

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

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BIOMED²¹
Bethesda, MD
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The Tox 21 Collaborative



National Toxicology Program
Department of Health and Human Services



National Institute of
Environmental Health Sciences



National Center
for Advancing
Translational Sciences

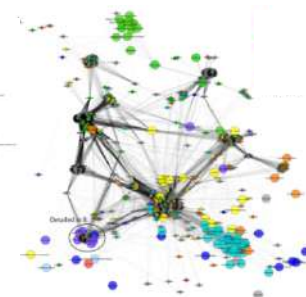
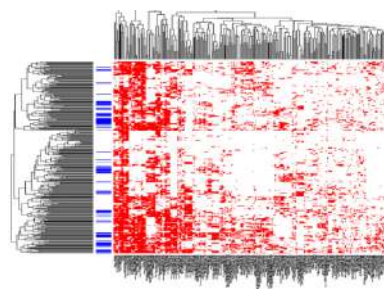


NIH CHEMICAL GENOMICS CENTER

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



Tox21 10K Compound Library

EPA

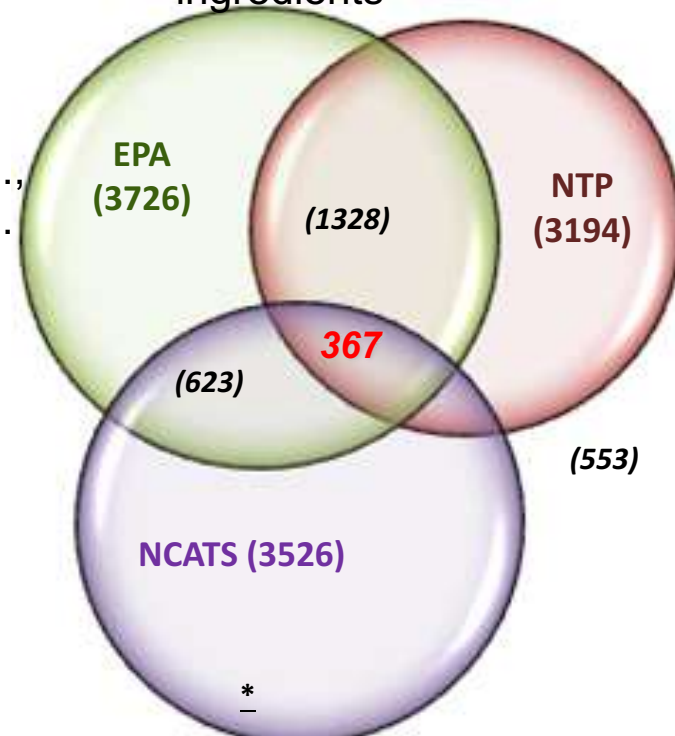
- ToxCast I and II compounds
- Antimicrobial Registration Program
- Endocrine Disruptor Screening Program
- OECD Molecular Screening Working Group
- FDA Drug Induced Liver Injury Project
- Failed Drugs

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

NCATS

- 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: 

