The Tox21 partnership: an overview and evolution of *in vitro* screening models

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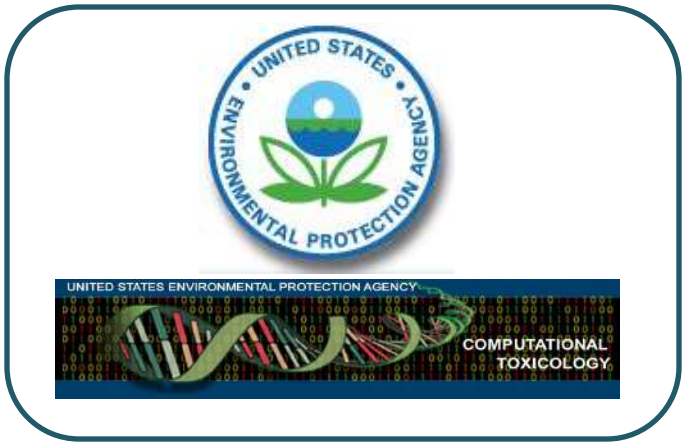
NCATS

BIOMED21
Bethesda, MD
June 27, 2017

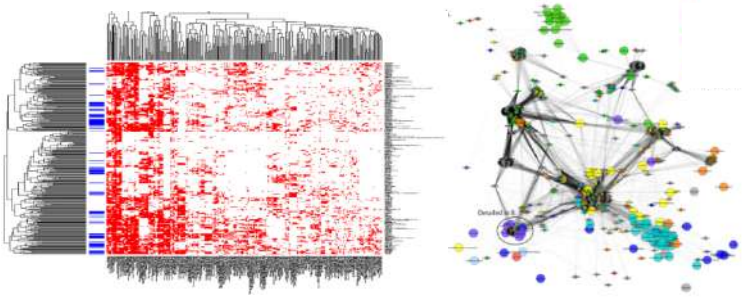
NIH National Center for Advancing Translational Sciences

The Tox 21 Collaborative



The Tox21 Collaborative

- Identify patterns of compound-induced biological response to characterize toxicity/disease pathways
- Prioritize compounds for more extensive toxicological evaluation
- Guide optimization of new compounds
- Develop predictive models for biological response in humans



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Tox21 10K Compound Library

EPA

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

NTP

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

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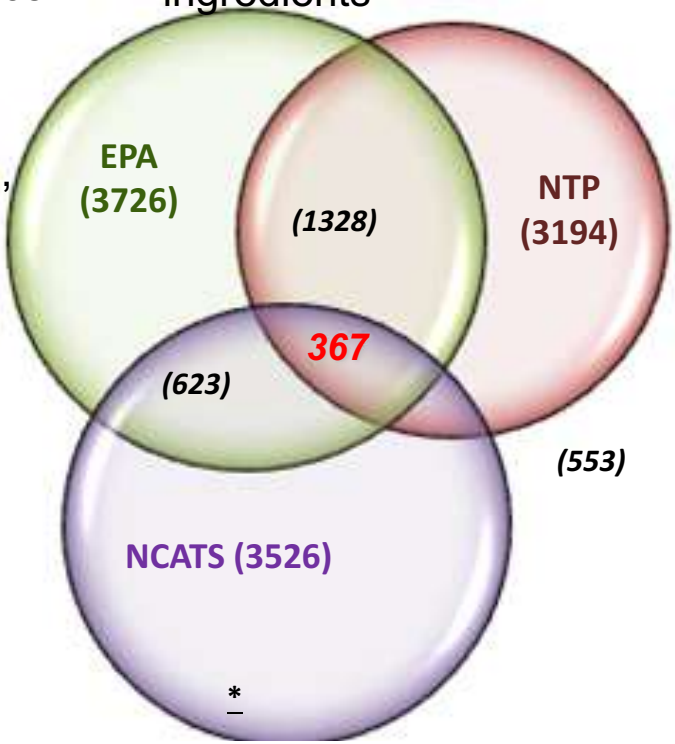
- Approved Drugs
- Investigational Drugs
- Active pharmaceutical ingredients



- *88 single-sourced compounds in duplicate on each plate*
- *Three library replicates, compounds positionally-varied*

Entire-Library QC Project

- Multi-year undertaking using a range of LC-/GC-MS and NMR methods.
- >7000 analytical chromatograms in PDF format available through PubChem: http://www.ncbi.nlm.nih.gov/pcsubstance

