CPC Comprehensive Pneumology Center

From high-throughput screenings for Toxicology to Clinical trials

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Outline

I. Overview of High-Throughput Screening (HTS)
   A. HTS experimental workflow
   B. Conventional high-throughput screening assays for toxicology:
      1. Biochemical
      2. Cell-Based assays

II. Applications- HTS research platforms for Toxicology

III. Conclusions

What is HTS?

(High Throughput Screening)

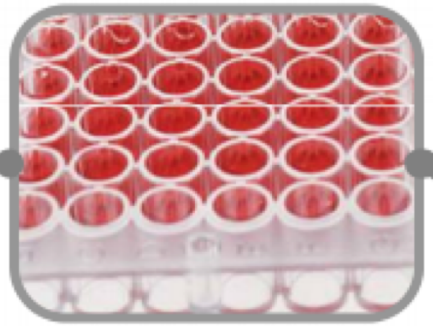
HTS started in the early to mid 90's

Automated tools to facilitate rapid execution of a large number and variety of biological assays that may include several substances in each assay.

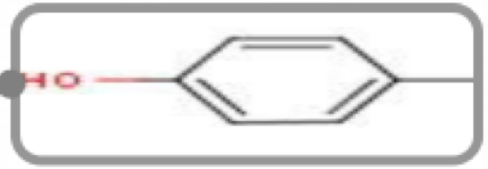
Screening mode	Number of samples tested per day	Examples
Low-throughput screening	1–500	Animal models, assays for CYP-mediated metabolism combined with LC/MS/MS
Medium-throughput screening	500–10,000	Fluorescent cellular microscopic imaging assay, assays for determination of catalytic activities of oxygen-consuming enzymes
High-throughput screening	10,000–100,000	Fluorescent enzymatic inhibition assay, luciferase reporter gene assays
Ultra-highthroughput screening	>100,000	β-lactamase cell reporter assay, assay for quantification of 5-HT_{2C} receptor editing

HTS uses robotics to more efficiently predict how chemicals may affect human health

Robotic Platform

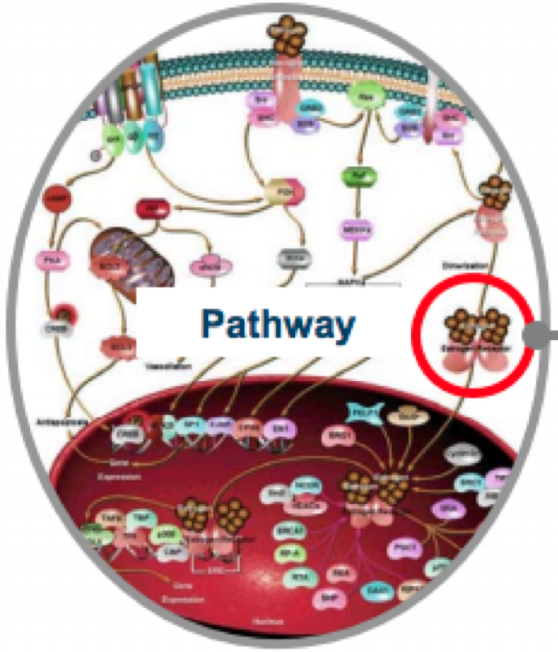


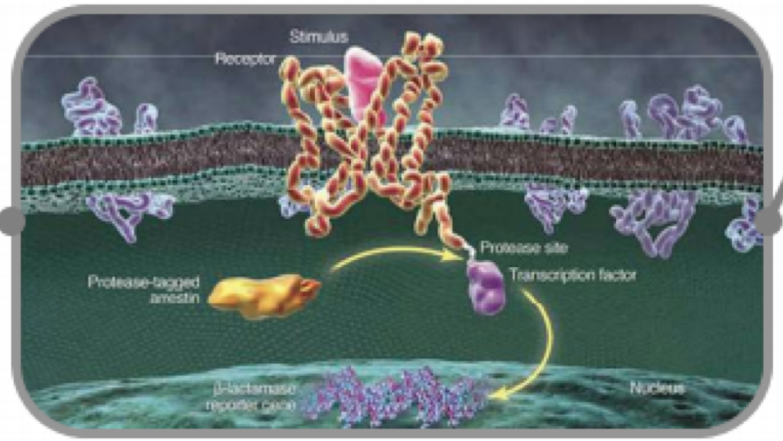
96-, 384-, 1536 Well Plates



Chemical Exposure

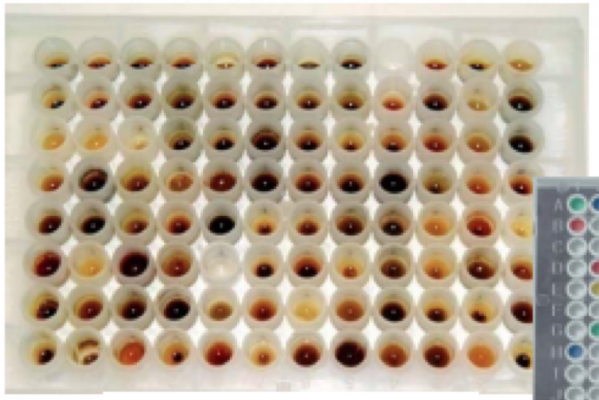
Cell Population





Target Biology (e.g., Estrogen Receptor)

The trend to Miniaturization

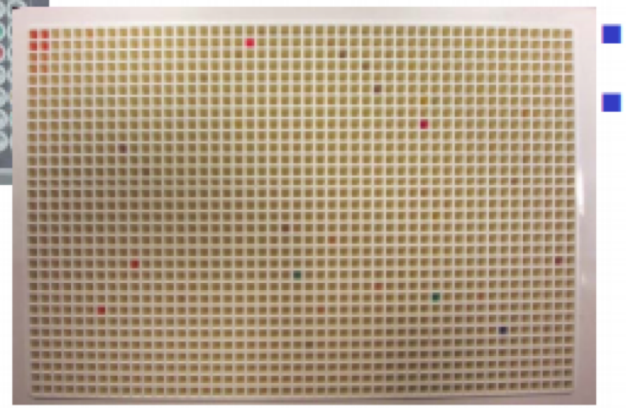


- 8 rows x 12 columns
- 88 test samples

96-well plate

- 16 rows x 32 columns
- 352 test samples

384-well plate
4 x 96-well plates



- 32 rows x 48 columns
- 1,408 test samples

At 100 plates/day, how long would it take to screen 1 MM samples?

