NTP
National Toxicology Program

Tox21: A U.S. Federal Collaboration to Improve the Human Hazard Characterization of Chemicals

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National Institute of Environmental Health Sciences

SACATM
September 24, 2013

Tox21: A collaboration of many…….

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NCATS NCCT

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Formation of the U.S. Tox21 Community

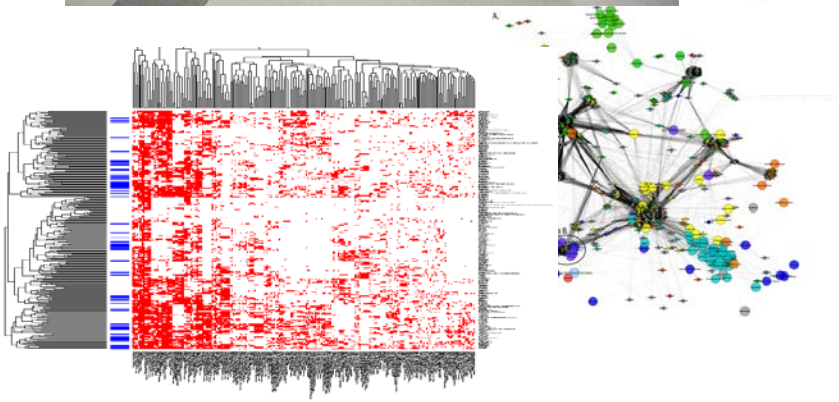
- 5-year Memorandum of Understanding (MoU) on “High-Throughput Screening, Toxicity Pathway Profiling, and Biological Interpretation of Findings” released on Feb 14, 2008 signed by NHGRI (F.S. Collins), NIEHS/NTP (S.H. Wilson), and EPA (G.M. Gray).

- Revised 5-year MoU to add FDA signed on July 19, 2010 http://ntp.niehs.nih.gov/go/28213) by NHGRI (E.D. Green), NIEHS/NTP (L.S. Birnbaum), EPA (P.T. Anastas), and FDA (J. Woodcock).

- A “community resource” project

Tox21 Goals

- Identify patterns of compound-induced biological response in order to:
  - characterize toxicity/disease pathways
  - facilitate cross-species extrapolation
  - model low-dose extrapolation
- Prioritize compounds for more extensive toxicological evaluation
- Develop predictive models for biological response in humans



Area of Expertise	NIEHS/NTP	NCATS	EPA	FDA
Lab Animal Toxicology	✓		✓	✓
Human Toxicology/Exposure Assessment	✓		✓	✓
Ultra High Throughput Screening		✓		
Low to Mid Throughput Assays	✓	✓	✓	✓
Stem Cell Assay Development	✓	✓	✓	✓
Epigenetic Assays	✓	✓		
Engineered Tissue Models	✓	✓	✓	✓
‘Omic Based Systems	✓	✓	✓	✓
Lower Organism Models	✓		✓	✓
Genetic Variability in Response	✓	✓		
Databases & Informatic Tools	✓	✓	✓	✓
Validation Experience	✓	✓	✓	✓

Agency Points of Contact

FDA - David Jacobson-Kram, Ph.D., **Thomas Colatsky, Ph.D.**
NCGC/NCATS – Christopher Austin, Ph.D., **Anton Simeonov, Ph.D.**
EPA/NCCT – Robert Kavlock, Ph.D., David Dix, Ph.D., **Russell Thomas, Ph.D.**
NIEHS/NTP - **Raymond Tice, Ph.D.**

Assays & Pathways Working Group

Co-Chairs
Kevin Gaido, Ph.D. (FDA)
Keith Houck, Ph.D. (EPA)
Kristine Witt, M.S. (NTP)
Menghang Xia, Ph.D. (NCGC)

- Identify toxicity pathways & corresponding assays
- Review nominated assays and prioritize for use at the NCGC

Chemical Selection Working Group

Co-Chairs
William Leister, Ph.D. (NCGC)
Donna Mendrick, Ph.D. (FDA)
Ann Richard, Ph.D. (EPA)
Suramya Waidanatha, Ph.D. (NTP)

- Establish compound libraries for qHTS (10K, mixtures, water-soluble)
- Establish QC procedures for compound identity, purity, concentration, and stability

Informatics Working Group

Co-Chairs
Ruili Huang, Ph.D. (NCGC)
Richard Judson, Ph.D. (EPA)
Jennifer Fostel, Ph.D. (NIEHS)
Weida Tong, Ph.D. (FDA)

- Evaluate assay performance
- Develop prioritization schemes and prediction models
- Make all data publicly accessible

Targeted Testing Working Group

Co-Chairs
Michael DeVito, Ph.D. (NTP)
David Gerhold, Ph.D. (NCGC)
Timothy Shafer, Ph.D. (EPA)
James Weaver, Ph.D. (FDA)

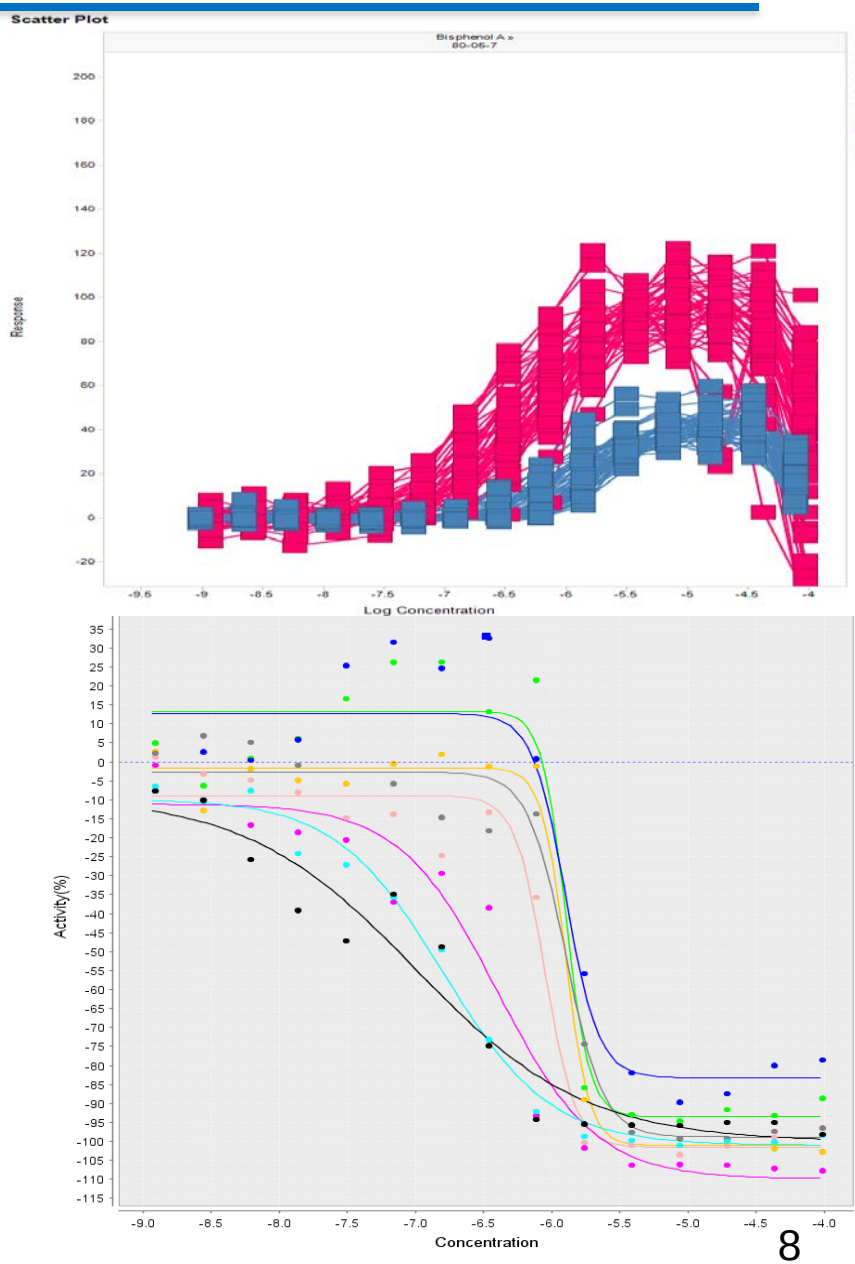
- Evaluate relevance of prioritization schemes & prediction models
- Extrapolate *in vitro* conc to *in vivo* dose

Tox21 Phase I – Proof of Principle (2005 – 2010)

