

Challenges and Opportunities in Disease and Toxicity Screening

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Environmental Measurement Symposium
New Orleans, LA
August 8, 2018

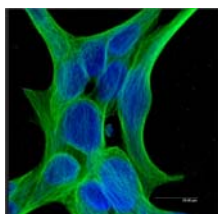


Therapeutic Discovery and Development

Identify disease mechanism → Create testing system → Test chemicals in system → Modify (chemistry) for human use → Test in animals → Test in humans



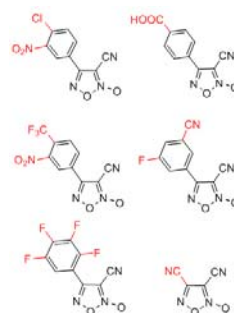
Basic Research



Assay Development



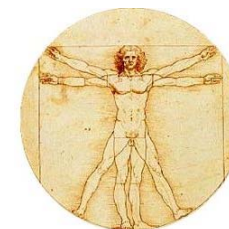
Screening



Medicinal Chemistry



Preclinical Development



Clinical Development



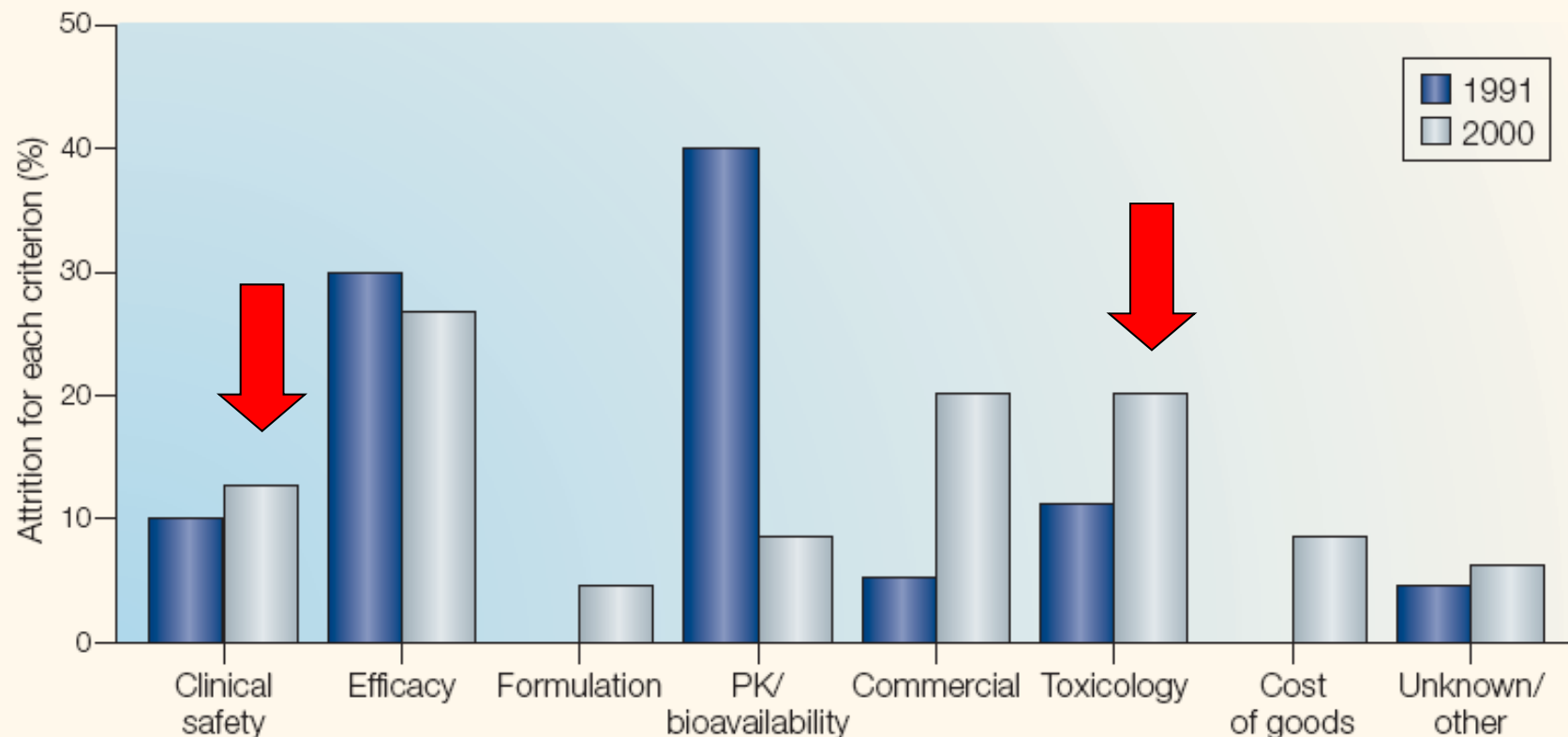
NIH National Center for Advancing Translational Sciences

The common causes of translational inefficiency are NCATS' focus

- Predicting safety and effectiveness of new drugs
- Scalable approaches to the >6000 untreatable diseases
- Data interoperability
- Biomarker qualification process
- Clinical trial networks
- Patient recruitment
- Electronic Health Records for research
- Harmonized IRBs
- Clinical diagnostic criteria
- Clinical outcome criteria (e.g., PROs)
- Adaptive clinical trial designs
- Shortening time of intervention adoption
- Methods to better measure impact on health (or lack thereof)
- Cross-sector collaborative structures
- Translational education/workforce development



