EPA
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Environmental Protection
Agency

Strategies for Integrating Transcriptional Profiling into High-Throughput Toxicity Testing

Society of Toxicology Annual Meeting
March 25, 2015

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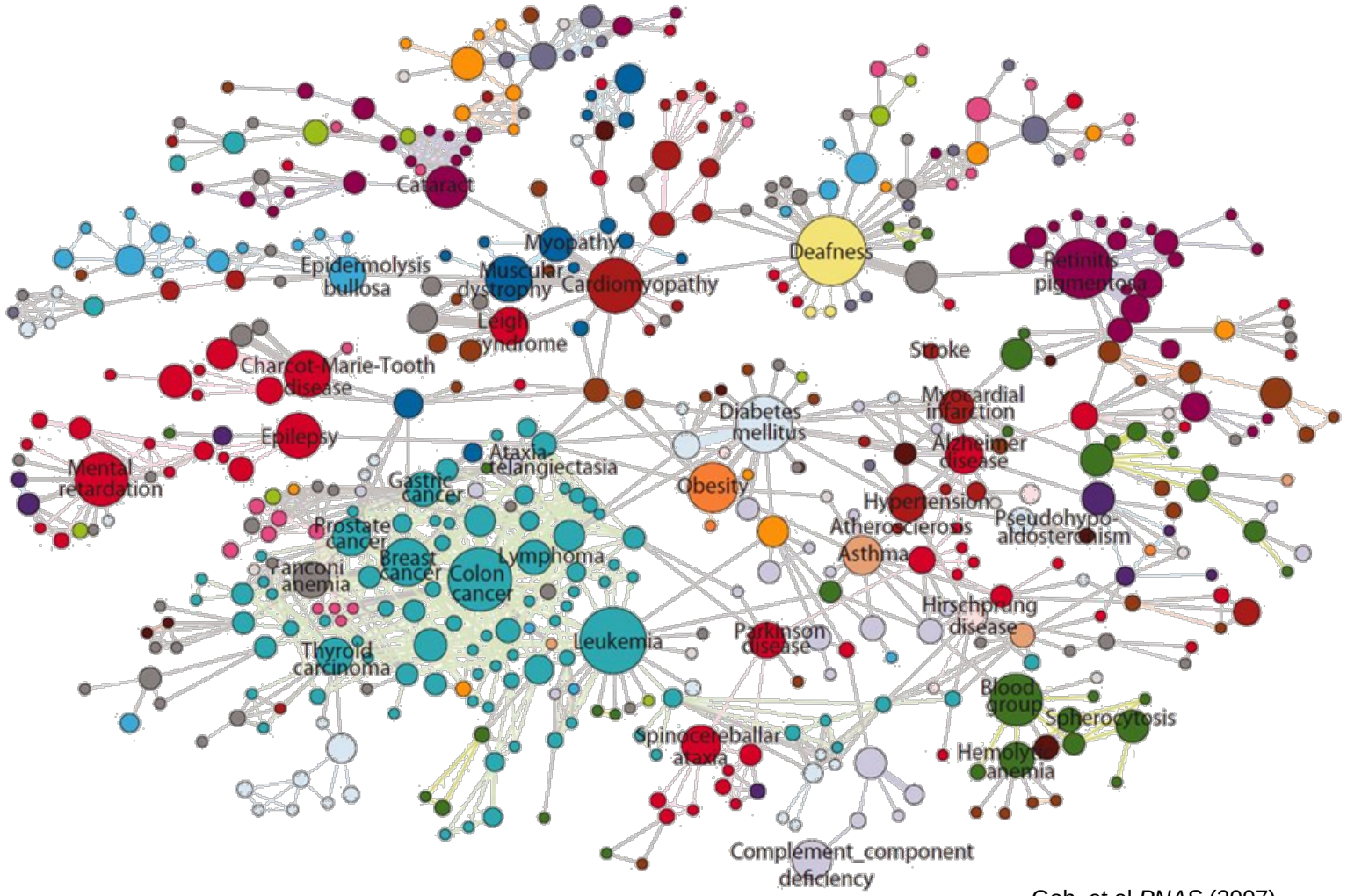
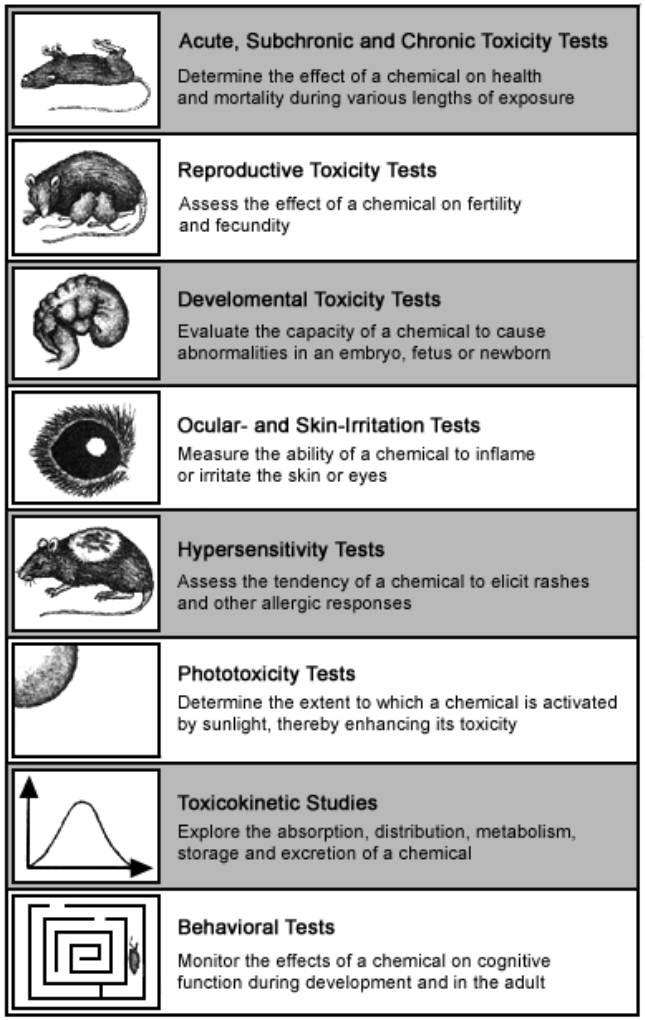
Director
National Center for Computational Toxicology

The Biological Scope for Toxicology is Necessarily Broad



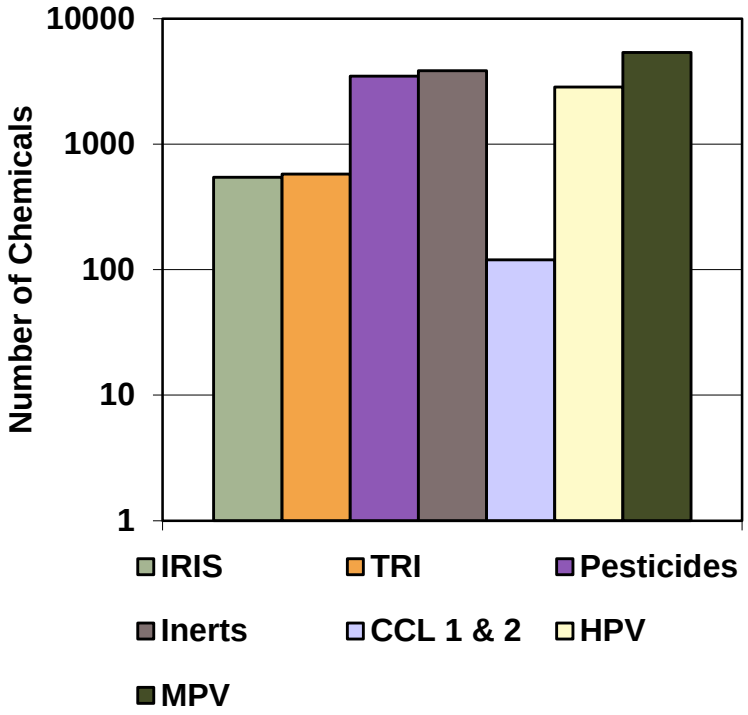
Goh, et al *PNAS* (2007)