- EPA via ToxCast™ screened 320 compounds (309 unique, primarily pesticide actives and some endocrine active compounds) in ~550 assays.
  - Data made public via ACToR (Aggregated Computational Toxicology Resource; http://epa.gov/actor)
- NCGC screened 1408 compounds (1353 unique) from NTP and 1462 compounds (1384 unique) from EPA in 140 qHTS assays representing 77 predominantly cell-based reporter gene endpoints.
- Data made public via PubChem (http://pubchem.ncbi.nlm.nih.gov/) and will be available in CEBS (Chemical Effects in Biological Systems; http://www.niehs.nih.gov/research/resources/databases/cebs/)

Quantitative High Throughput Screening (qHTS) at NCATS

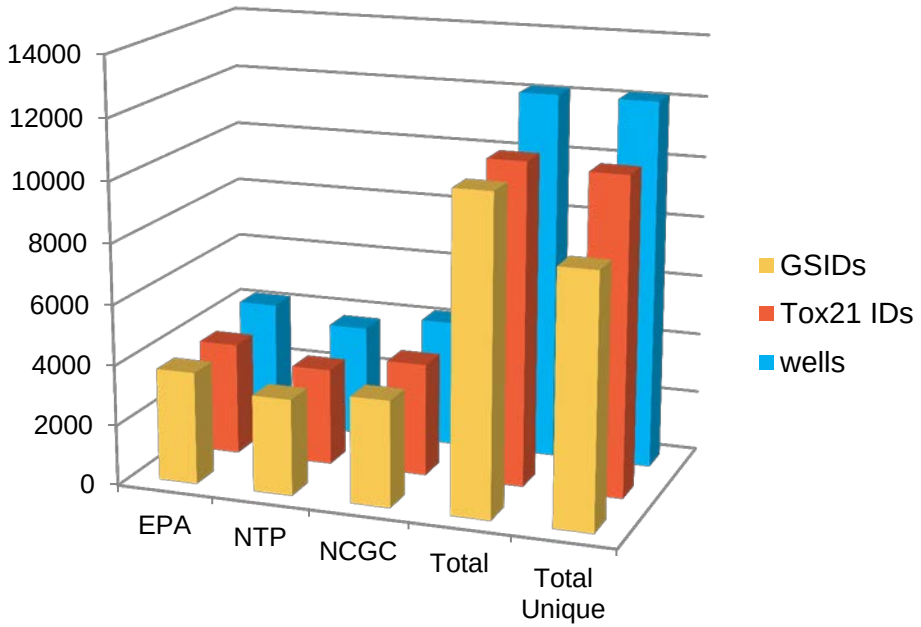
- DMSO soluble compounds
- homogeneous assays
- 1536-well plate format
- 15-point concentration-response curve
- 5 nM to 92 μM typical
- ~5 μL assay volume
- ~1000-2000 cells/well

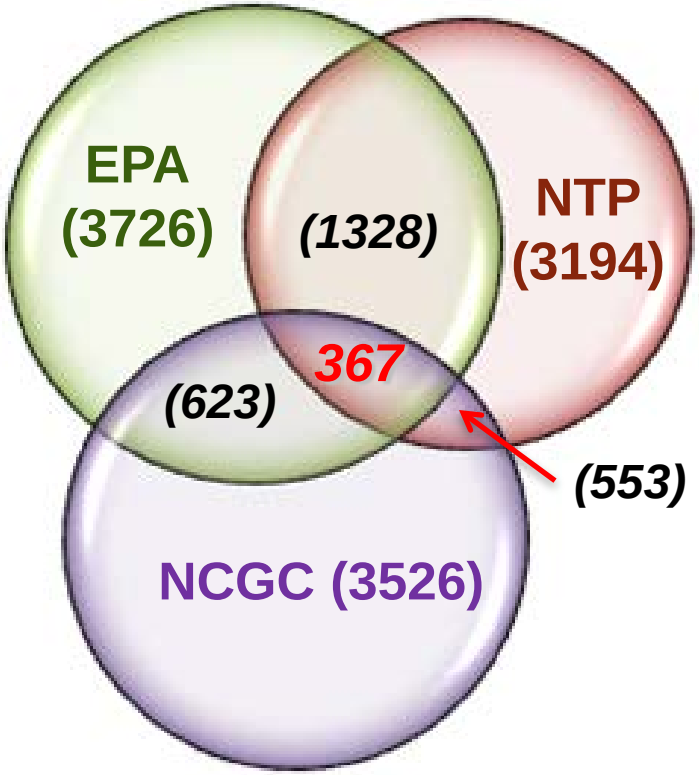


Tox21 Phase II – Expanded Compound Screening (2011 – 2014)

- EPA's ToxCast™ Phase II: ~700 compounds in ~700 assays, ~1000 compounds in endocrine activity assays
- NCGC qHTS Phase II:
  - 10K compound library screened 3 times at 15 concentrations in each qHTS assay
  - qHTS assays focused on:
    - nuclear receptor activation or inhibition
    - induction of cellular stress response pathways
    - characterizing human variability in response
- Partner-lead projects
  - cardiotoxicity (FDA)
  - endocrine disruptors (EPA)
  - genotoxicity (NIEHS/NTP)
  - mitochondrial toxicity (NCATS)

Tox21 10K Compound Library





Library tested 3x in each assay

Unique	EPA	NTP	NCGC	Total	Total Unique
GSIDs	3726	3194	3524	10444	8307
Tox21 IDs	3729	3210	3733	10672	10496
wells	4224	3726	4224	12174	12174

unique substances

unique solution IDs

total number of test cmpd wells

2255 replicate substances (GSIDs) across 3 inventories

88 single-sourced cmpds in duplicate on each plate

Compound identity and structures available at http://www.epa.gov/ncct/dsstox/sdf_tox21s.html

Tox21 10K Compound Library

NCGC

- Drugs
- Drug-like compounds
- Active pharmaceutical ingredients

EPA

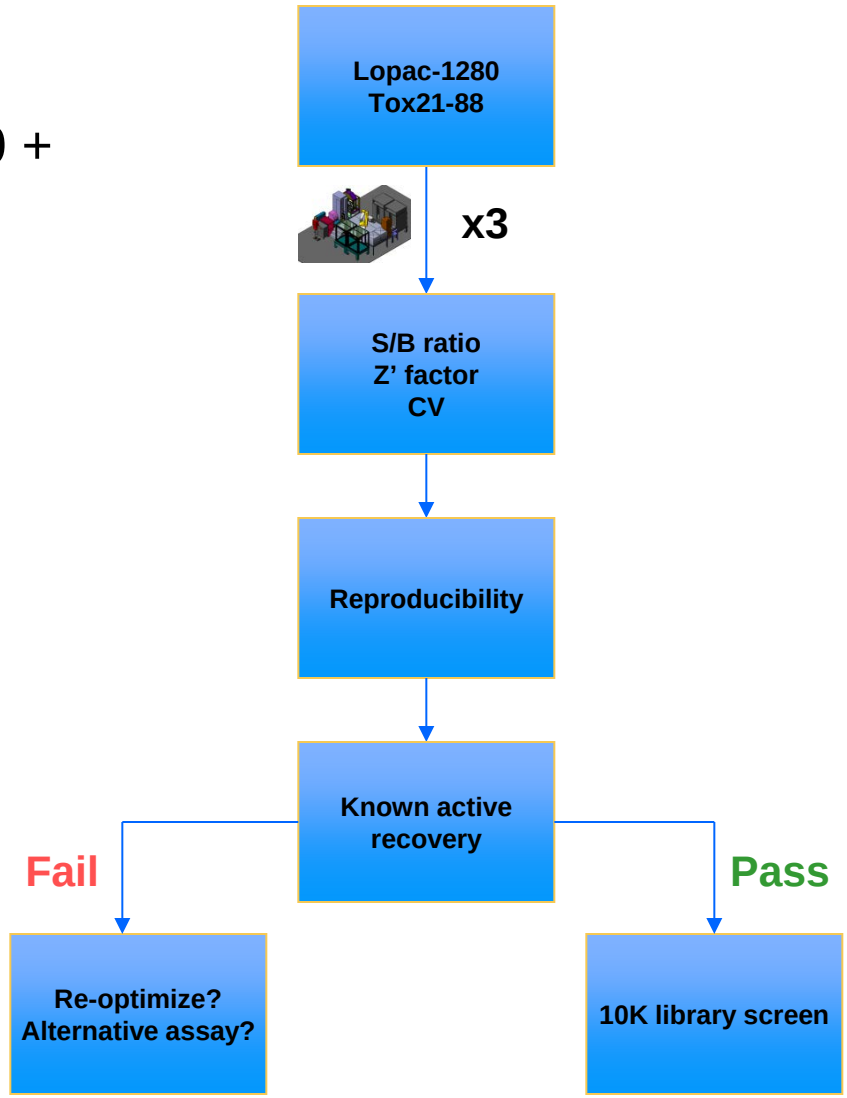
- ToxCast I and II compounds
- Antimicrobial Registration Program
- Endocrine Disruptor Screening Program
- OECD Molecular Screening Working Group List
- FDA Drug Induced Liver Injury Project
- Failed Drugs

NTP

- NTP-studied compounds
- NTP nominations and related compounds
- NICEATM/ICCVAM reference compounds from *in vivo* regulatory tests
- External collaborators (e.g., Silent Spring Institute, U.S. Army Public Health Command)
- Formulated mixtures

qHTS Assay Validation Process

- Online validation on Tox21 Robot
  - Tox21 validation plate (Lopac-1280 + 88 Tox21 replicates)
  - Triplicate runs
- Acceptance criteria consideration
  - Performance metrics - S/B ratio, Z' factor, CV
  - Reproducibility
  - Ability to identify reference compounds/known actives
- Pass
  - Proceed to 10K library screening
- Fail
  - Go back to optimization?
  - Select alternative assay?