Toxicity is (still) a common reason for drug development failure

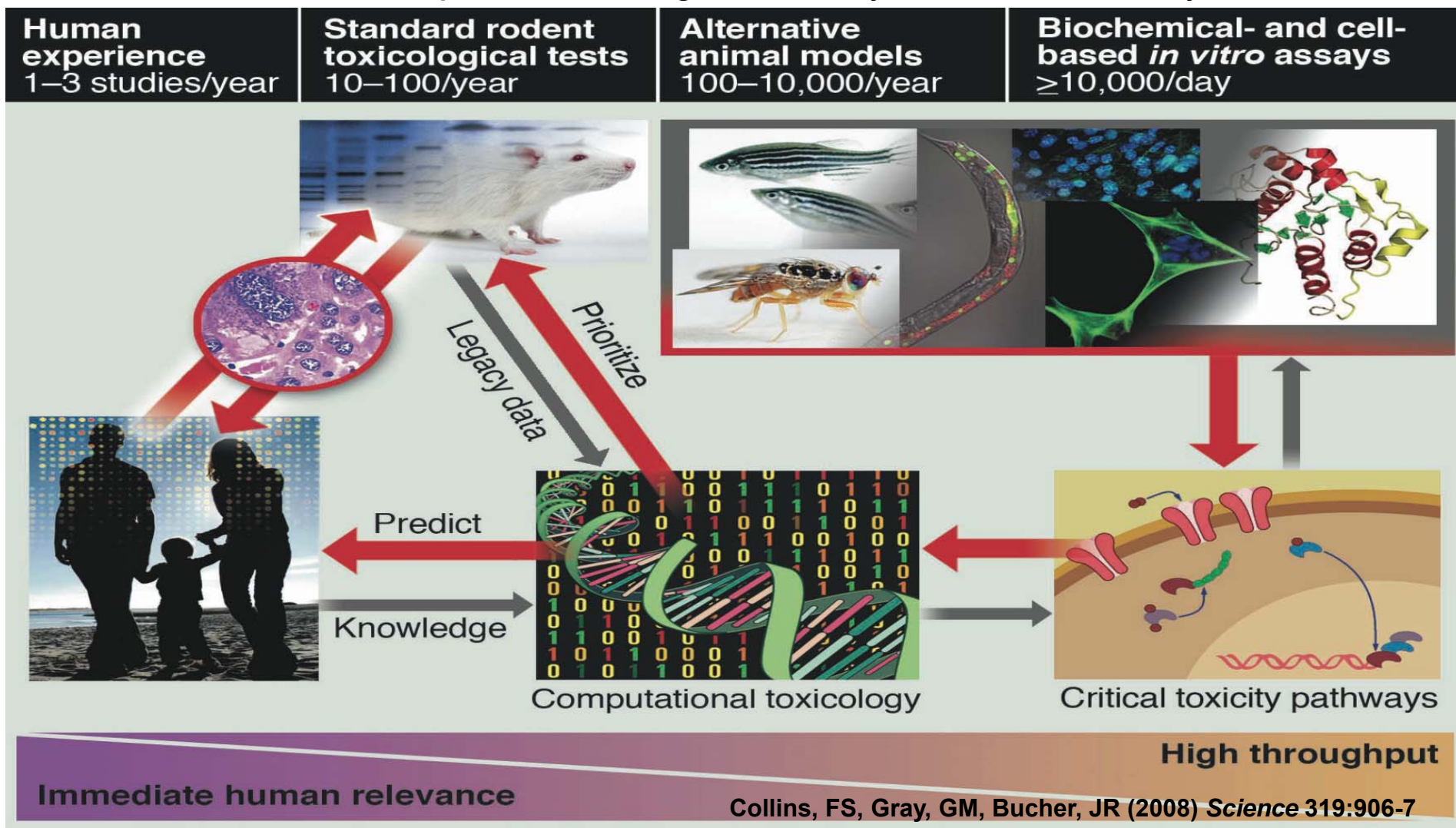


Preclinical (21%) + Clinical (12%) Tox = 33% of all failures

Kola and Landis, *Nature Reviews Drug Discovery* 3, 711-716, 2004.

Why do we need to prioritize compounds for testing?

- There are over 80,000 chemicals in commerce, the majority with little to no toxicological data.
- We cannot solve the problem using laboratory animal tests only.



The Tox21 Collaborative



National Toxicology Program
Department of Health and Human Services

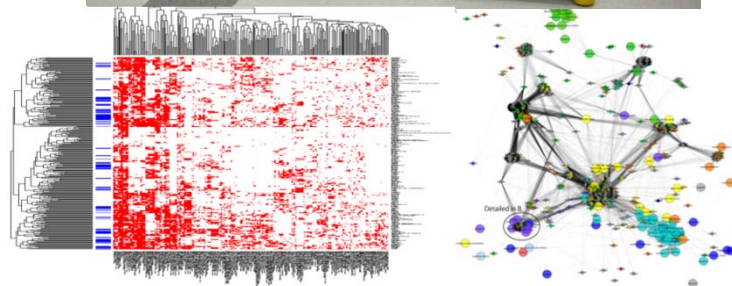


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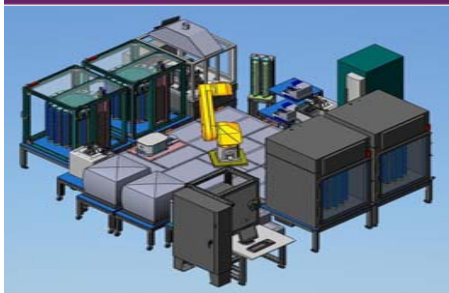


National Institute of
Environmental Health Sciences

- Develop predictive models for biological response to new chemicals in humans, reducing use of animals
- Identify patterns of chemical-induced biological response to understand adverse effects
- Prioritize chemicals for more extensive toxicological evaluation, guide optimization



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The Tox21 10K Library Screening Project



Collection of
diverse
chemicals

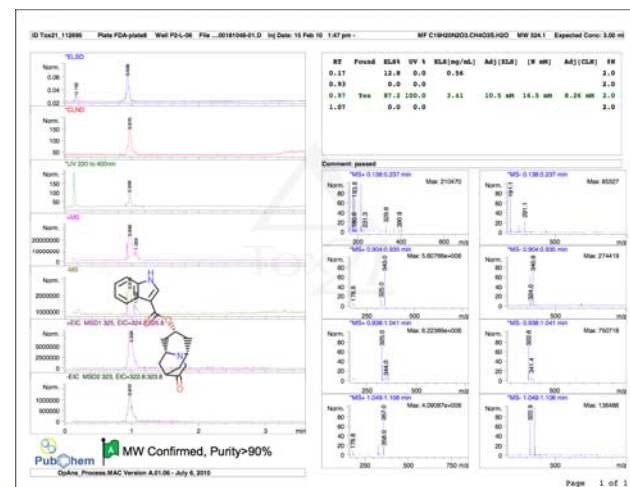
In vitro test
methods,
screening

High quality
bioactivity
data

Predictive
models
(of bioactivity
of a new
chemical *in vitro* and, one
day, *in vivo*)

>50 screening
campaigns of the 10K
Collection

**Tox21 10K Chemical Collection: ~10,000
chemicals (nominated and procured by EPA,
NIEHS, and NCATS) comprising approved drugs,
failed drugs, pesticides, industrial chemicals, etc.
Extensive Quality Control →**



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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

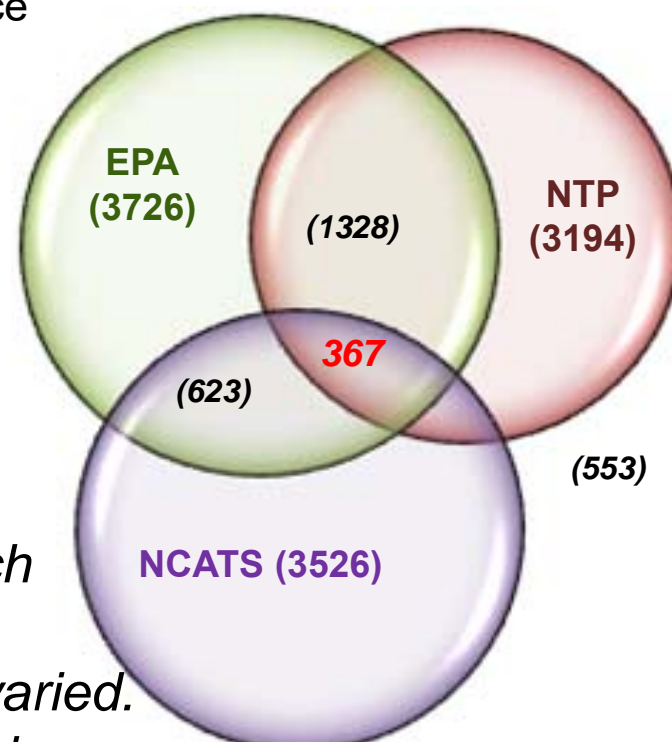
NTP

- 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

NCATS

- Approved Drugs
- Investigational Drugs

- *88 single-sourced compounds in duplicate on each plate.*
- *Three library replicates, compounds positionally-varied.*
- *Each sample arrayed in 15 concentrations over 4 logs.*