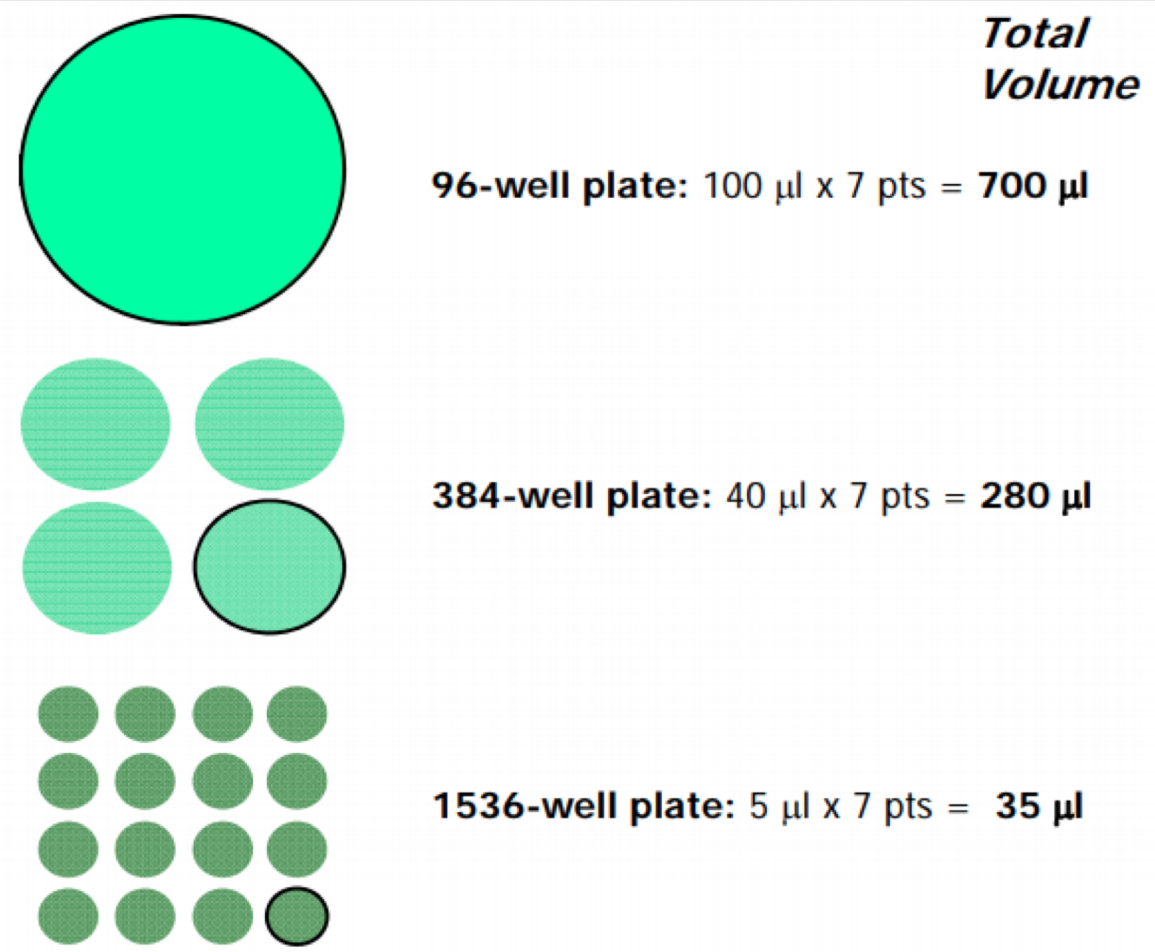
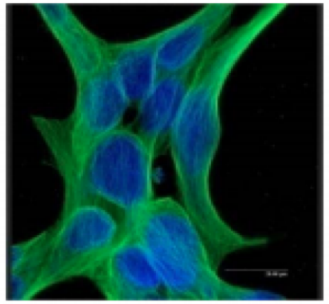


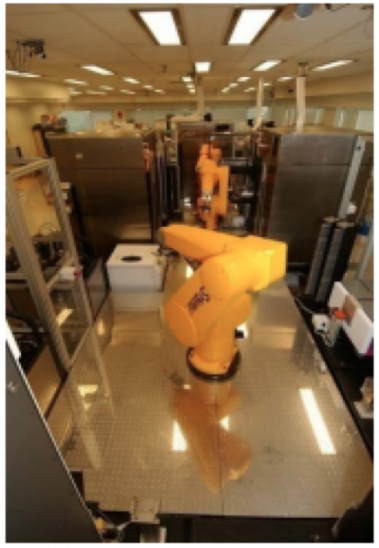
Plate format	samples/day (wells/day)	Time to screen 1 MM samples
96-well	8,800 (9,600)	
384-well	35,200 (38,400)	
1536-well	140,800 (153,600)	

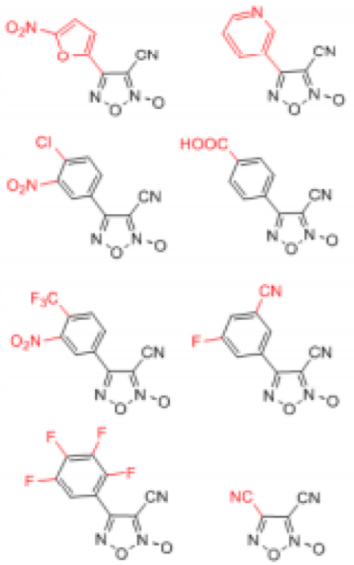
How is drug-discovery HTS different from Toxicology HTS?

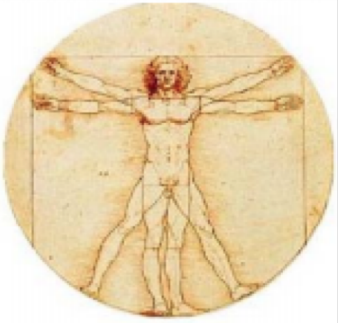
HTS for Drug Development

Identify target, pathway, or cellular phenotype → Create testing system (aka, "assay") → HTS tests >100,000 chemicals with *no known activity* for effect on target → Make modifications to most active chemicals to make suitable for in vivo testing → Test in animals for safety, effectiveness → Test in humans for safety, effectiveness









HTS for Toxicology

False negatives are of greater concern!

Chemicals with known or suspected toxicity/ activity

Identify toxicity pathways, cellular phenotypes

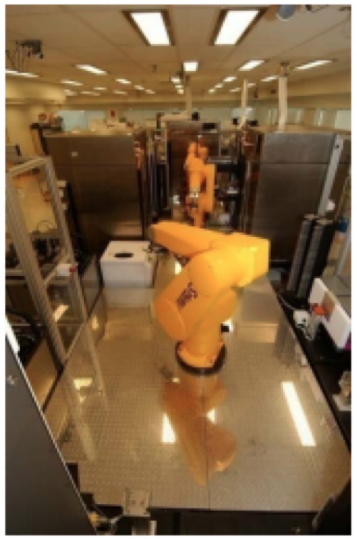
Obtain or create testing systems ("assays")

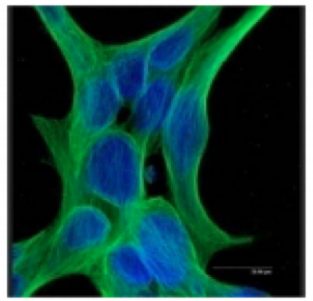
HTS tests chemicals for effect on assays

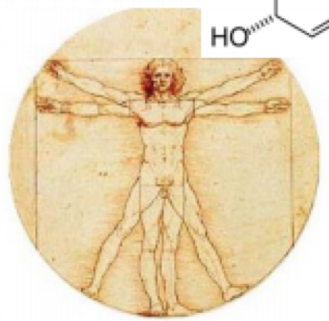
Computational analysis & Synthesis of HTS results

