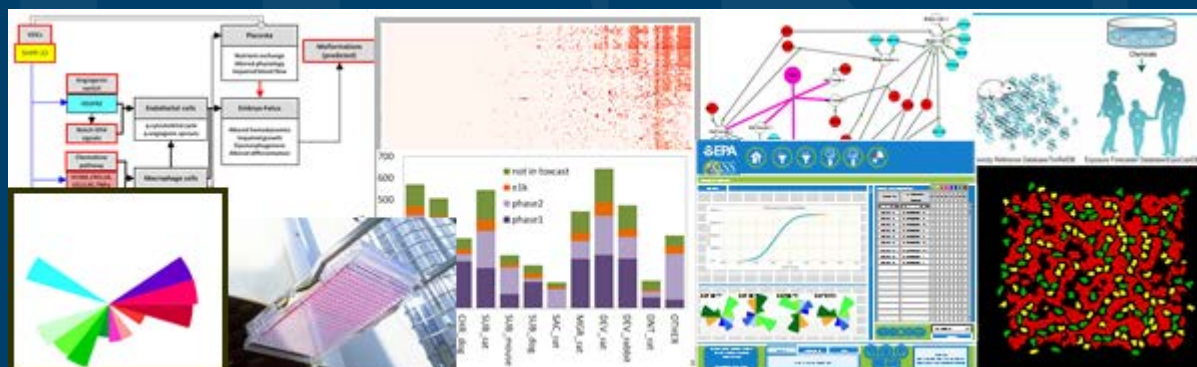


EPA Activities for Integrating Genome Technology into High-Throughput Chemical Screening



GlobalChem
February 28, 2018

Rusty Thomas
Director
National Center for Computational Toxicology

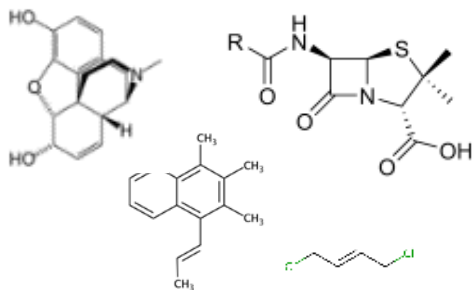
What Do Grandmothers and Toxicologists Have in Common?



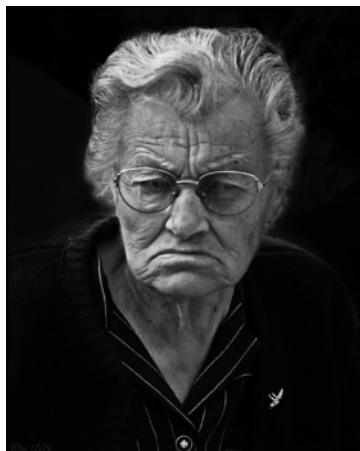
<http://talesfromthecircus.com/hissing-grandmother-roaming-hands/>

Push To Use Alternative Methods Comes From Multiple Drivers

Number of Chemicals /Combinations



Can We Satisfy
Grandma Too?