Entire-Library QC Project

- Multi-year undertaking using a range of LC-/GC-MS and NMR methods.
- >10,000 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:

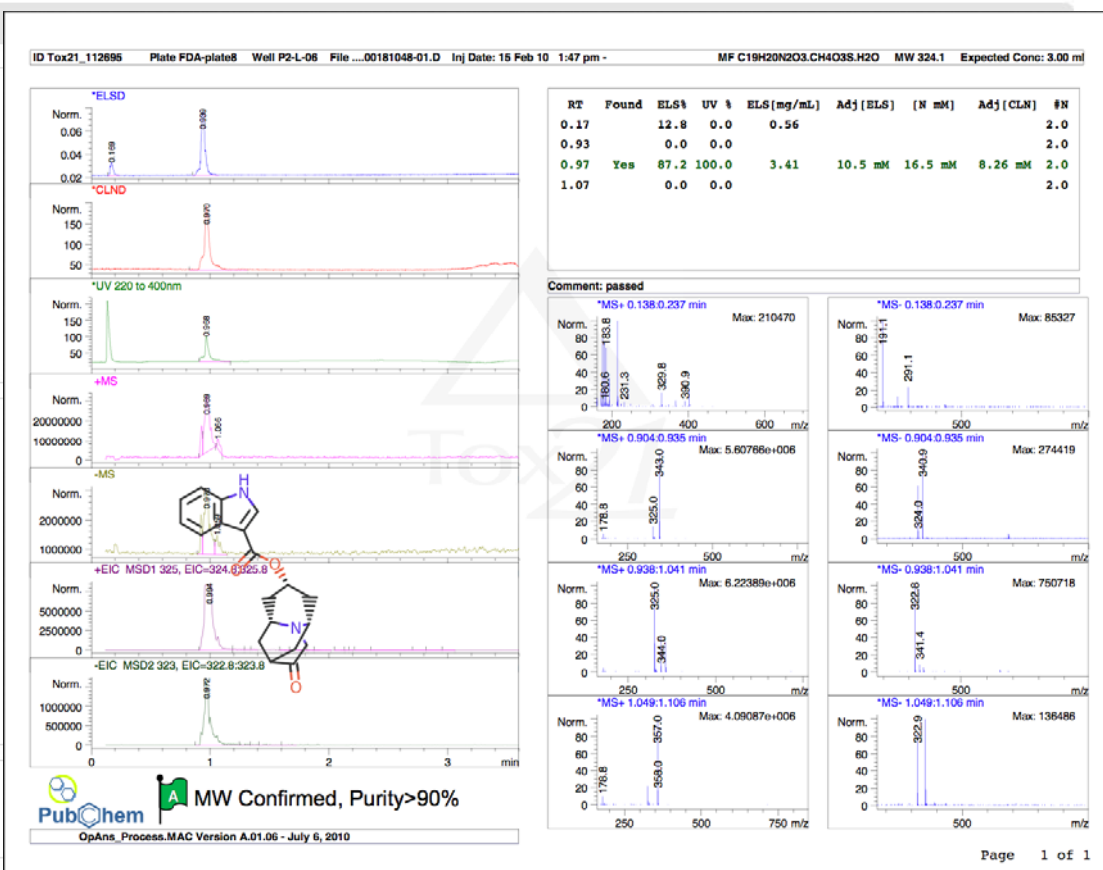
Depositor: Tox21

External ID: NCGC00181048-01

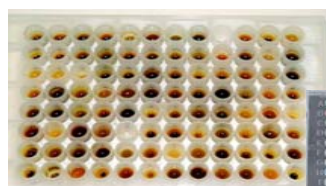
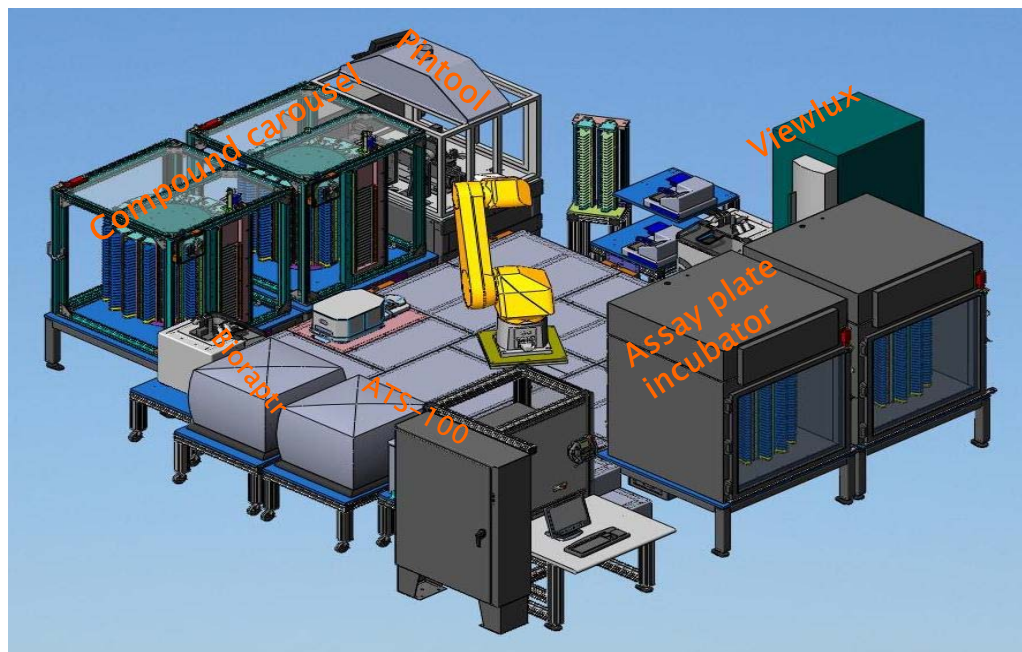
Compound CID: 3033817