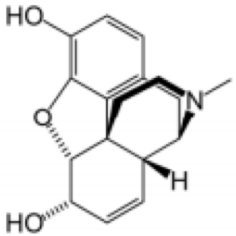
Test prioritized chemicals in animals

Categorize as inactive subject to further testing





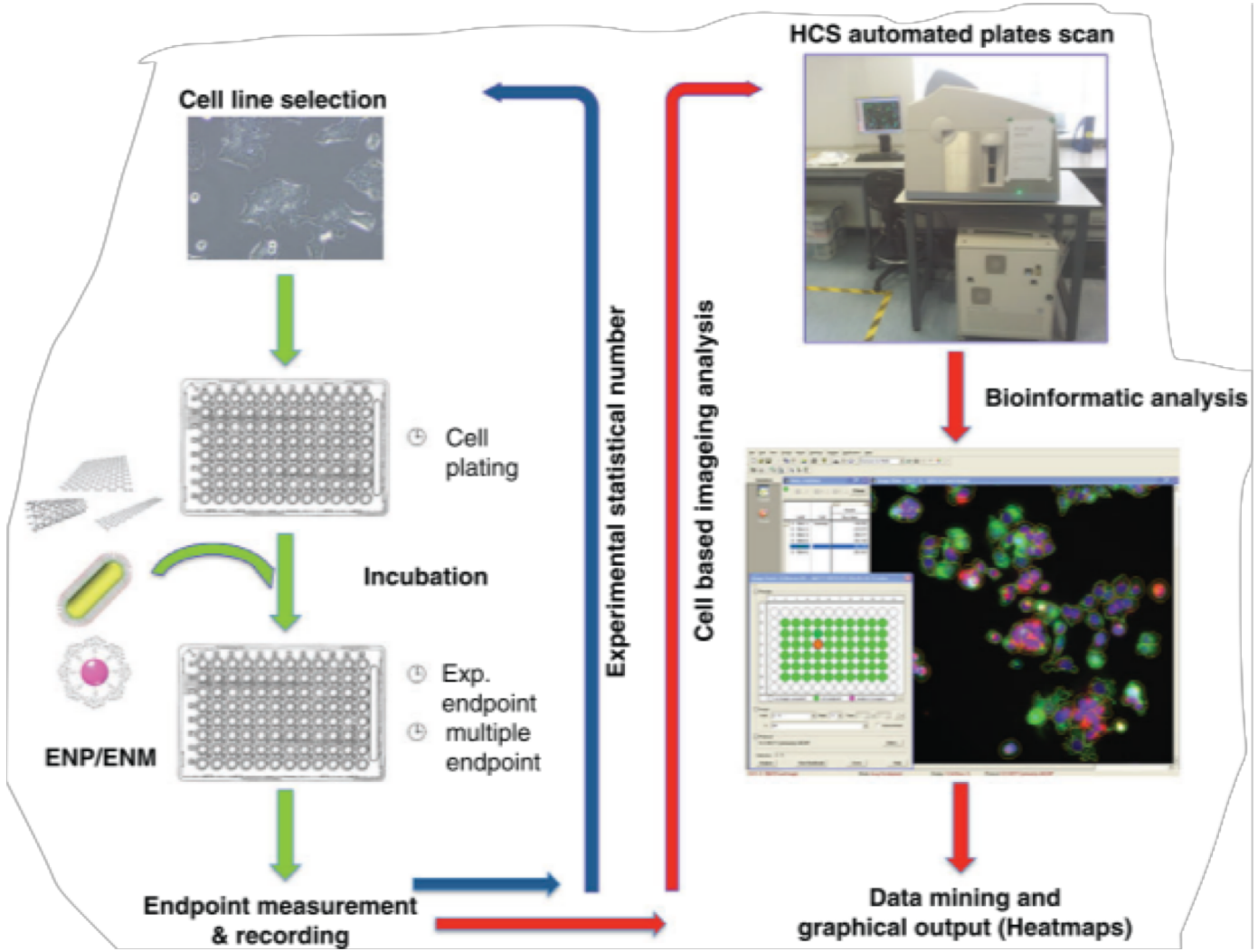




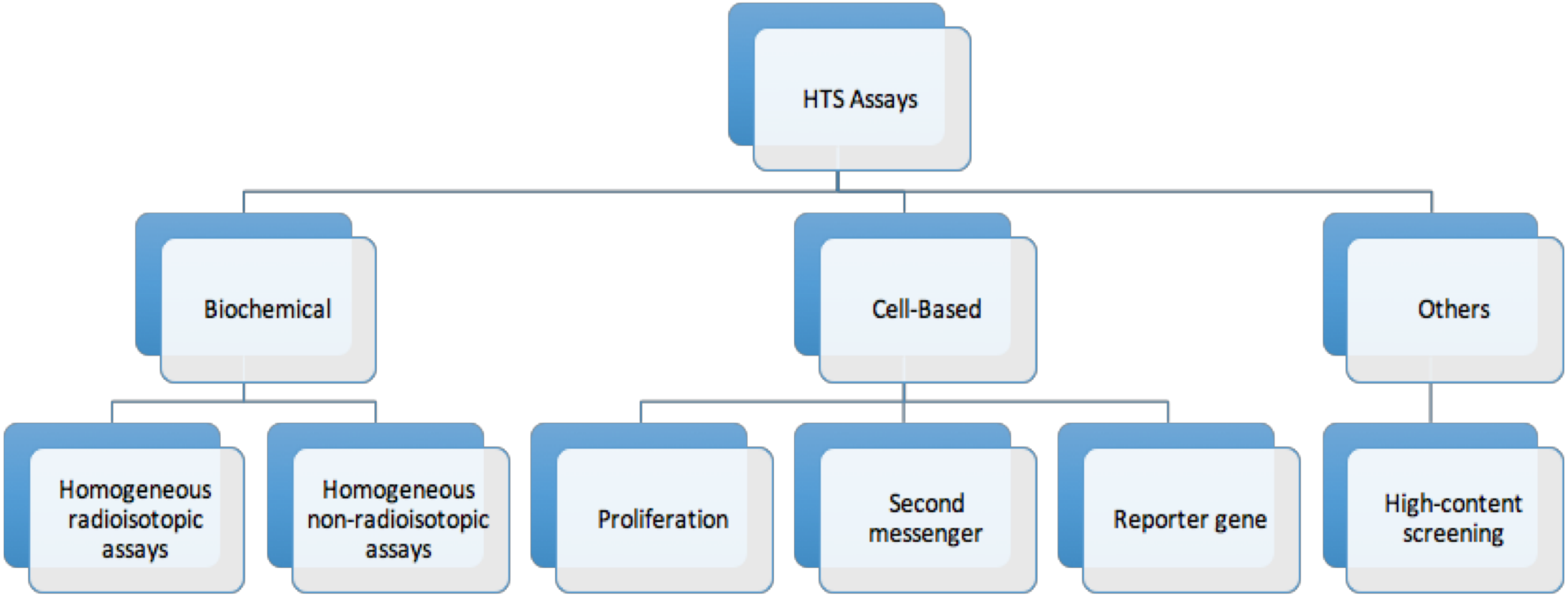
Why was HTS adapted for Toxicity testing?