<http://talesfromthecircus.com/hissing-grandmother-roaming-hands/>

Legislative Mandates

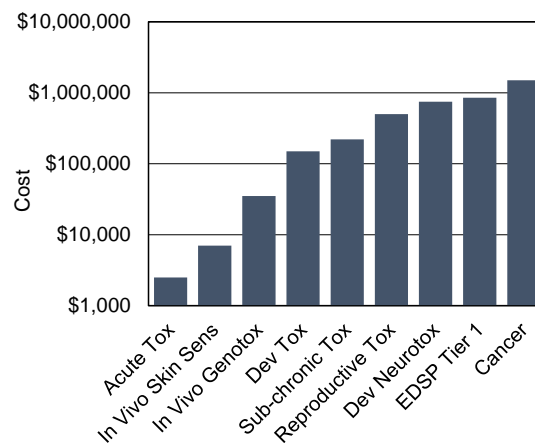


Ethics Concerns

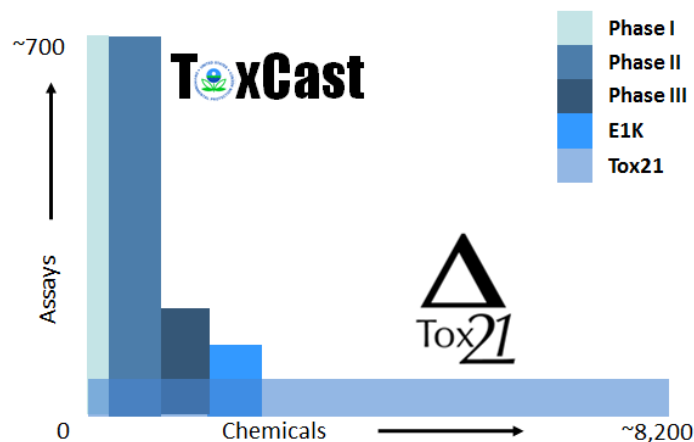


<http://www.victorpest.com>

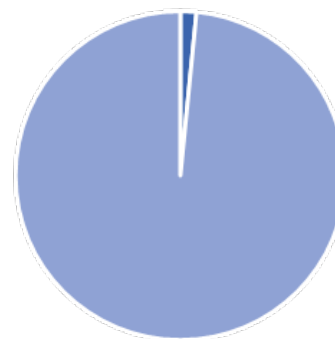
Economics



High-Throughput Screening Efforts Attempt to Address Worries, but...

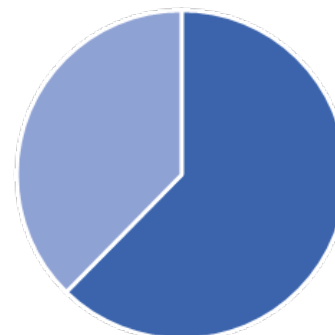


Gene Coverage



■ ToxCast
■ Not in ToxCast

Pathway Coverage*



*At least one gene from pathway represented

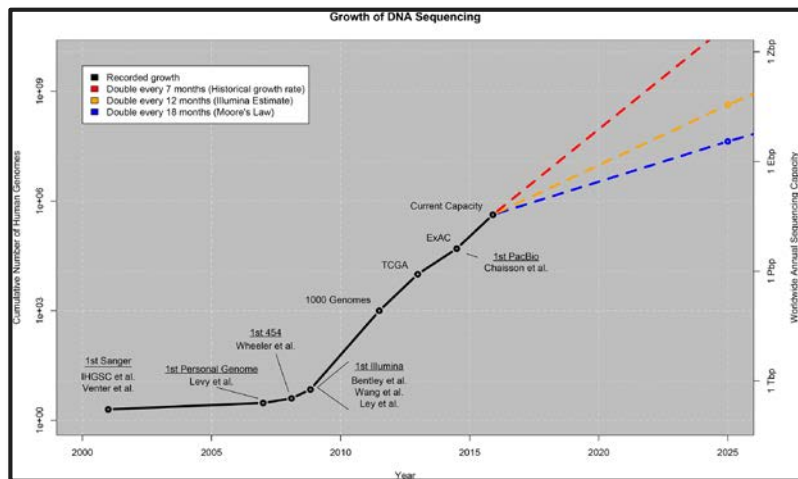
Using Technology from Genome Revolution to Increase Coverage



