

# Strategies for Integrating Transcriptional Profiling into High-Throughput Toxicity Testing











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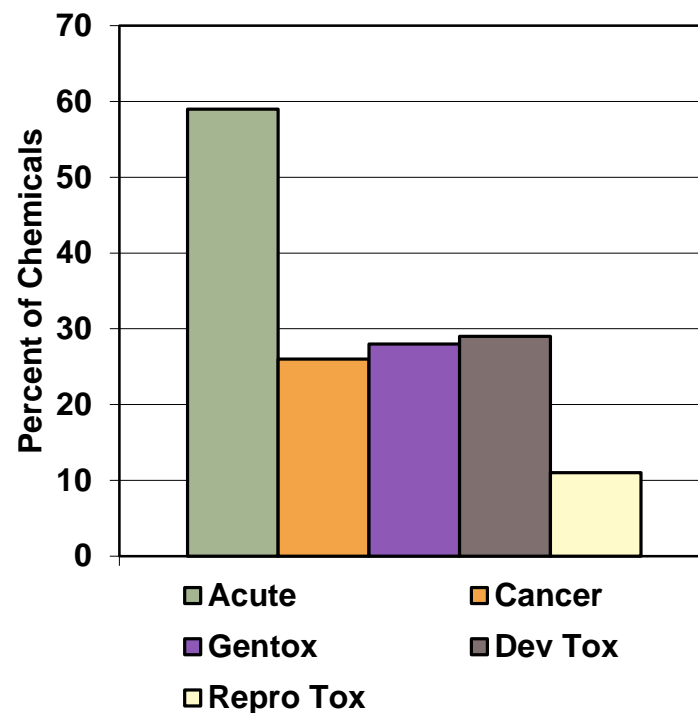
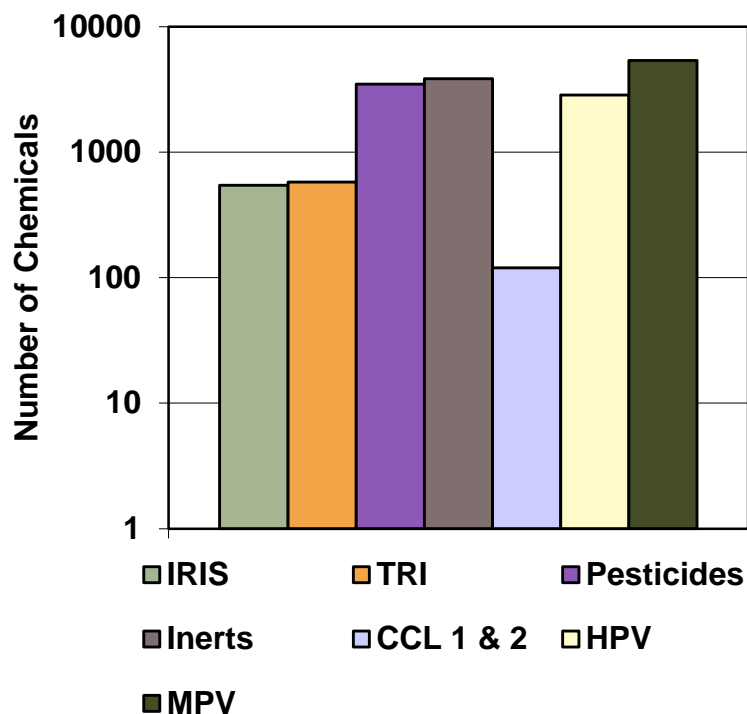


# Traditional Studies Attempt to Cover Range of Potential Adverse Responses

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



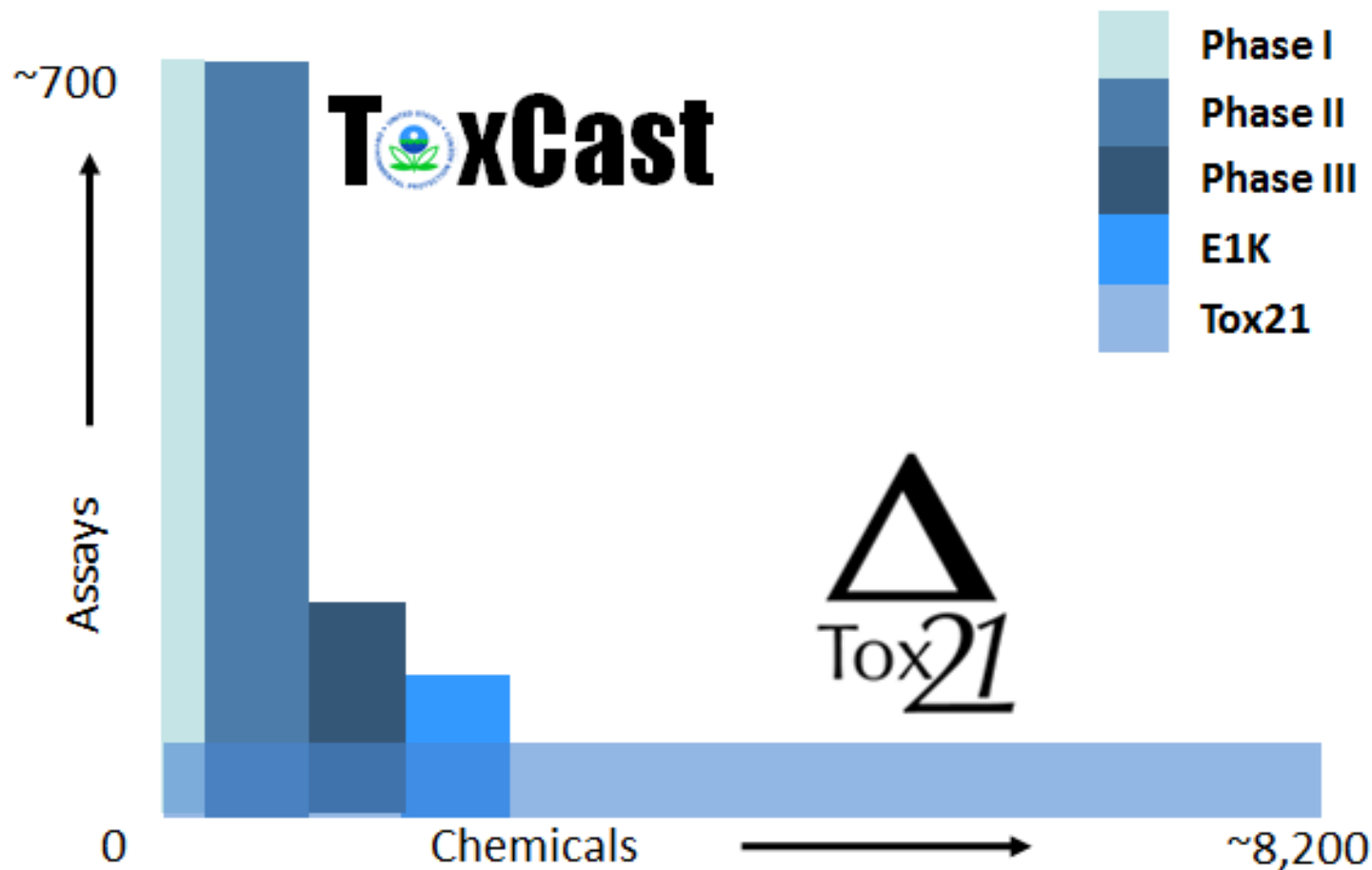
Judson, et al *EHP* (2010)