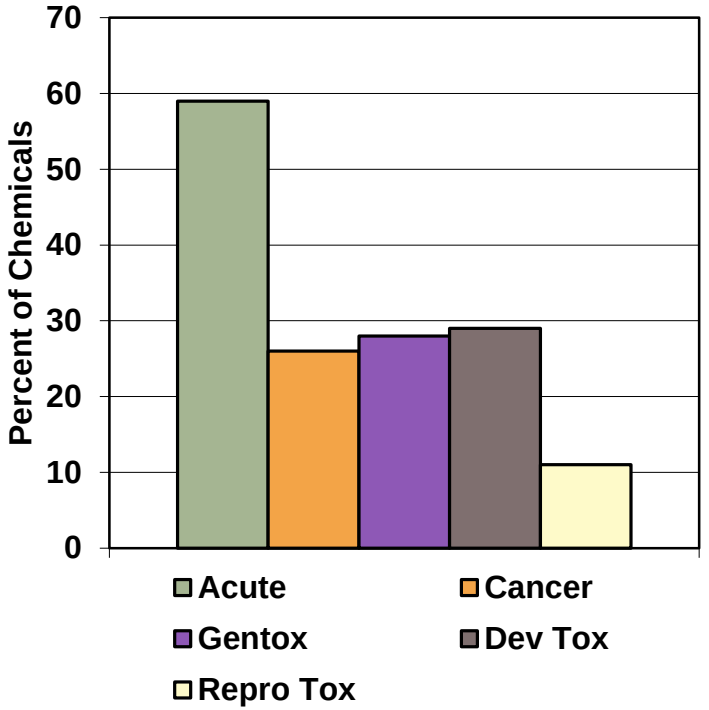
Traditional Studies Attempt to Cover Range of Potential Adverse Responses



Goldberg and Frazier (1989)

Current System for Chemical Safety Testing Has Not Kept Pace





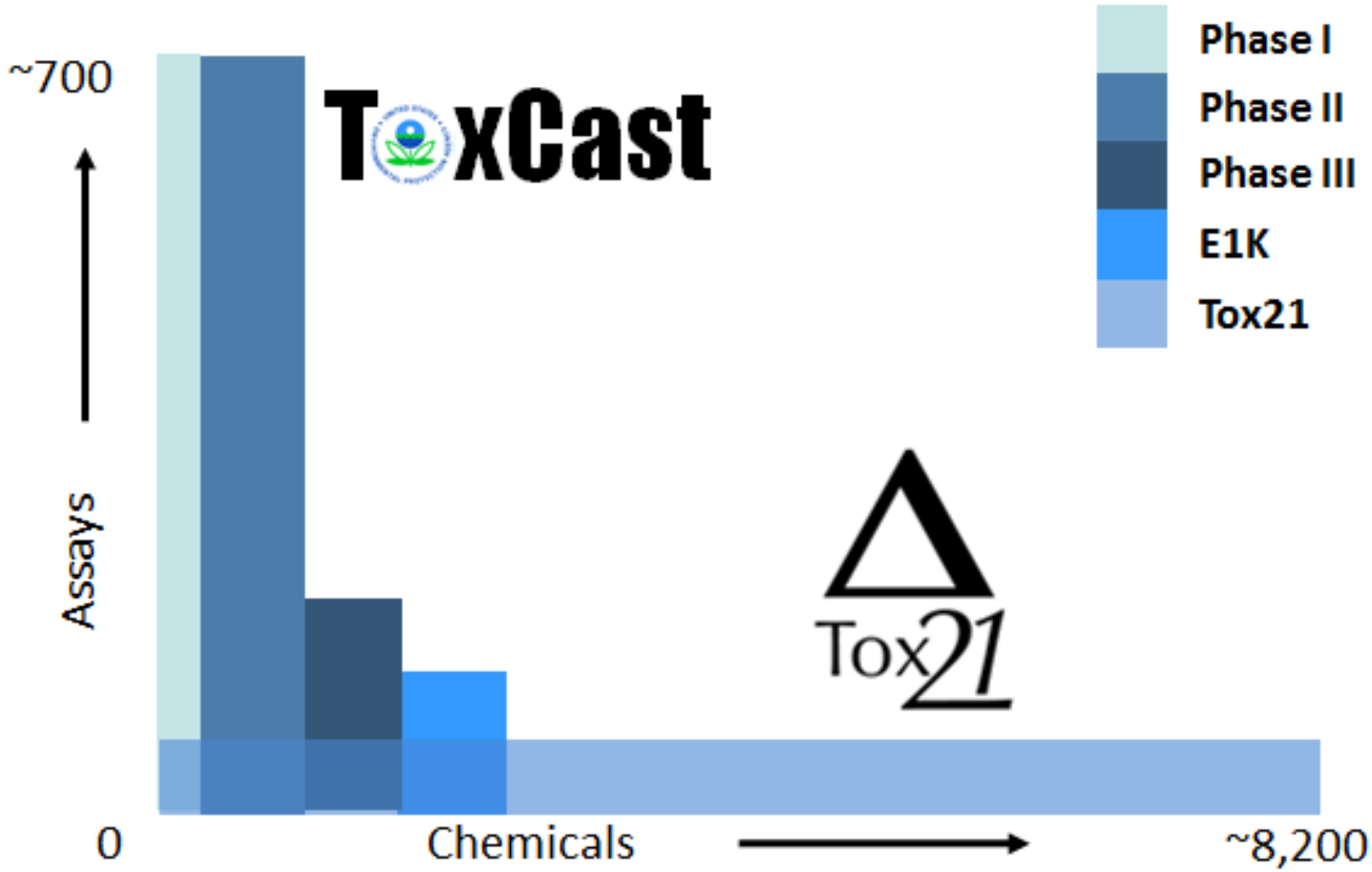
Judson, et al *EHP* (2010)

Significant Economic and Animal Costs Associated with Testing

Toxicity Study	Number of Animals	Approx. Cost
Skin sensitization (*in vivo*)	20	$7,000.00
Acute toxicity by oral route	20	$2,500.00
Repeated dose toxicty (one species, male and female (28 d), most appropriate route) (OECD407)	40	$100,000.00
In vivo 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