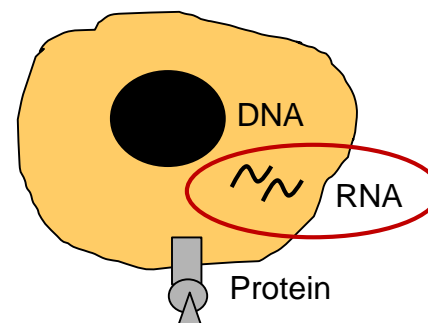
April, 2003



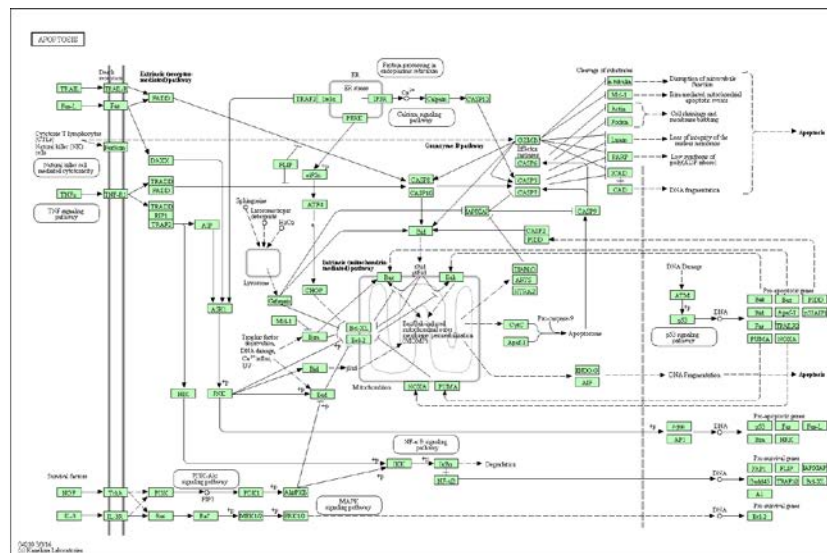
Dec, 2012



<https://doi.org/10.1371/journal.pbio.1002195>

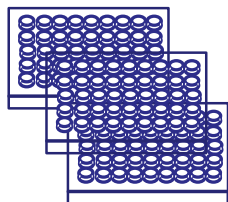


Pathway

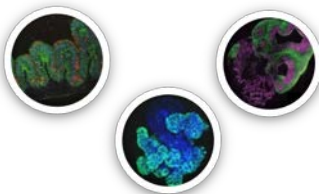


KEGG Pathway - hsa04210

Developing a High-Throughput Transcriptomic Toolbox



Thousands of
Chemicals



Multiple Cell
Types

High-Throughput Transcriptomic Screening Platform

- Low cost, 384-well, cell lysate compatible
- Whole transcriptome
- Workflow integration of reference materials and controls, development of performance standards
- Portable platform/workflow for collaborative data generation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	MAQC-A (Us)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	non-treated
B	MAQC-A (Us)	10	10	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	non-treated
C	MAQC-B (Us)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	non-treated

Reference RNAs

Bulk Lysates

IN PROGRESS

- Completed first large scale screen of 2,200 chemicals (ToxCast I/II/III) in single cell type
- Additional screens across multiple cell types/lines
- Tox21 cross-partner project to add reference chemical database

T

characterized cells

- Concentration resp
- Parallel imaging/cy
- RNA and lysate reference materials
- Positive and negative controls