# 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

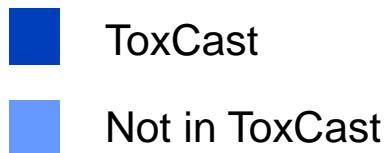
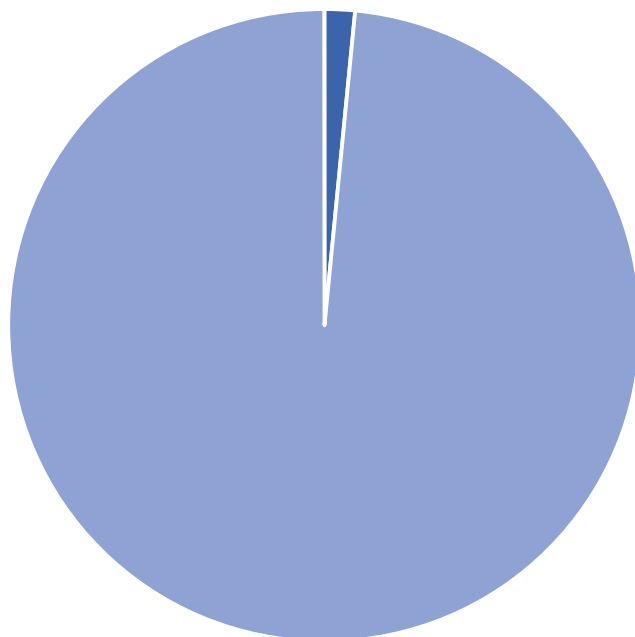
\*Offspring not counted

# Multiple Federal Efforts Have Begun to Address the Data Gap

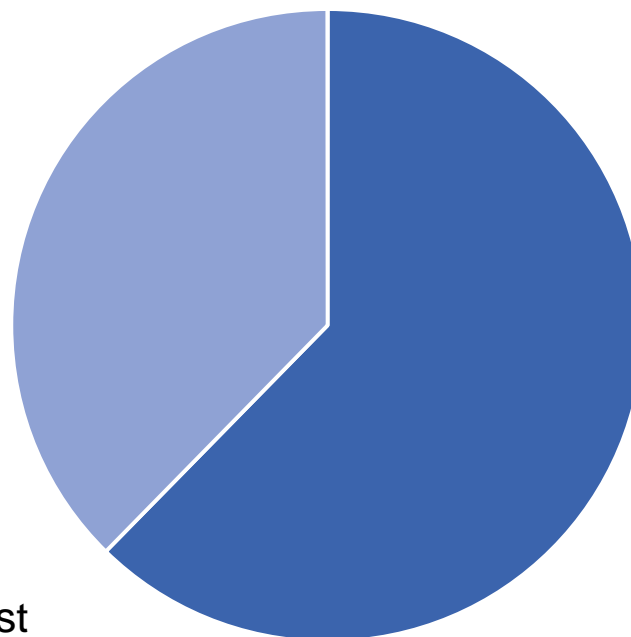


# Current Coverage of Biological Space is Less Than Optimal

ToxCast Gene Coverage

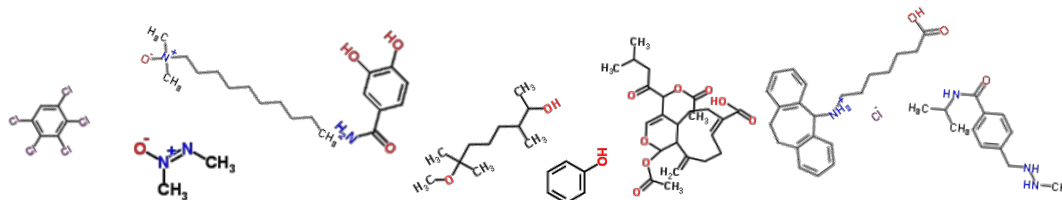


ToxCast Pathway Coverage\*



\*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



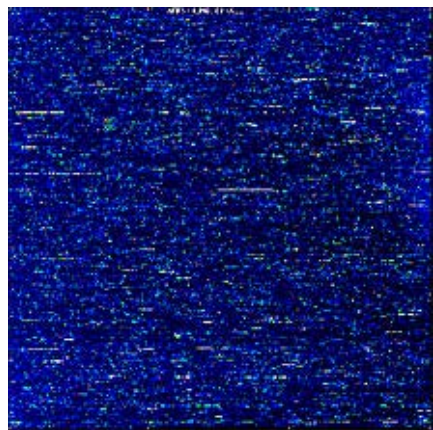
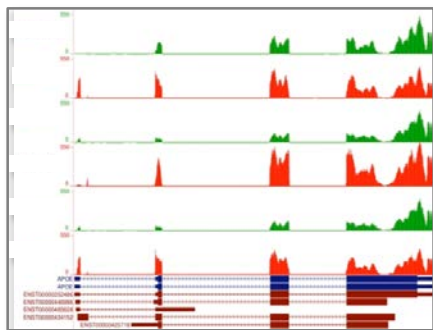
# Requirements and Potential Platforms for HT Transcriptomics