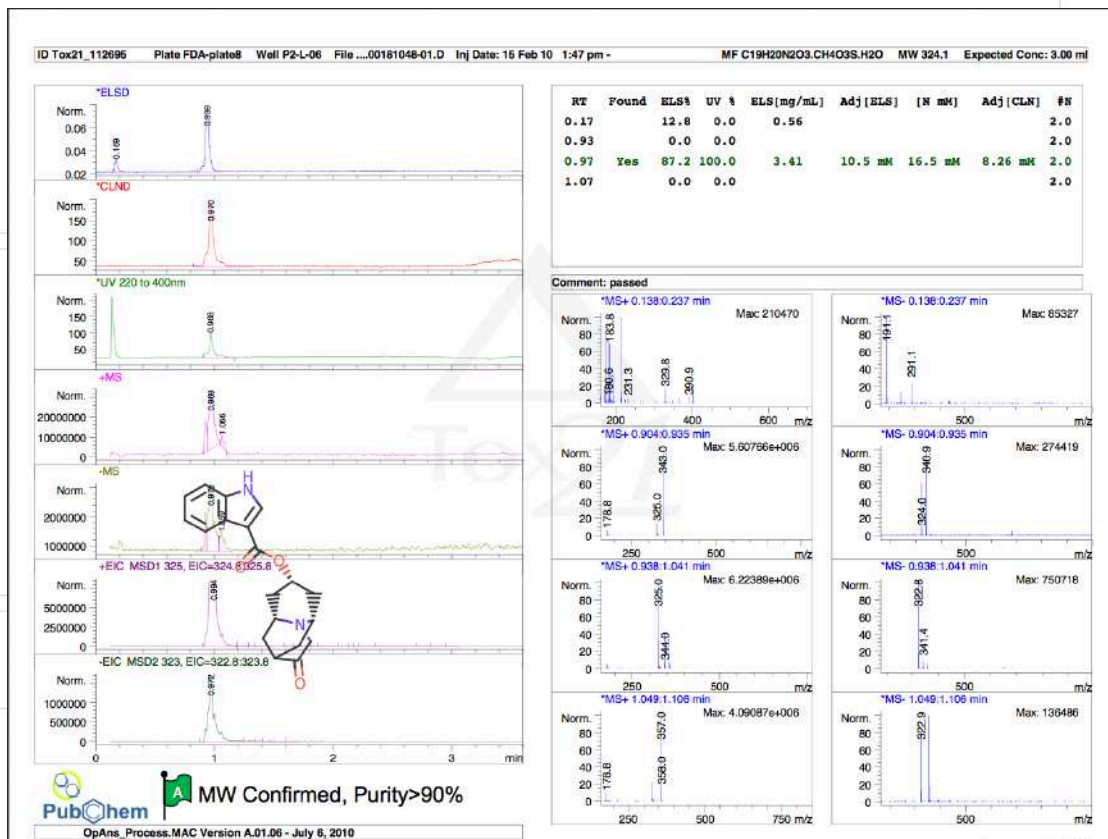
Depositor: Tox21

External ID: **NCGC00181048-01**

Compound CID: 3033817

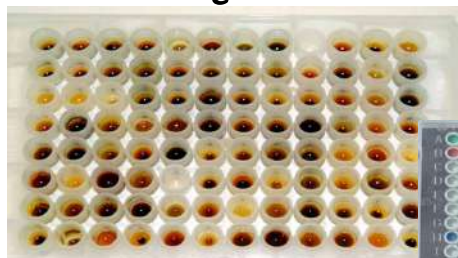
Depositor Comments

TOX21S_v5a



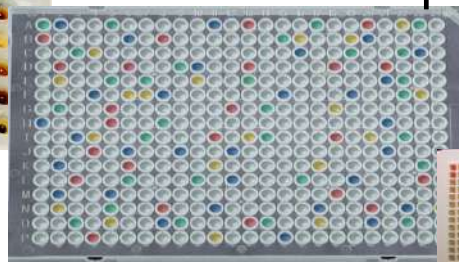
Leveraging modern technologies: shrinking reaction volumes, inclusion of multiple doses

C



- 8 rows x 12 columns
- 88 test samples

96-well plate



384-well plate
4 x 96-well plates

- 16 rows x 32 columns
- 352 test samples



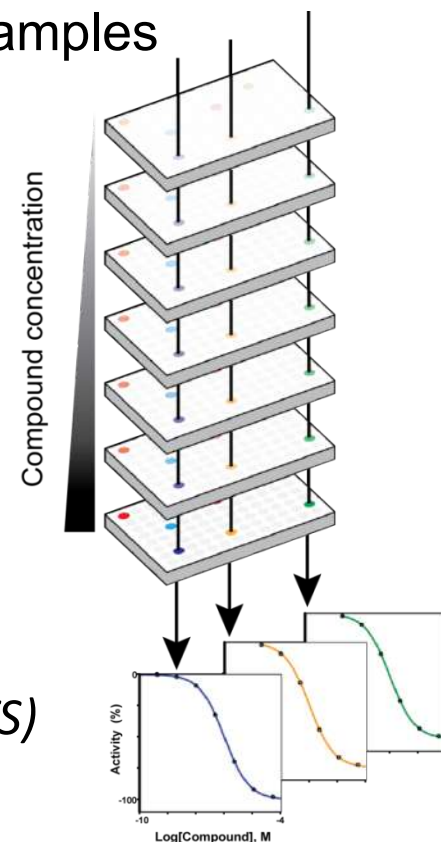
1536-well plate
16 x 96-well plates

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

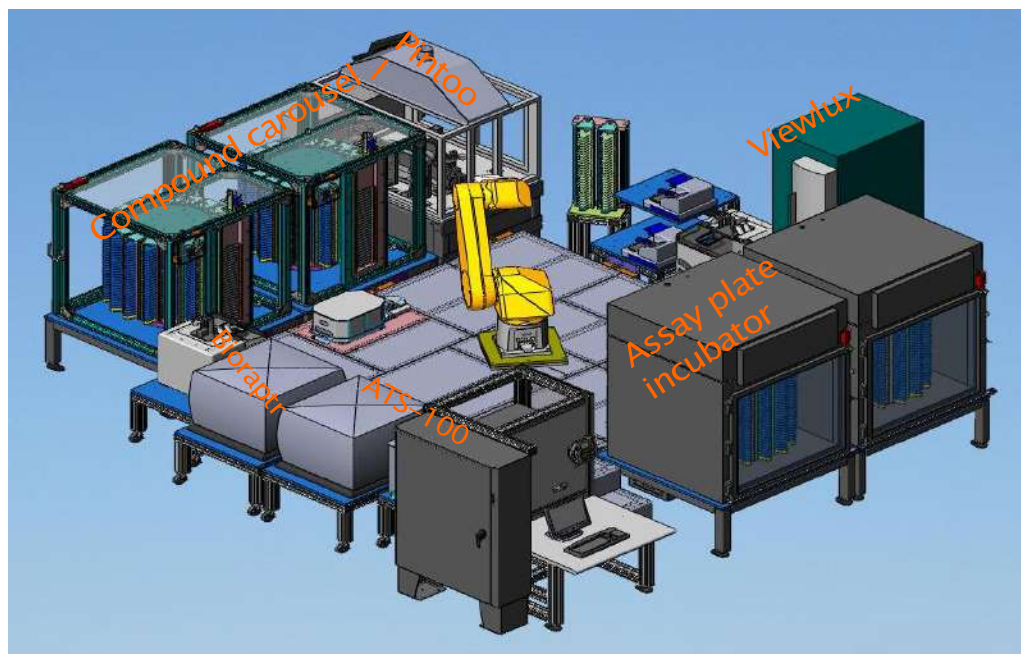
If @ 100 microtiter plates per day:

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



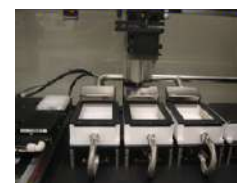
- Transfer size: 0.2 - 10 ul
- 0.5 ml dead volume
- 4 reagents