Phase II Nuclear Receptor and Related qHTS Assays*

hAhR full length receptor in HepG2 cells
hAR full length receptor in MDA kb2 cells; partial receptor in HEK293 cells
hERα full length receptor in BG1 cells; partial receptor in HEK293 cells
hFXR partial receptor in HEK293 cells
hGR full length receptor in HeLa cells
hPPARδ partial receptor in HEK293 cells
hPPARγ partial receptor in HEK293 cells
hPXR full length receptor in HepG2 cells
hRORγ partial receptor in CHO cells
rTRβ full length receptor in GH3 cells; partial human receptor in HEK293 cells
hVDR partial receptor in HEK293 cells
Inhibition of aromatase using MCF-7 cells

NR assays conducted in agonist and antagonist modes

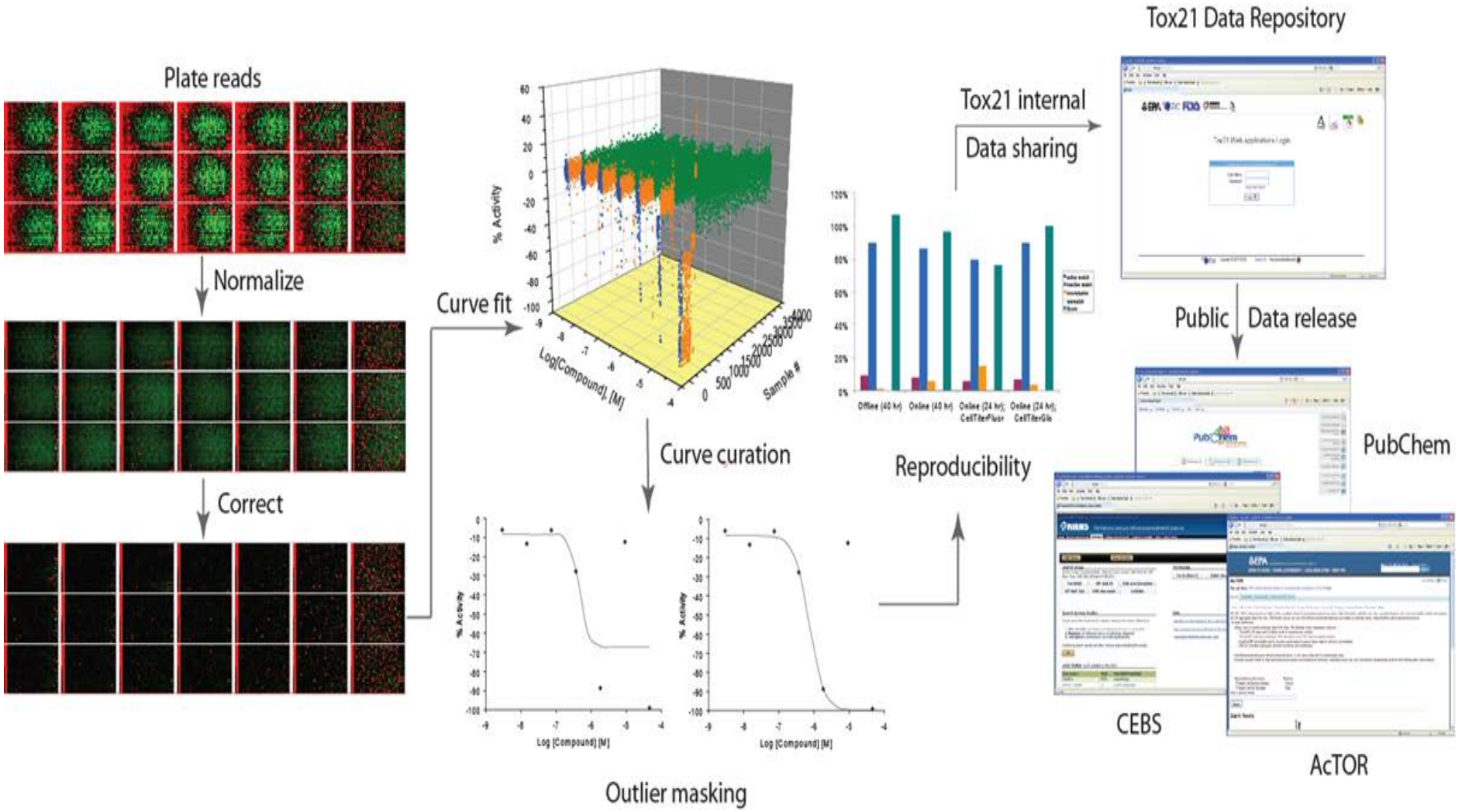
**Bolded text indicates completed assays*

Phase II Stress Response qHTS Assays

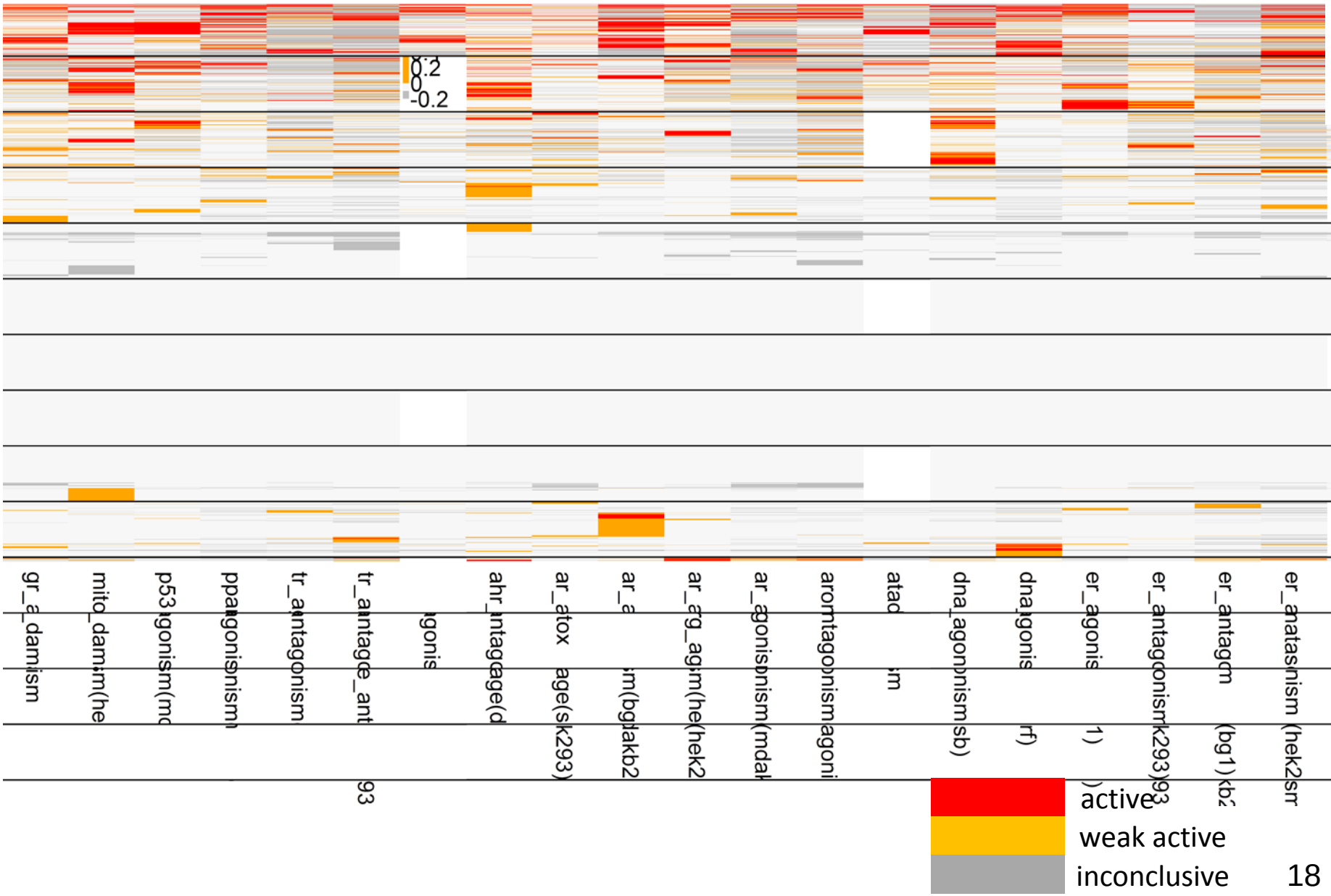
Oxidative stress	**ARE/Nrf2 in HepG2**
Genotoxic stress	**P53 activation in HCT-116 colon cancer cells**
	ATAD5 levels in HEK293 cells (ATPase family AAA domain-containing protein 5 – a DNA damage response element)
	DT40 (DNA-repair mutant isogenic chicken cell clones) (Rev3 (-/-), rad54/ku70 (-/-), wild type)
	pH2AX induction in CHO cells
Heat shock	Hsp70 in HeLa or HepG2 cells
ER stress	ESRE (lipid damage) in HeLa cells
Hypoxia	HRE (HIF-1α) in ME-180 cervical carcinoma cells
Inflammation	NFκB in ME-180 cells
Multiple stresses, cell death, specific toxicities	AP-1 activation in ME-180 or HepG2 cells
	Caspase 3/7 activation
	LDH release, **ATP levels**
	mitochondrial membrane potential in HepG2 cells
	hERG (ion channel effects) in U2OS cells (cardiotoxicity)