## 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)

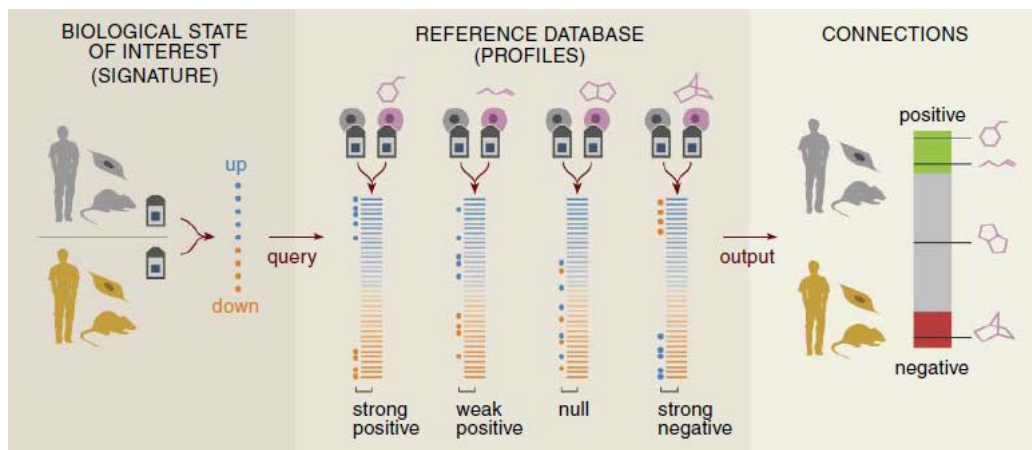


# How Would a HT Transcriptomic Platform be Deployed?

High-Throughput  
Transcriptomic  
Assay

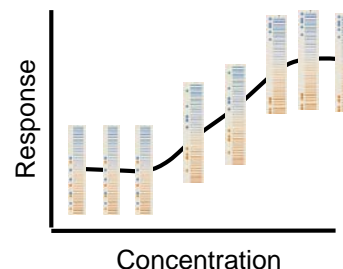
Tier 0

AOP/MOA

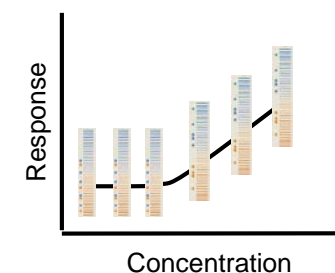


Lamb et al. *Science* (2006)