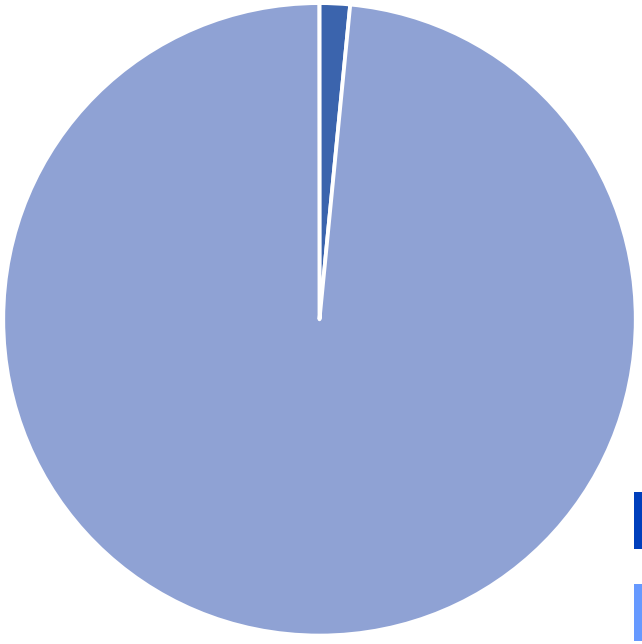
*Offspring not counted

Multiple Federal Efforts Have Begun to Address the Data Gap



Current Coverage of Biological Space is Less Than Optimal

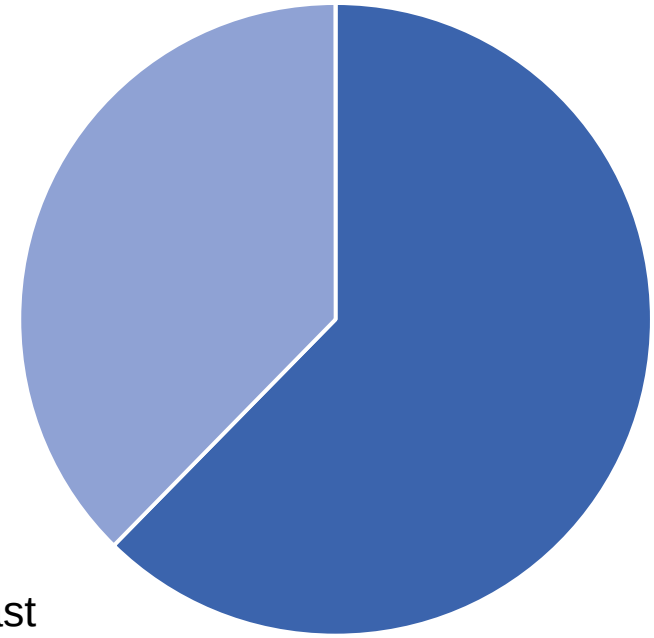
ToxCast Gene Coverage



ToxCast

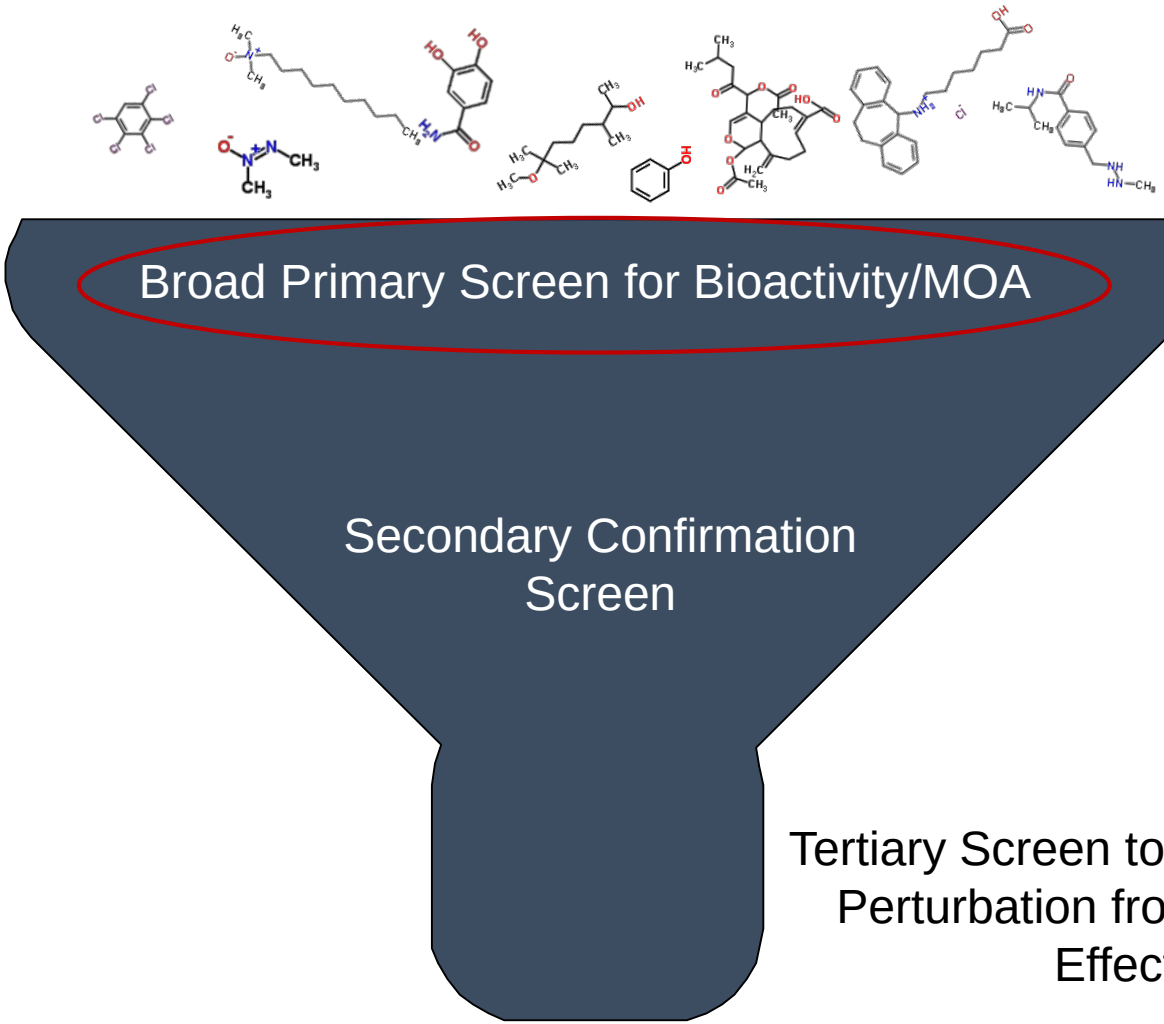
Not in ToxCast

ToxCast Pathway Coverage*

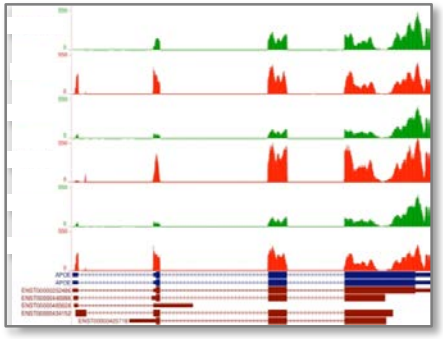


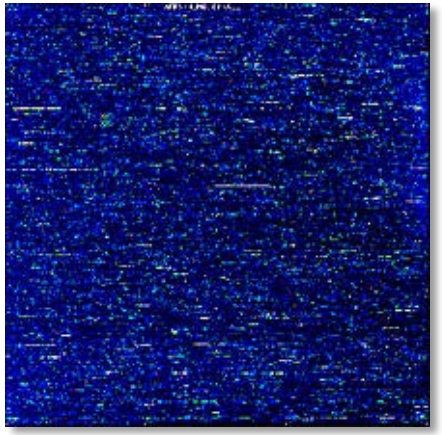
*At least one gene from pathway represented