Identification

Depositor-Supplied Synonyms

DOLASETRON MESYLATE
DSSTox_CID_26827
DSSTox_RID_81939
DSSTox_GSID_46827
Tox21_112695
NCGC00181048-01
CAS-115956-13-3

... see more options

Substance Information

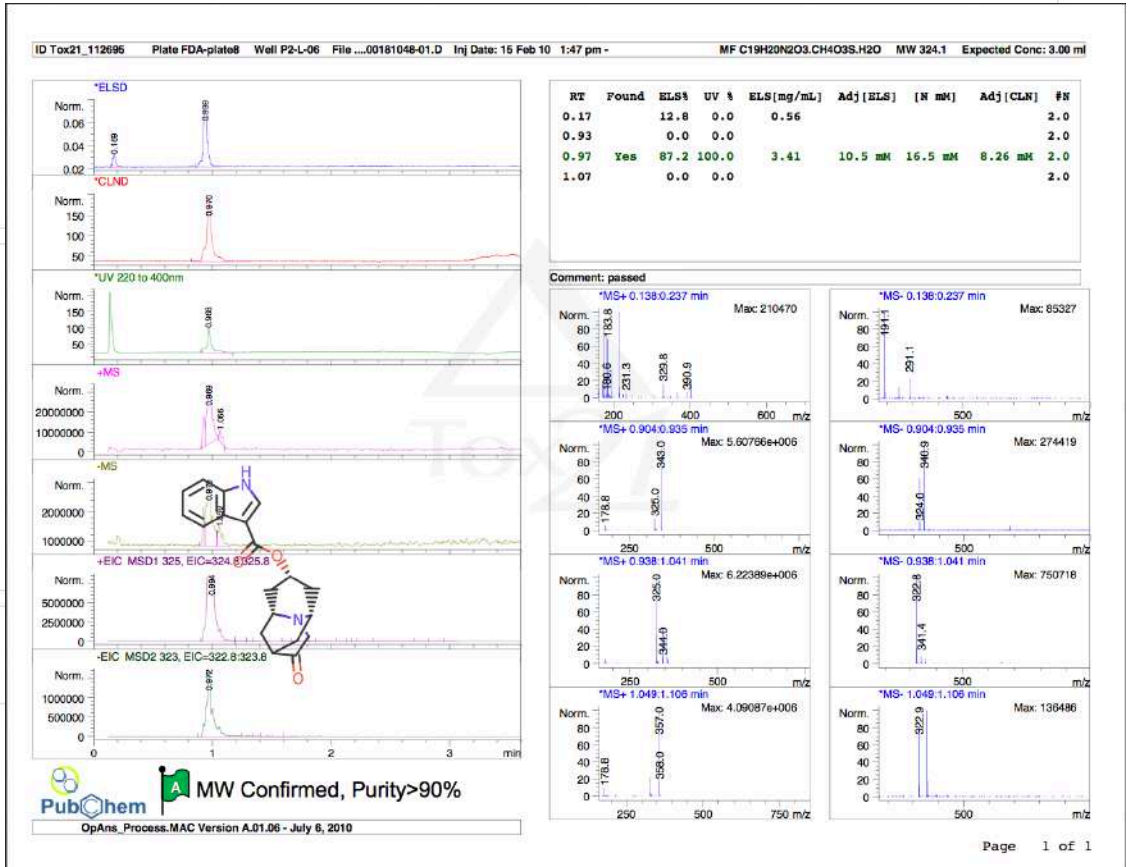
SID 144206248
Deposit Date: 2012-10-06
Modify Date: 2014-12-12
Substance Version: 2

Data Source:
Depositor: Tox21
External ID: NCGC00181048-01

Compound CID: 3033817

Depositor Comments

TOX21S_v5a



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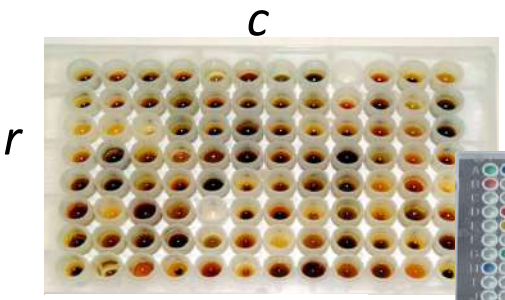
Leveraging modern technologies: shrinking reaction volumes, inclusion of multiple doses



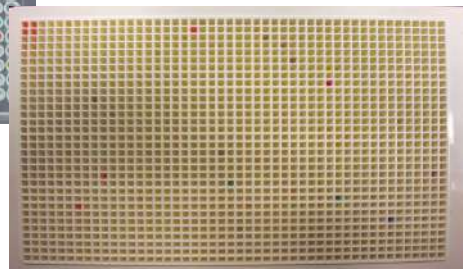
96-well plate

- 8 rows x 12 columns
- 88 test samples

384-well plate

4 x 96-well plates

- 16 rows x 32 columns
- 352 test samples



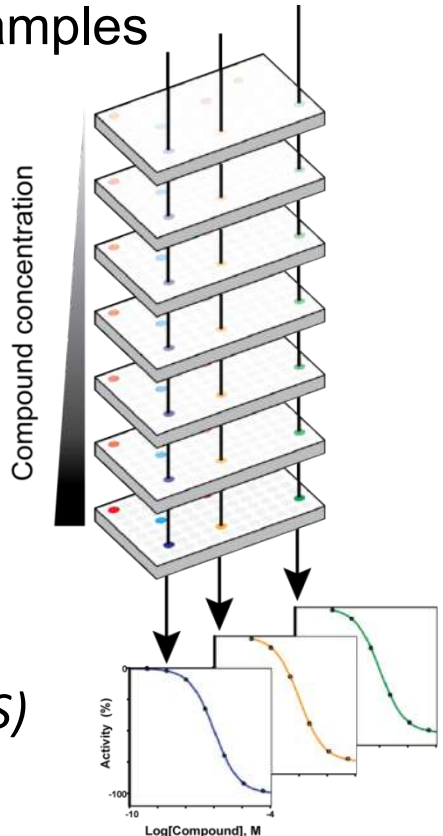
1536-well plate

16 x 96-well plates

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

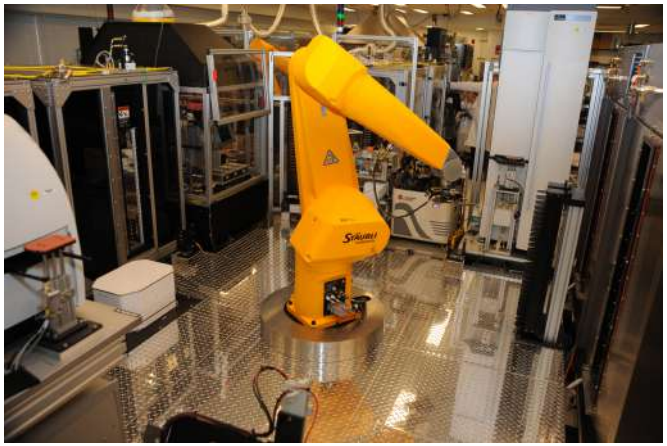
If @ 100 microtiter plates per day:

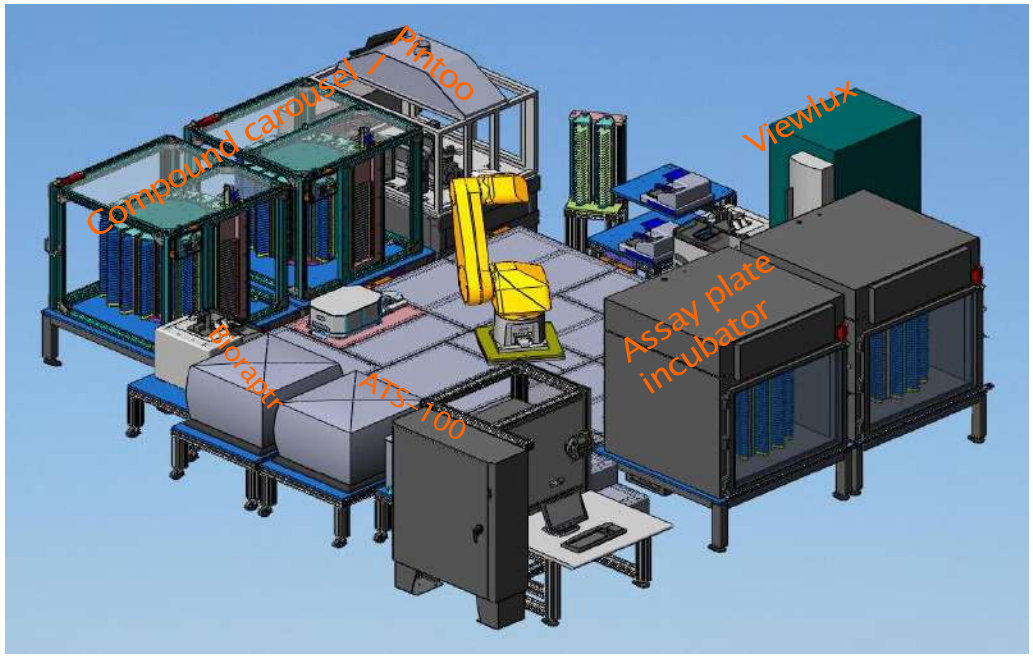
Plate format	samples§/day (wells/day)	Time to screen 1 M samples
96-well	8,800 (9,600)	4 months
384-well	35,200 (38,400)	4 weeks
1536-well	**140,800** (153,600)	**7 days**



Dose-response-based screening (qHTS)
Proc Natl Acad Sci 103:11473 (2006)

Tox21 Robot Platform





ViewLux Multilabel Reader

- Absorbance
- Fluorescence
- F.P.
- Luminescence
- TR-FRET
- Top reading

EnVision Multilabel Reader

- Absorbance
- Fluorescence
- F.P.
- Luminescence
- TR-FRET
- AlphaScreen
- Top/Bottom reading