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

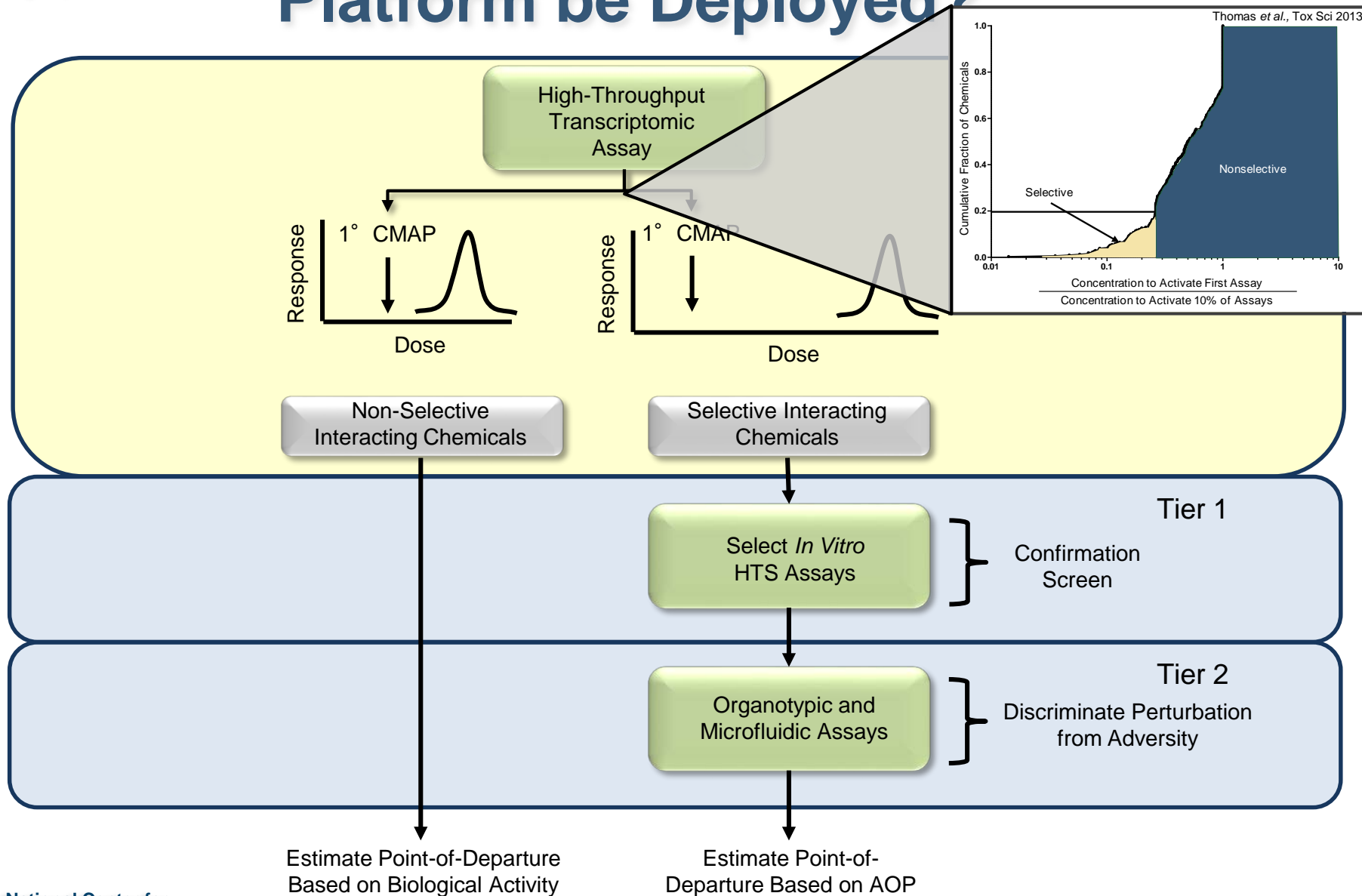


Cell Type #2

Cell Type #3

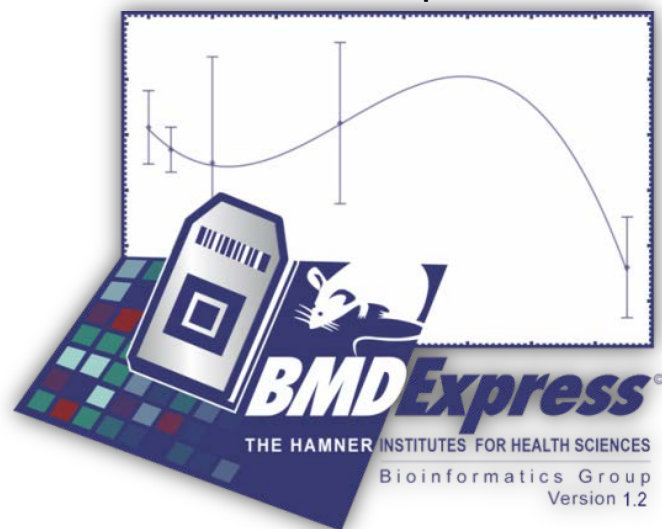
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# How Would a HT Transcriptomic Platform be Deployed?



# Approaches for Estimating a Transcriptomic Point of Departure

## BMDExpress



<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**

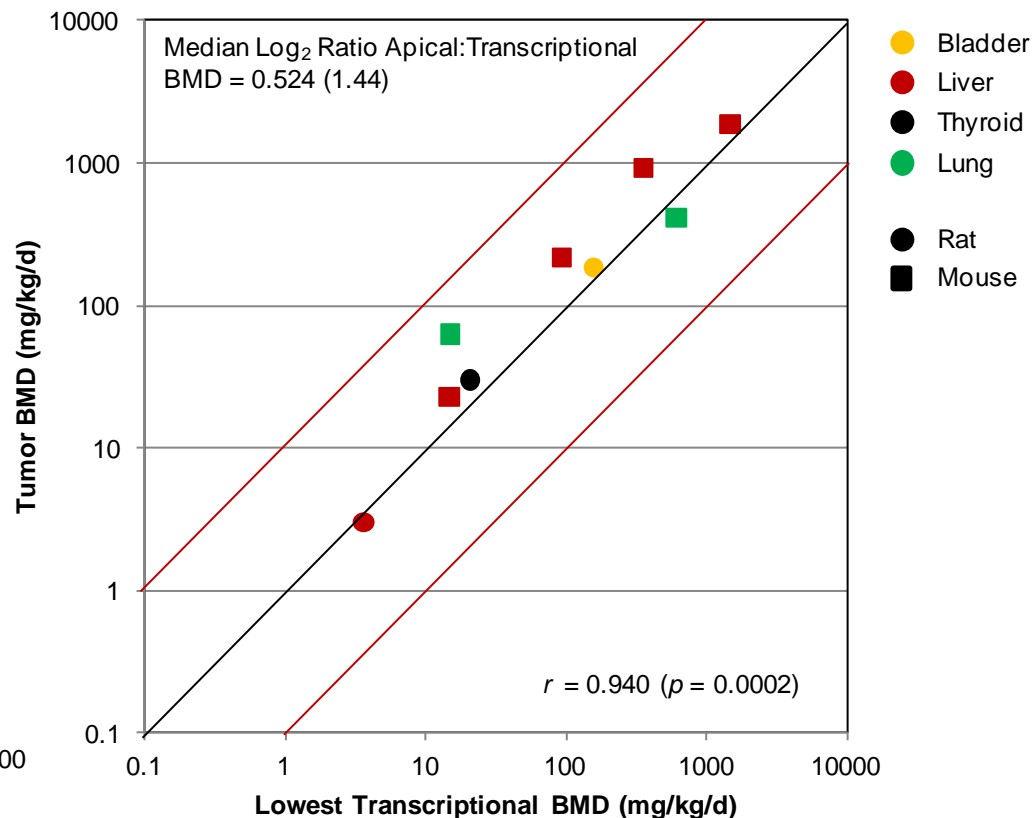
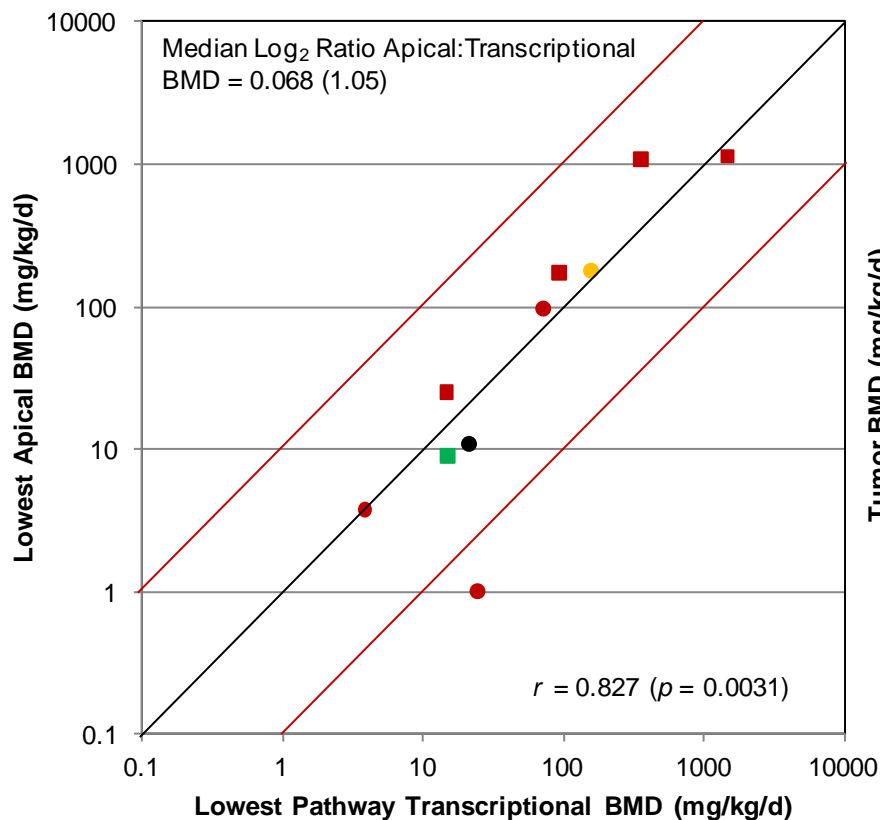
P-value threshold for inclusion: 0.1  
Number of data re-samples: 100  
Pathway FDR threshold: 0.05