- Too many **chemicals** and too **little data**
- Very **high cost**
- HTS is used to identify signatures to **predict hazard**
- To rely **less** on **animal toxicity data**
- Integration of data with **bioinformatics** to generate predictive tools

HTS experimental workflow



Conventional HTS assays

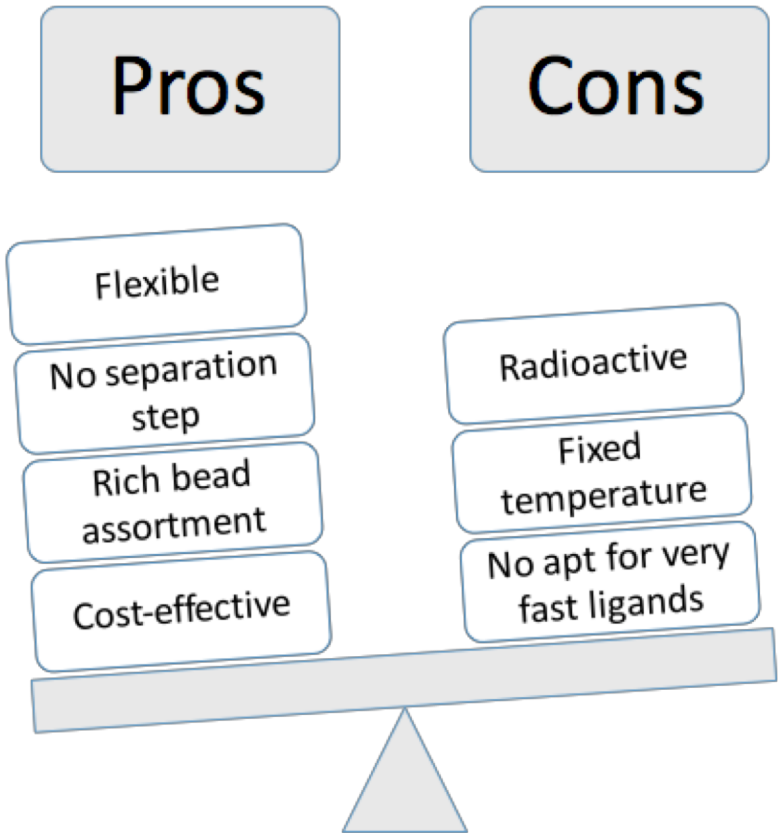


Biochemical assays

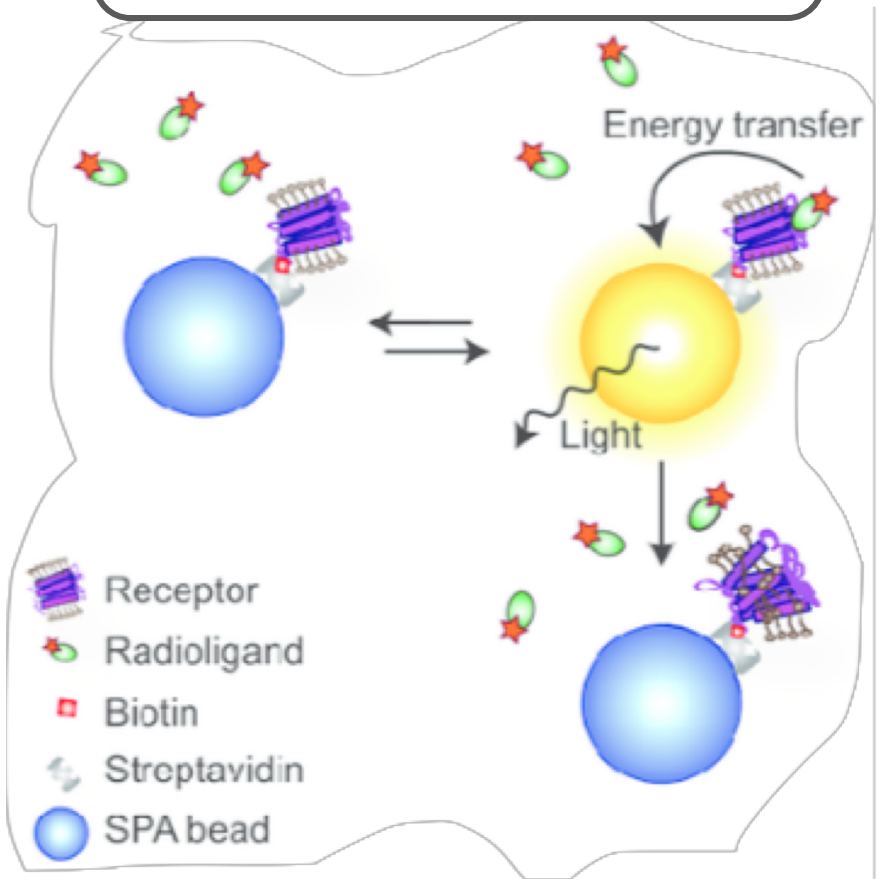
	Assay classification	Specific assay type
Biochemical assays (e.g. enzyme inhibition, receptor–ligand binding)	Homogeneous radioisotopic assays	Scintillation proximity assay
	Homogeneous non-radioisotopic assays	Colorimetric- or absorbance-based assay – enzyme-linked immunosorbant assay Luminescence-based assay – chemiluminescence – electrochemiluminescence Fluorescence-based assay – fluorescent intensity – fluorescence polarization – fluorescence resonance energy transfer – homogeneous time-resolved fluorometry – fluorescence correlation spectroscopy