**Bolded text indicates completed assays*

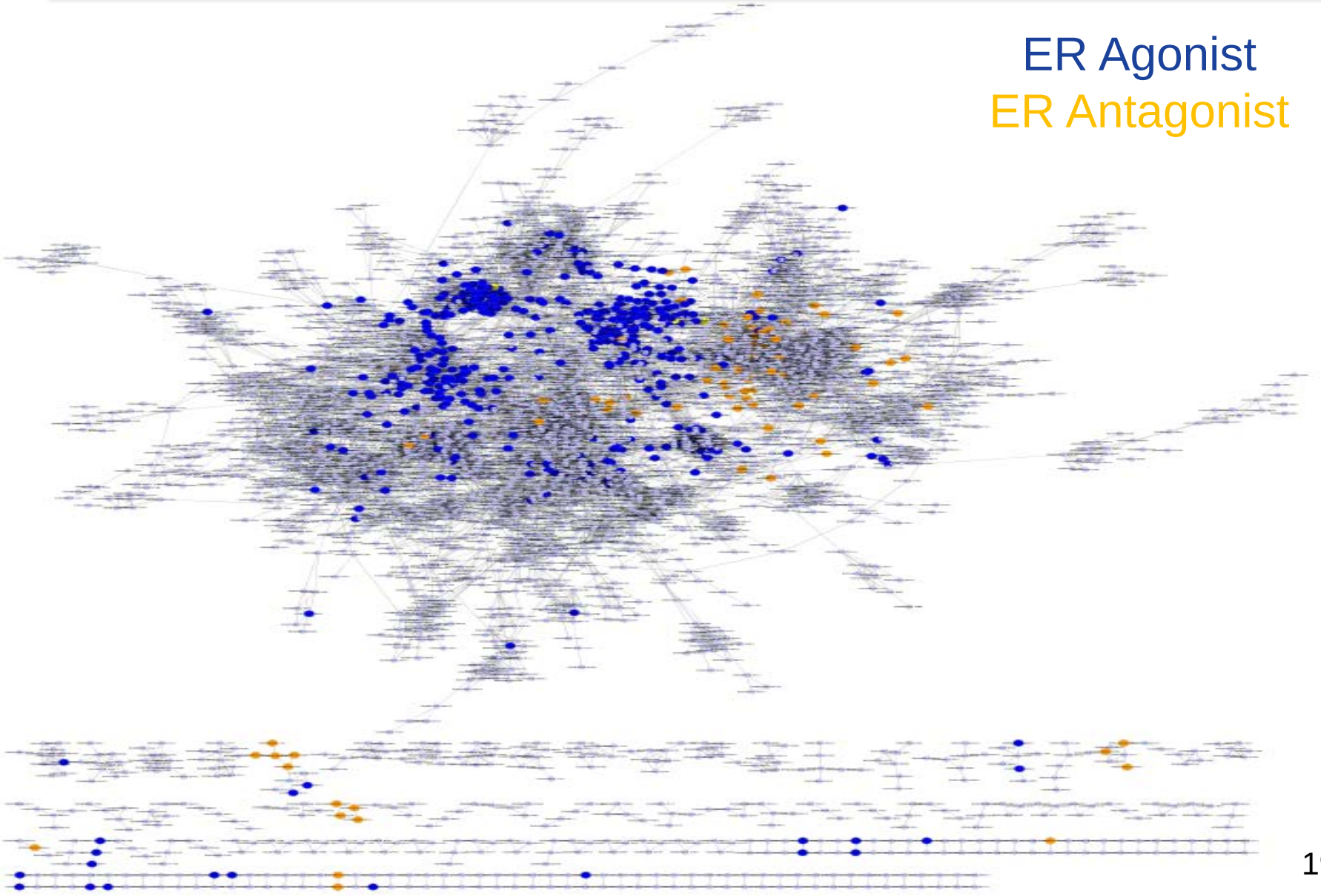
Tox21 Screening Informatics Analysis Process