Multidrop Combi



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

Pintool Station

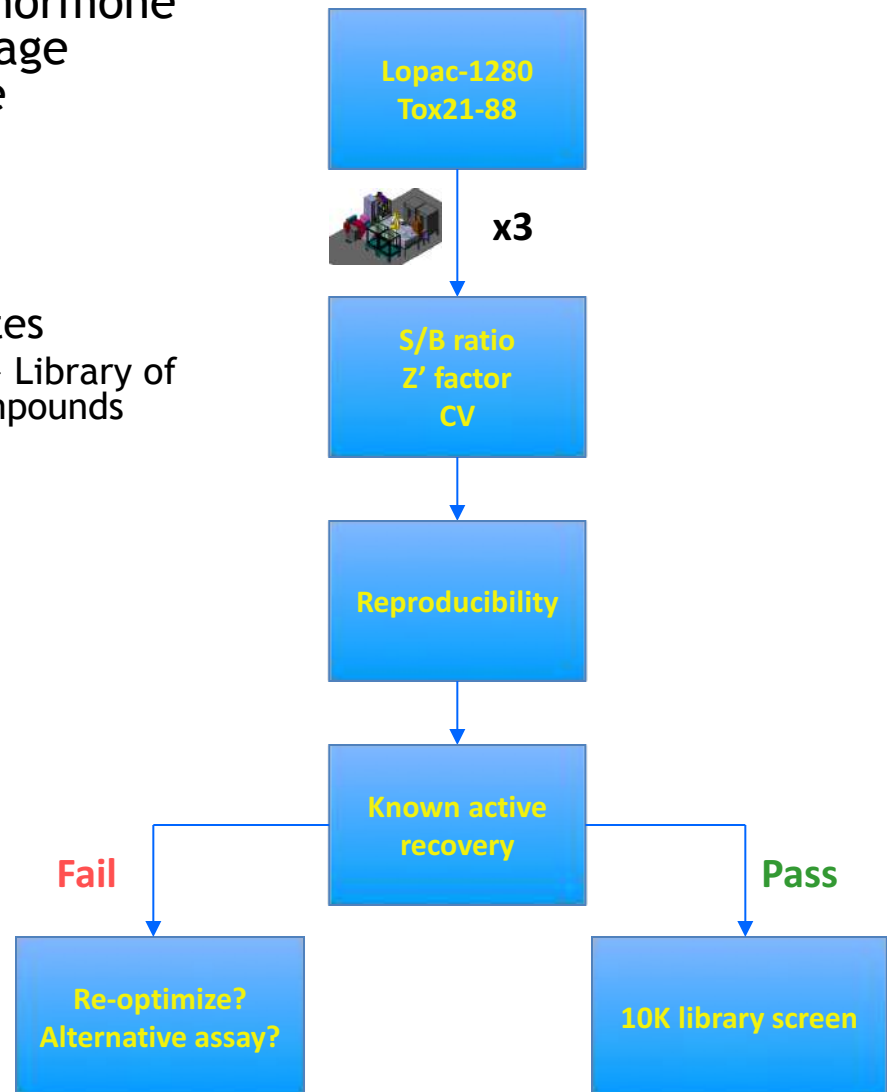


- Transfer size: 20 nl
- Pins washed in 3 solvents

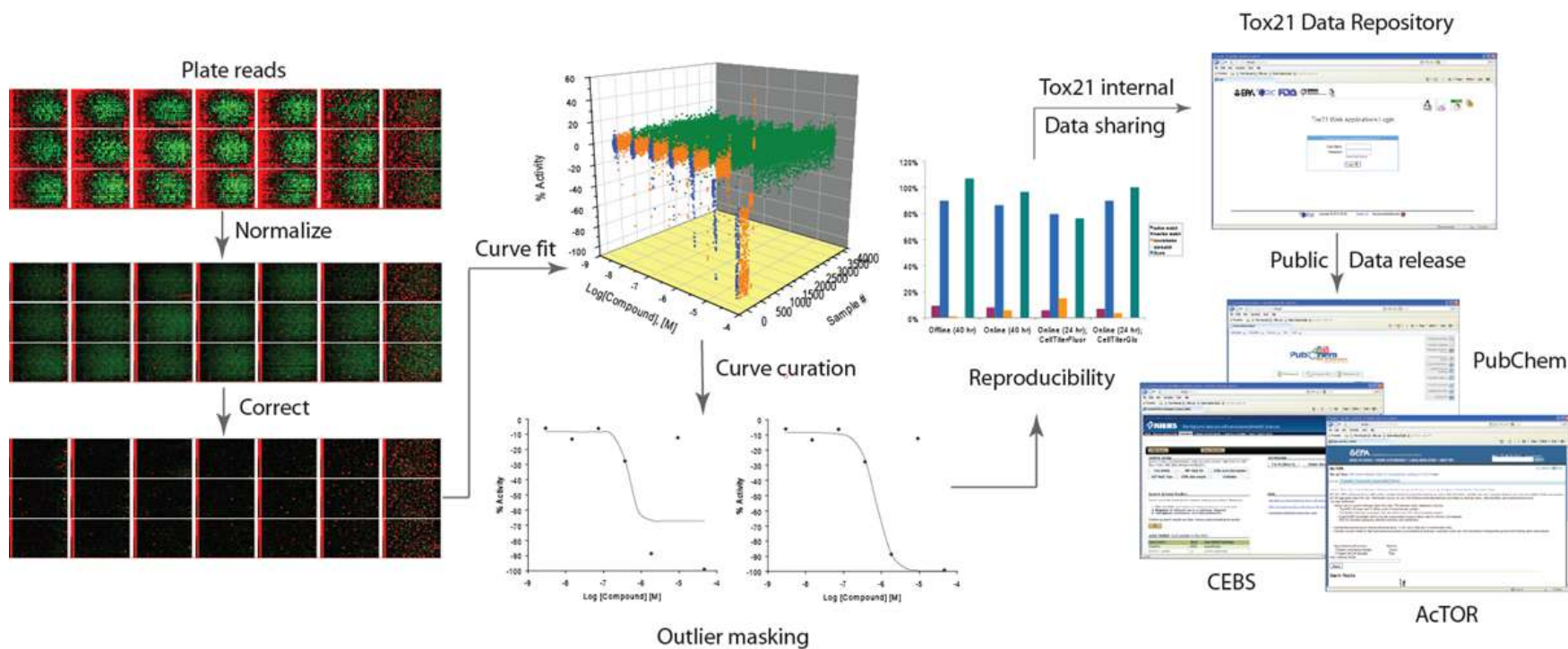
- Compound plate storage and assay plate incubator
- Pintool station and acoustic dispenser for nanoliter compound transfer
- Reagent dispensers (BioRAPTR, Multidrop)
- Centrifuge (V-spin)
- Plate readers (ViewLux and EnVision)

