

# NCATS update on Tox21 activities

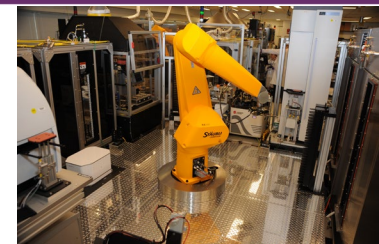
Anton Simeonov, Ph.D.

*Scientific Director, Division of Preclinical Innovation, National Center for Advancing Translational Sciences (NCATS), NIH*

**ICCVAM Virtual Meeting  
May 21, 2020**



# The Tox21 Library Screening Project



Collection of diverse chemicals

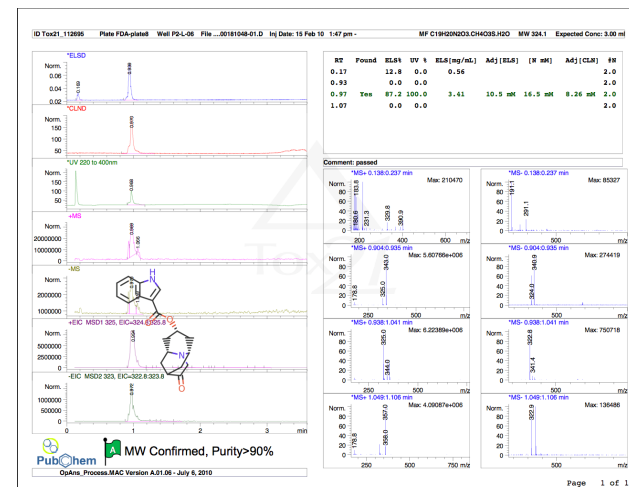
*In vitro* test methods, screening

High quality bioactivity data

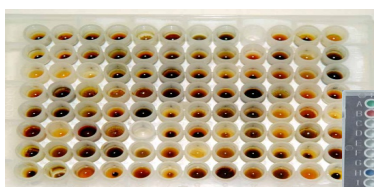
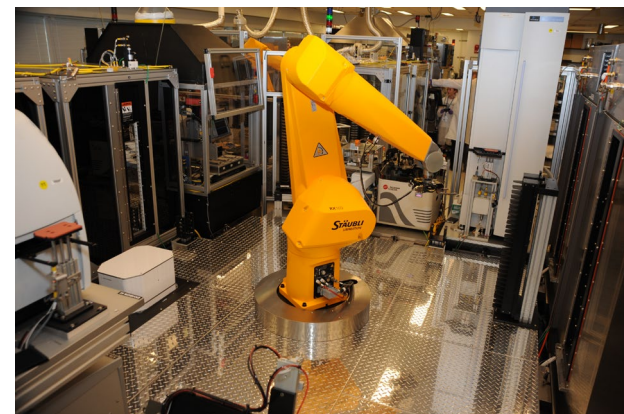
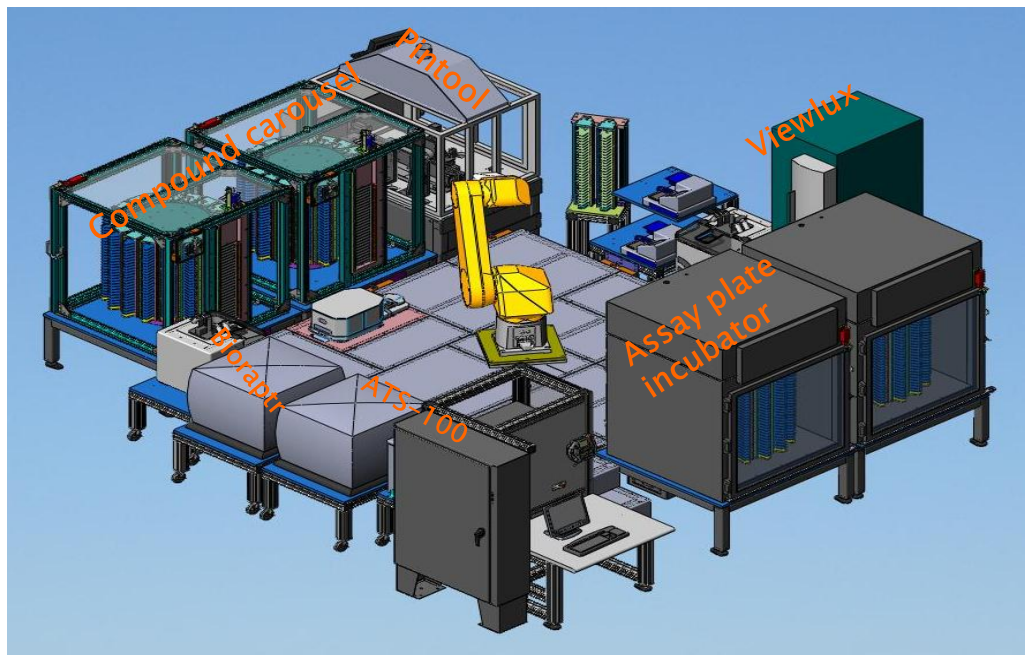
Predictive models  
(of bioactivity of a new chemical *in vitro* and, one day, *in vivo*)