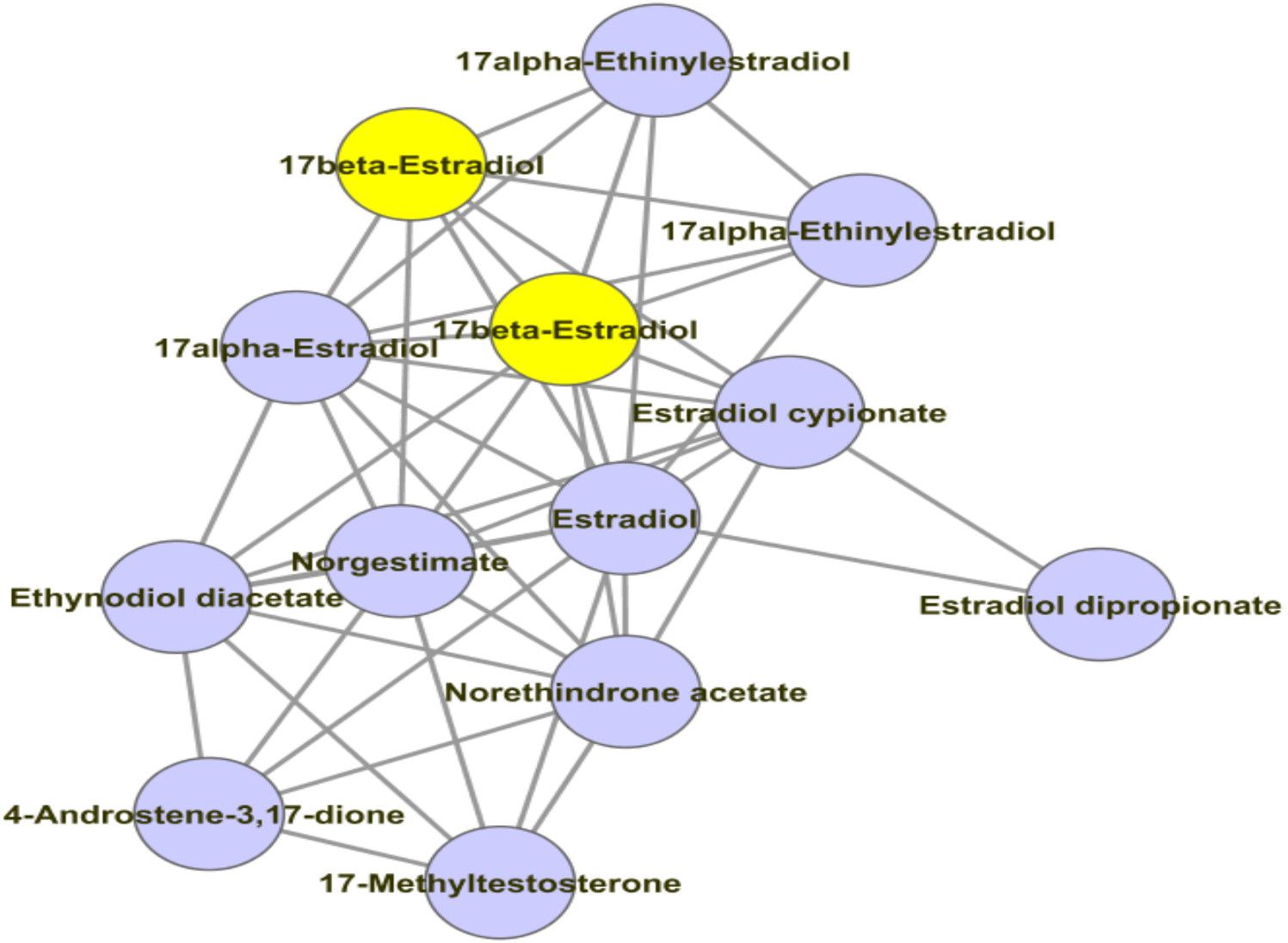
Tox21 10K Library qHTS Activity Profile



ER Actives (pAC50 with Pearson Correlation >0.7) – All Data Network



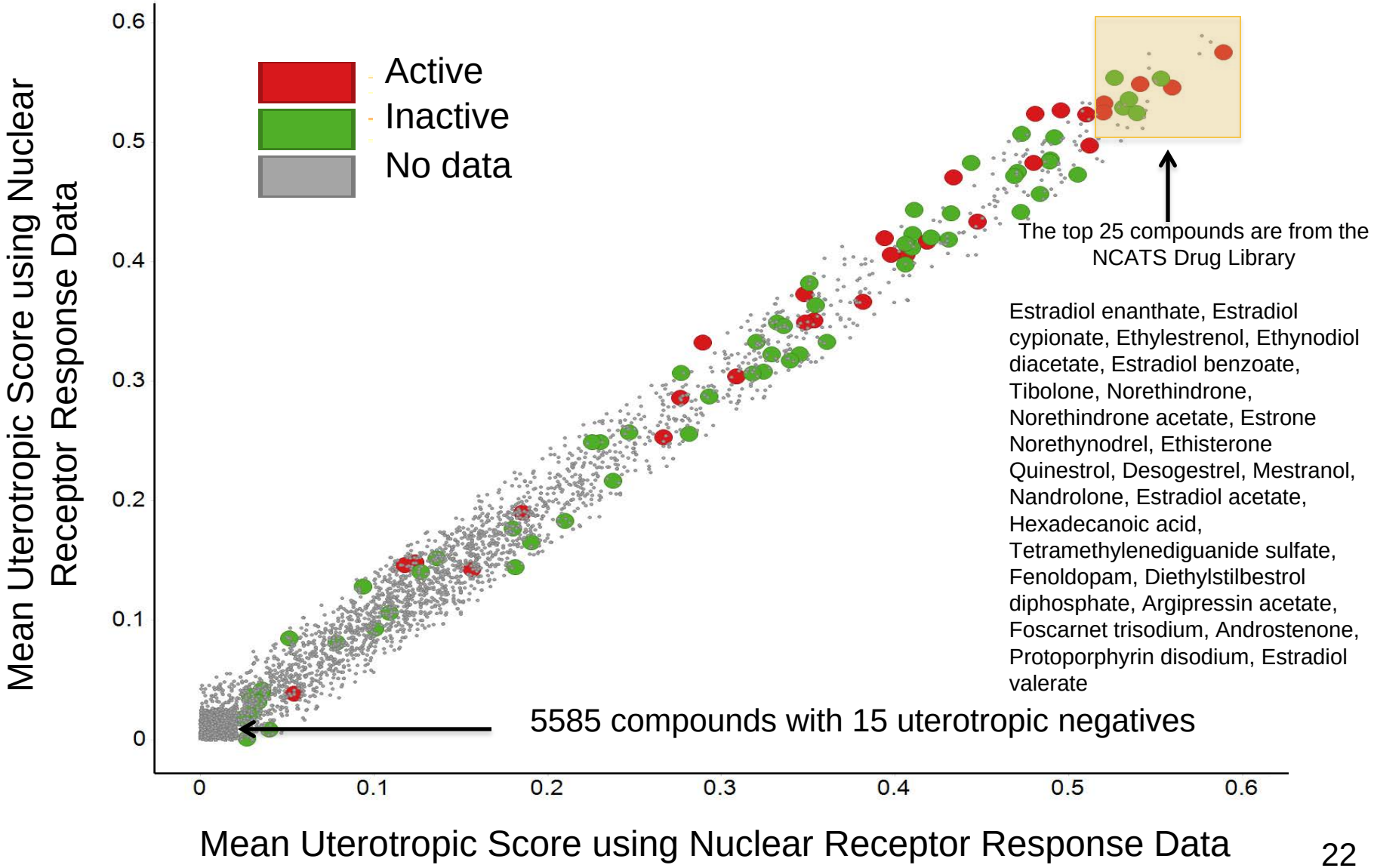
17β-Estradiol (Pearson >0.7) in All Data Network – Level 1



Identification of untested chemicals with increased likelihood of *in vivo* reproductive toxicity

- Identified 10 chemicals in NICEATM ER validation set that are positive in uterotropic assay
  - 17α-estradiol
  - 17α-ethynyl estradiol
  - bisphenol A
  - bisphenol B
  - daidzein
  - o,p'-DDT
  - dihydrotestosterone
  - estrone
  - genistein
  - 4-tert-octylphenol
- Determine mean correlation between each selected uterotropic positive and all other chemicals using all nuclear receptor data only
- Rank chemicals by mean correlation

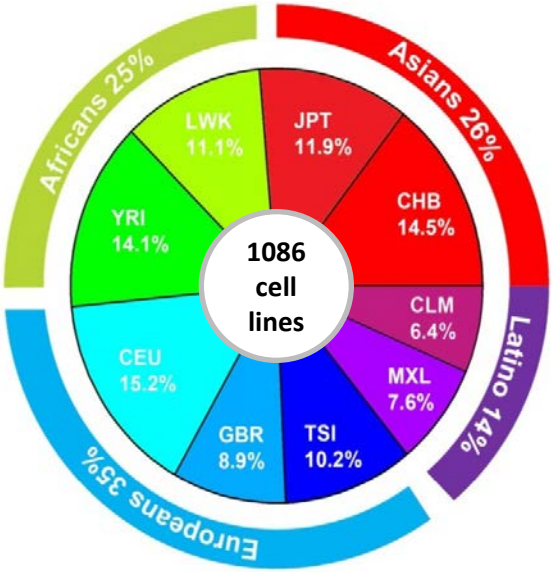
Identification of untested chemicals with increased likelihood of *in vivo* reproductive toxicity

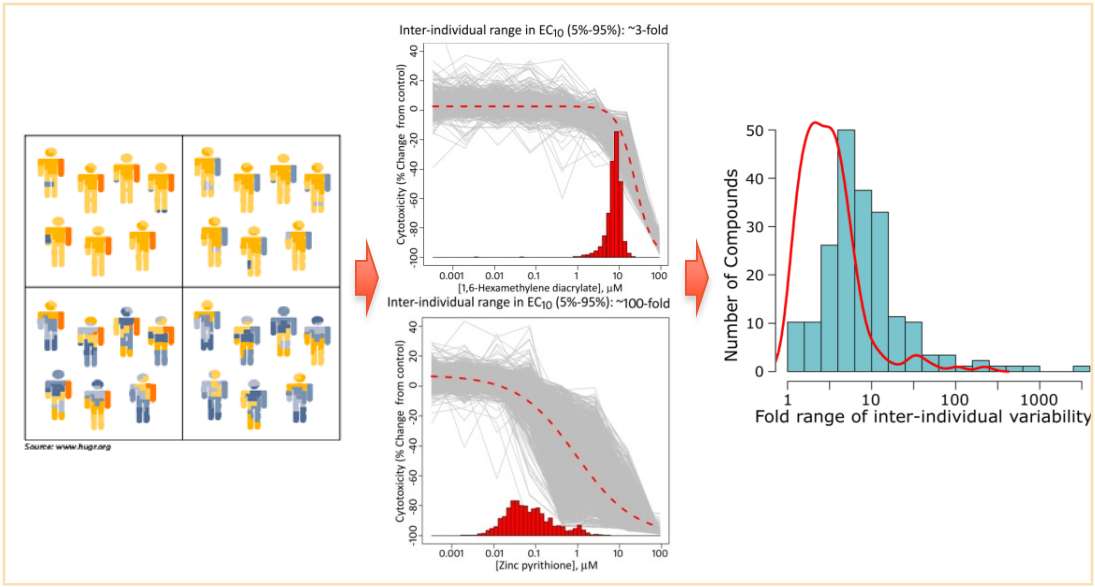


NIEHS-NCATS-UNC TOXICOGENETICS PROJECT:

qHTS for Cytotoxicity in a Population-Based *in vitro* Model

POPULATION-WIDE STUDY DESIGN:





- To understand how genetic variation affects individual response to common environmental and pharmaceutical chemicals
- The largest ever population-based ex-vivo cytotoxicity study
  - 1086 cell lines
  - 179 common, pharmaceutical, or important environmental chemicals (9 duplicates)
  - 8 concentrations (0.33 nM – 92 μM)
  - 1-3 plate replicates
  - ~2,400,000 data points + 2-5x10^6 SNPs

NIEHS-NCATS-UNC TOXICOGENETICS PROJECT:
qHTS for Cytotoxicity in a Population-Based *in vitro* Model

Goal: use crowdsourcing to better predict the toxicity of chemicals

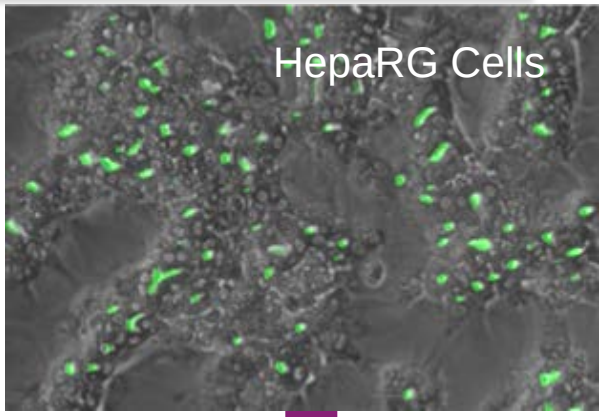
1. Use the biological data (SNPs, basal gene expression) to develop a model that accurately predicts ***individual responses*** to compound exposure
2. Use the intrinsic chemical properties to develop a model that accurately predicts how a particular ***population*** will respond to ***certain types of chemicals***

Tox21 Phase II Limitations

- Extent of pathway coverage
- Focus on the use of reporter gene assays using immortal cell lines
- Extent of chemical coverage
- Focus on single compounds
- Limited capability for xenobiotic metabolism
- Focus on simple biological systems
- Limited to acute exposure scenarios
- Limited availability of “big” data analysis tools

Tox21 Phase III – Improving on Biological Coverage and Relevance (2013 - ?)

- Focus on high content assays and high throughput transcriptomics platforms using:
  - cells capable of hepatic metabolism
  - ES/iPSC derived differentiated cell populations (e.g., cardiomyocytes, neurocytes, hepatocytes) from human and mouse representing healthy and disease models.
- Increased use of *in silico* models (e.g., metabolite prediction) and extrapolation models (e.g., reverse toxicokinetics)
- Expanded utilization of lower organism model systems (zebrafish, *C. elegans)*
- Use of 3D tissue models
- Integrate AOP concept into Tox21
- Expand collaborations and interactions



Targeted Assays

•High Content screening
- Hoechst: Cell loss & nuclear size
- DHE: Oxidative stress/ROS
- p53: DNA damage
- pH2A.X: Genotoxicity
- JC-10: Mitochondrial damage (MMP)
- Caspase 3: Apoptosis
- Lipitox: Steatosis & Phospholipidosis
- Reactive metabolites/ROS: GSH depletion

•Receptor Activation via Induction of gene expression
- AhR, CAR, PXR, PPARα, FXR

•Necrosis
- miR-122 leakage or LDH leakage

High Throughput Transcriptomics Workshop On Gene Prioritization Criteria

September 16-17, 2013
National Institute of Environmental Health Sciences

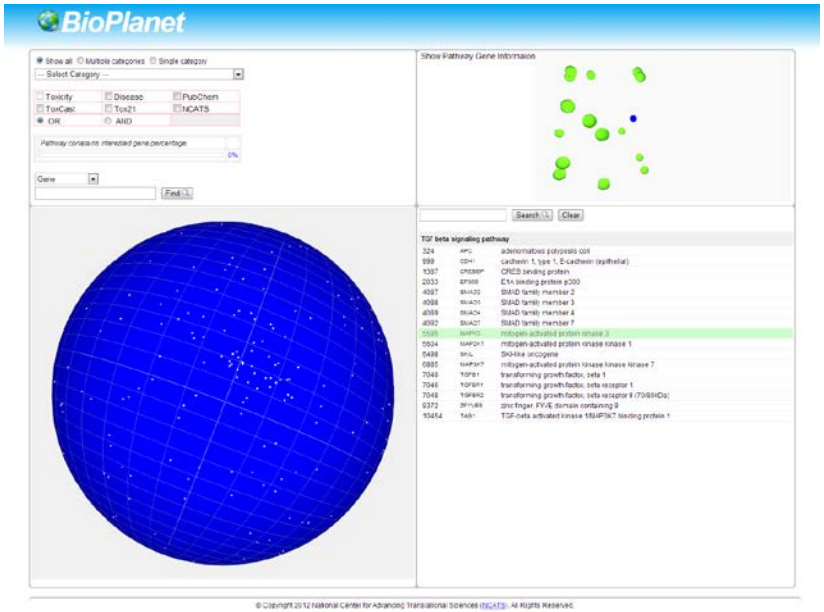
29 July 2013 Federal Register Request for Information

- The nomination and prioritization of ~1000 environmentally responsive genes per species for use in screening large numbers of substances in cells or tissues from human, rat, mouse, zebrafish, and *C. elegans*, using high throughput toxicogenomic technologies.

- Recommendations on criteria to use for prioritizing the genes that potentially would be the most useful in a screening paradigm, with a focus on effects that reflect general cellular responses, independent of cell type, and gene expression changes that are specific by cell type.

The NCATS BioPlanet: the universe of biological pathways for assay selection and prioritization

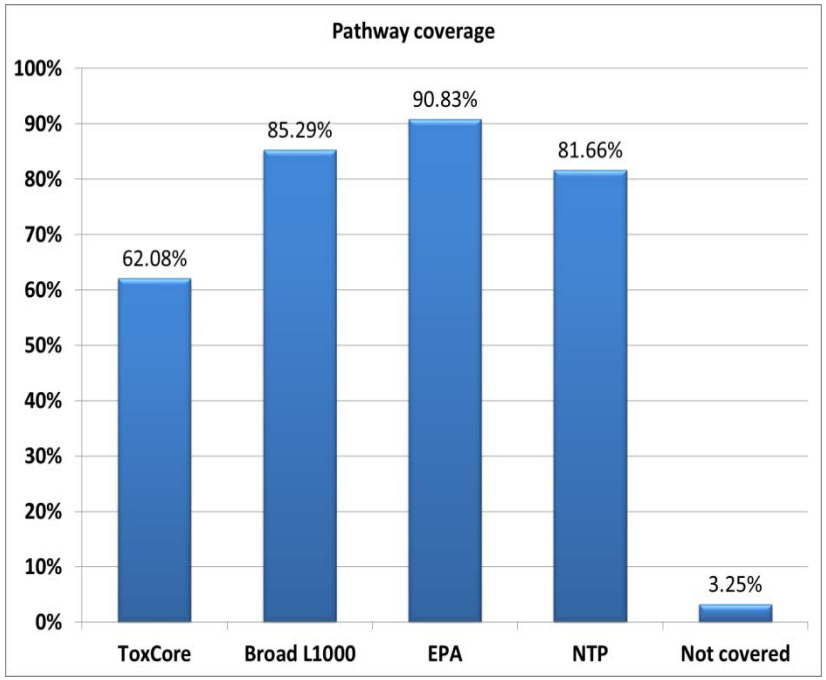
- Hosts the universe of human pathways (~1100 unique)
- All pathway annotations from manually curated, public sources (e.g., KEGG, WikiPathways, Reactome, Science Signaling)
- Integrates pathways from >10 different data sources



- Annotates pathways by source, biological function/process, disease/toxicity relevance, assay availability
- Easy visualization, browsing, analysis of pathways
- Facilitates pathway assay selection/prioritization for Tox21 production phase
- Web version in process for public release