**Results**

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

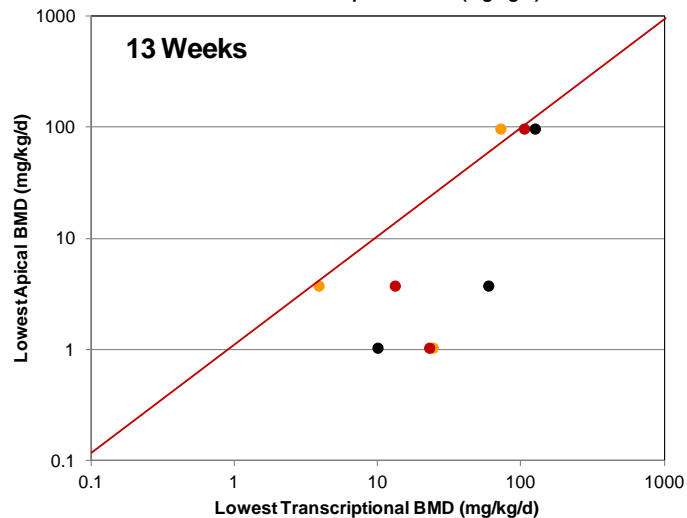
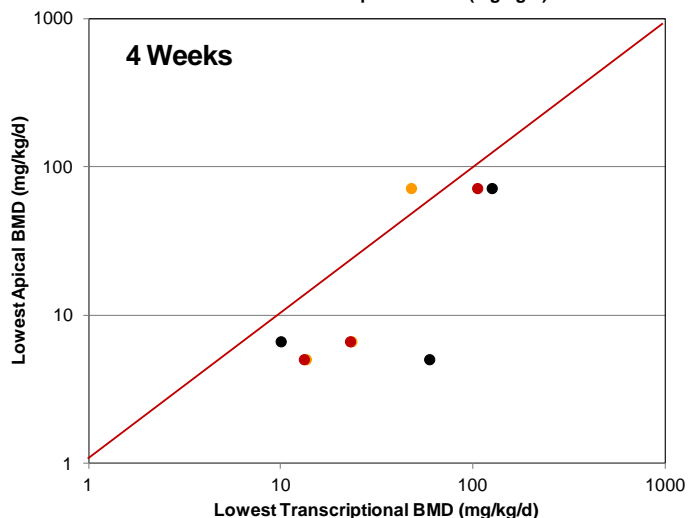
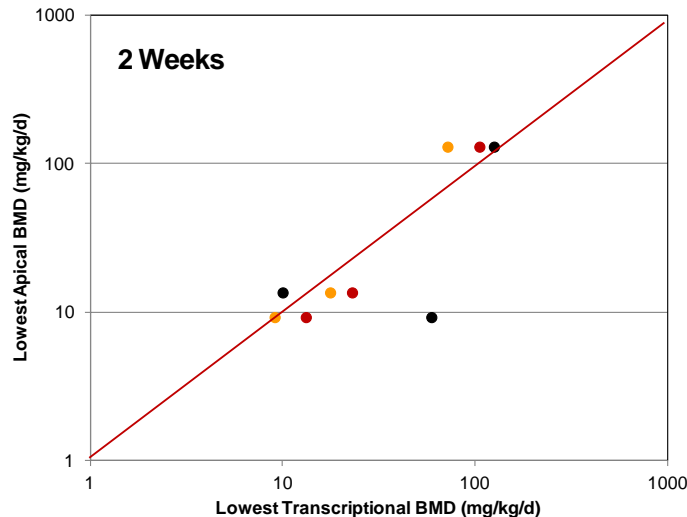
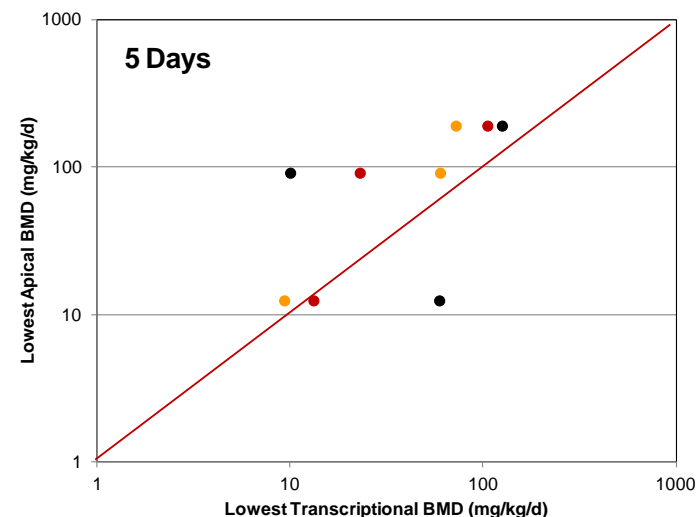
<http://comptox.unc.edu/DRPathway.php>