Depositor Comments

TOX21S_v5a



Tox21 Robot Platform



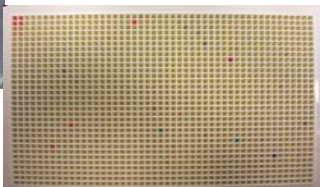
96-well plate

- 8 rows x 12 columns
- 88 test samples



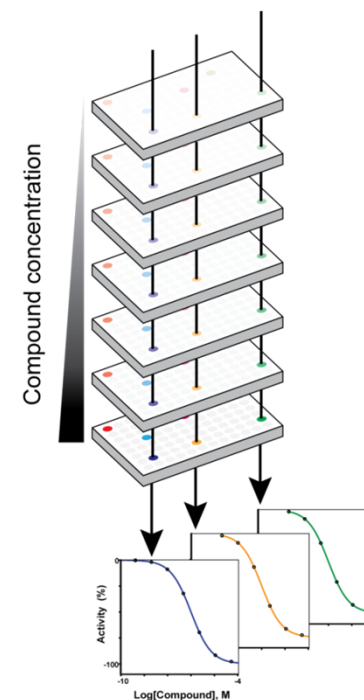
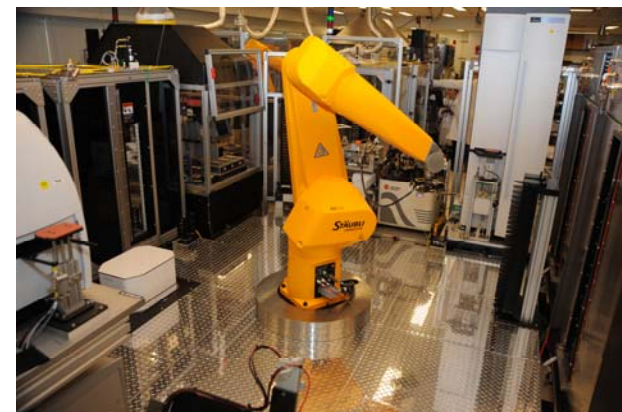
384-well plate
4 x 96-well plates

- 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



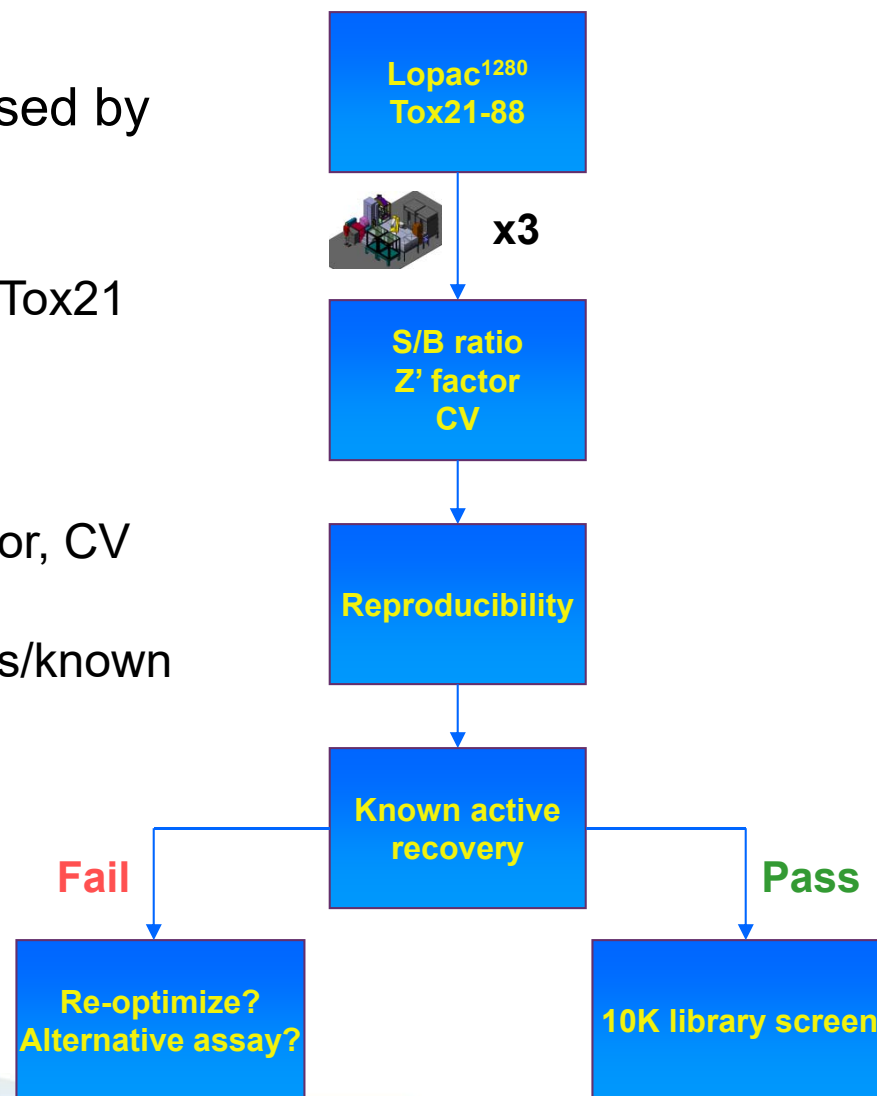
Dose-response-based screening
Proc Natl Acad Sci 103:11473



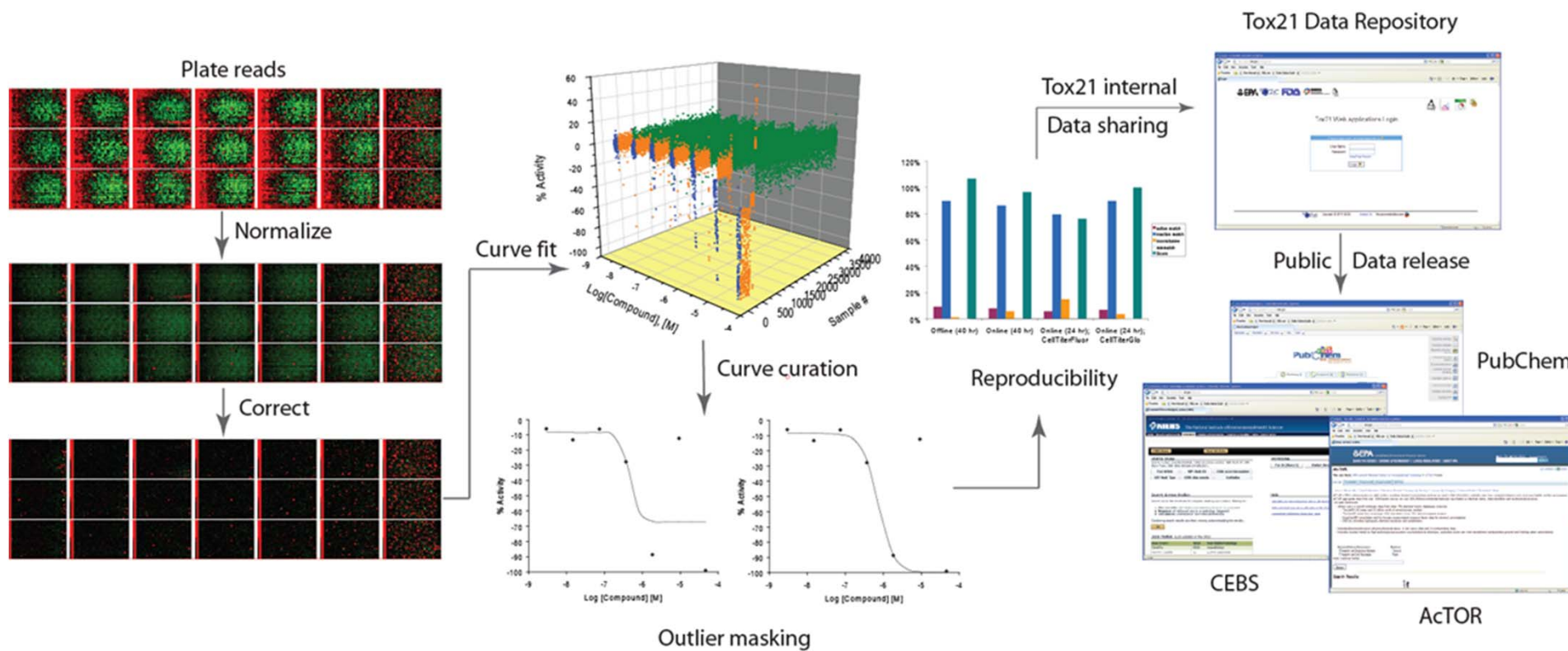
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Assay Nomination and Validation Process