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?

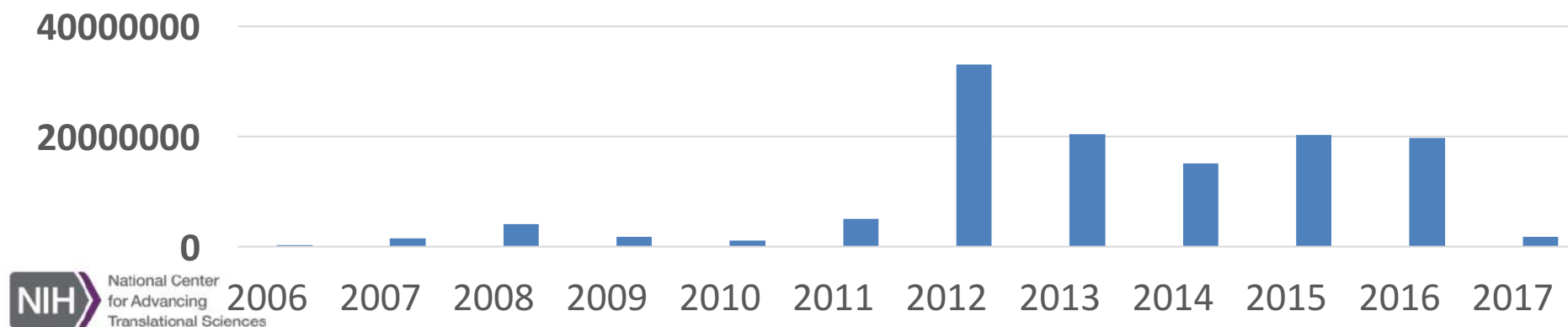


Informatics Analysis Process



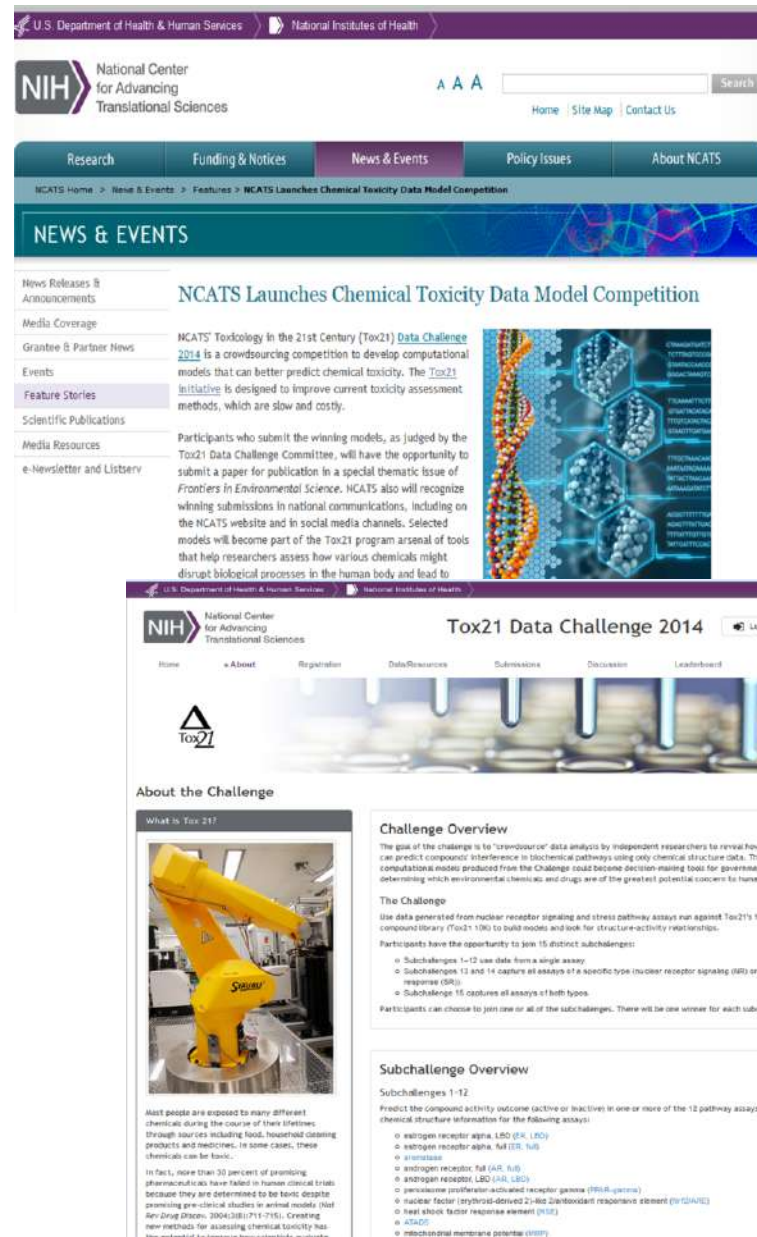
Tox21 Milestones

- 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.