Biochemical assays- Scintillation Proximity assay

Applications: Enzyme assays, molecular interactions, receptor binding

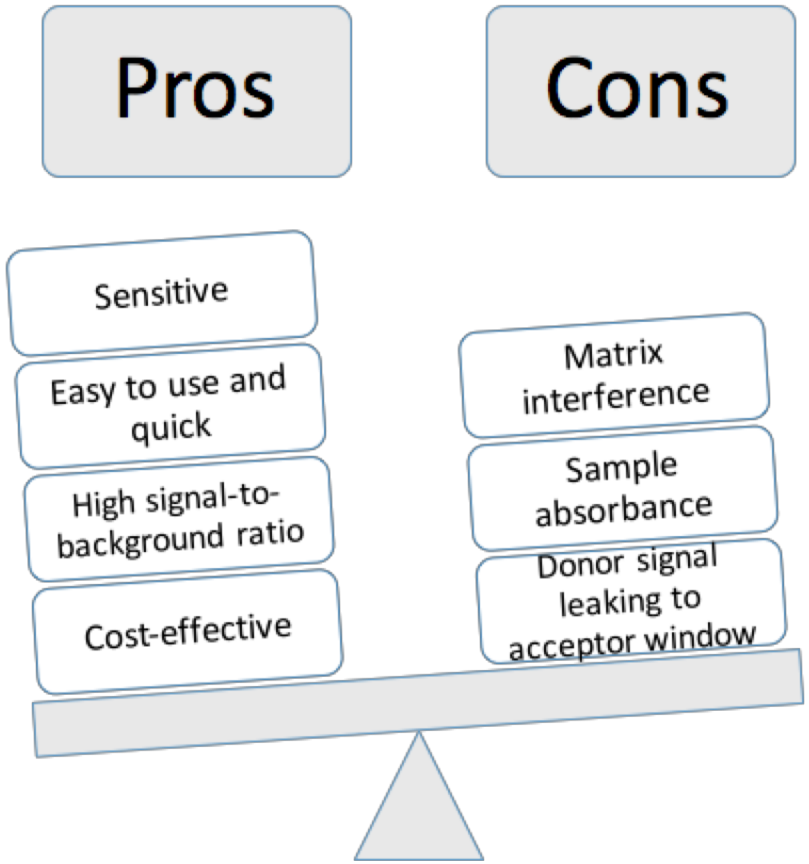


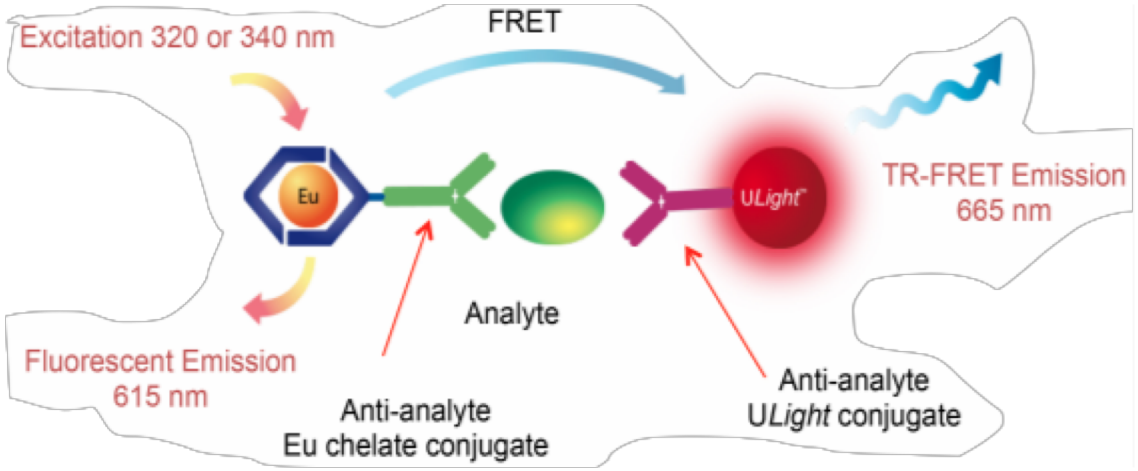
Principle
Radioligand in close proximity stimulates bead to produce light

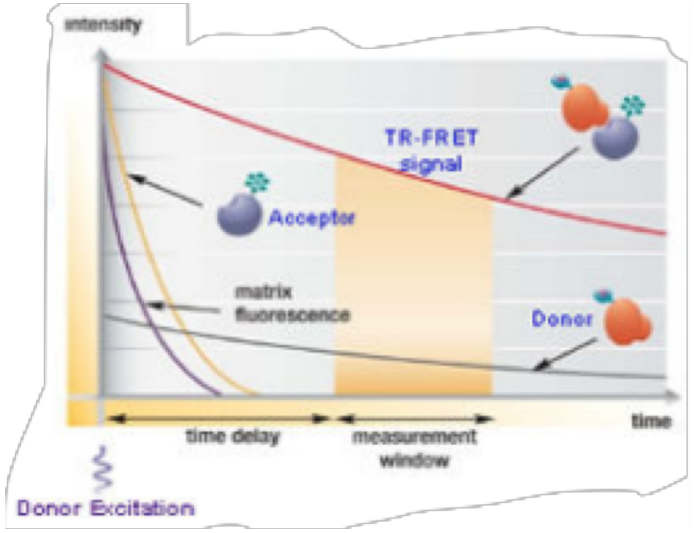


Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) Assay

Applications: receptor-ligand or protein-protein interactions







Most commonly used cell-based assays

Cellular assays	Cell proliferation assays	Dye uptake (e.g. Alamar blue, MTT) Oxygen sensor Radioactive isotope uptake
	Second messenger assays (e.g. ion channel)	Ion flux assay Fluorescence-based assay – fluorometric imaging plate reader Automated patch clamp
	Reporter gene assays (e.g. GPCR)	Enzymatic assay – luciferase, β-lactamase, β-galactosidase Immunoassay Direct protein measurement – green fluorescent protein
	High-content screening	Multiple endpoint assay using fluorescent probes

MTT assay

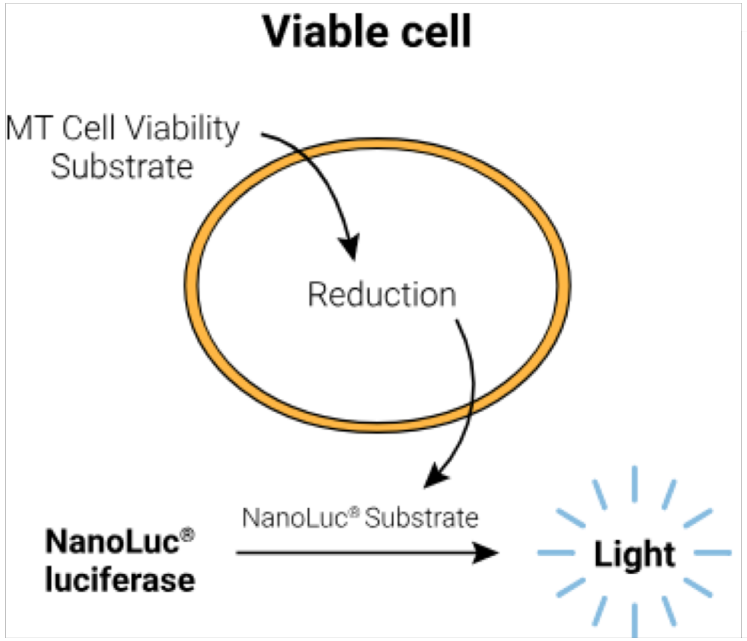
Application:

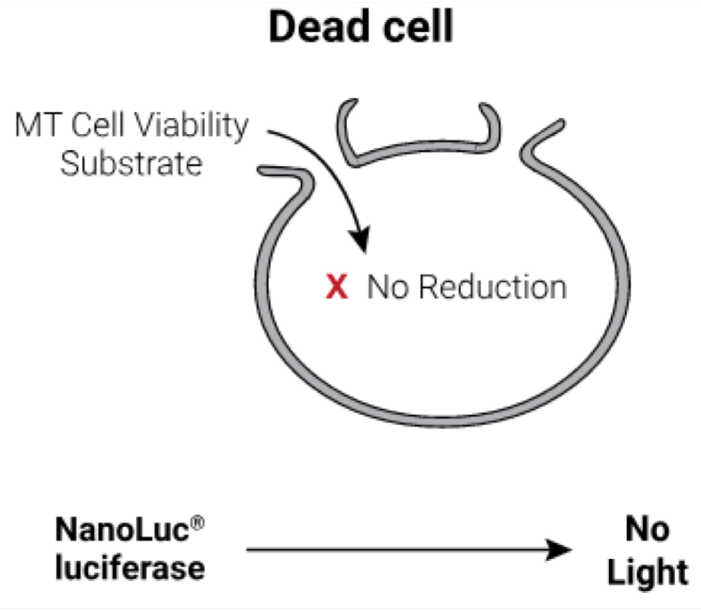
Cell viability, proliferation

Pros: easy

Cons:

- Not very sensitive
- Does not distinguish between apoptosis and necrosis
- Based on mitochondrial activity



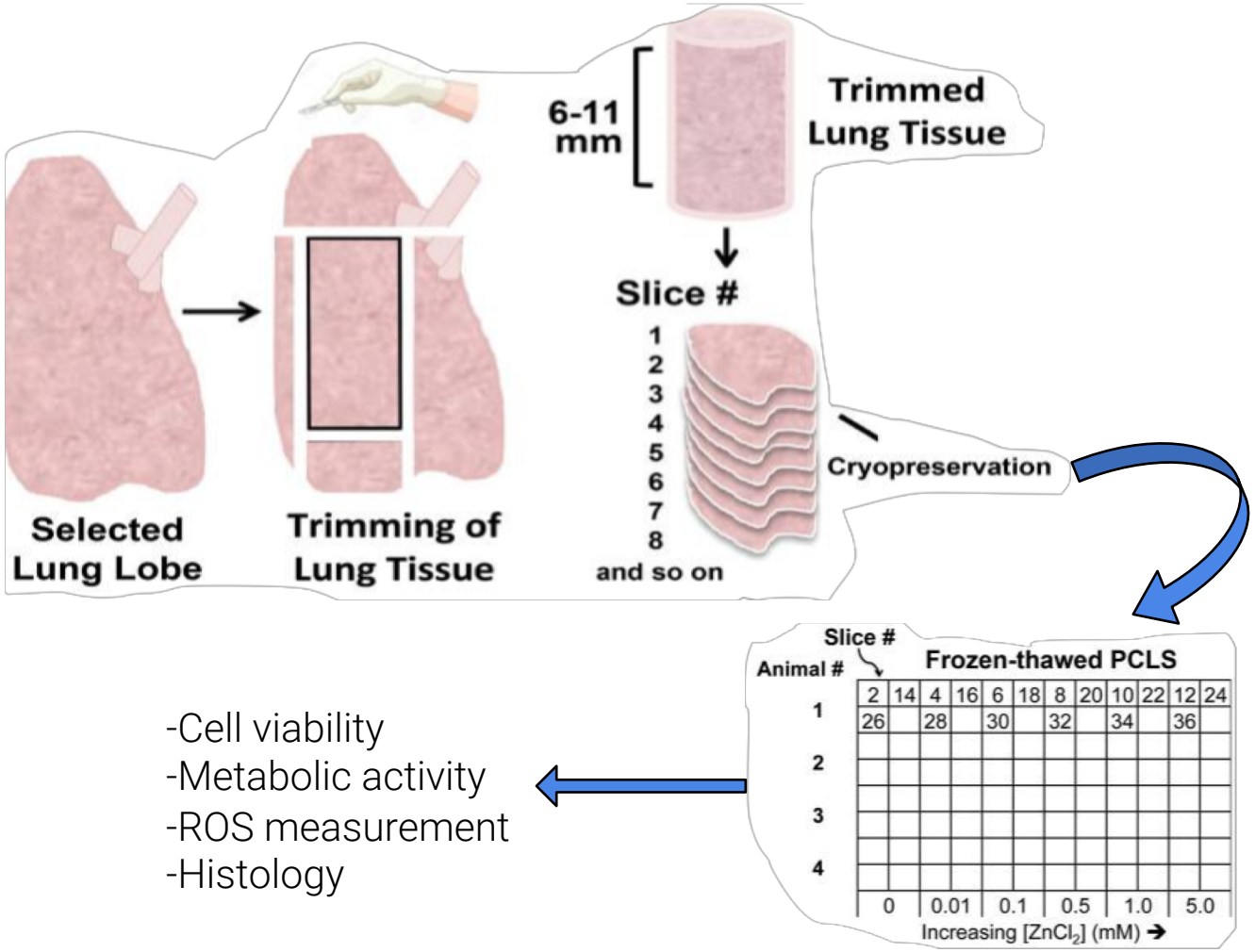


Cryopreserved Precision Cut Lung Slices (PCLS)

Application:

Toxicity of chemical allergens, biotoxins, nanomaterials, chemotherapeutic agents

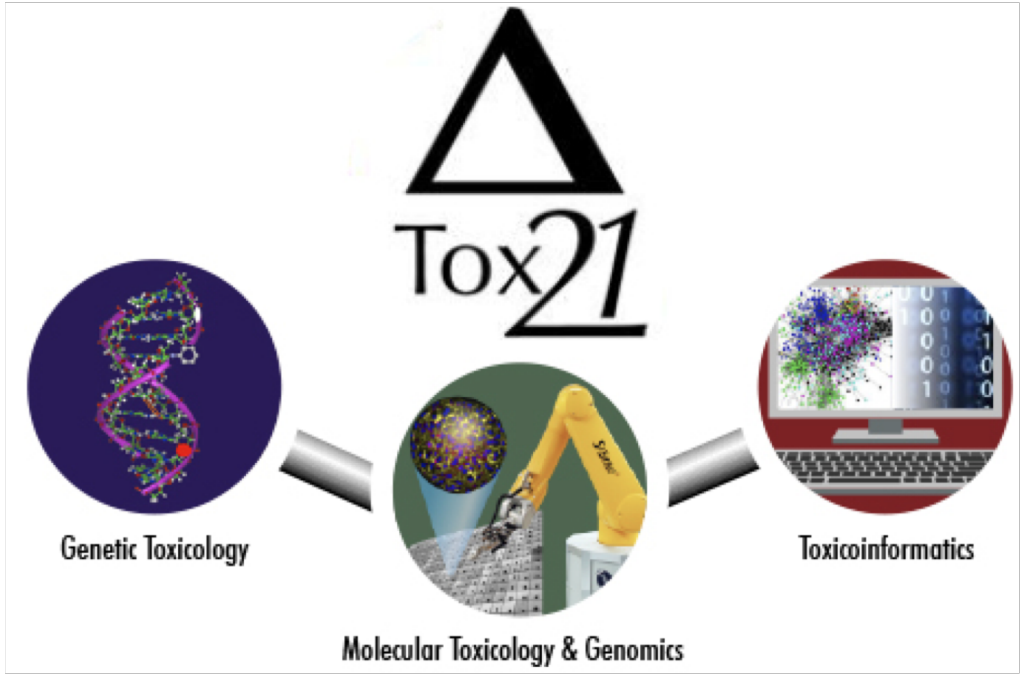
Pros	Cons
Better picture of organ complexity	Small reduction metabolic activity
No time-limited viability	Cryopreservation can damage cell membrane
Similar to conventional PCLS	Complex



-Cell viability
-Metabolic activity
-ROS measurement
-Histology

HTS Platforms for Toxicology

TOX 21 Initiative



3-Phase Project
Several HTS assays

Tox21 screened a **10K** chemical library using more than **42** assays, most of which tested immortal cancer cells, and produced more than **65 million** measurements