Incorporating a Broad Biological Screening Platform



Requirements and Potential Platforms for HT Transcriptomics





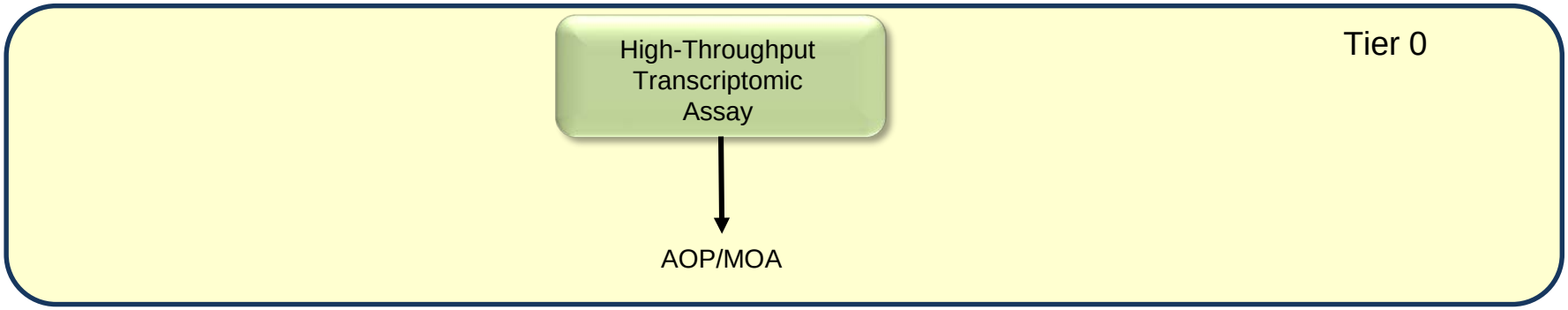
Requirements

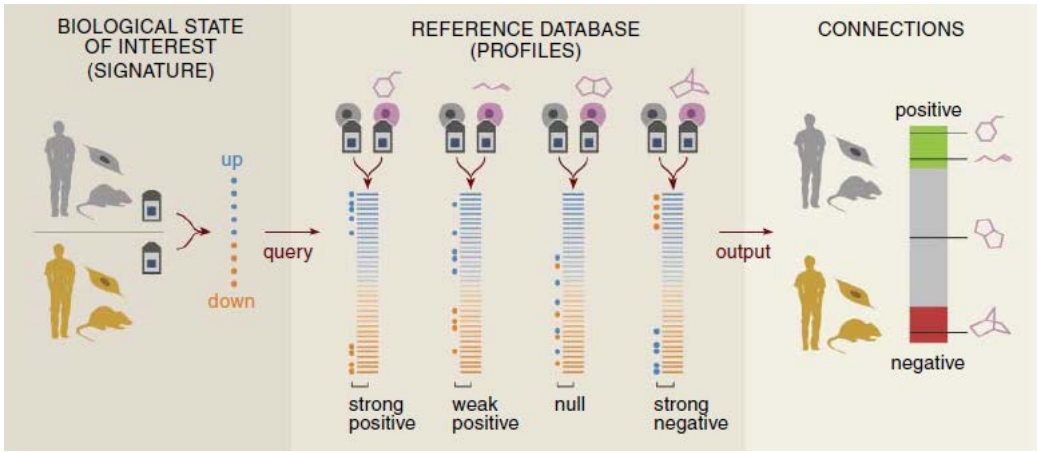
- Measure or infer transcriptional changes across the whole genome (or very close to it)
- Compatible with 96- and 384-well plate formats (maybe 1536?) and laboratory automation
- Work directly with cell lysates (no separate RNA purification)
- Compatible with multiple cell types and culture conditions
- 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)

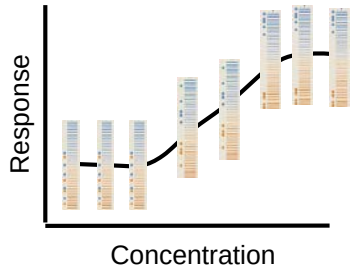
How Would a HT Transcriptomic Platform be Deployed?



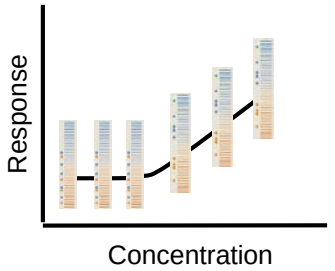


Lamb et al. *Science* (2006)

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)