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



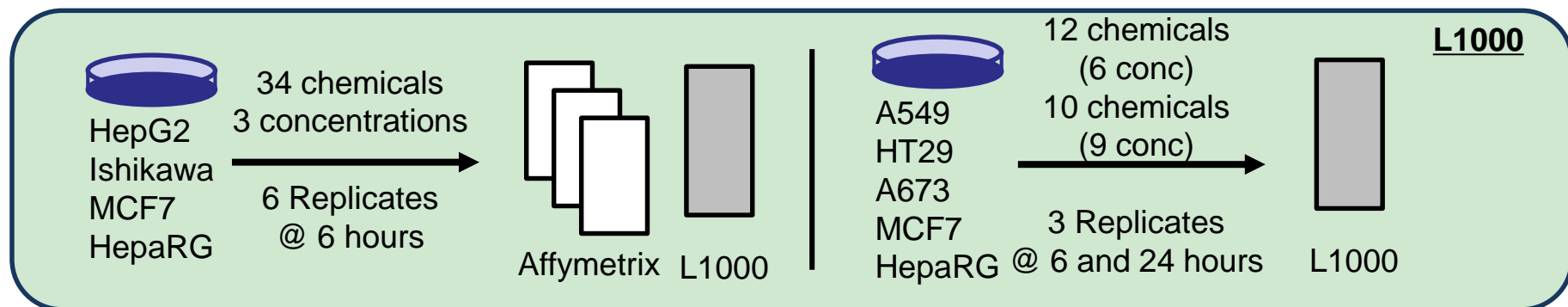
Thomas *et al.*, *Toxicol Sci*, 2013

# What About *In Vitro* Transcriptional Responses?



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

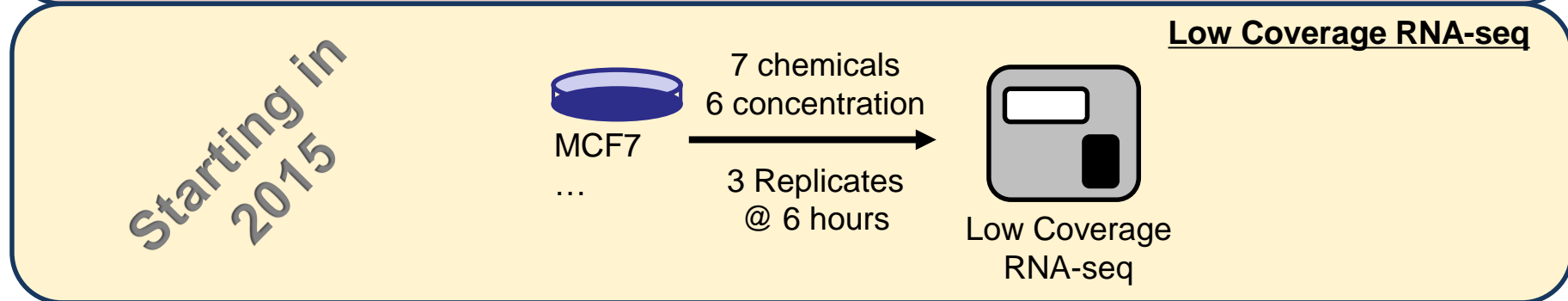
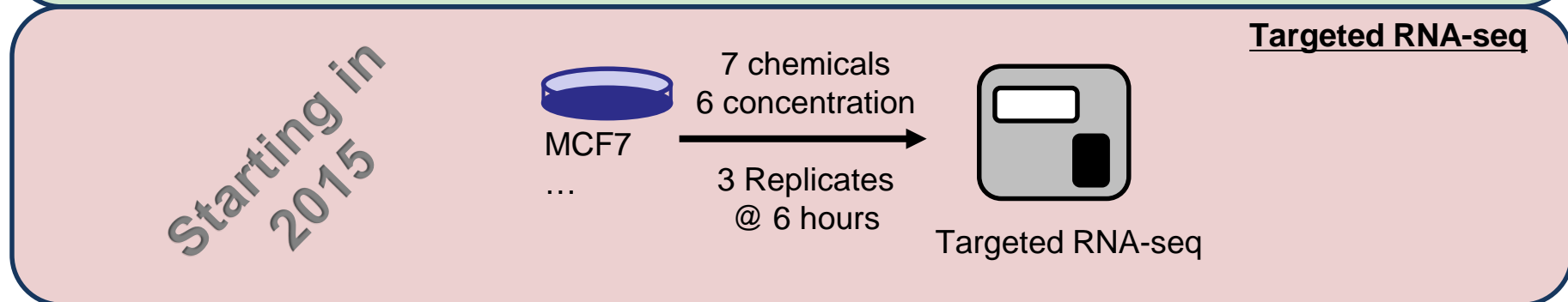
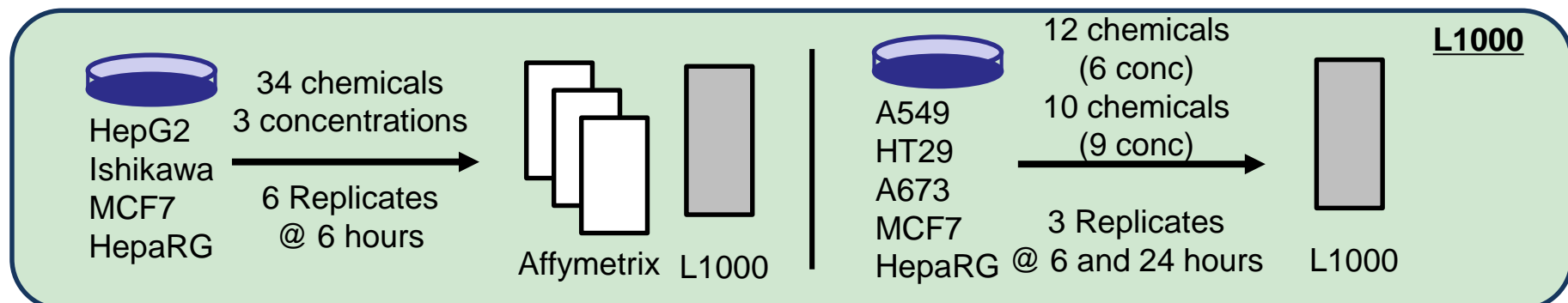
# Beginning the Search for a Platform