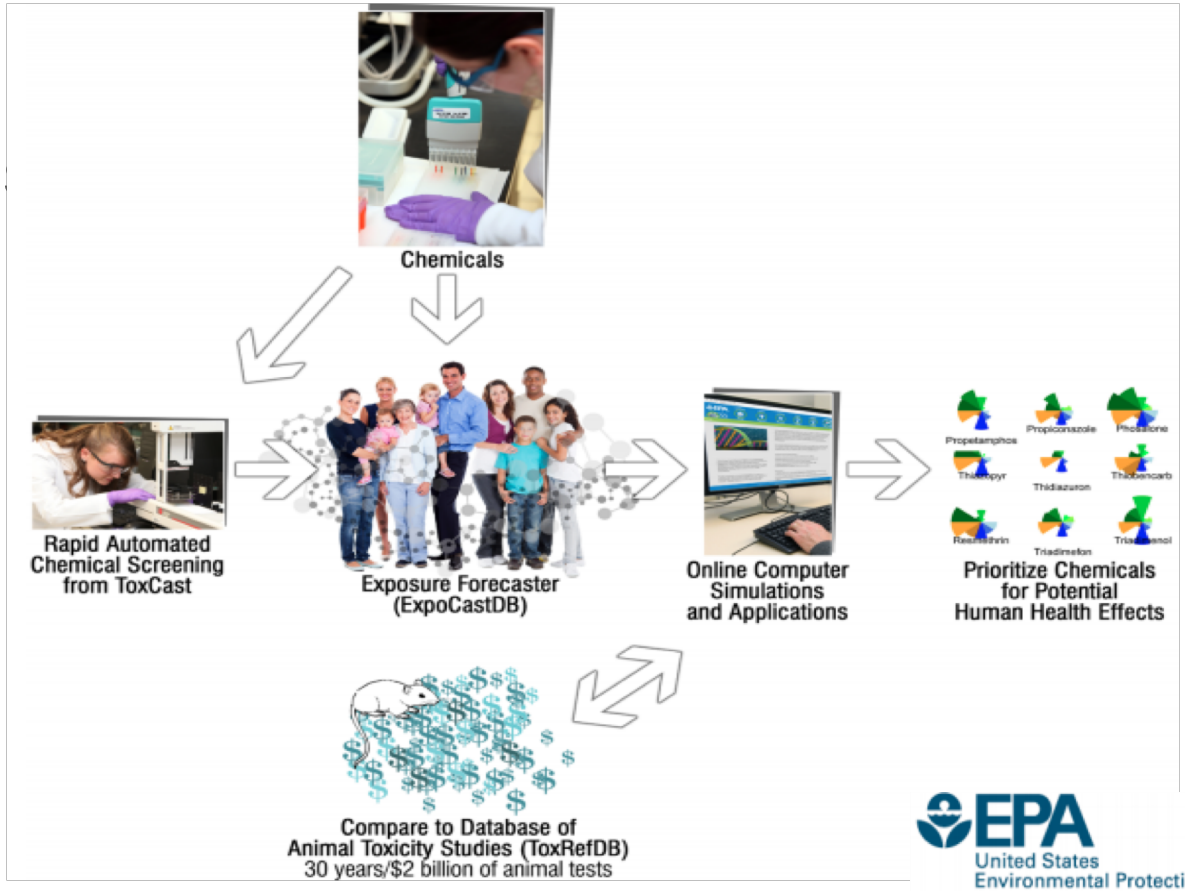
EPA NIH National Institute of Environmental Health Sciences NIH National Center for Advancing Translational Sciences FDA NTP National Toxicology Program U.S. Department of Health and Human Services

ToxCast (EPA HTS Platform)

Phase I: More than 2000 chemical evaluated in 700 different HTS assays, covering about 300 signaling pathways

Phase II (Tox21): testing 1800 chemicals for potential endocrine disruption

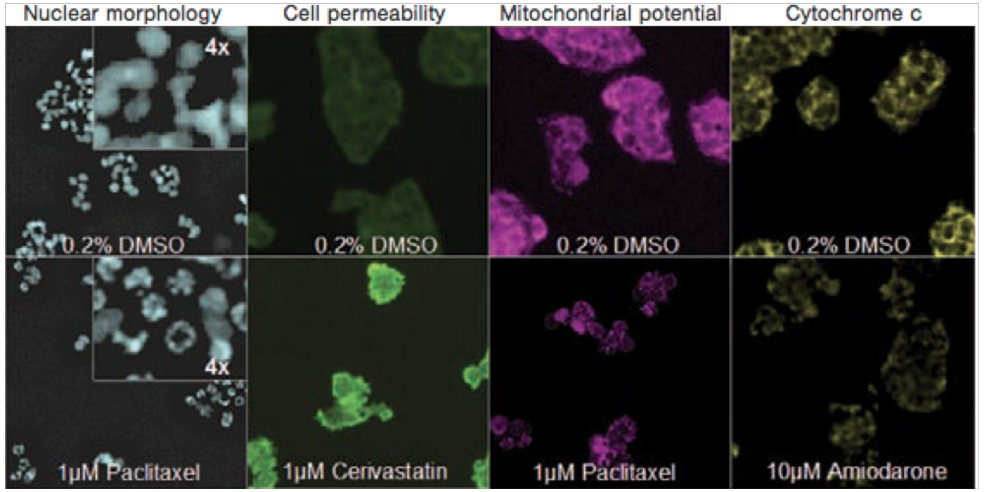
Phase III: HTS transcriptomics
Human primary cells and stem cells
Animal models in zebrafish



Cell-Based HTS and HCS Cytotoxicity screening panel

Analysis Method	High Content Screening
Toxicity Markers	Cell loss Nuclear size Nuclear morphology Cell membrane permeability Mitochondrial membrane potential Mitochondrial mass Cytochrome c release
Cell Type	HepG2 (others available on request)
Test Article Concentration	8 point dose response curve up to 500 µM or solubility limit (different concentrations available)
Number of Replicates	3 replicates per concentration
Quality Controls	0.5% DMSO (vehicle control) Chlorpromazine (positive control) Valinomycin (positive control)
Test Article Requirements	3-5 mg solid (depending on molecular weight) or equivalent DMSO solution
Data Delivery	Minimun toxic concentration Dose response curves

Cellomics ArrayScan® VTI or Cellomics ToxInsight (Thermo Scientific)