Cell Type #1

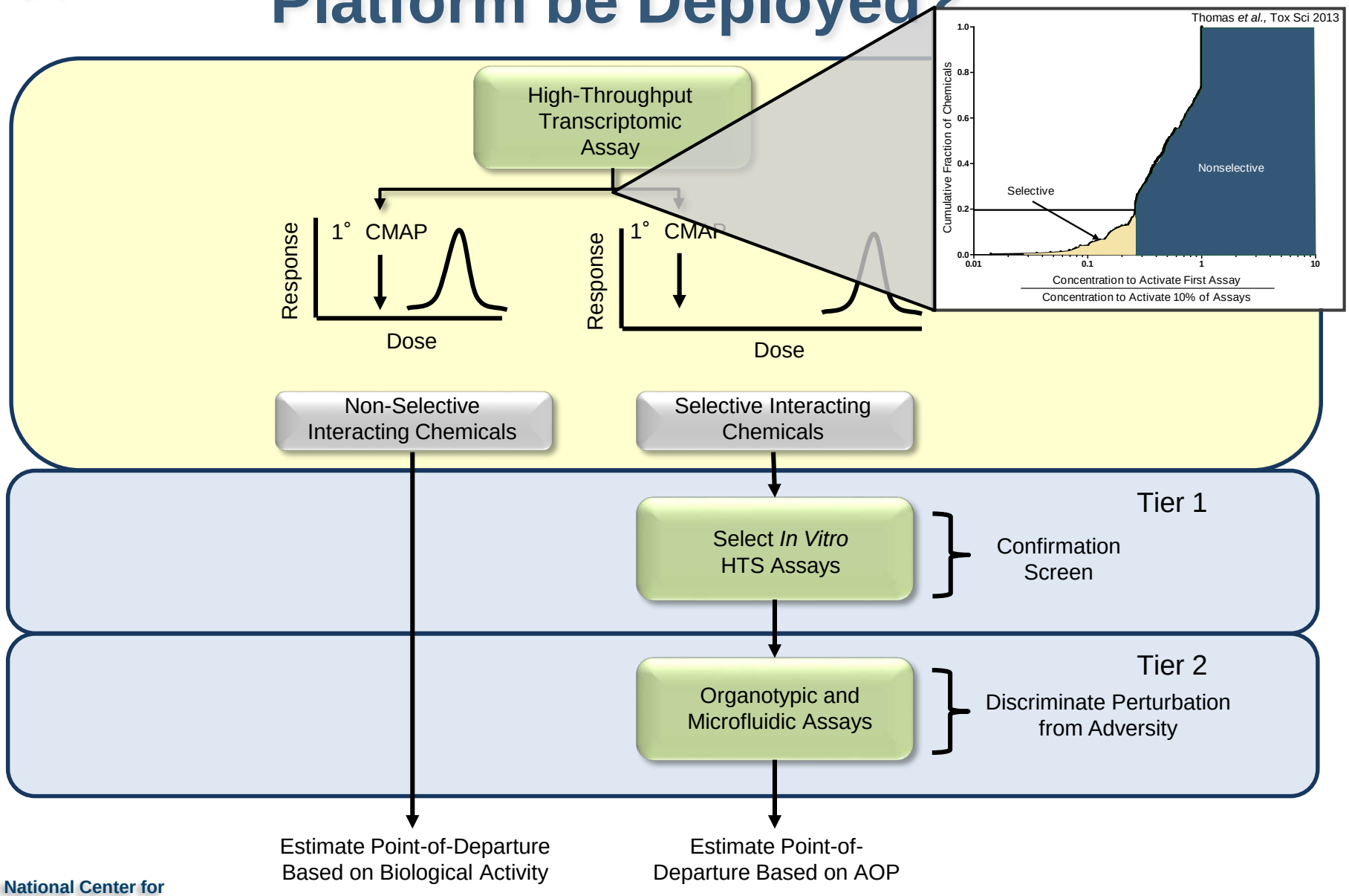


Cell Type #2

Cell Type #3

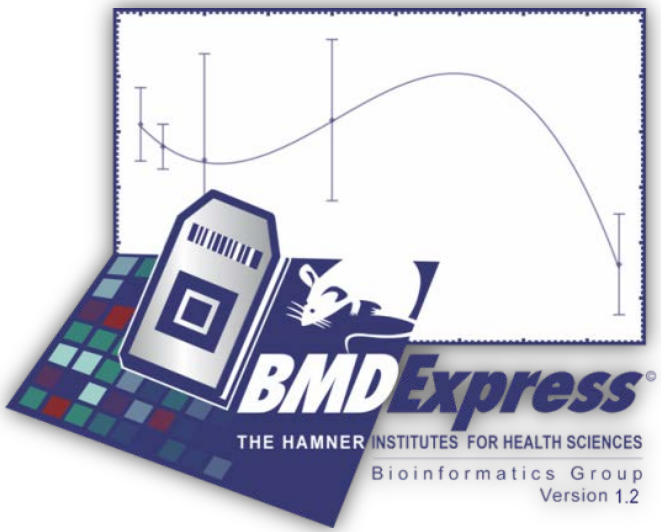
…

How Would a HT Transcriptomic Platform be Deployed?



Approaches for Estimating a Transcriptomic Point of Departure

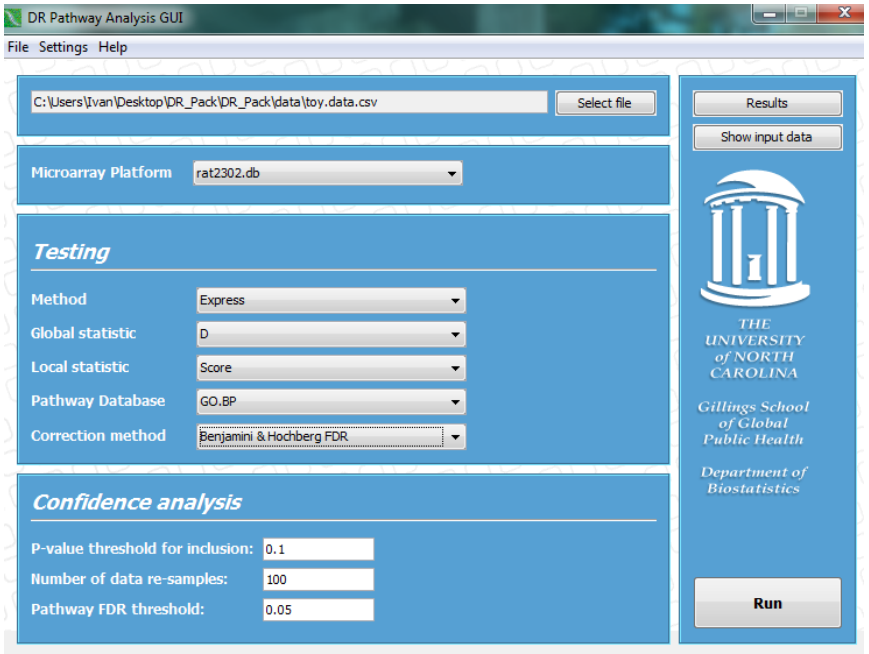
BMDExpress



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

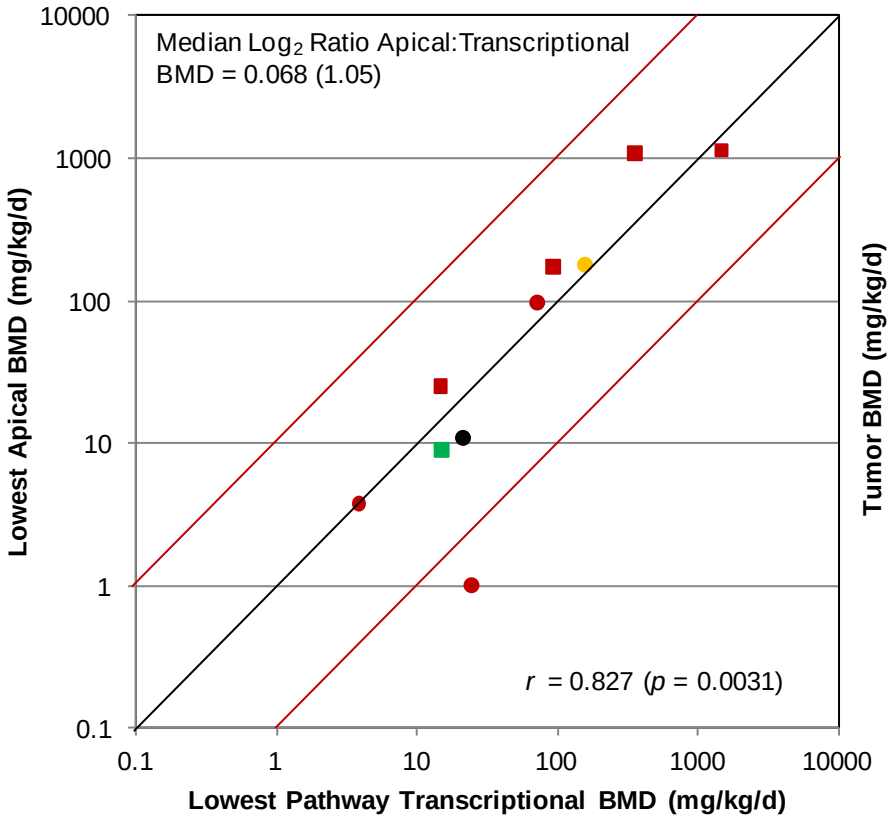
Yang *et al., BMC Genomics*, 2007
Thomas *et al., Toxicol Sci.*, 2007

