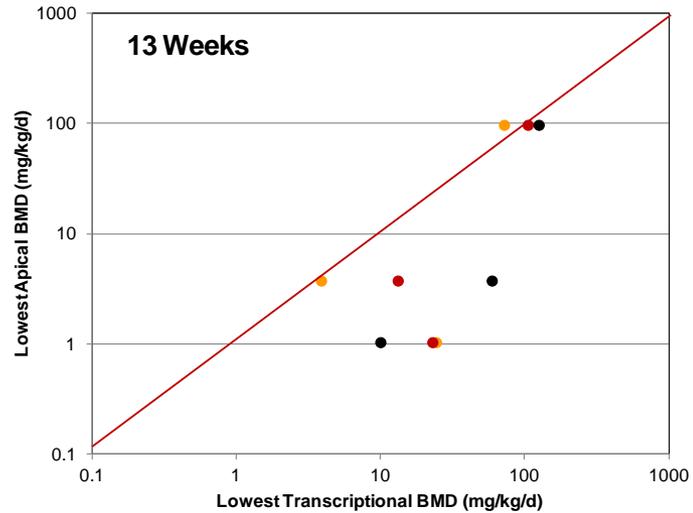
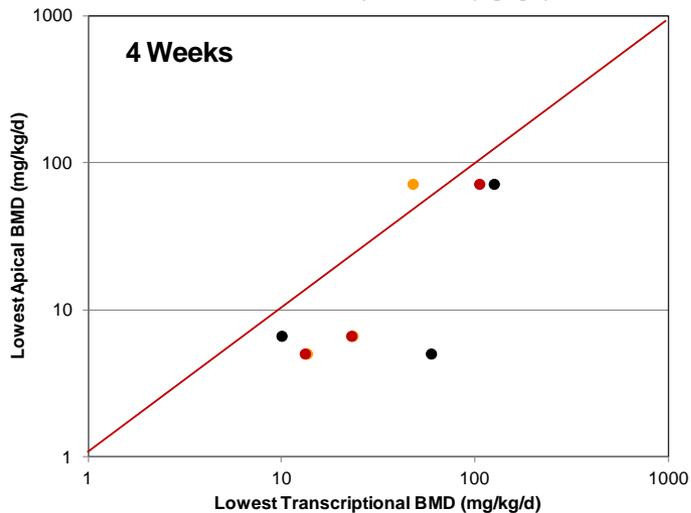
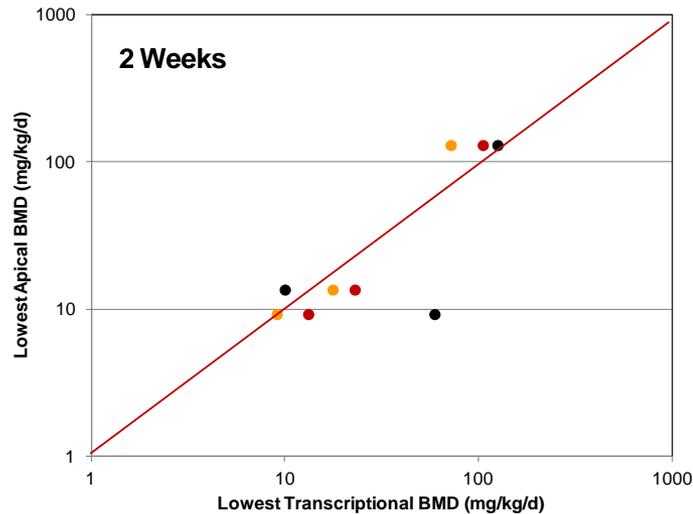
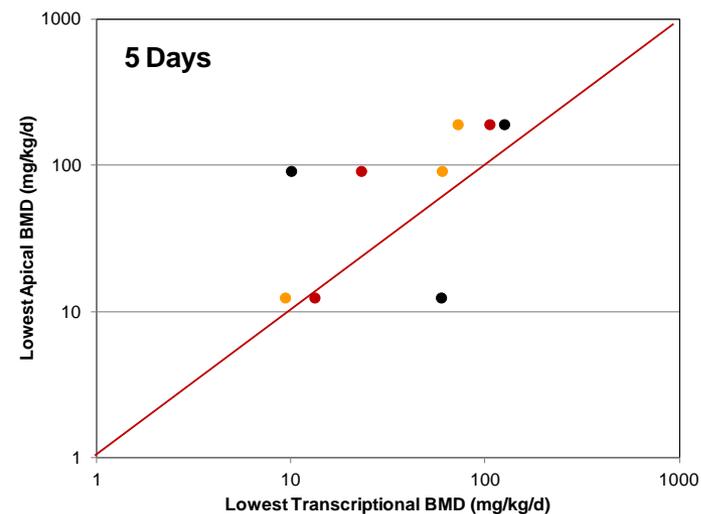