- Screening assay proposed and discussed by Assays and Pathways WG.
- Online validation on Tox21 Robot
 - Tox21 validation plate: Lopac¹²⁸⁰ + 88 Tox21 replicates
 - Triplicate runs
- Acceptance criteria
 - 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 Screening Outcomes

- Rapid testing of chemicals enabled through robotic screening, largest collection of environmental chemicals and drugs assembled, multiple Quality Control (QC) measures in place.
- Deposition into the public domain of the largest-ever toxicology dataset (>90M datapoints).
- Using crowdsourcing to move from data to knowledge.
- Estrogen receptor *in vitro* data being used by the EPA for regulatory purposes (<https://www.epa.gov/endocrine-disruption/use-high-throughput-assays-and-computational-tools-endocrine-disruptor>).
- Multiple organizations and consortia worldwide using Tox21 data (e.g., eTox/IMI, Tiley *et al. Environ Int* 101:19-26 used Tox21 data to rank chemicals of concern at Superfund sites).



Tox21 Rapid Response to a Public Health Emergency: the EPA Oil Spill Dispersants Project

Task: using *in vitro* tests, evaluate as rapidly as possible the potential toxicities of dispersants to use in cleaning the Deepwater Horizon oil spill



- Project completed in 30 days from start to finish, including preparation of tests and dispersants, carrying out the tests, data analysis, and reporting.
- Corexit 9500, ultimately used for cleaning the oil spill in the Gulf of Mexico, did not show estrogenic and androgenic activity.
- Team received an Appreciation Award from the EPA.



Dissemination and utilization of data: model-building through “challenge” 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 Jan 2015, presentations at SOT2016
- Papers describing top models published in *Frontiers in Environmental Science*
- >80% accuracy of prediction for most top-scoring models

The image displays two screenshots of web pages related to the Tox21 Data Challenge 2014. The top screenshot shows the NCATS (National Center for Advancing Translational Sciences) website, specifically the 'NEWS & EVENTS' section. It features a headline 'NCATS Launches Chemical Toxicity Data Model Competition' and a detailed description of the challenge, which is a crowdsourcing competition to develop computational models that can better predict chemical toxicity. The bottom screenshot shows the 'Tox21 Data Challenge 2014' website, which includes a 'Challenge Overview' section. This section explains the goal of the challenge: to 'crowdsource' data analysis by independent researchers to reveal how well they can predict compound interference in biochemical pathways using only chemical structure data. It also lists the subchallenges, which involve building models to predict the outcome of 12 pathway assays based on chemical structure information.

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

ALTEX 2018 Mar 8; 35(2): 163-168. doi: 10.14573/altex.1803011



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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.

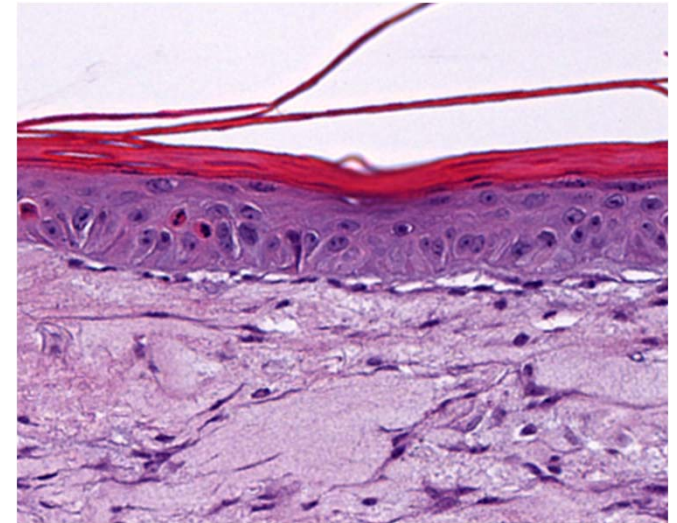
Potential Impact: If successful, the Tox Testing Challenge will improve the relevance and predictive capacities of automated tests that can quickly and simultaneously evaluate hundreds, even thousands, of chemicals.

<http://www.transformtoxtesting.com/>

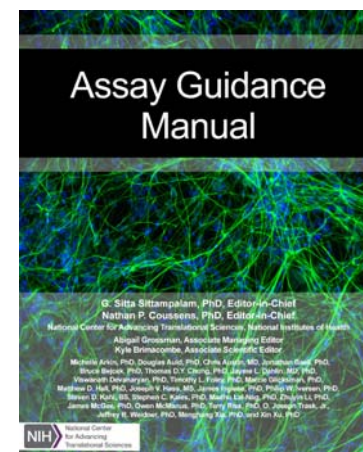
Transform Tox Testing Challenge Innovating For Metabolism	
	Stage 1: Concept Development Call for Proposals: Submission period, January 8, 2016 – April 8, 2016 Chemical test designers and other companies, universities, government scientists and non-governmental organizations submit ideas for retrofitting 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.

Tox21 Next Phase Focus Areas for NCATS

- Work with partners on the continuing evolution of the chemicals test set(s).
- Maintain supply of the approved-drugs (NPC) portion of the screening library.
- Increased use of physiologically relevant cells (e.g., primary and iPS-derived) in HTS.
- Collaboration with EPA and NTP on improving the metabolic competence of HTS assays.
- Introduction and use of 3D models, such as those derived through 3D bioprinting.
- Continue building predictive models using Tox21 datasets.
- Dissemination: improved web site, AGM.