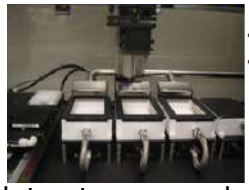
BioRAPTR FRD Workstation

- Transfer size: 0.2 - 10 ul
- 0.5 ml dead volume
- 4 reagents

Multidrop Combi

- Transfer size: 2 - 10 ul
- 10 ml dead volume
- 1 reagent

Pintool Station

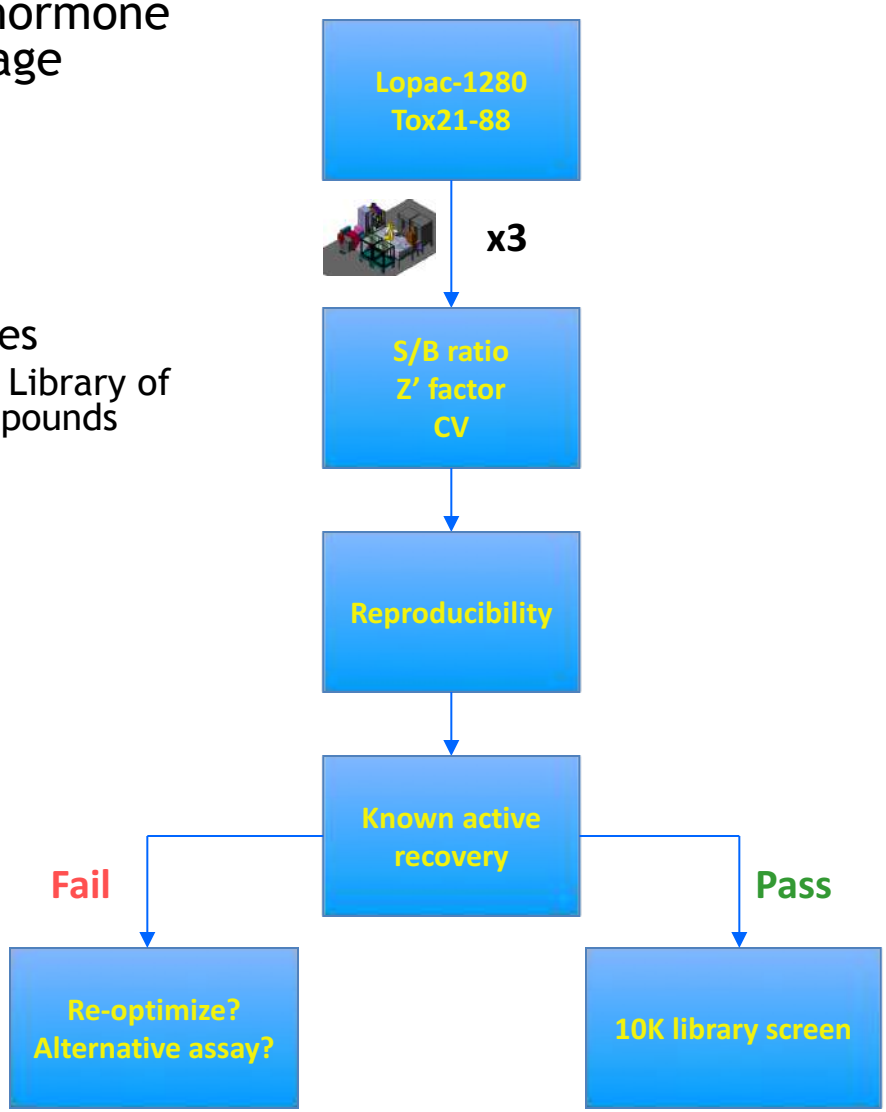


- Transfer size: 20 nl
- Pins washed in 3 solvents

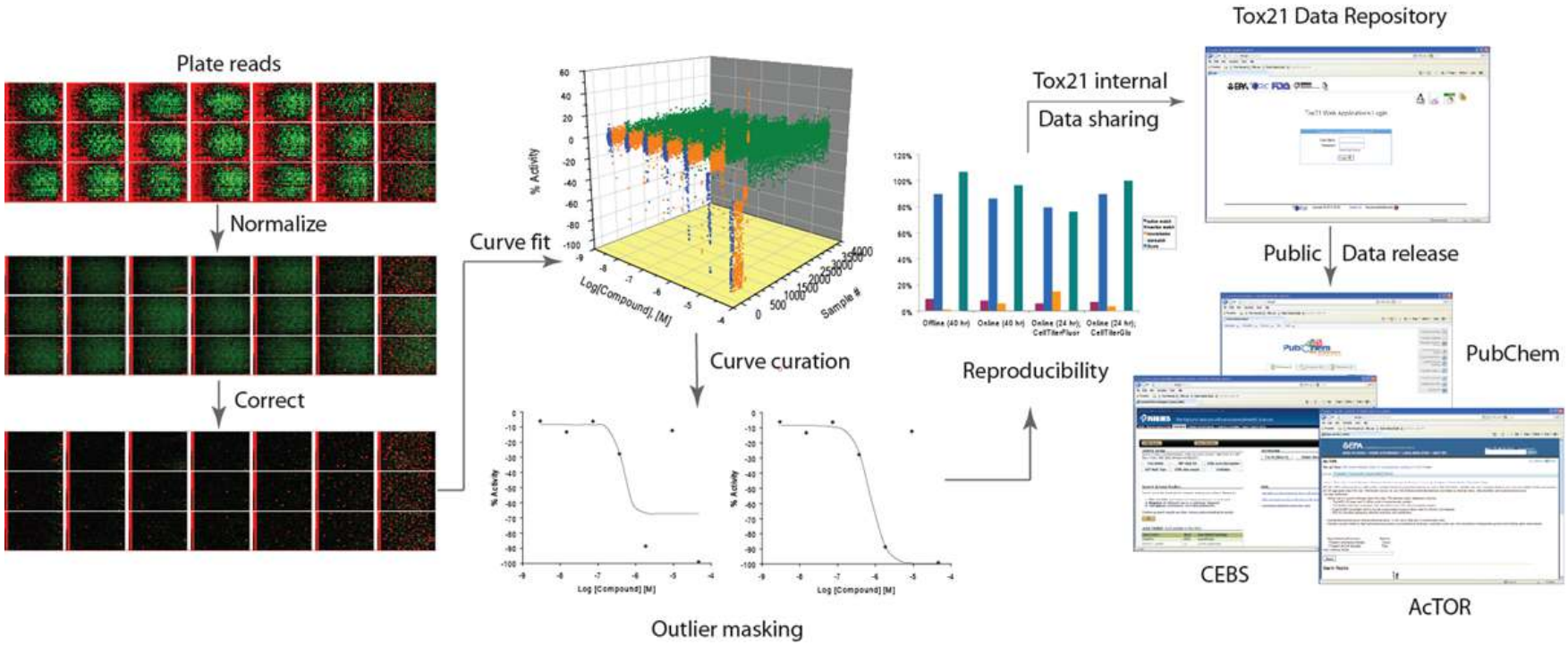
- Compound plate storage and assay plate incubator
- Pintool station and acoustic dispenser for nanoliter compound transfer
- Reagent dispensers (BioRPTR, Multidrop)
- Centrifuge (V-spin)
- Plate readers (ViewLux and EnVision)

Assay Nomination and Validation Process

- Specific screening assay proposed and discussed through joint working group: major assay types include nuclear hormone receptors and cell stress (DNA damage response, mitochondrial membrane potential, ER stress).
- Online validation on Tox21 Robot
  - Tox21 validation plate
    - Lopac-1280 + 88 Tox21 replicates
      - Lopac®1280 (Sigma-Aldrich) – Library of Pharmacologically Active Compounds
  - Triplicate runs
- Acceptance criteria consideration
  - Performance metrics
    - S/B ratio, Z' factor, CV
  - Reproducibility
  - Ability to recover reference compounds/known actives
- Pass
  - Proceed to 10K library screening
- Fail
  - Go back to optimization?
  - Select alternative assay?