UHRR

HBRR

BL_DMSO

BL_TSA

Sample_Type

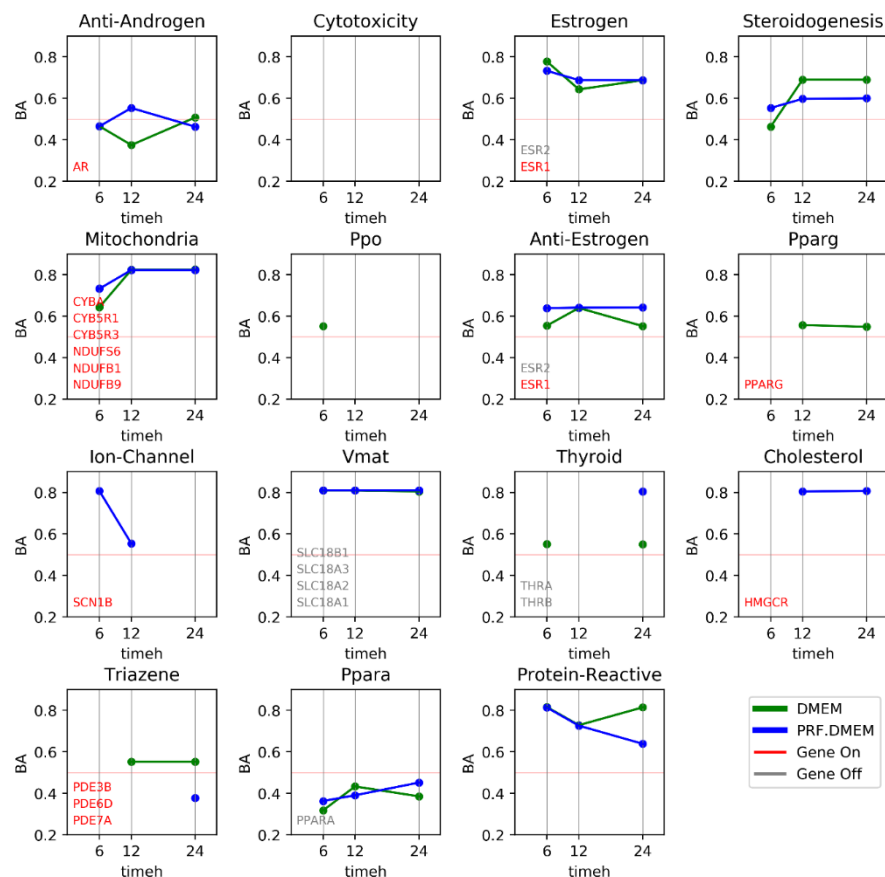
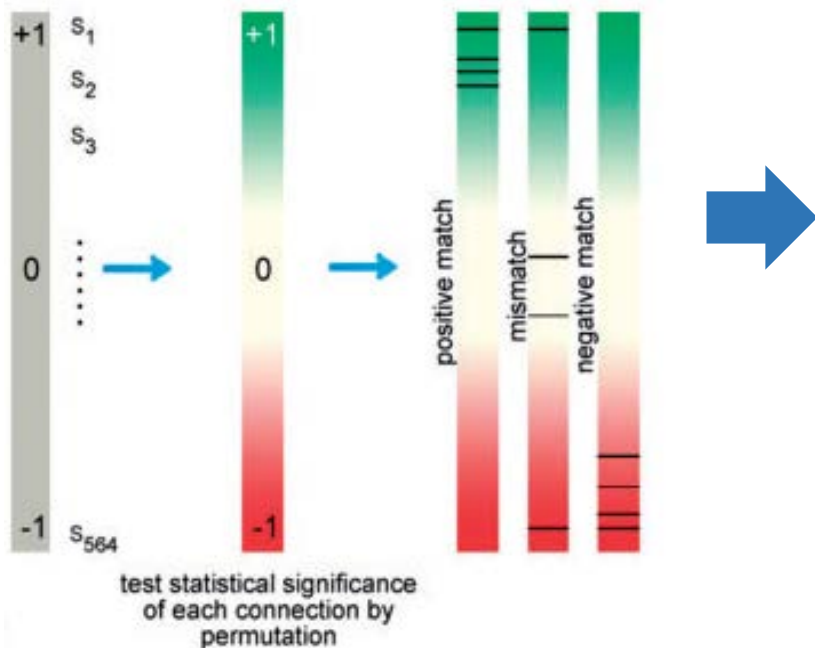
Sample_Type