3D-bioprinted skin produced at NCATS



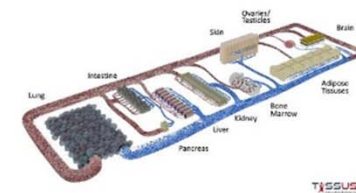
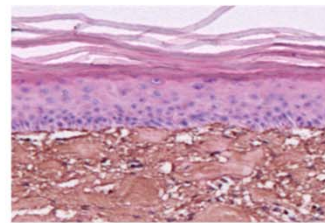
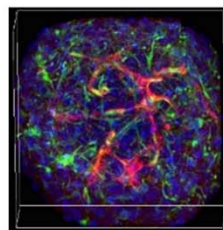
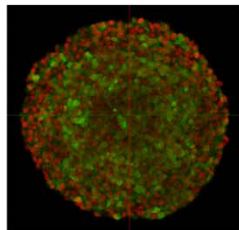
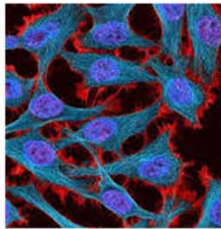
<https://ncats.nih.gov/expertise/preclinical/agm>



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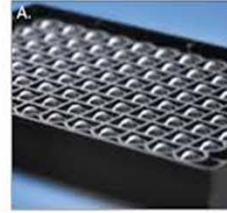
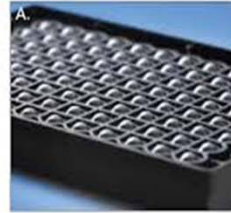
Increasing the predictivity of HTS: a continuum of 3D models of healthy and diseased tissues

2D Spheroids Organoids Printed Tissues Organ-on-a-chip



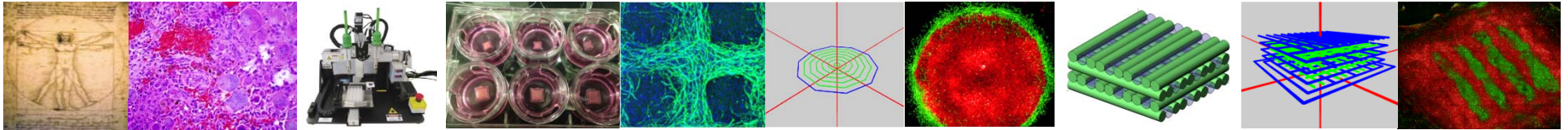
HTS compatibility

Physiological complexity



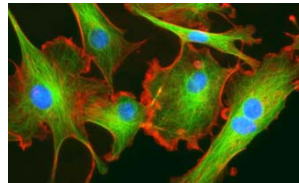
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3D Tissue Bioprinting



Gel

+



Cells



Syringe

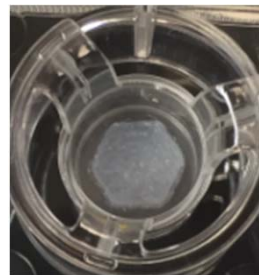


Printer

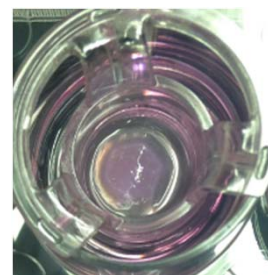
Hydrogel polymer is mixed with cells and loaded into syringe.



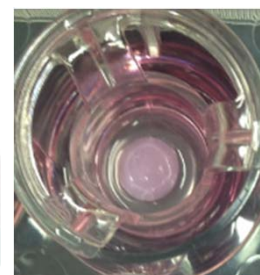
The printer “3D prints” the cell/gel mixture in a layer by layer approach.



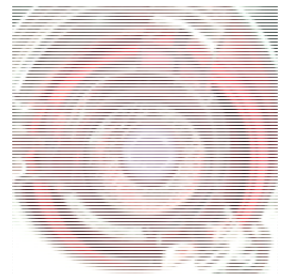
Printed construct



1 day



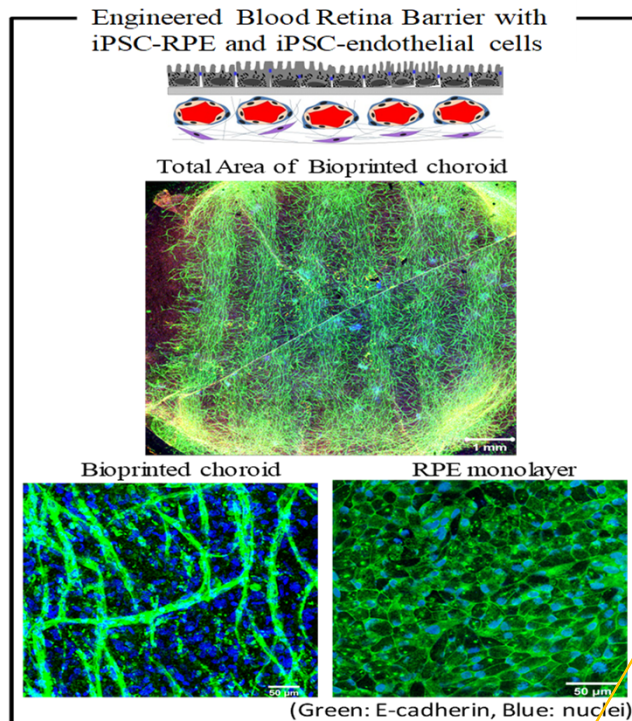
1 week



2 weeks

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

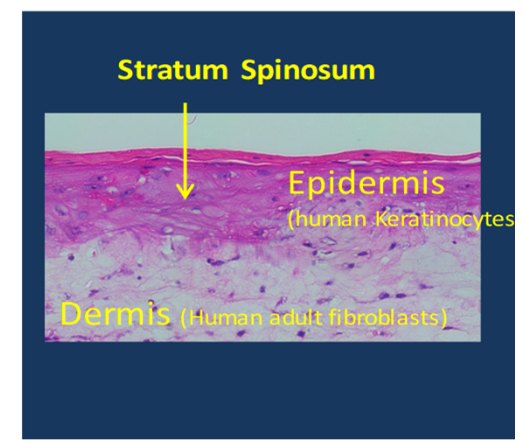
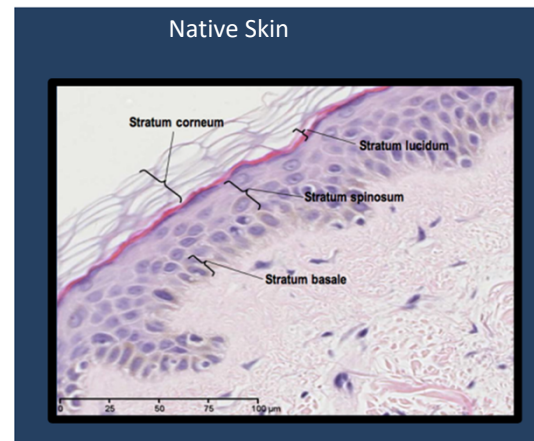
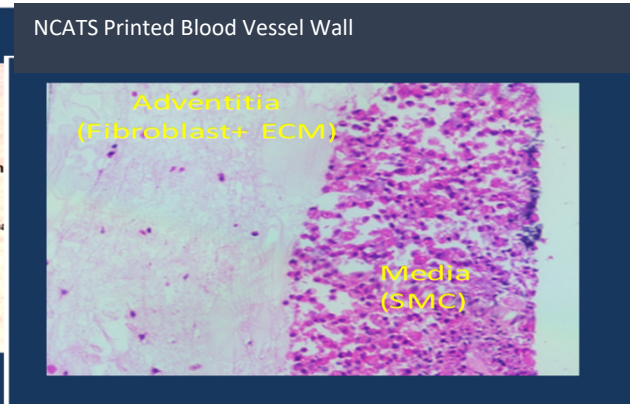
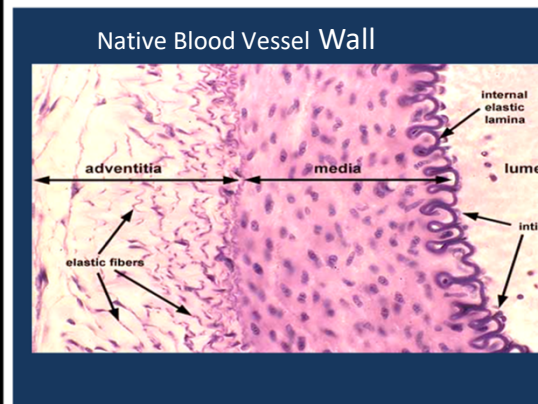
3D Bioprinting Pilot Projects