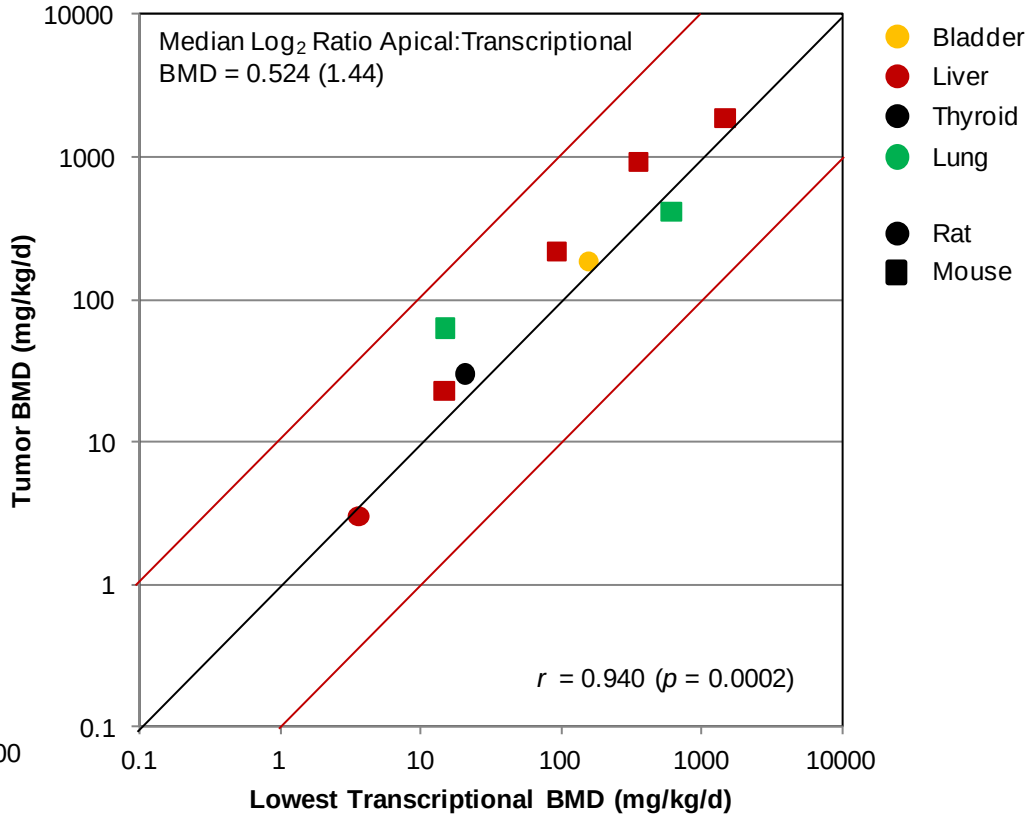
DR Pathway



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

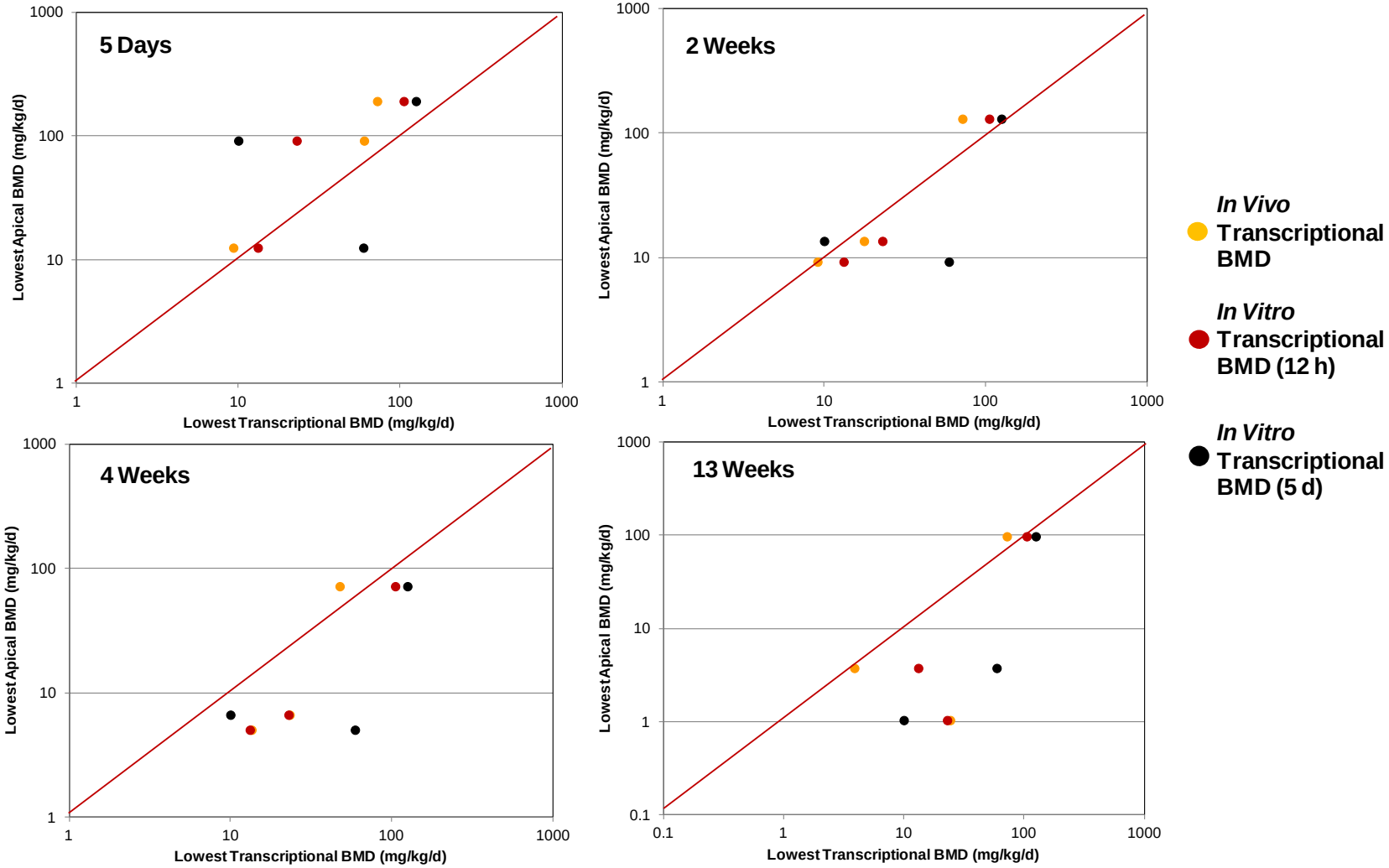
Correlation of *In Vivo* Apical and Transcriptional Points of Departure



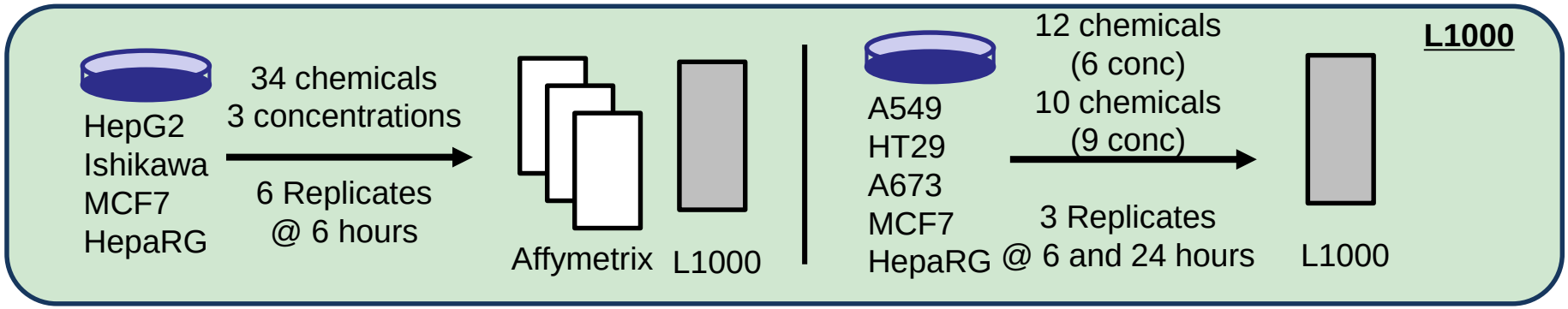


Thomas *et al., Toxicol Sci,* 2013

What About *In Vitro* Transcriptional Responses?