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

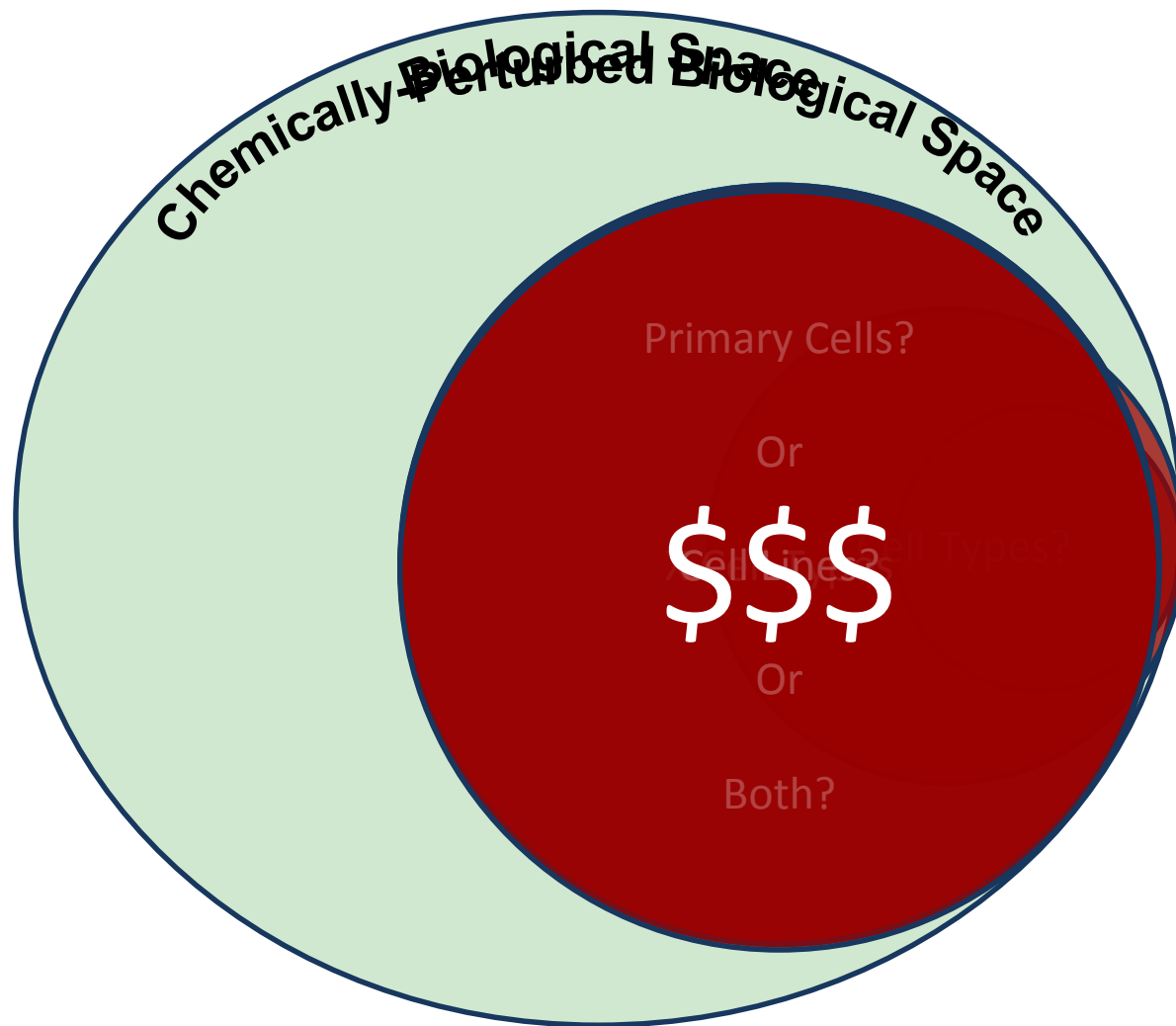
# Beginning the Search for a Platform





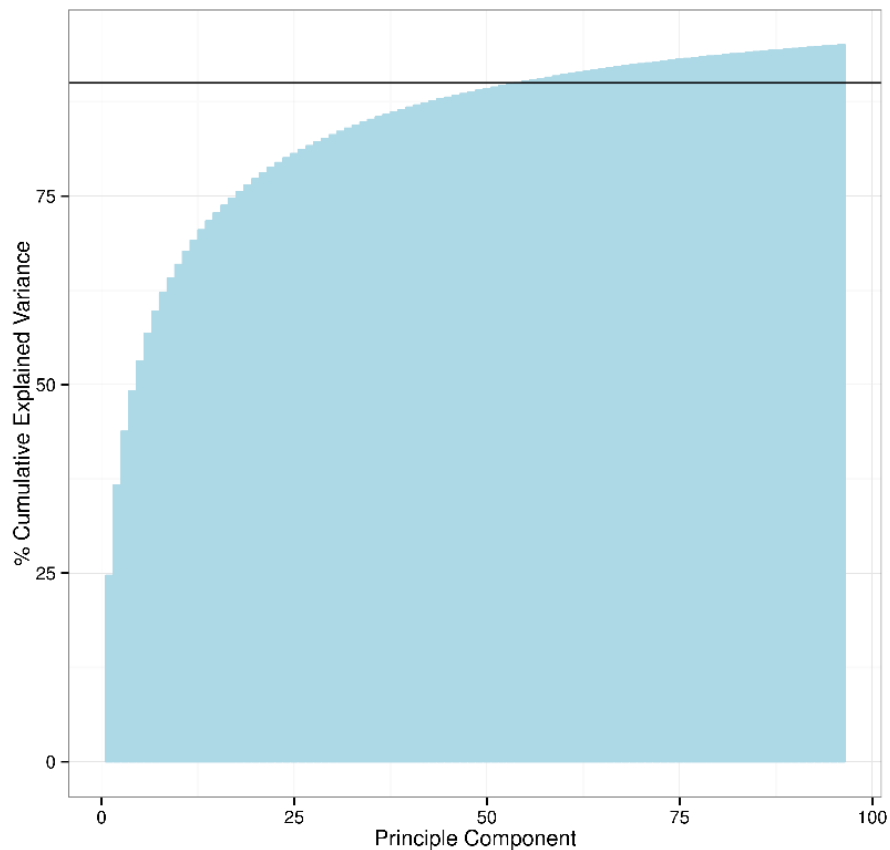


# Beginning the Search for the Cell Types/Lines



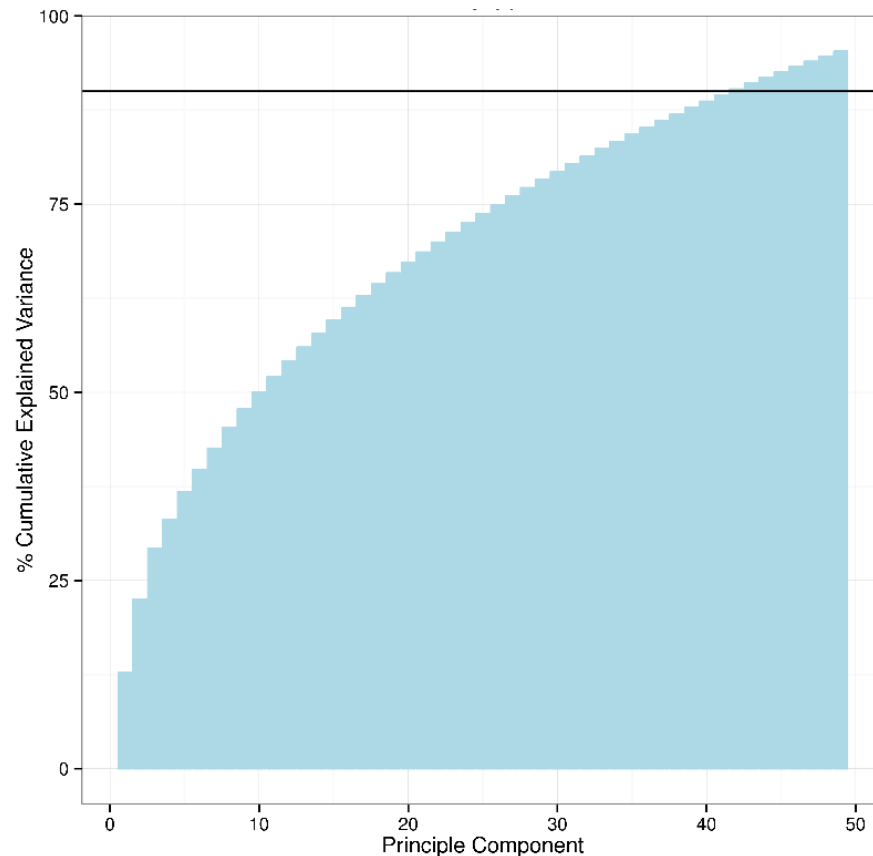