Retina
(Kapil Bharti, National Eye Institute)

Blood vessel wall
(Kan Cao, U of Maryland)

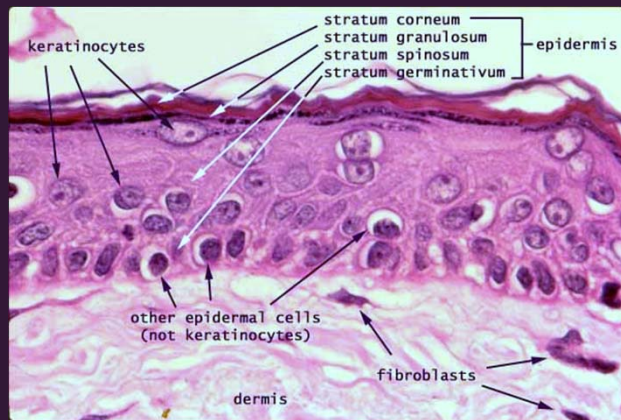
Skin (Angela Christiano, Columbia University)



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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

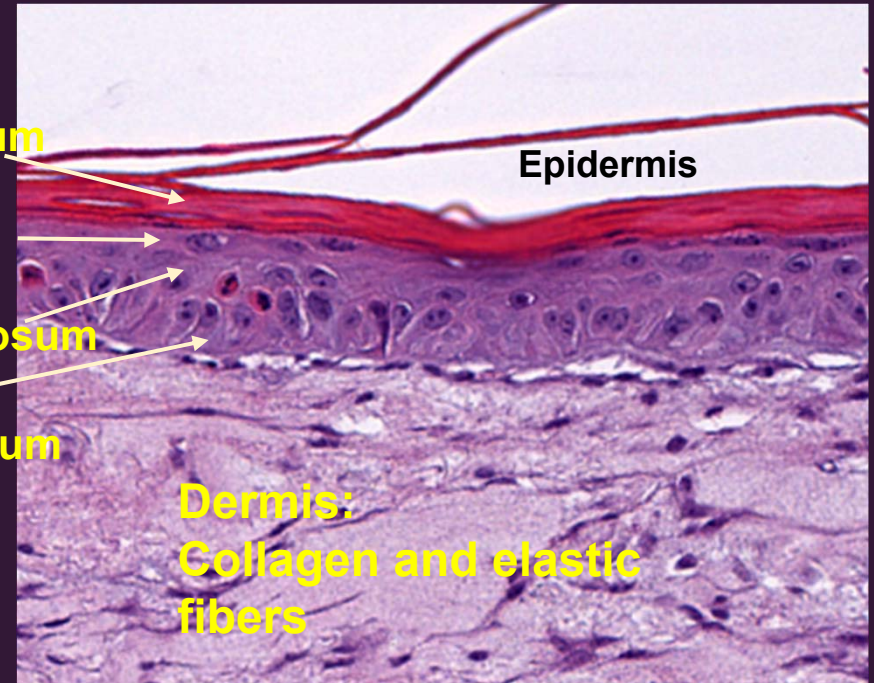
Stratum corneum

Stratum granulosum

Stratum spinosum

Stratum germinativum

Dermis:
Collagen and elastic
fibers



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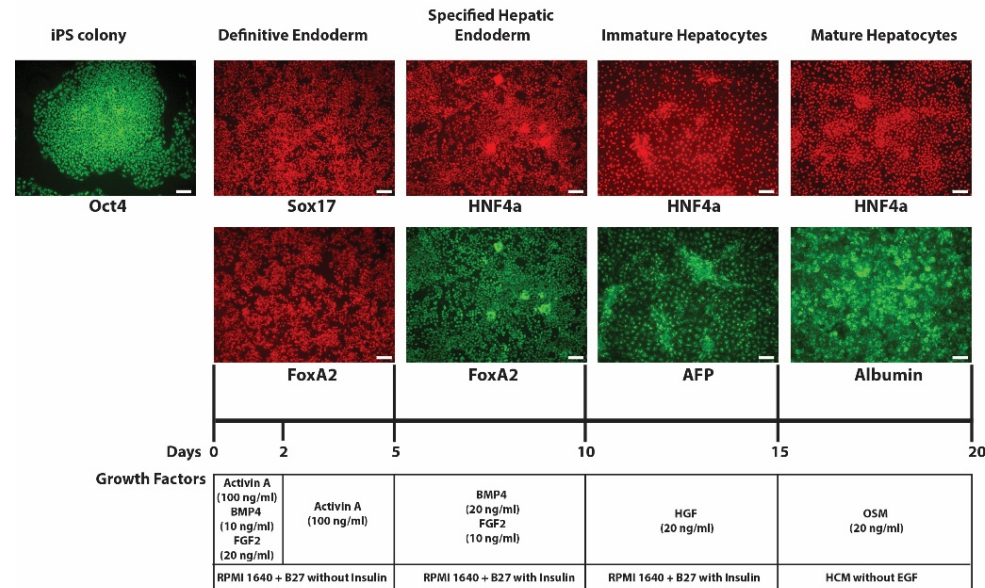
Improving chemical testing models to better predict effect in humans

Addressing roadblocks to utilization of induced pluripotent stem cells (iPSC)

- Problem: present methods for production of cells for drug screening and regenerative medicine are not cheap, standardized, and scalable.
- Solution: create a Stem Cell Translation Laboratory that uses cutting-edge technologies (single-cell proteomics, next-gen sequencing, screening technologies and chemistry) in order to:
 - Derive and disseminate Quality Control standards.
 - Dramatically improve methods for cell production by making them cheaper and more efficient, and demonstrating scalability, reproducibility, and transferability.