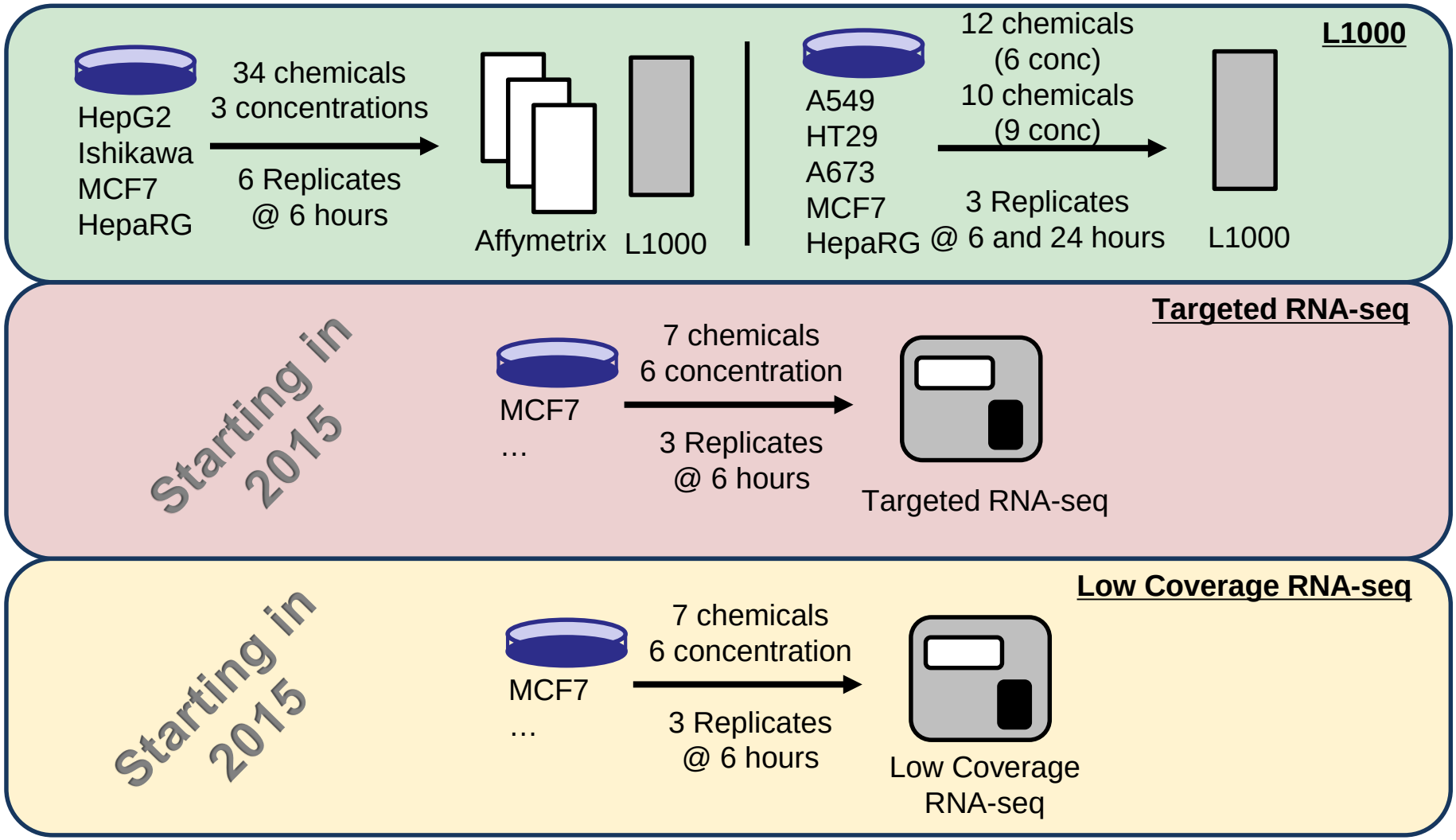
Beginning the Search for a Platform



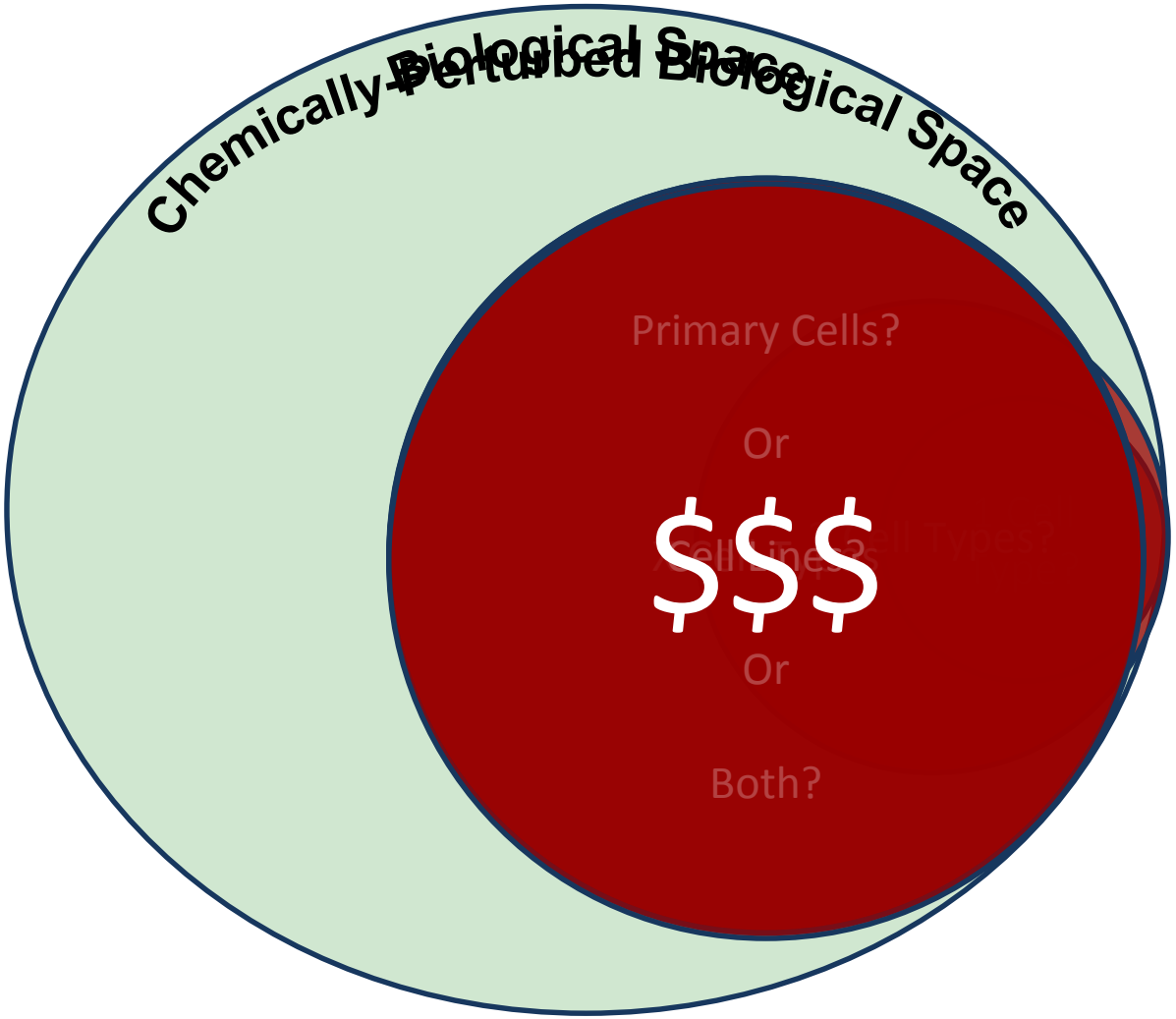
Collaboration with Proctor & Gamble (G. Daston and J. Naciff)
and Hamner Institutes (B. Wetmore and M. Black)