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*



The image displays two screenshots of websites related to the Tox21 Data Challenge 2014. The top screenshot shows the NCATS (National Center for Advancing Translational Sciences) website, which is part of the U.S. Department of Health & Human Services and the National Institutes of Health. It features a navigation bar with links to Research, Funding & Notices, News & Events, Policy Issues, and About NCATS. A prominent banner reads "NCATS Launches Chemical Toxicity Data Model Competition". Below this, a sidebar lists various content types like News Releases, Media Coverage, and Grants. The main content area details the competition's goal: to develop computational models to predict chemical toxicity, improve current assessment methods, and recognize winning submissions for publication and recognition. It also mentions that participants will have the opportunity to submit a paper for publication in a special thematic issue of *Frontiers in Environmental Science*.

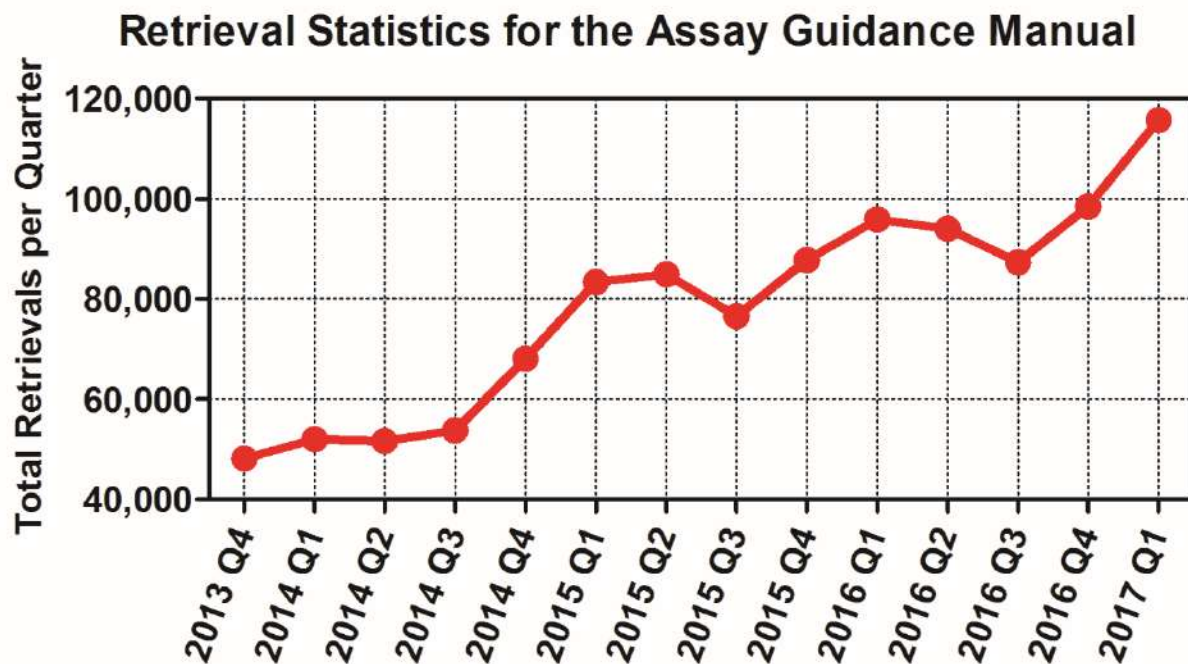
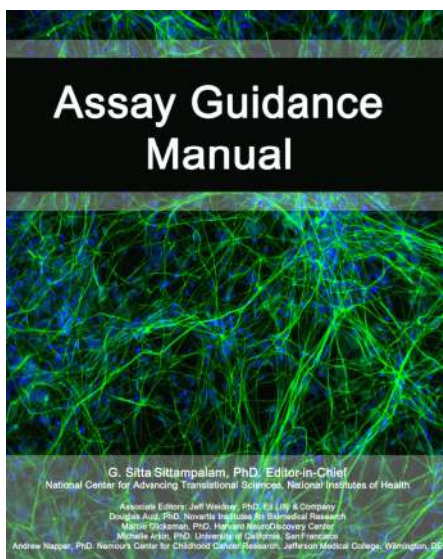
The bottom screenshot shows the "Tox21 Data Challenge 2014" website. It features a navigation bar with links to Home, About, Registration, Data/Resources, Submissions, Discussion, Leaderboard, and Contact Us. A large banner at the top reads "Tox21 Data Challenge 2014". Below this, a section titled "About the Challenge" includes a video player and text explaining the challenge's goal: to develop computational models to predict chemical toxicity, improve current assessment methods, and recognize winning submissions for publication and recognition. It also mentions that participants will have the opportunity to submit a paper for publication in a special thematic issue of *Frontiers in Environmental Science*. A "Challenge Overview" section provides details about the challenge, including the goal, the data generated, and the subchallenges. The "Subchallenge Overview" section lists the 12 subchallenges and the assays used to evaluate the models.

