NIEHS National Toxicology Program Tox21 Update

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Acting Chief, Biomolecular Screening Branch

ICCVAM Public Forum
NIH Natcher Conference Room Center, Room D
Bethesda, MD

25 May 2016
Tox21 qHTS Phase II:

• 10K compound library (8,948 unique; 13,129 unique solution IDs), ~ 3,000 each from EPA, NTP, NCATS (drugs)

• Screened 3 times at 15 concentrations in each “quantitative High Throughput Screen” (qHTS) assay

• qHTS assays (~ 60 assays) focused on:
  — Nuclear receptor activation or inhibition
  — Cellular stress response pathways and cytotoxicity
Tox21 Public Outreach

Multiple workshops, webinars, meeting presentations, reports, publications, and websites for distribution of information rapidly and transparently to stakeholders

Data Released to Public as Rapidly as Possible
- via EPA Safer Chemicals Research website
  [https://www.epa.gov/chemical-research/research-evaluating-chemicals-adverse-effects](https://www.epa.gov/chemical-research/research-evaluating-chemicals-adverse-effects)
- via Tox21 Data Browser ([https://tripod.nih.gov/tox21/index](https://tripod.nih.gov/tox21/index))

Transparent Data Processing and Analysis Pipeline
- **Pay Attention to Warning Flags – Compounds & Calls**
Quality Control of Tox21 Compounds

MW Confirmed, Purity > 90%
<table>
<thead>
<tr>
<th>Structure</th>
<th>Tox21 ID</th>
<th>Name</th>
<th>QC Grade T0</th>
<th>QC Grade T4</th>
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<tr>
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</tbody>
</table>
Tox21 Phase III

Improving on Biological Coverage and Human Relevance

- Increased use of computational models to predict toxicity and metabolism.
- Increased focus on human cells with known ability to metabolize chemicals.
- Increased focus on genetic variation to understand susceptibilities - Toxicogenetics.
- Increased use of stem cells (both embryonic and iPS) to investigate the effects of chemicals on developmental processes.
- Enhanced testing of compounds in lower organisms (e.g. zebrafish, C. elegans).
- Expand our understanding of biology by developing and implementing a high throughput and low cost approach to measure the entire transcriptome – HT-Transcriptomics.
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Human HepaRG Polarized Spheroids

B-catenin

Nuclei

Merge

MRP2

Nuclei

Merge

Sreenivasa Ramaiahgari and Steve Ferguson, NTP 100 um
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Distribute sets of reference compounds
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Paules_2016
SEAZIT – Systematic Evaluation of the Application of Zebrafish in Toxicology

• A multipronged, multi-year program for the systematic evaluation of the application of zebrafish in toxicology (SEAZIT) studies by the DNTP.

• Aims:

  – to provide the scientific basis on which to make a programmatic decision on the further use of zebrafish in toxicological screening of chemicals to which humans are exposed during development and into adulthood.

  – provide fundamental knowledge on the use of zebrafish in toxicology, which will support further research endeavors by the academic community.

Contact: Nigel Walker, NIEHS/DNTP
Aims (cont.):

- Determine the influence of various protocol parameters on distribution within the zebrafish during development and into adulthood.
- Assess the developmental origins of health and disease in zebrafish.
- Determine optimal methods for evaluating zebrafish data to link it to phenotypic outcomes in mammals.
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- Expand our understanding of biology by developing and implementing a high throughput and low cost approach to measure the entire transcriptome – *HT Transcriptomics*.
Tox21 HT Transcriptomics

The Assumption:

- Global “Omic” (Whole System) approaches can link perturbations with alterations in biological processes that result in toxicity and/or disease.

The Hypothesis:

- Alterations in the transcriptome following exposures can provide:
  - linkage between chemical exposures and adverse biological outcomes (signatures/biomarkers),
  - linkage between *in vitro* and *in vivo* model systems, and
  - linkage between *in vitro* models & human pathobiology
The Ideal Solution:
A rapid and low-cost High Throughput (HT) method to measure expression levels of ALL GENES

The Reality:
• At this time, whole transcriptome technologies are prohibitively expensive for HT applications.
  - Target cost of $25 - $50 per sample.
• It will be necessary to focus on a subset of genes to use in a rapid, low-cost technology suitable for HT studies.
Attributes of a Tox21 S1500+ Gene Set

1. **Diversity**: Capture the maximal expression variability and dynamics.

2. **Co-Expression**: Capture the Sentinel genes with maximal co-expression information to represent members of nodes or networks.

3. **Maximal Pathway Coverage**: Genes are included to ensure maximal biological pathway coverage.
   
   *1500 Sentinel Genes Bioinformatically Selected – S1500*

4. **Inclusion of toxicity and disease related genes**: Specific genes will be selected for their reported roles in toxicity-related and disease-related processes. Also include L1000 gene set from LINCS program.

5. **“Extrapolatability”**: This property refers to the ability to extrapolate or infer or impute with some accuracy the expression changes in all genes from those observed in this reduced set of sentinel genes.
Where Are We Now?

Develop Approach & Train on Rat Data Sets – Complete

- Pathway Concordance between Extrapolated & Measured Data ~ 0.9

Generate Human “S1500+” Gene Set –

- Affymetrix microarray human data sets (HG-U133plus2) were downloaded from GEO and 3339 series were manually curated.

- Human gene expression data sets that passed curation were subjected to our hybrid bioinformatic gene selection process

- **1500 gene set** identified bioinformatically in a strictly data-driven approach

- Additional genes were added from knowledge-based, nominated gene lists for a total of **2871 genes**

Hosts the universe of public, curated human pathways (2314 Pathways)
Where Are We Going with HT Transcriptomics?

**Short Term**
Application to Cell Systems used in Tox21 Phase II Assays with a subset of the 10k set of chemicals
Application to Metabolically-Competent Human Cells (e.g. primary hepatocytes, 3D HepaRG spheroids, organoids)
Application to Human iPS cells undifferentiated and induced to differentiate along specific lineages

**Mid Term**
Application to other species (rat, mouse, zebrafish, etc.)
Application to NTP archived material from rat and mouse studies

**Longer Term**
Application of HT Transcriptomics to Human samples from molecular epidemiological studies and clinical studies
Tox21: A Collaboration of Many …

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