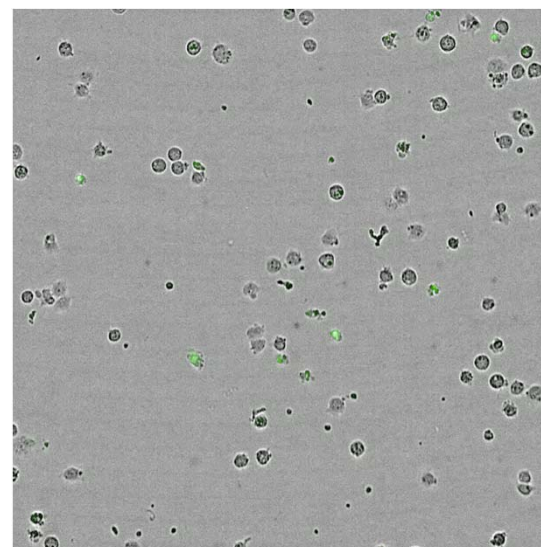
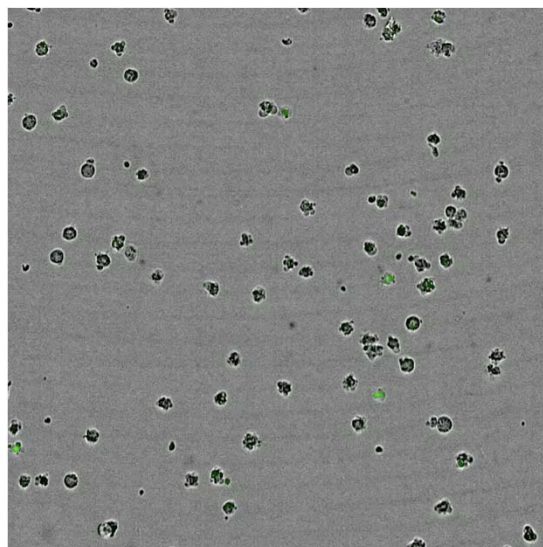
Example: Fully-automated production of drug screen-ready liver cells from iPSCs



Example: A novel small molecule combination to promote stress-free scale-up of iPS cells

Culturing of cells using the current methods:

Green=stress



Culturing of cells using the novel reagent combination to minimize stress.



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Sharing internal know-how: Assay Guidance Manual (47 chapters/ 1,338 printed pages)

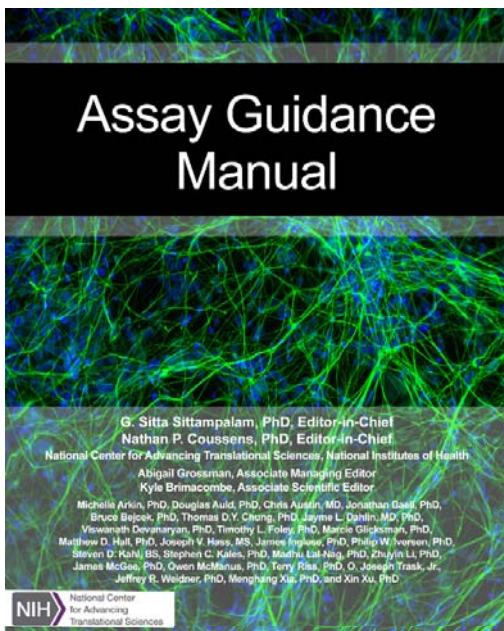
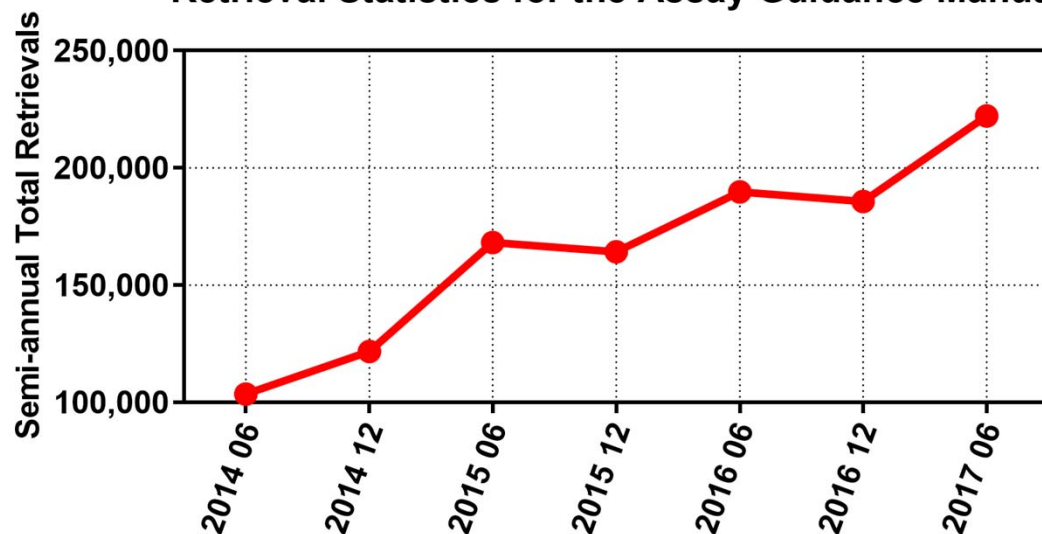


Table of Contents	
Preface	
Considerations for Early Phase Drug Discovery	1 Chapter
<i>In Vitro</i> Biochemical Assays	10 Chapters
<i>In Vitro</i> Cell Based Assays	19 Chapters
<i>In Vivo</i> Assay Guidelines	2 Chapters
Assay Artifacts and Interferences	4 Chapters
Assay Validation, Operations and Quality Control	5 Chapters
Assay Technologies	2 Chapters
Instrumentation	2 Chapters
Pharmacokinetics and Drug Metabolism	1 Chapter
Glossary of Quantitative Biology Terms	1 Chapter

Retrieval Statistics for the Assay Guidance Manual



Website: <https://ncats.nih.gov/expertise/preclinical/agm>

Email us: NCATS_AGM_Editors@mail.nih.gov



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LinkedIn: www.linkedin.com/groups/7437344



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Q-State Biosciences
Promega Corporation
PerkinElmer, Inc.
QualSci Consulting, LLC
Merck Research Laboratories
Novartis Institute for Biomedical Research
NCATS

Lectures/Workshops to bring AGM to the community:

2017- October 23

University of North Carolina Chapel Hill, NC

2018- February 3

Society for Laboratory Automation and Screening
Annual Conference
San Diego, CA

2018- March 26 and September 10

William F. Bolger Center
Potomac, MD

2019- February

Society for Laboratory Automation and Screening
Annual Conference
Washington, DC



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