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/>

Transform Tox Testing Challenge Innovating For Metabolism



Stage 1: Concept Development

Call for Proposals: Submission period,
January 8, 2016 – April 8, 2016

Chemical test designers and other companies, universities, government scientists and non-governmental organizations submit ideas for retrofitting high throughput screens (HTS) to include metabolism. Up to 10 proposal submissions may receive an award of \$10,000 each and an invitation to continue on to the next stage.

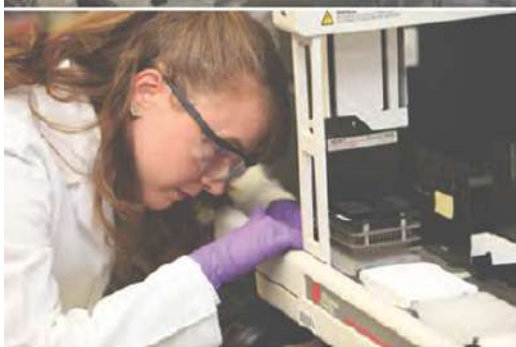
Semi-Finalists to be Announced May 27



Stage 2: Prototype Development

Submission period to be determined

Semi-finalists submit prototypes demonstrating their HTS in use. Up to five participants may be awarded up to \$100,000 each and invited to participate in the final stage.



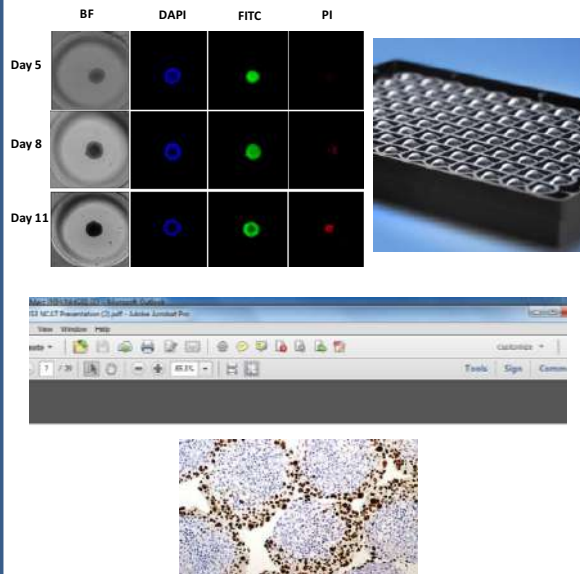
Stage 3: Assay Testing

Submission period to be determined

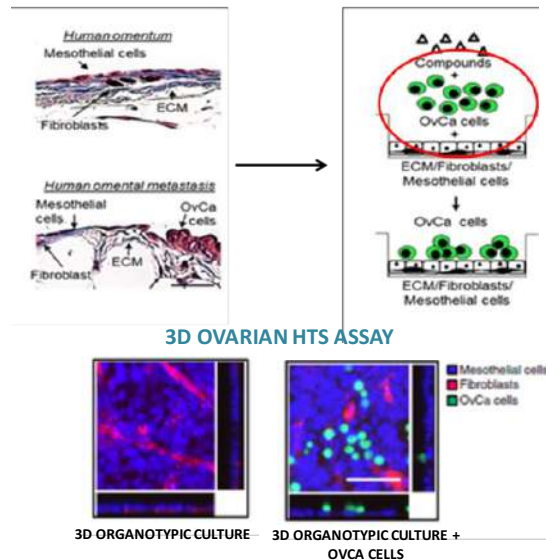
Invited participants propose a commercially viable test method or technology for EPA and its partners to demonstrate and evaluate. Based on this evaluation one participant may be awarded up to \$400,000 to complete the development of a method or device that can provide metabolic competence to HTS assays.

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

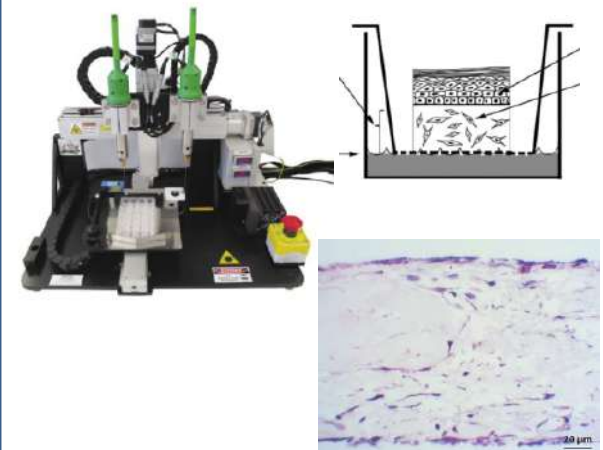
3D Tumor Spheroids



3D Layered Tissues

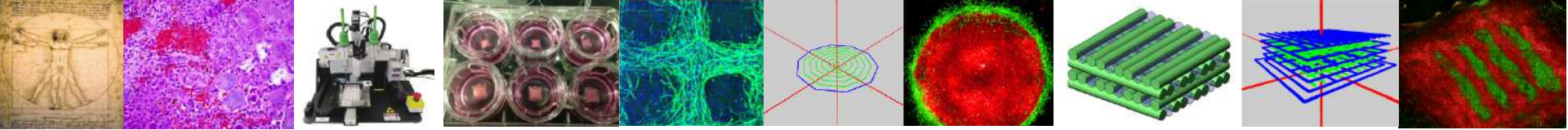


3D Bioprinting of architecturally defined tissues



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

Whole-organism (animal, human)

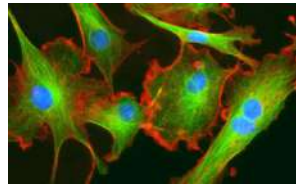


3D Bioprinting

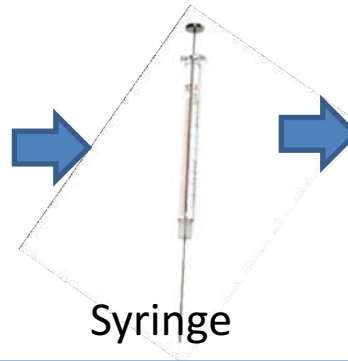


Gel

+



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.