From Ruili Huang, NCATS

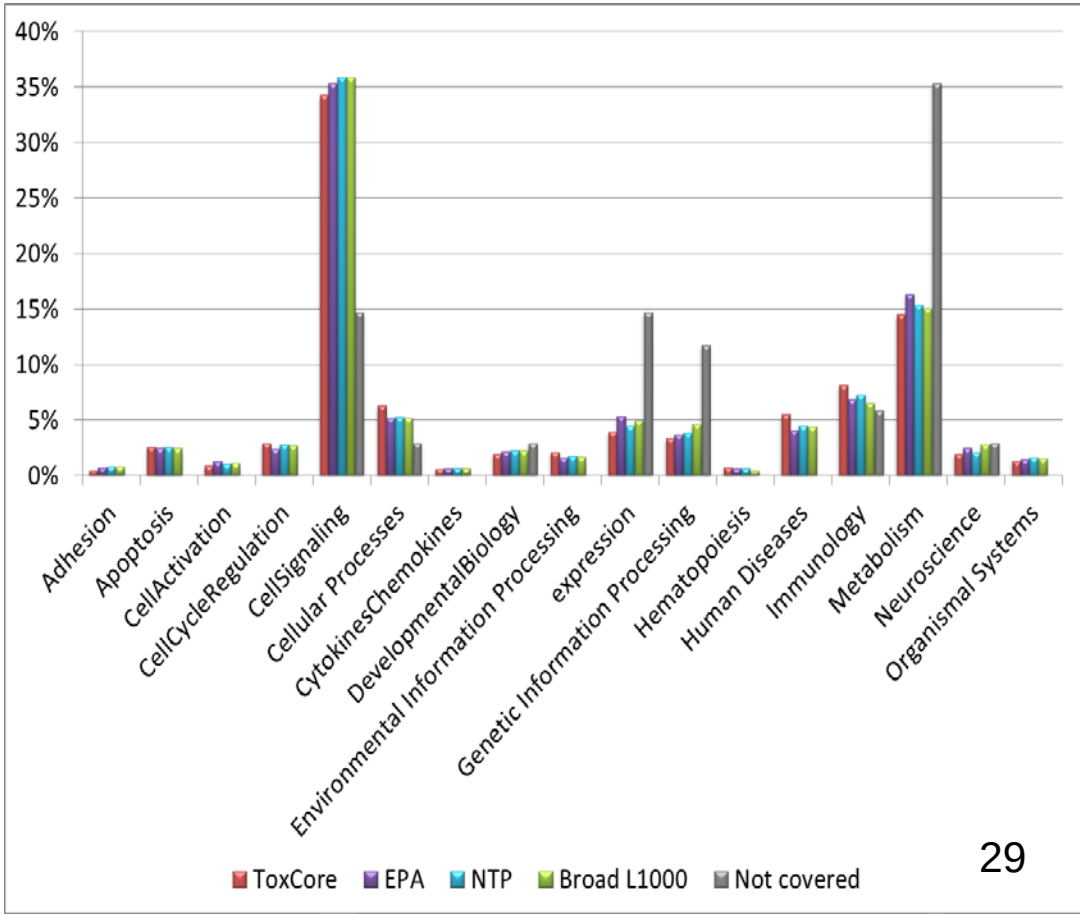
Pathway coverage by proposed gene lists



- NCATS 320: 62%
- EPA 1000: 91%
- NTP 1000: 82%
- Broad L1000: 85%
- Not covered by any: 3%

From Ruili Huang, NCATS

Pathway category coverage



In Silico Xenobiotic Metabolism Approaches to Bin the 10k Library

Identify Practicable Approaches

- Enzyme Substrate Predictions
- Metabolite Structure Predictions
- Extent of Metabolism Predictions

Assess Predictivity of Approaches

- Identify/Assess established substrates
- Do we predict true metabolites
- Do models predict extent of high, moderate, low turnover compounds

Analyze Tox21 10k Library

- P450 & UGT Substrate Predictions
- Metabolite Structure Predictions
- CL_{INT} & Combined CL_{INT}

Bin 10k Library

- Predicted metabolite chemical structure alerts
- Extent of metabolism predictions

Identify Subset(s) of Chemicals to Study in Metabolically Competent Systems

- HepaRG cells
- Primary Hepatocytes
- Emerging models

Substrate Predictions

- 9 P450s & 9 UGTs
- Avg. Accuracy 88.3% with established substrates

Metabolite Structure Predictions

- 9 P450s
- Multiple levels/rounds of metabolism
- Predicted metabolites

Extent of Metabolism Predictions

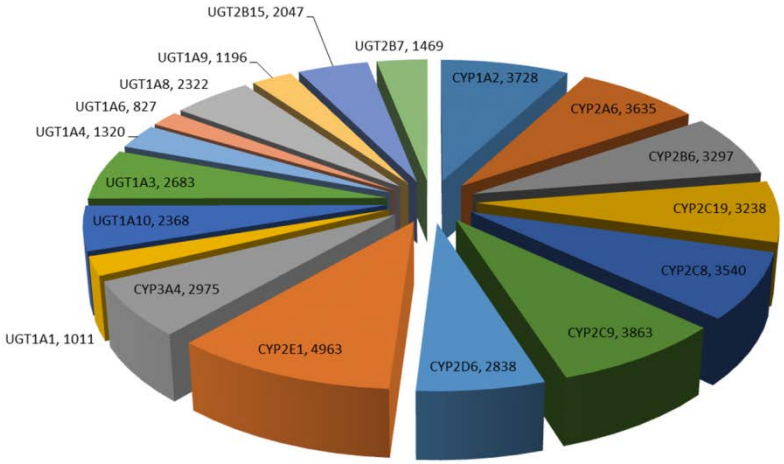
- CL_{INT} predictions 5 P450s

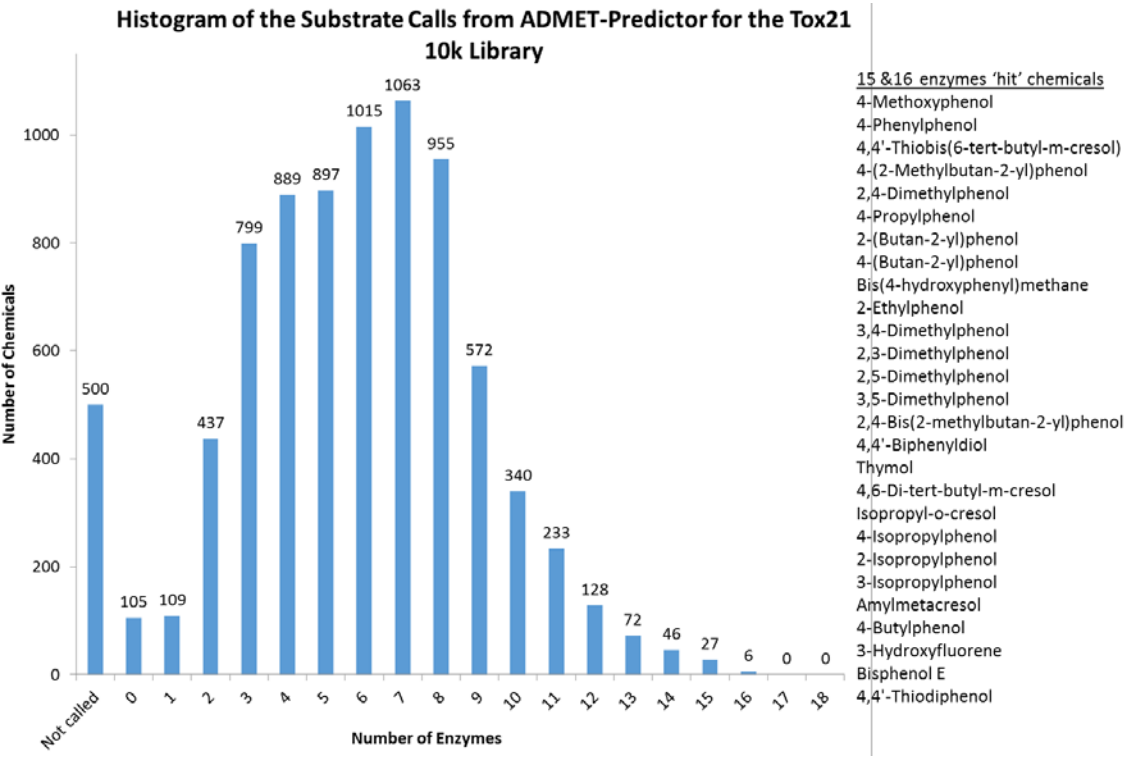
Toxicity Module

- 22 QSAR models for various toxicity endpoints (e.g., estrogenic, mutagenic)
- Analyzing 10k library and predicted metabolite structures