Av Corr >0.9

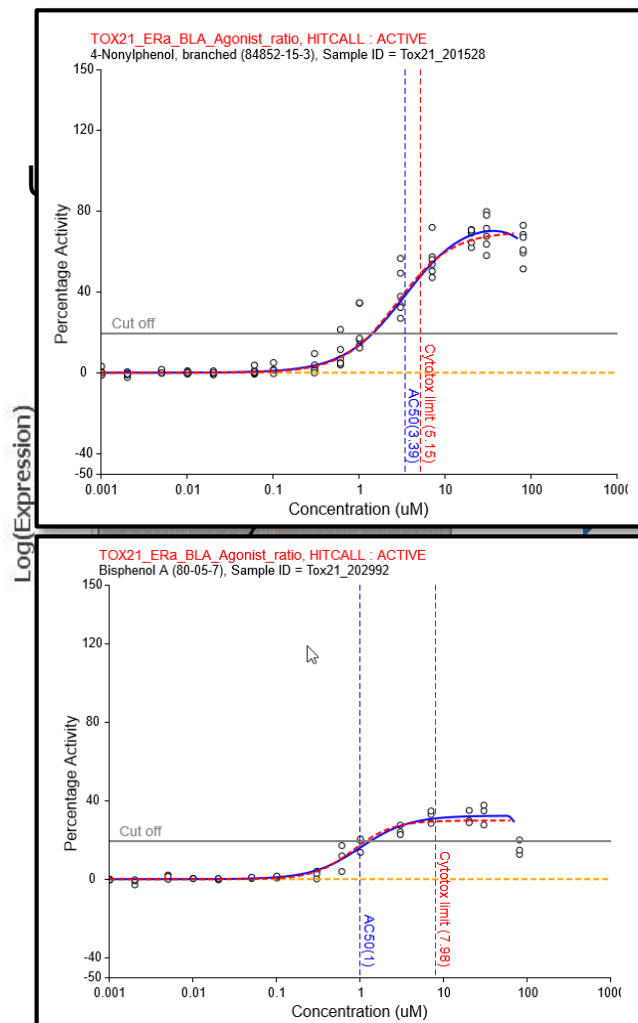
Randomized
treatment

Developing a High-Throughput Transcriptomic Toolbox

Using Connectivity Map to Identify Potential MOAs/MIEs



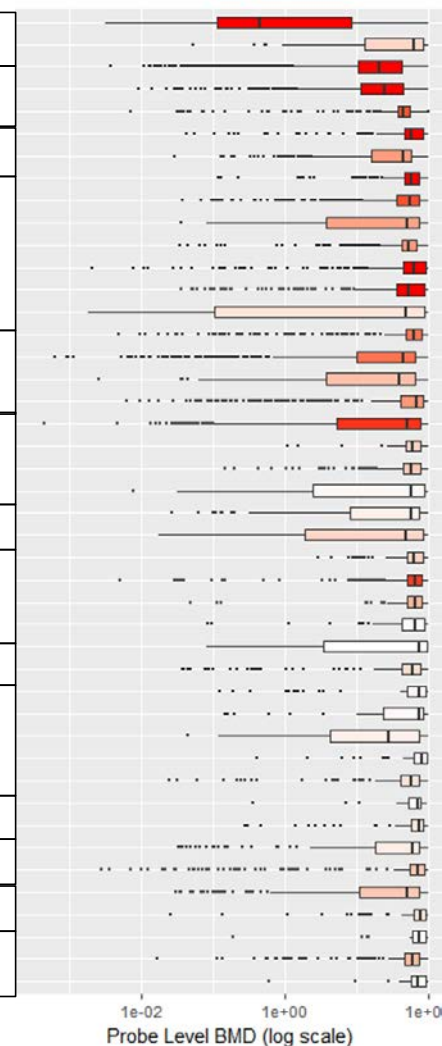
Developing a High-Throughput Transcriptomic Toolbox