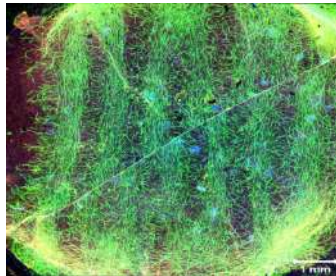
The printed construct is incubated to allow the cells to form a tissue, and to enable proper cell differentiation.

3D Bioprinting Pilot Projects

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

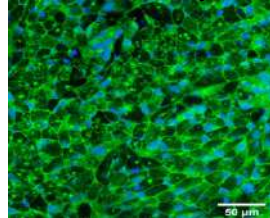
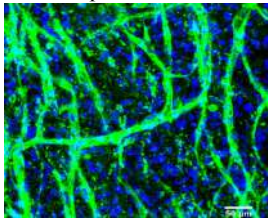


Total Area of Bioprinted choroid



Bioprinted choroid

RPE monolayer



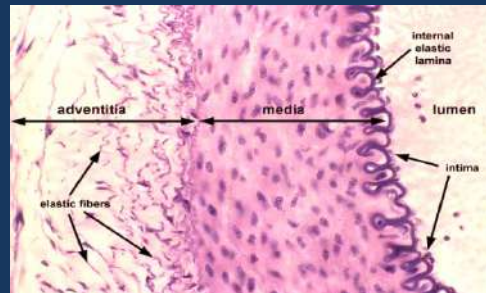
(Green: E-cadherin, Blue: nuclei)

Retina
(Kapil Bharti, National Eye Institute)

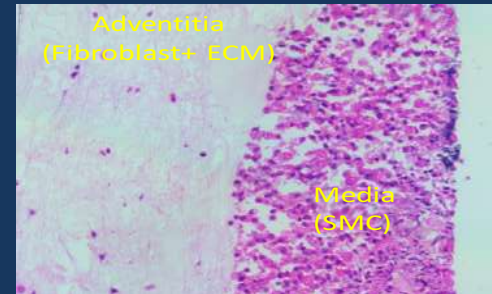
Blood vessel wall
(Kan Cao, U of Maryland)

Skin (Angela Christiano, Columbia University)