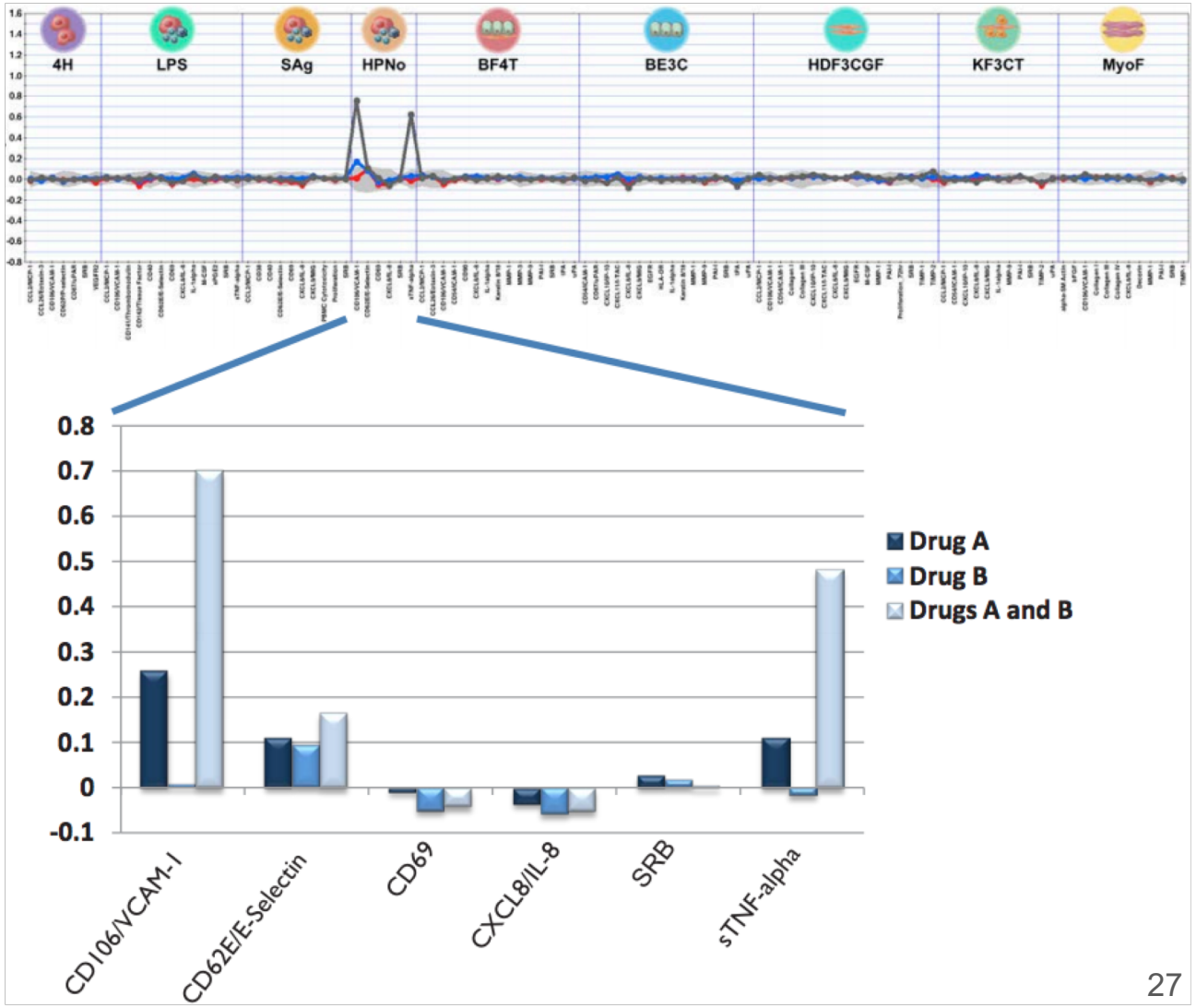


Bridging the Gap from HTS to Clinical Trials

Testing for adverse effects of drugs

Three hypotheses:

1. The AE was caused by the client's investigative drug, Drug A;
2. The AE was caused by prior courses of Drug B;
3. low residual levels of Drug B in patients could synergize with Drug A to induce the AE.



Final Remarks

3 days

vs.

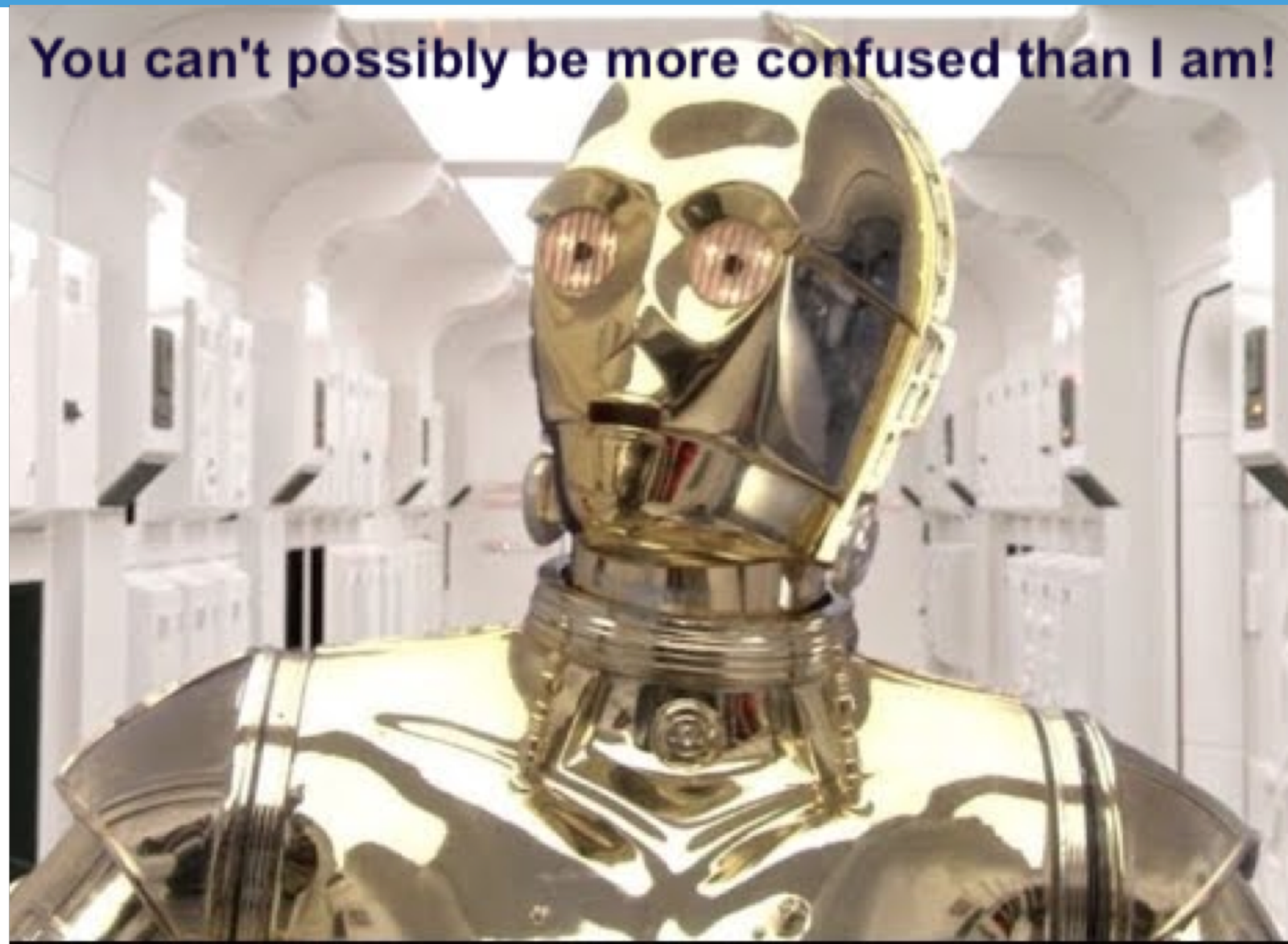
12 years

Conclusions

- HTS techniques to rapidly and efficiently test chemicals for toxicity have the potential to assist regulators in assessing the risk novel compounds
- The Tox21 and ToxCast collaboration is combining technology, biology, and computational methods in order to advance in vitro testing for toxicology

CPC

Thank you!



Tox21 Robot

https://www.youtube.com/watch?time_continue=513&v=CjQTVfXQ8N4