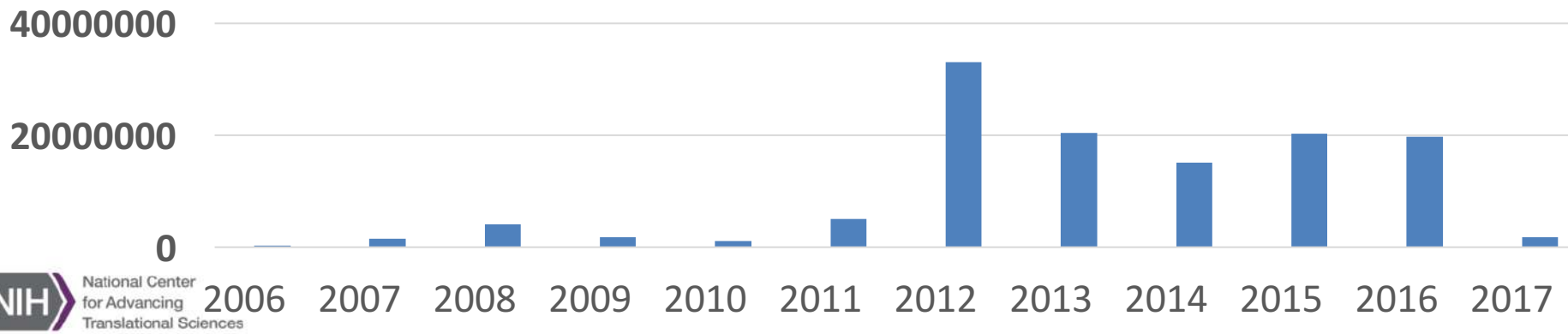


Informatics Analysis Process



Tox21 Milestones

- Infrastructure and processes for rapid testing of chemicals enabled through robotic HTS in miniaturized format, largest collection of environmental chemicals and drugs assembled, multiple QC measures in place.
- Deposition into the public domain of the largest-ever concentration-response dataset: ~90 M experimental datapoints, >130 assay datasets.
- Using crowdsourcing to move from data to knowledge.



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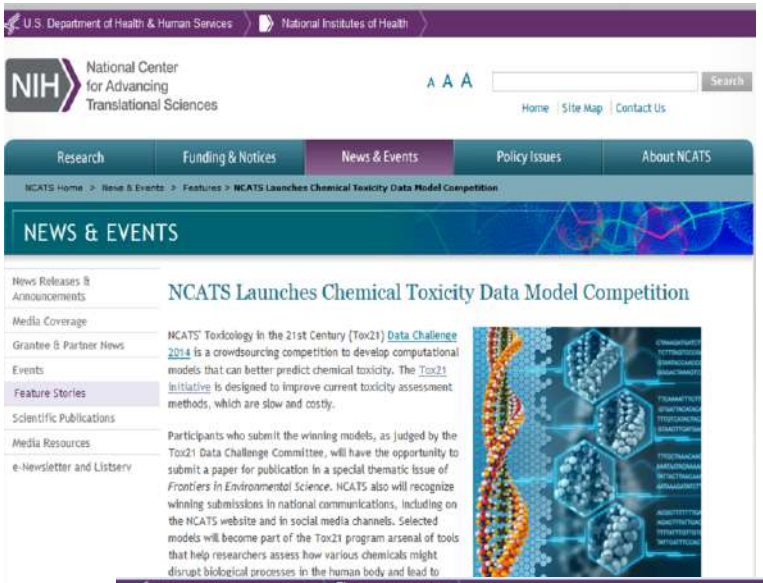
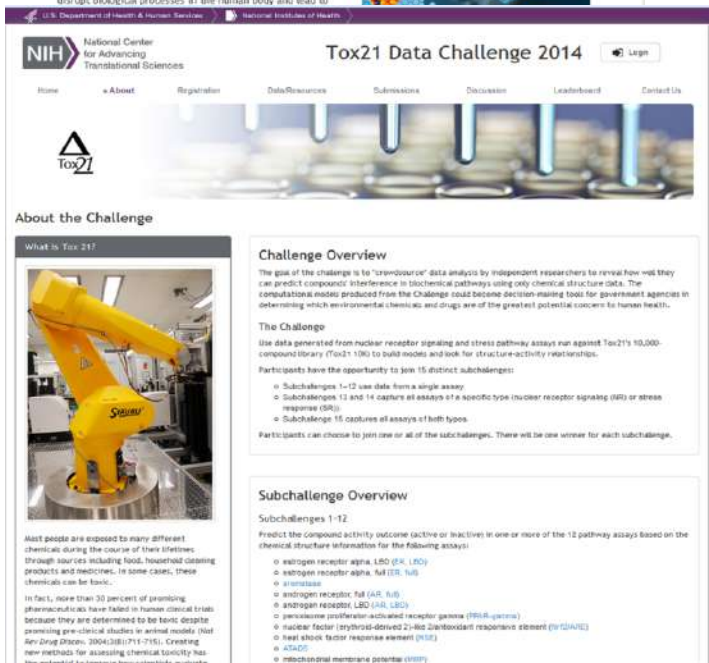
Dissemination of data: model-building through crowdsourcing competitions

- Data:
  - 30 nuclear receptor signaling and stress pathway assays
  - 50M data points (15 pt CRs)
- Goal: Models to predict toxicity assay response based on chemical structure
- 125 participants from 18 countries
- Winners announced 26 Jan 2015, presentations at SOT2016
- Papers describing top models published in *Frontiers in Environmental Science*