10k LibraryXenobiotic Metabolism Predictions

Substrate Calls

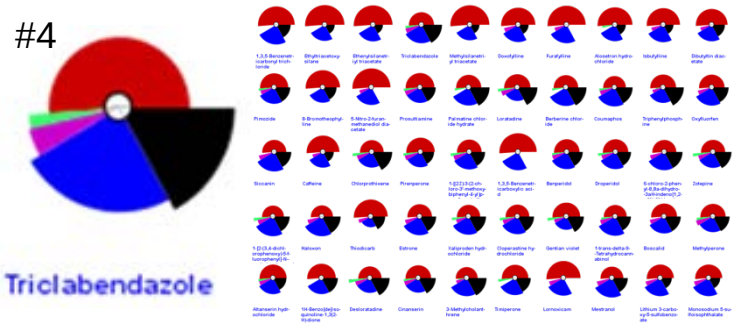




15 &16 enzymes 'hit' chemicals
4-Methoxyphenol
4-Phenylphenol
4,4'-Thiobis(6-tert-butyl-m-cresol)
4-(2-Methylbutan-2-yl)phenol
2,4-Dimethylphenol
4-Propylphenol
2-(Butan-2-yl)phenol
4-(Butan-2-yl)phenol
Bis(4-hydroxyphenyl)methane
2-Ethylphenol
3,4-Dimethylphenol
2,3-Dimethylphenol
2,5-Dimethylphenol
3,5-Dimethylphenol
2,4-Bis(2-methylbutan-2-yl)phenol
4,4'-Biphenyldiol
Thymol
4,6-Di-tert-butyl-m-cresol
Isopropyl-o-cresol
4-Isopropylphenol
2-Isopropylphenol
3-Isopropylphenol
Amylmetacresol
4-Butylphenol
3-Hydroxyfluorene
Bisphenol E
4,4'-Thiodiphenol

Extent of Metabolism Predictions

- CL_{INT} predicted for 5 individual P450s
- Combined CL_{INT} from 5 Enzymes in ToxPi
  - Weighted each enzyme CL_{INT} contribution by relative P450 expression levels



- **168,805 unique metabolites predicted**
- Evaluating ability to predict known metabolites in library
- Assessing 10k library & 169k metabolites with various toxicity prediction models

Transcriptomics Efforts

NTP DrugMatrix Rat Database*

- Integrated Collection of Data
  - 637 unique chemicals (mostly drugs)
  - 5600 drug-treatment transcript profiles in rat organs
  - 127,000 histopathology measurements
  - 100,000 blood chemistry measurements
  - 60,000 literature facts
- Over 500 validated signatures
  - Mode of action and pathology
- Comprehensive data mining
  - Formulate 100,000's questions (phenotypes)
  - Test for ability to classify using transcript data only
- ~122,000 frozen tissues
- Automated genomics analysis

NTP Archives

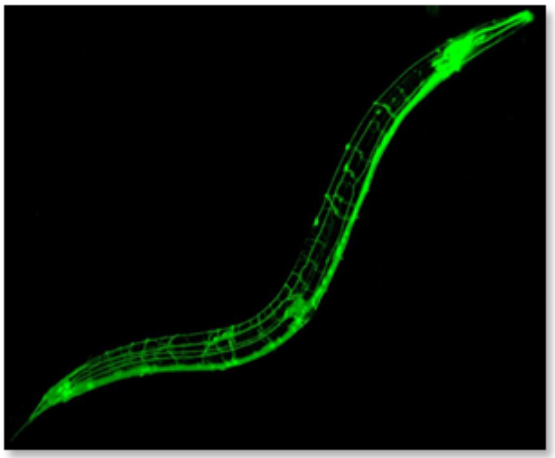
- 250,000 frozen tissues
- 5 million formalin fixed, paraffin embedded tissues
- Chemical-phenotype known
- Additional pathological insight and predictive power to histopath/clin chem
- Retrospective comparison across NTP studies for molecular markers

*Drugmatrix website: https://ntp.niehs.nih.gov/drugmatrix/

*ToxFx website: https://ntp.niehs.nih.gov/toxfx/

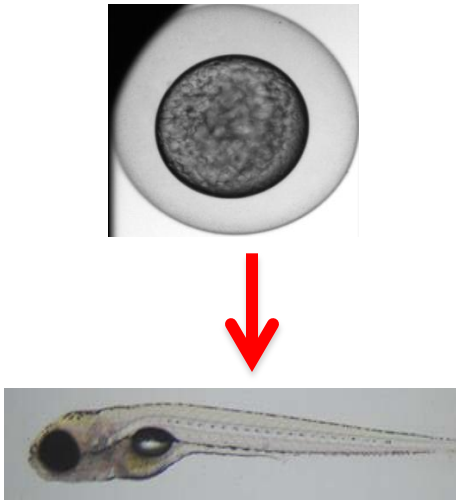
Alternative Organisms – *C. elegans* and Zebrafish

C. elegans (NIEHS/NTP) - J. Freedman/W. Boyd



- Screened ToxCast Phase II compounds in growth assay
- Screening subsets of compounds in assays that measure
  - feeding
  - larval lethality
  - reproduction

Zebrafish – R. Tanguay (Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, OR)



- Screened Toxcast Phase II compounds
- Screening 3455 NTP compounds at ~ 64 µM
- Assays include
  - 1 day photo induced behavior
  - 1 day assessment of mortality/developmental progression
  - 5 day photo motor response
  - 5 day assessment of 20 morphological endpoints

Stem Cell Related Projects

- Collaboration with Cellular Dynamics and Molecular Devices to screen 80 compounds (focus on neurotoxicants, cardiotoxicants, mitochondrial toxicants) in:
  - Neurite outgrowth assay/mitochondrial membrane potential
  - Beating cardiomyocyte assay/mitochondrial membrane potential
- Collaboration with QPS, PhoenixSongs Biologicals, & the Hamner Institutes to evaluate biological activity of the same 80 compounds in various human and rat neuronal cell culture systems (e.g., primary, embryonic stem cell–derived, induced pluripotent stem cell-derived, transformed neural cell lines)
- Collaboration with XCell to characterize response of iPSC-derived neural populations (e.g., dopaminergic) from Parkinson's disease (familial & sporadic) to mitochondrial/neuro toxicants
- *In vitro* genetics with ES cell lines from Diversity Outbred Mice (Ted Choi, Predictive Biology, Inc.)

Novel Assays for Screening the Effects of Chemical Toxicants on Cell Differentiation RFA-ES-13-003

- RFA supports the development of assays that can be adapted to a high-throughput format to evaluate the effects of toxicants on cell differentiation using multi-potent or pluripotent cells.
- Could entail developing assays to detect changes in differentiation into specific lineages and/or molecular changes in the differentiated cells
- Applicants could develop assays using human or mouse ES or iPS cells
- Introduce genetic diversity into tox testing
- Could engineer stem cell lines to incorporate common genetic variants associated with diseases
- 11 SBIR Phase I Awarded (e.g., neuro differentiation, cardiomyocyte, hematopoietic, epigenomics)

Milestones Reached

- Successfully characterized the qHTS data structure and identified the artifacts that lead to false results
- Made progress in data analysis and in the development of tools for prioritization
- Made all ToxCast and Tox21 Phase I data public
- Making chemical libraries available to investigators to expand the breadth of toxicological information
- Exchanging assays and data with other organizations/efforts (e.g., EU Joint Research Centre, Health Canada, Seurat, OpenTox)
- Working with NICEATM and ICCVAM to evaluate the utility of Tox21 assay data for use by regulatory agencies

Tox21/ToxCast Data Release Activities

Tox21 10K library data

•Phase II data being released in PubChem, CEBS, ACToR (34 assay data available currently, all data collected to date to be released in 2013)

ToxCast Phase II data (More information: http://epa.gov/ncct/workshops)

•Oct 15 – Release of Phase II data and launch of **iCSS (I**nteractive **C**hemical **S**afety for **S**ustainability) Web Application

•Late October: Public webinar (EPA Communities of Practice)

•Dec. 2-3: Stakeholder Workshop in Potomac Yards

•January 14, 2014: Stakeholder workshop in RTP

•May 13-14, 1014: Data Summit

Success depends on

- Robust scientific collaborations
- Well-characterized chemical libraries
- Well-characterized assays in terms of reliability and relevance, with broad biological coverage
- Incorporating xenobiotic metabolism into *in vitro* assays
- Informatic pipelines/tools that integrate and mine diverse data streams
- Understanding the relationships between pathways and disease in humans and animal models
- Making all data public
- Outreach to the scientific community on the usefulness and limitations of Tox21 data

What will success bring?

- Test methods for toxicity testing that are scientifically sound and more economically efficient
- An increased ability to evaluate the large numbers of chemicals that currently lack adequate toxicological evaluation
- Models for risk assessment that are more mechanistically based
- Reduction and/or replacement of animals in regulatory testing

Discussion Question

The potential utility for regulatory purposes of the collective data generated through the Tox21 program is just beginning to be appreciated. Traditional notions of validation appear insufficient or inappropriate in many respects for dealing with this new data stream. Please provide advice and comment to assist ICCVAM in designing an appropriate approach to evaluate the information generated by these new technologies.