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

Beginning the Search for a Platform

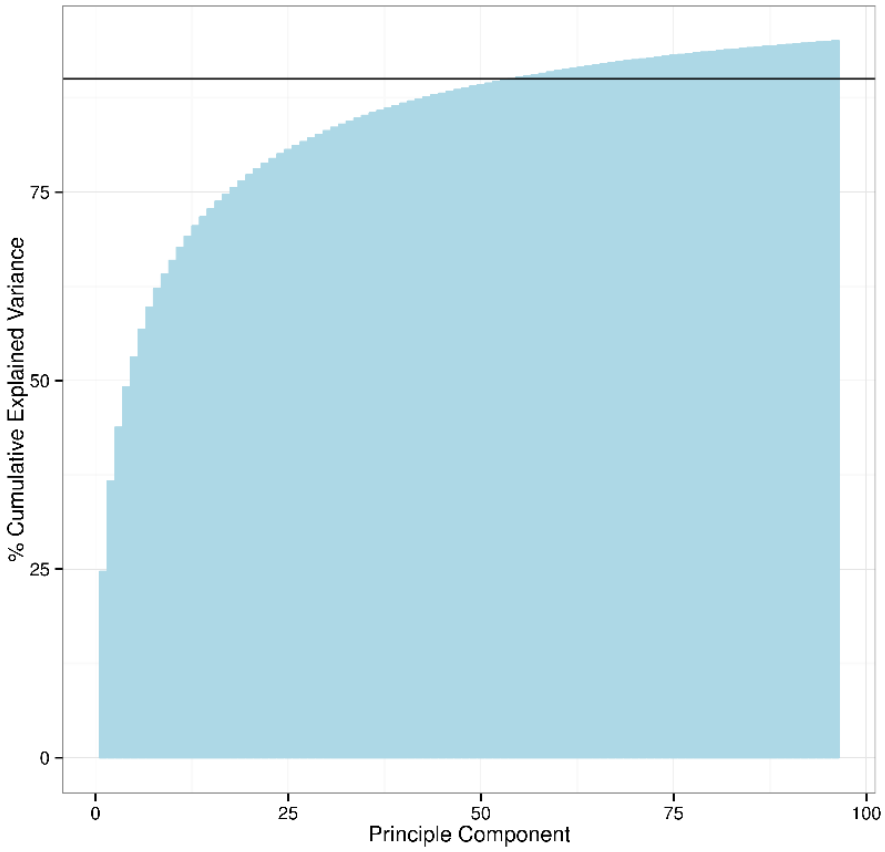


Beginning the Search for the Cell Types/Lines



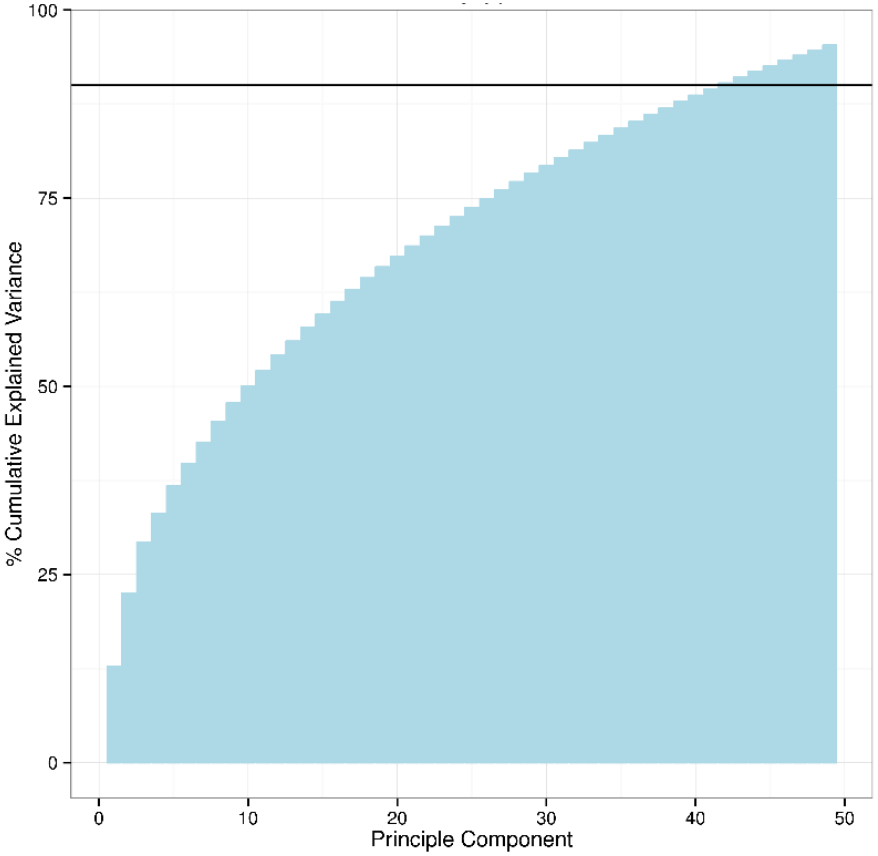
Exploring Cell Line Requirements

Primary Cell Atlas (302 cell types)

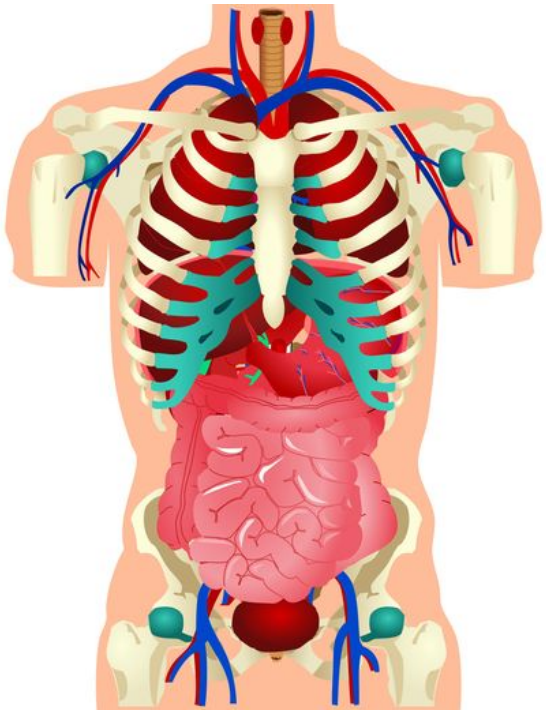


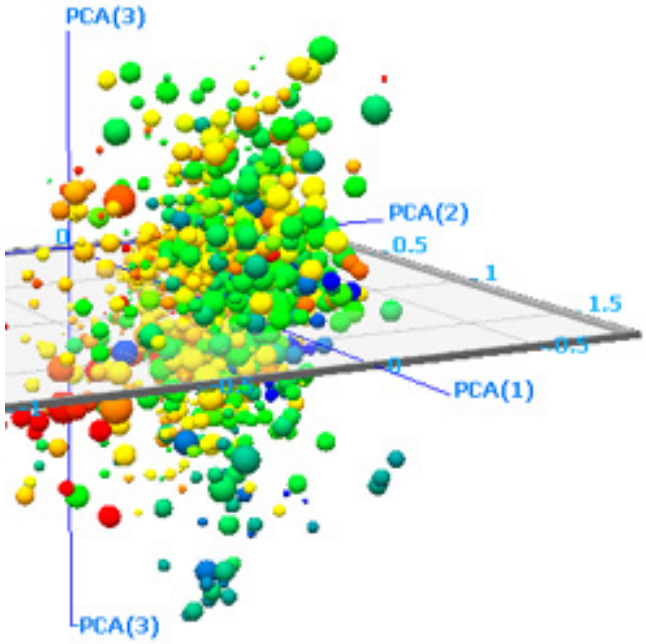
(GSE49910)

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