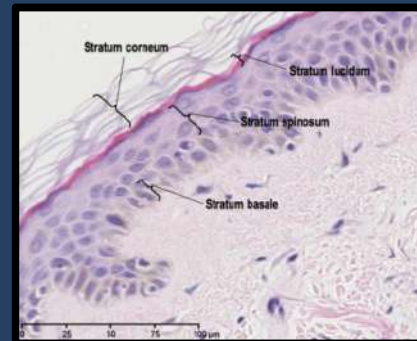
Native Blood Vessel Wall



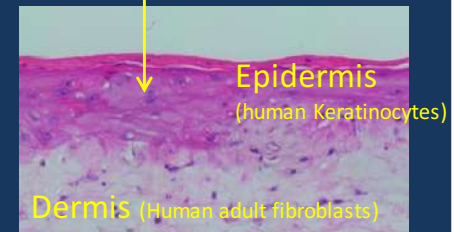
NCATS Printed Blood Vessel Wall



Native Skin

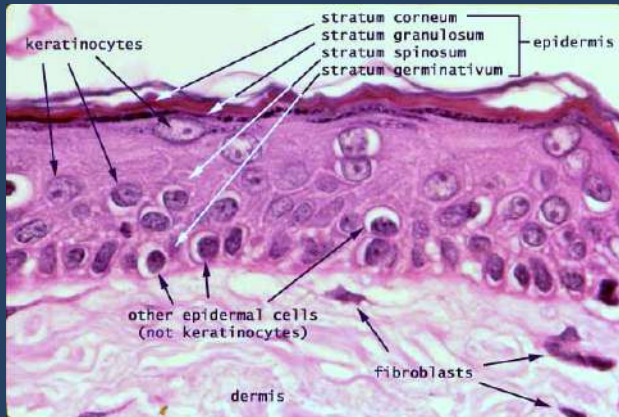


Stratum Spinosum

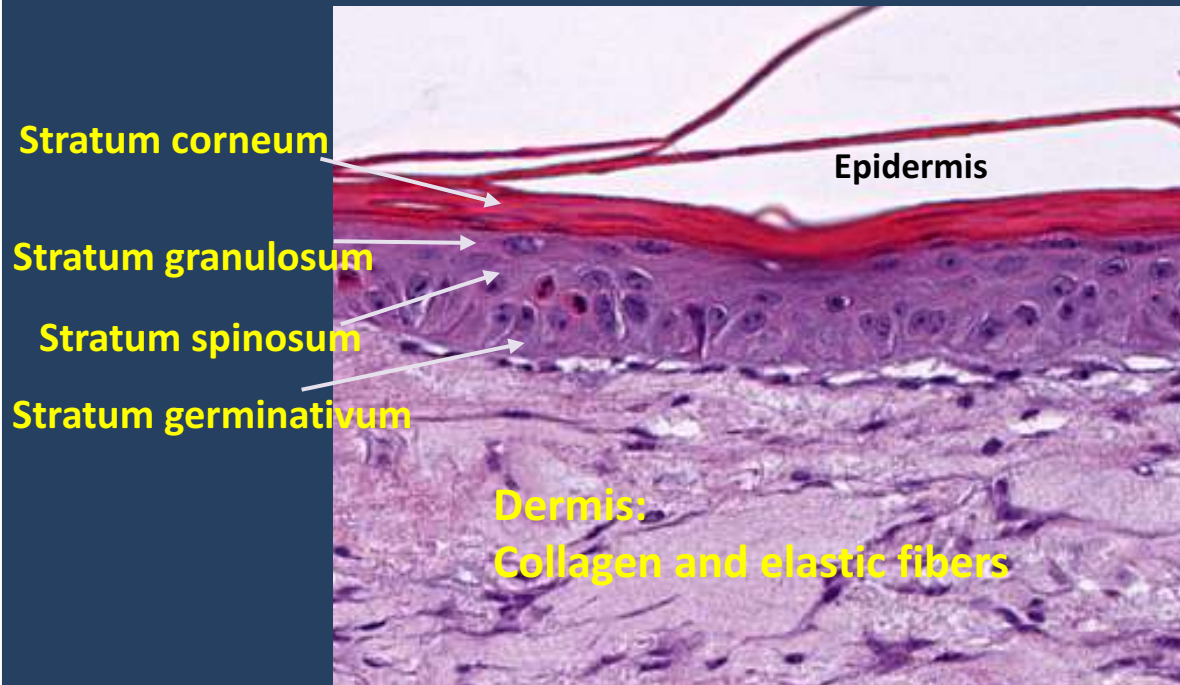


Layers of the Epidermis: native skin *versus* 3D-bioprinted skin

Native Skin



3D-Bioprinted Skin



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

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

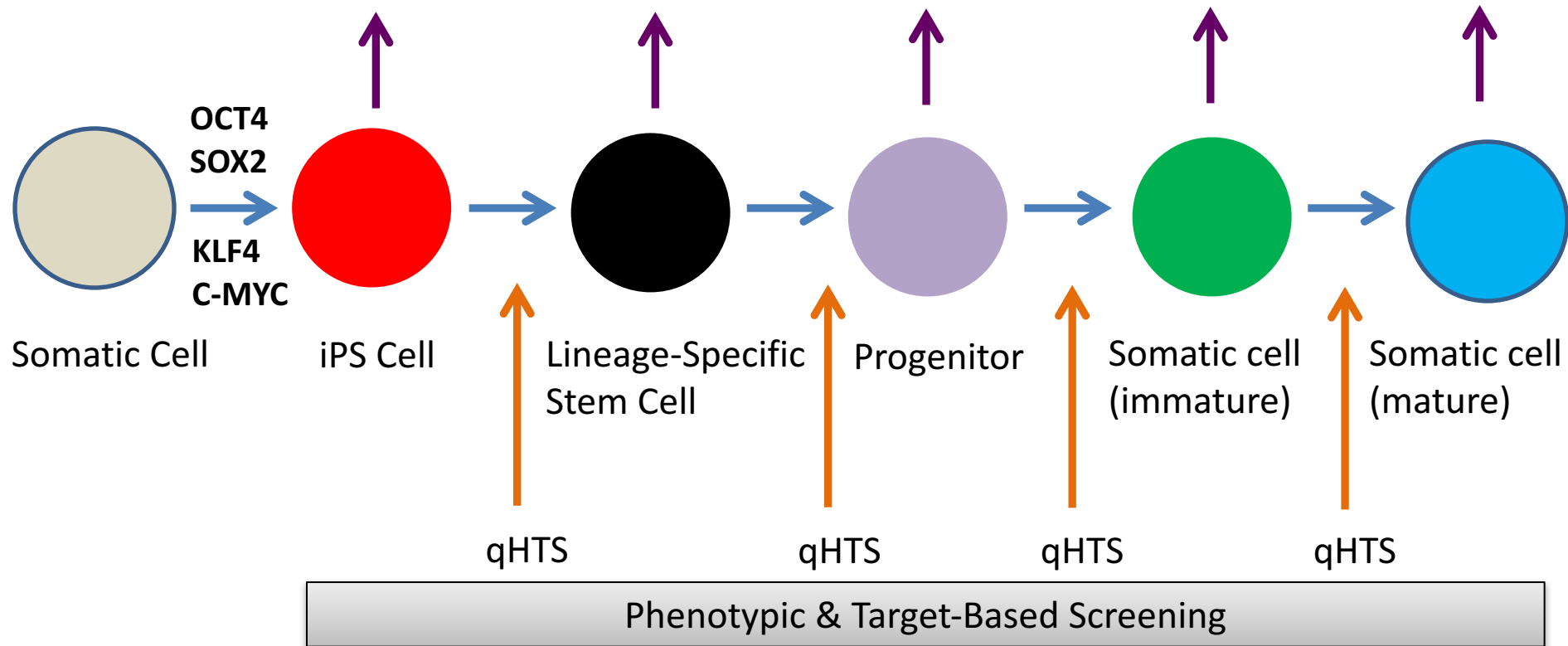
<https://grants.nih.gov/grants/guide/rfa-files/RFA-TR-17-007.html>

- Represents a unique NCATS intramural-extramural partnership model.



Feeding the bioprinter: Stem Cell Translation Laboratory at NCATS

Genomics, Epigenomics, Proteomics, Metabolomics, Imaging, Functional Assays



- 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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