THE UNIVERSITY of NORTH CAROLINA
Gillings School of Global Public Health
Department of Biostatistics

<http://comptox.unc.edu/DRPathway.php>

Correlation of *In Vivo* Apical and Transcriptional Points of Departure

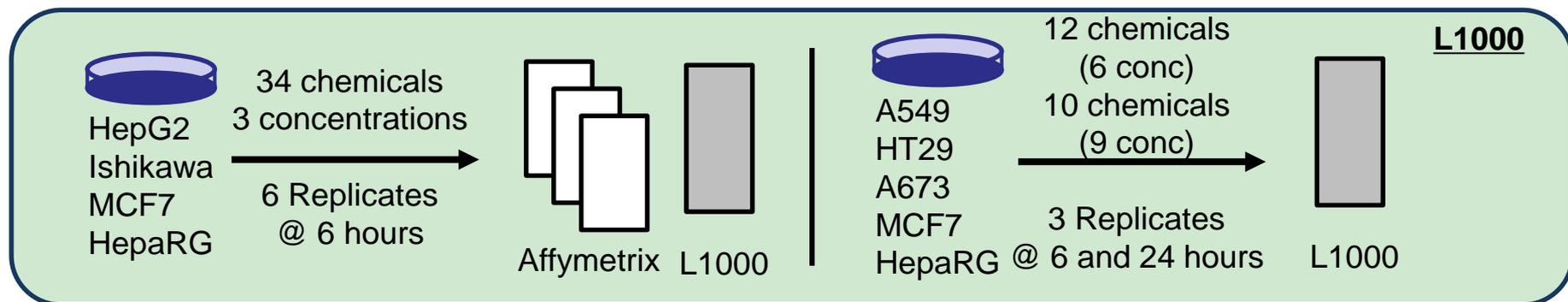


What About *In Vitro* Transcriptional Responses?



- *In Vivo* Transcriptional BMD
- *In Vitro* Transcriptional BMD (12 h)
- *In Vitro* Transcriptional BMD (5 d)