# Exploring Cell Line Requirements

Primary Cell Atlas (302 cell types)

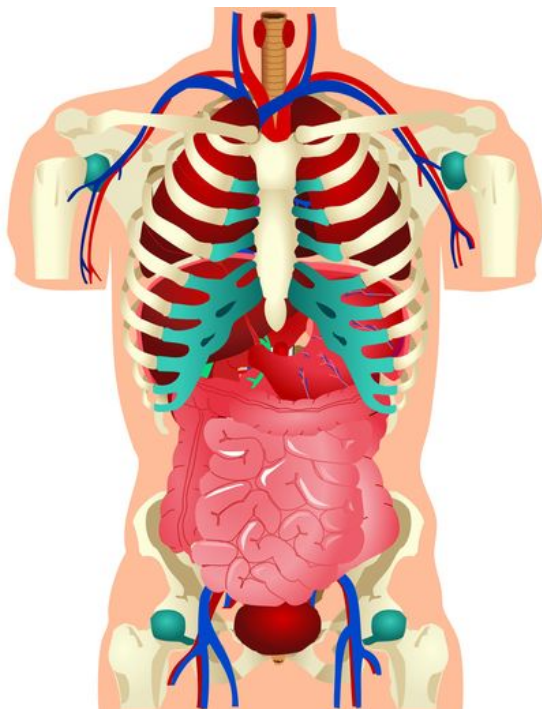


(GSE49910)

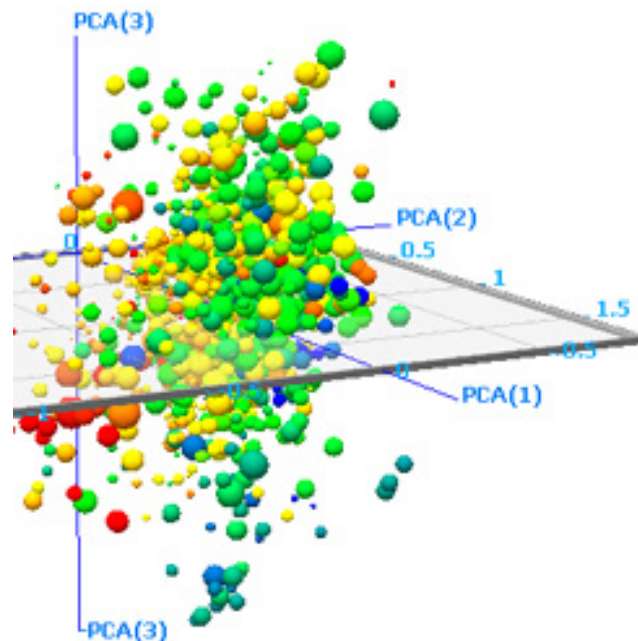
NCI-60



# 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**