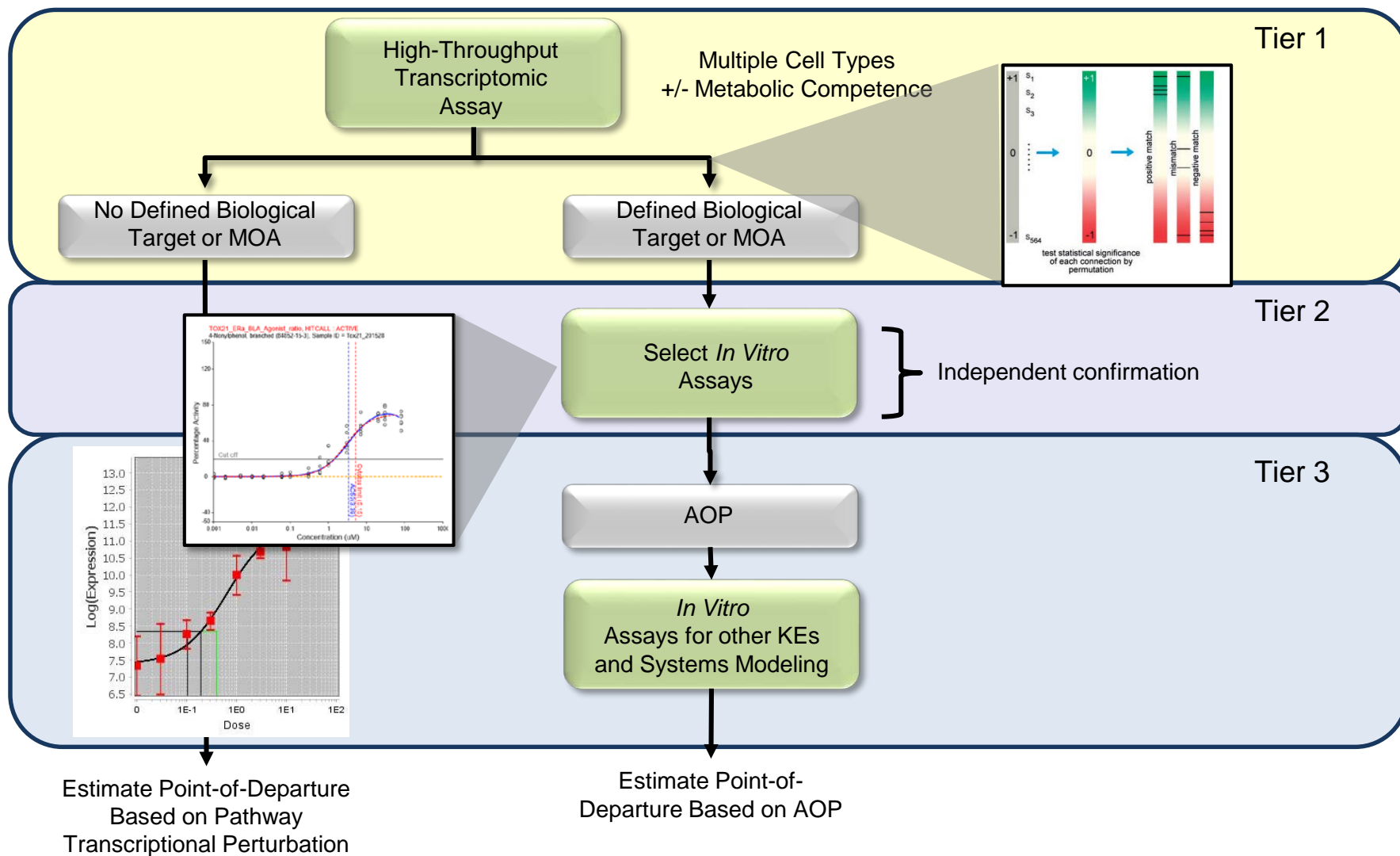
Chemical Name

	Target Expression (ON / OFF / NA)
Cycloheximide - Cladribine - Ziram - Thiram - Maneb -	CYTOTOXICANT
iodarone hydrochloride - Reserpine - Nonylphenol, branched - 4-Cumylphenol - Bisphenol A - Bisphenol B - 4-Hydroxytamoxifen - Clomiphene citrate - Fulvestrant - Rotenone -	PROTEIN REACTIVE
Fenpyroximate (Z,E) - Trifloxystrobin - Pyraclostrobin - Imazalil - Prochloraz - Propiconazole - Cyproconazole - Lovastatin - Simvastatin - Flutamide - Nilutamide -	SLC18A2 / A3 / A1 / B1
Cyproterone acetate - Vinclozolin - Troglitazone - Farglitazar - PFOA - PFOS - Lactofen - Clofibrate - Fenofibrate - Fomesafen - Butafenacil - Tetrac -	ESR1 / ESR2
3,5,3'-Triiodothyronine - Cypermethrin - Bifenthrin - Atrazine - Cyanazine - Simazine -	NDUFB9, NDUFB1, NDUFS6, CYB5R3, CYP5R1, CYBA
	STEROIDOGENESIS
	HMGCR
	AR
	PPAR γ
	PPAR α
	PPO INHIBITOR
	THRA / THR β
	SCN1B
	PDE7A, PDE6D, PDE3B