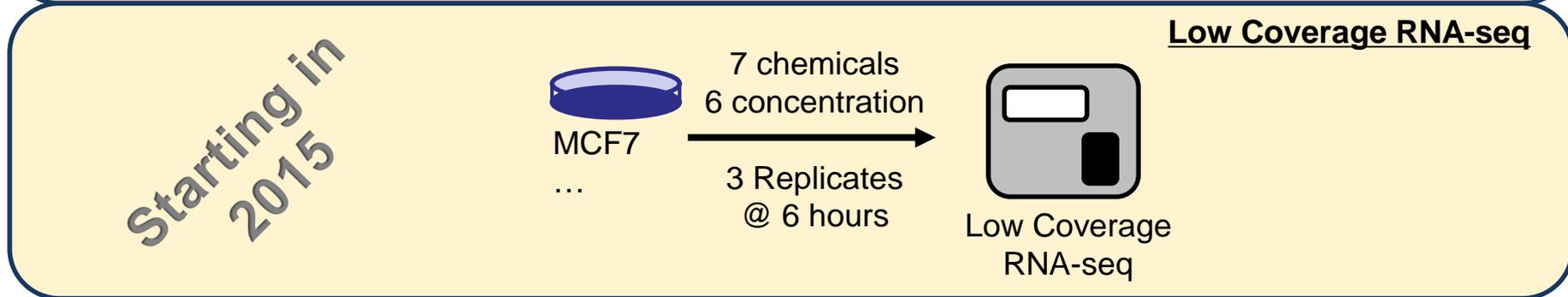
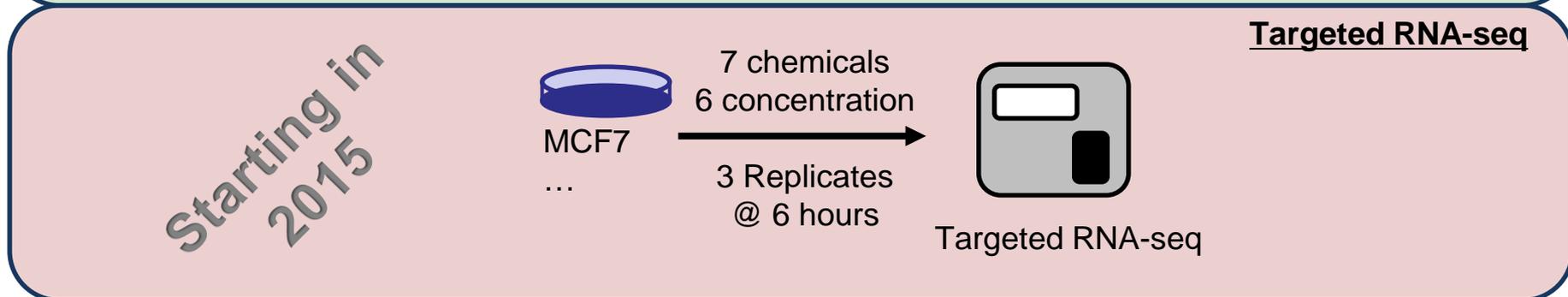
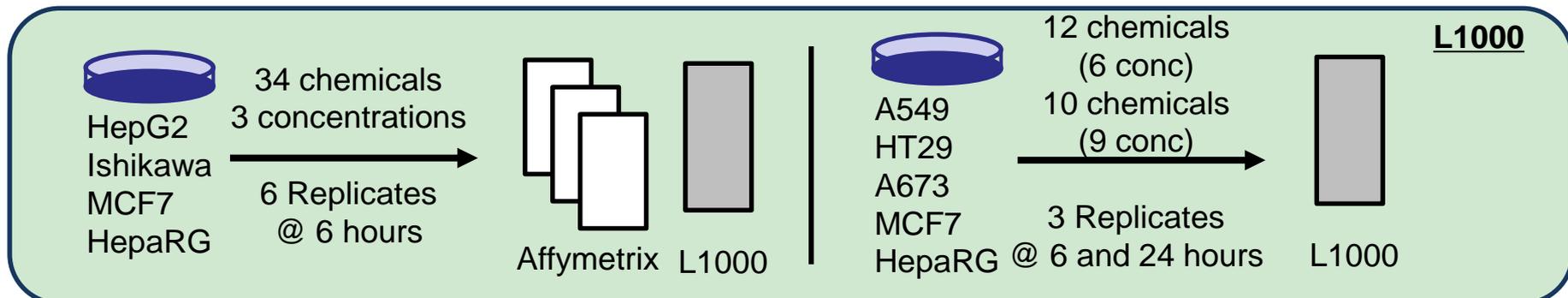
Beginning the Search for a Platform