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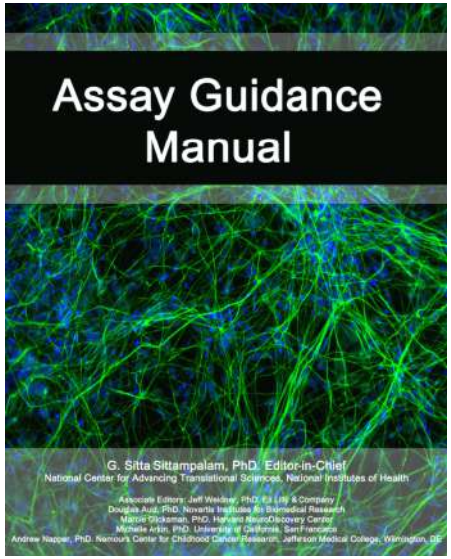
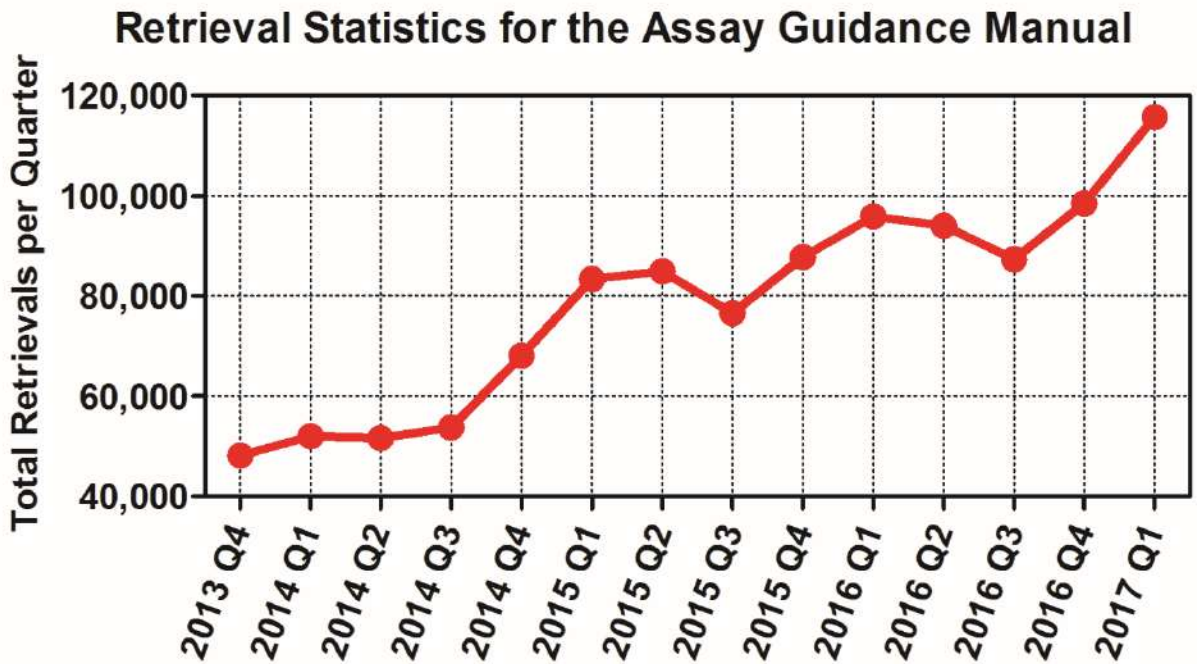
Dissemination of Assay Know-how : The Assay Guidance Manual eBook

Originally, the Eli Lilly Quantitative Biology Manual for HTS and Lead Optimization

- **Freely-available resource for the drug discovery community.**
- **"Tribal knowledge" of over 100 scientists at Lilly and other organizations, edited by >15 experts worldwide, plus *ad hoc* contributors.**
- **eBook at NLM/NCBI, contributions to expand content being continually added.**
- **Recently, a workshop series started based on the AGM.**

http://www.ncbi.nlm.nih.gov/books/NBK53196/





Tox21 Limitations Being Addressed in the Next Phase

- 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
- Limited to acute exposure scenarios

TOX21 ISSUES NEW CHALLENGE COMPETITION

TRANSFORM TOX TESTING CHALLENGE: INNOVATING FOR METABOLISM

Key Development: Three federal agencies are offering toxicity test developers up to $1 million to modify high throughput screens to predict the toxicity of chemical metabolites.

Potential Impact: If successful, the Tox Testing Challenge will improve the relevance and predictive capacities of automated tests that can quickly and simultaneously evaluate hundreds, even thousands, of chemicals.

http://www.transformtoxtesting.com/

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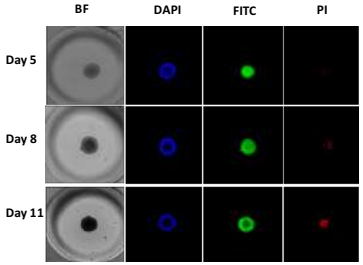
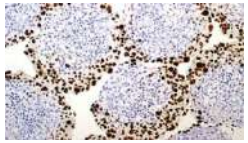
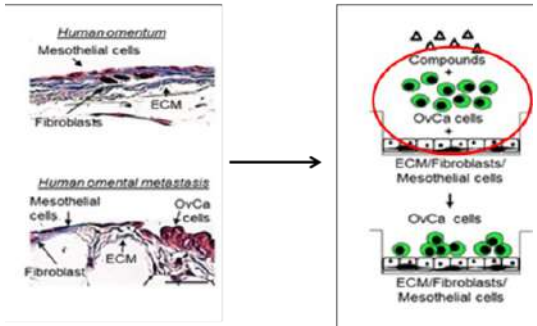
Increasing the Sophistication of Testing Models: A Continuum of 3D Models of Healthy and Diseased Tissues

3D Tumor Spheroids

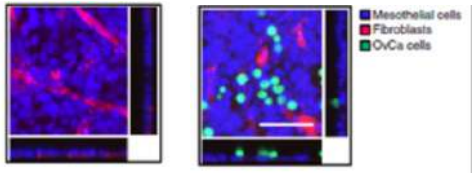




3D Layered Tissues



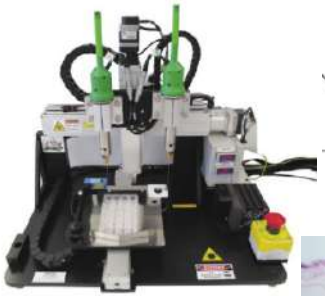
3D OVARIAN HTS ASSAY

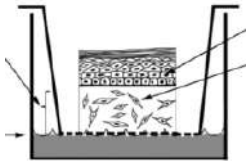


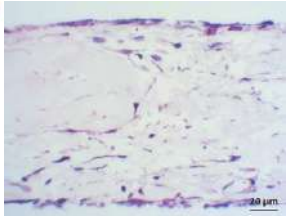
3D ORGANOTYPIC CULTURE

3D ORGANOTYPIC CULTURE + OVCA CELLS

3D Bioprinting of architecturally defined tissues







Tissue-chip (organ-on-a-chip)