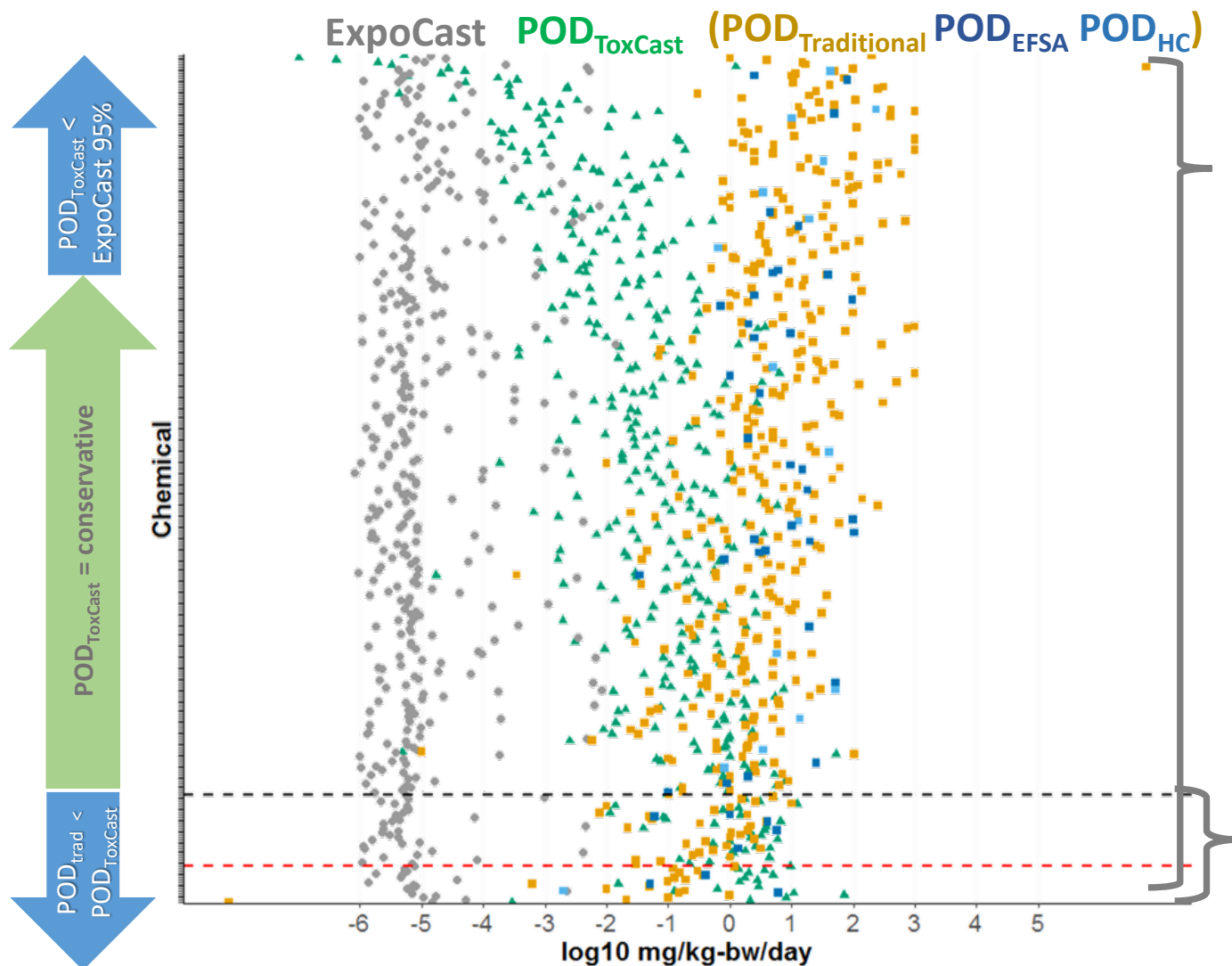
Probe Level BMD Results



Integrating Components Into a Tiered Testing and Assessment Strategy



Bioactivity Provides a Conservative Estimate of a NOAEL/LOAEL



**Total =
380 chemicals**

*httk, ToxCast data, and POD
value(s) currently available*

*For ~91.3% of the
chemicals,
POD_{ToxCast} was
conservative.
(~130-fold with
human HTKK
~40-fold with rat
HTTK)*

*Missing an
important
component
of biology?*

Take Home Messages...

Grandma
Approved



https://www.freepik.com/free-photo/senior-woman-with-a-thumbs-up_1014676.htm

- Using advances from the genome revolution to broadly capture chemical perturbations in biological pathways and processes
- High-throughput transcriptomic platform is being designed to include performance standards and as a portable platform/workflow for collaborative data generation
- Integrating bioinformatic tools to identify potential MOAs/MIEs and potency estimates
- The new transcriptomic platform is part of a larger tiered testing strategy for priority setting and other risk-based decisions

Acknowledgements and Questions

Tox21 Colleagues:

NTP
FDA
NCATS

EPA Colleagues:

NERL
NHEERL
NCEA

Collaborative Partners:

Unilever
A*STAR
ECHA
EFSA
Health Canada

EPA's National Center for Computational Toxicology