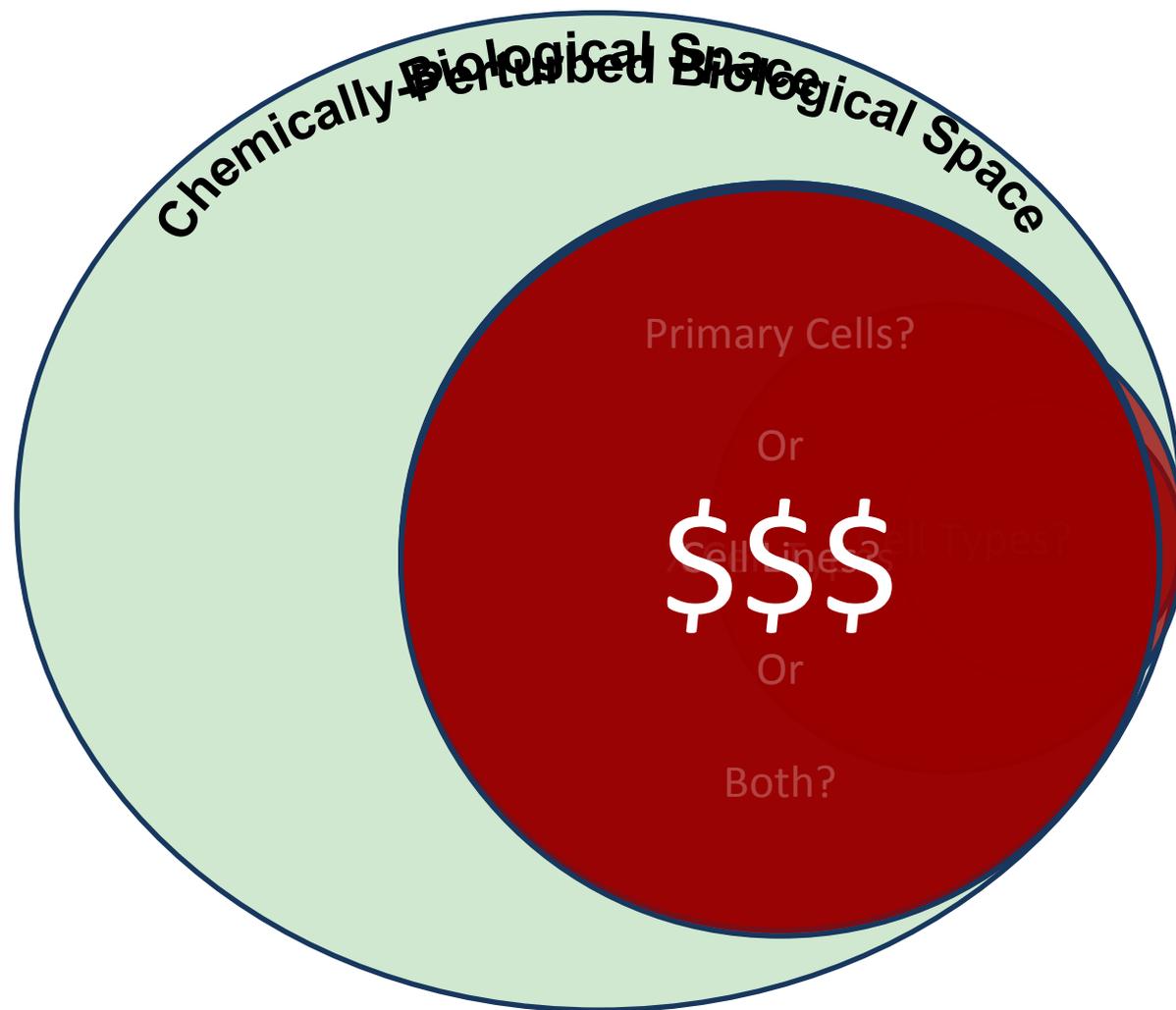
Collaboration with Proctor & Gamble (G. Daston and J. Naciff)
and Hamner Institutes (B. Wetmore and M. Black)

Visit Posters: M. Martin *et al.*, Poster #434; Wednesday afternoon
M. Black *et al.*, Poster #316; Thursday morning