Whole-organism (animal, human)

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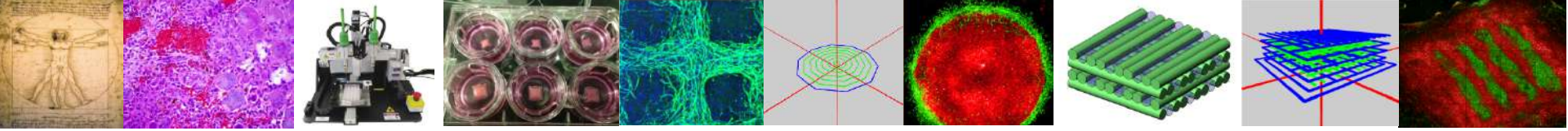
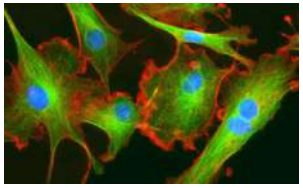


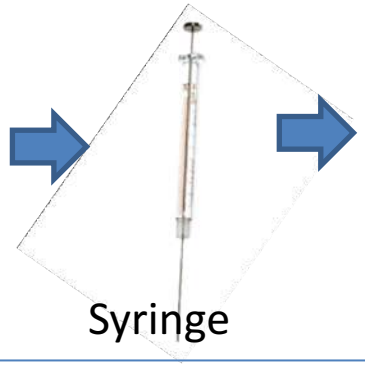
3D Bioprinting

Gel

+



Cells

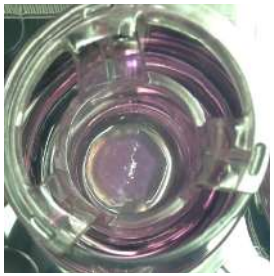


Syringe

Printer

Hydrogel polymer is mixed with cells and loaded into syringe.

Printed construct



1 day

1 week

2 weeks

The printer “3D prints” the cell/gel mixture in a layer-by-layer approach.

The printed construct is incubated to allow the cells to form a tissue, and to enable proper cell differentiation.

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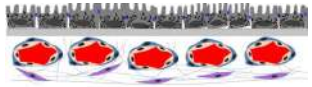
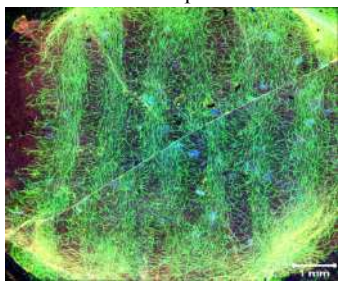
3D Bioprinting Pilot Projects

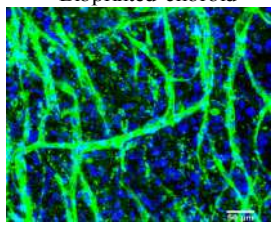
Engineered Blood Retina Barrier with iPSC-RPE and iPSC-endothelial cells



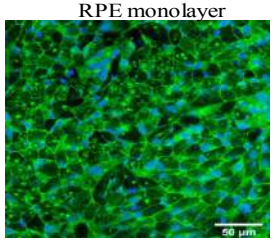
Total Area of Bioprinted choroid



Bioprinted choroid

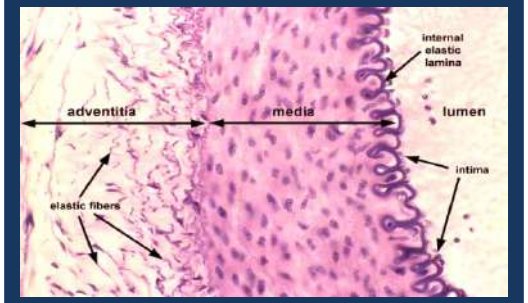


RPE monolayer

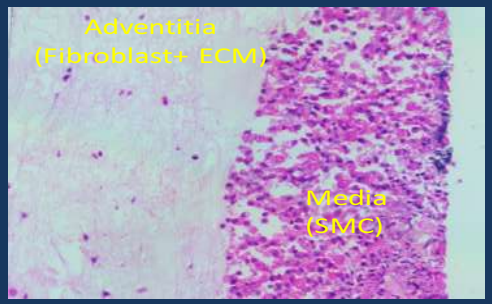


(Green: E-cadherin, Blue: nuclei)

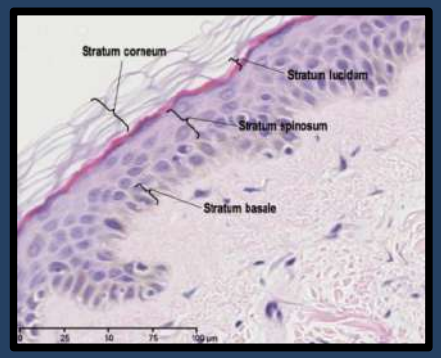
Native Blood Vessel Wall

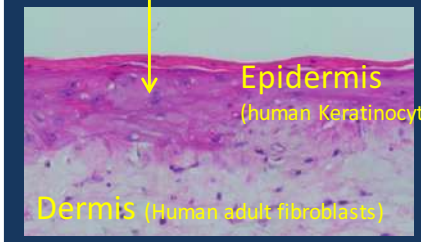


NCATS Printed Blood Vessel Wall



Native Skin





Blood vessel wall (Kan Cao, U of Maryland)

Retina (Kapil Bharti, National Eye Institute)

Skin (Angela Christiano, Columbia University)

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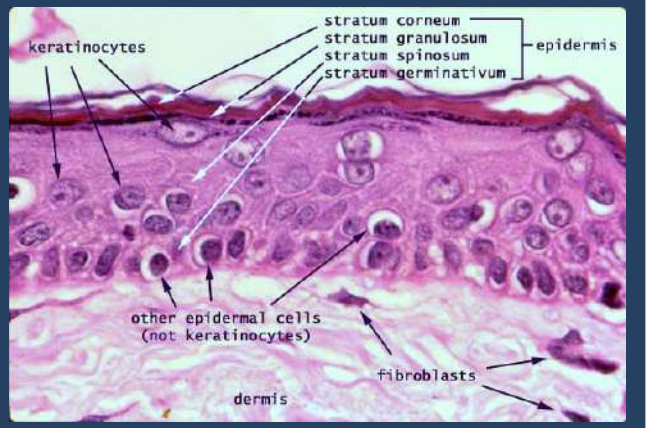
Layers of the Epidermis: native skin *versus* 3D-bioprinted skin

Native Skin



http://www.siumed.edu/~dking2/intro/IN005b.htm

3D-Bioprinted Skin

3D Bioprinting: Engaging the Community

U18 – *Pilot Program Collaborative Drug Discovery Research using Bioprinted Skin Tissue*

- Goal: enable development of bioprinted tissue for drug discovery and toxicity testing.
  - NCATS scientists provide expertise in bioprinting, assay development and drug screening
  - Extramural scientists provide cell resources, disease expertise, and model validation

https://grants.nih.gov/grants/guide/rfa-files/RFA-TR-17-007.html

- Represents a unique NCATS intramural-extramural partnership model.

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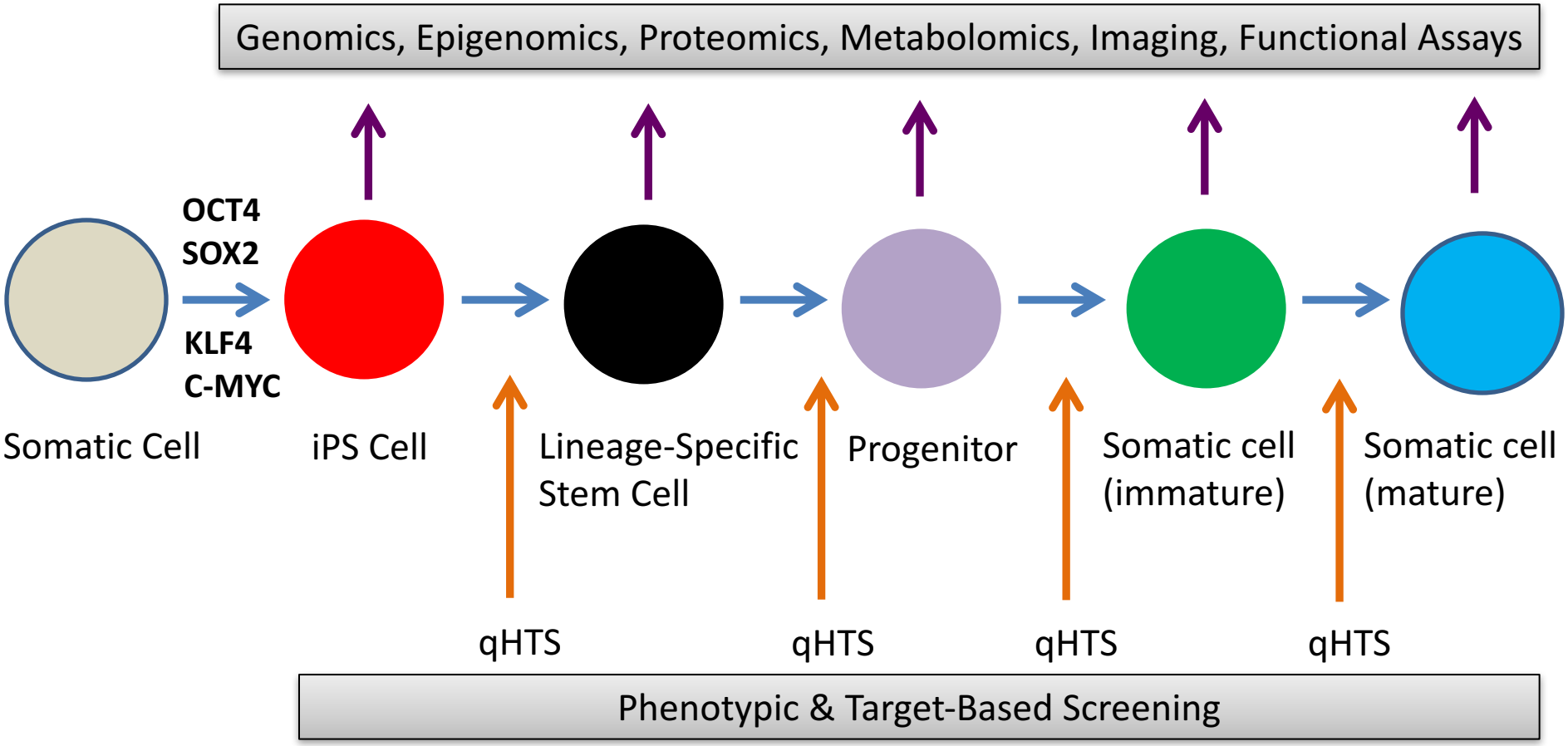
Feeding the bioprinter: Stem Cell Translation Laboratory at NCATS



- Establish QC standards to define pluripotency and differentiated cell types.
- Develop standardized methods to produce mature cells; discover, validate, and disseminate small molecule reagents to replace expensive recombinant proteins, xenogenic material, and undefined media components.

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