Beginning the Search for a Platform

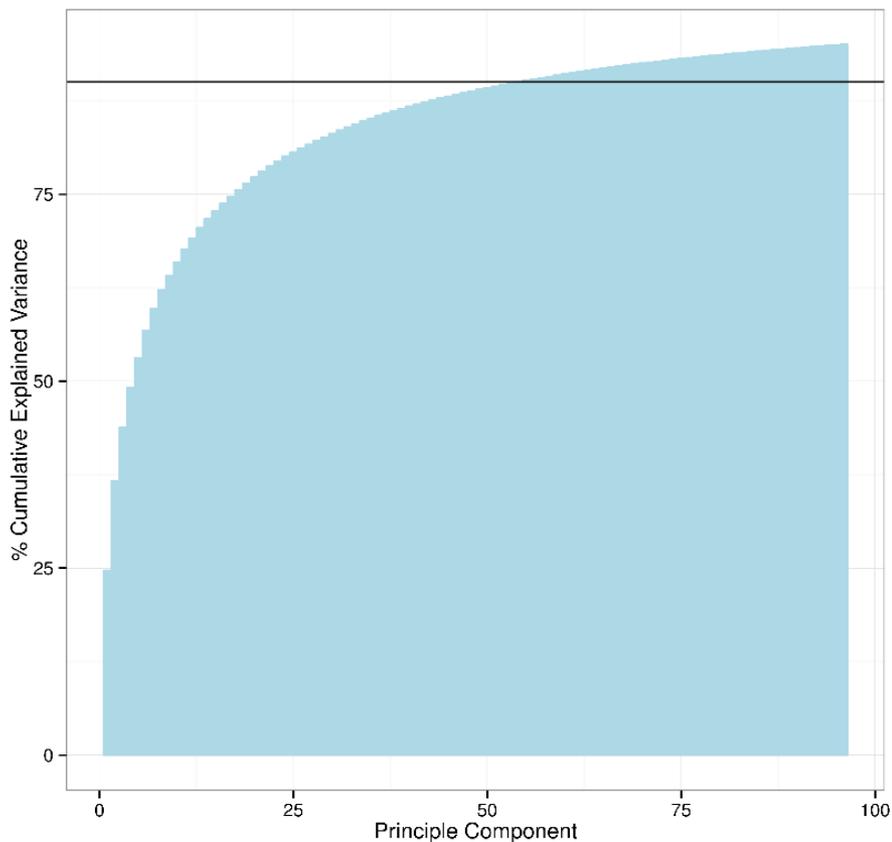


Beginning the Search for the Cell Types/Lines



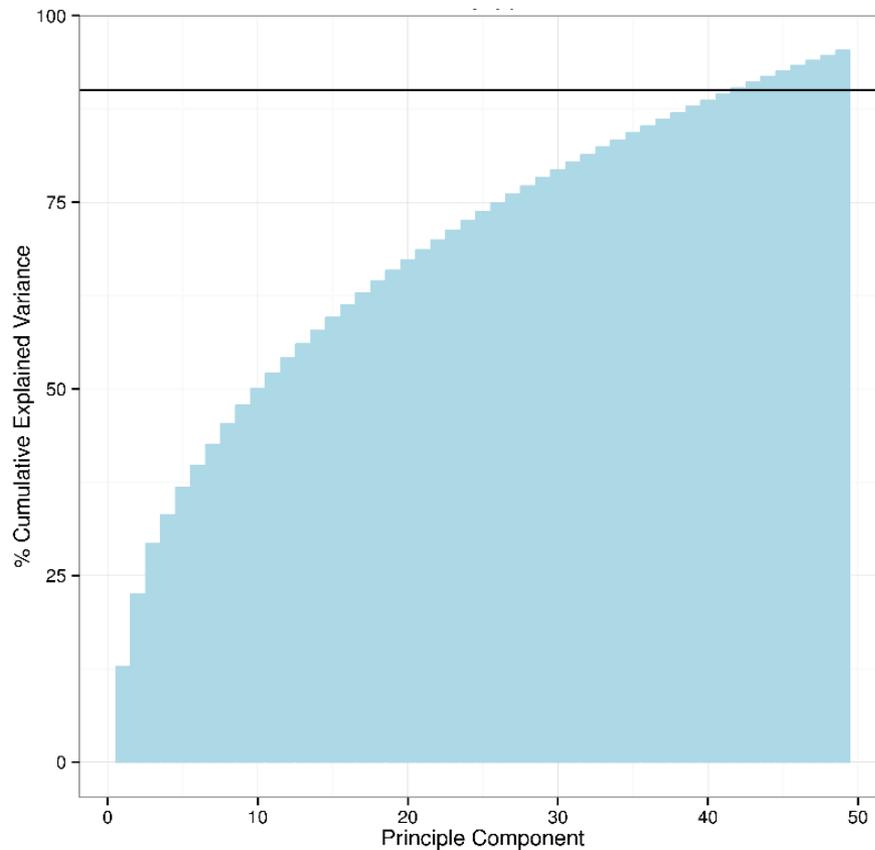
Exploring Cell Line Requirements

Primary Cell Atlas (302 cell types)

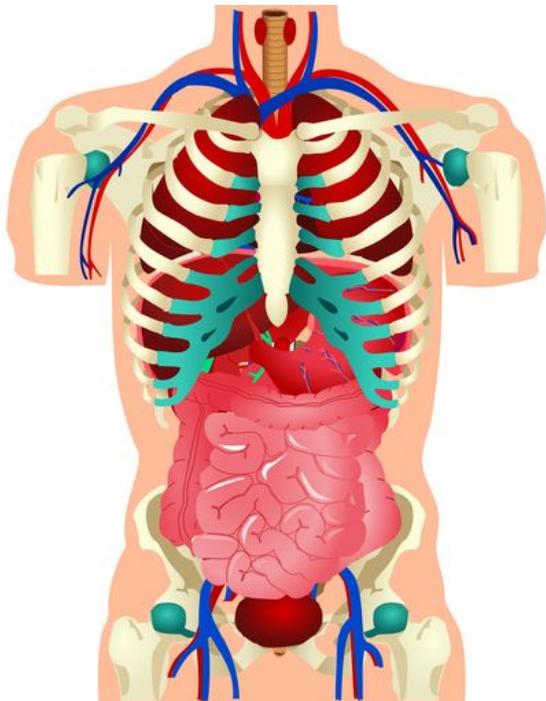


(GSE49910)

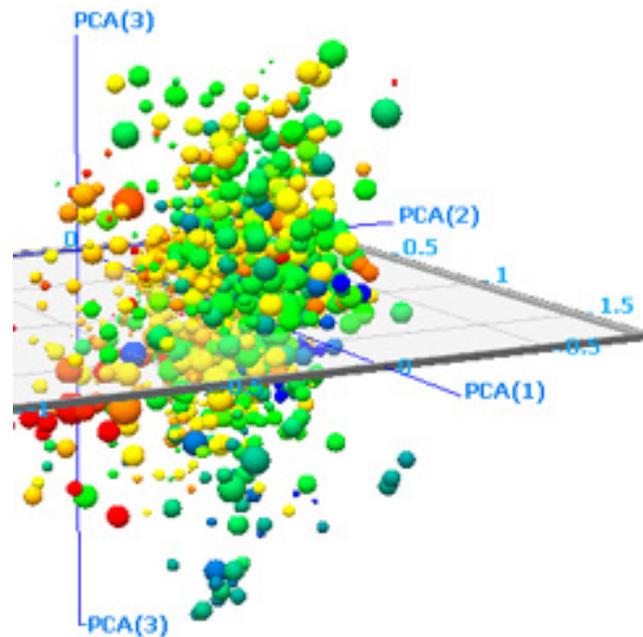
NCI-60



Scientific Rationale for Cell Type/Line Selection



Biologically-Driven?



Data-Driven?

See poster by N. Sipes *et al.*, Poster #349; Thursday morning



Summary

- High-throughput transcriptomics has the potential to 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 estimates of points-of-departure for both selective and non-selective chemicals
- Technical evaluations of multiple platforms are underway
- Cell type/line selection challenges remain

Acknowledgements

Tox21 Colleagues:

NTP Crew

FDA Collaborators

NCATS Collaborators

Hamner Collaborators:

Barbara Wetmore

Michael Black

P&G Collaborators:

George Daston

Jorge Naciff



EPA's National Center for Computational Toxicology