>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 →**



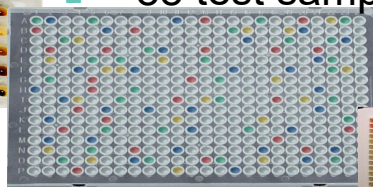
# Tox21 Robot Platform



96-well plate

- 8 rows x 12 columns

- 88 test samples

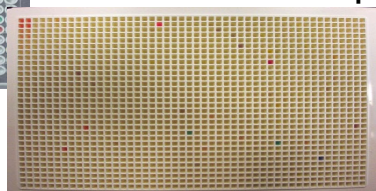


384-well plate

4 x 96-well plates

- 16 rows x 32 columns

- 352 test samples

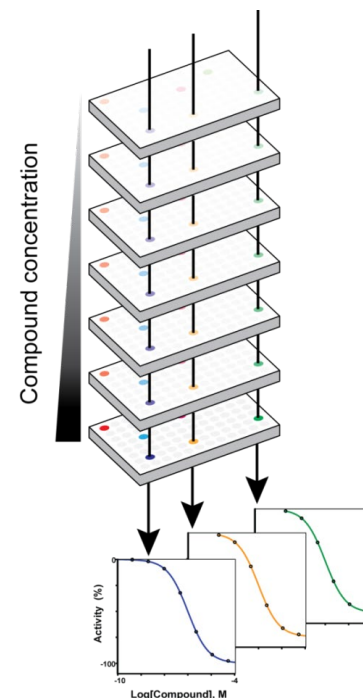


1536-well plate

16 x 96-well plates

- 32 rows x 48 columns

- 1,408 test samples



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



NIH National Center for Advancing Translational Sciences

# Screening outcomes, next steps

- 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 (~100M datapoints), using crowdsourcing to move from data to knowledge.
- Evolution of project ecosystem and partnership governance: cross-partner projects, emphasis on increased sophistication of test systems, improved data dissemination.



# Tox21 Public Data Browser - Chemicals



Structure Search

Search...



Home / Tox21 Samples

## QC Grade T0

<input type="checkbox"/>	A	6372
<input type="checkbox"/>	ND	4230
<input type="checkbox"/>	Fns	403
<input type="checkbox"/>	D	272
<input type="checkbox"/>	Ac	254
<input type="checkbox"/>	B	154
<input type="checkbox"/>	M	135
<input type="checkbox"/>	Fc	131
<input type="checkbox"/>	Z	122

## QC Grade T4

<input type="checkbox"/>	ND	6989
<input type="checkbox"/>	A	4385
<input type="checkbox"/>	B	207
<input type="checkbox"/>	D	139
<input type="checkbox"/>	M	134
<input type="checkbox"/>	Z	133
<input type="checkbox"/>	C	127
<input type="checkbox"/>	Ac	115
<input type="checkbox"/>	I	77

## QC Method T0

<input type="checkbox"/>	LCMS	5874
<input type="checkbox"/>	NMR	1977
<input type="checkbox"/>	GCMS	316
<input type="checkbox"/>	FIA	54

## QC Method T4

<input type="checkbox"/>	LCMS	5378
--------------------------	------	------

12495 << 1 2 3 4 5 6 7 ... 1249 1250 >>

Structure	Tox21 ID	Name	QC Grade T0	QC Grade T4
	<a href="#">Tox21_110001</a>	D-Xylo-5-hexulosonic acid...	ND Not Determined Analytical analysis is in progress	ND Not Determined Analytical analysis is in progress
	<a href="#">Tox21_110002</a>	D-Gluconic acid, barium salt	ND Not Determined Analytical analysis is in progress	ND Not Determined Analytical analysis is in progress
	<a href="#">Tox21_110004</a>	Thanite	ND Not Determined Analytical analysis is in progress	ND Not Determined Analytical analysis is in progress
	<a href="#">Tox21_110006</a>	tert-Butylhydroquinone	A MW Confirmed, Purity > 90%	ND Not Determined Analytical analysis is in progress
	<a href="#">Tox21_110005</a>	8-Quinololinol Salicylate		ND Not Determined
	<a href="#">Tox21_110007</a>	tert-Butylhydroquinone		
	<a href="#">Tox21_300081</a>	tert-Butylhydroquinone		
	<a href="#">Tox21_201998</a>	1,10-Phenanthroline		
	<a href="#">Tox21_202309</a>	tert-Butylhydroquinone		
	<a href="#">Tox21_110010</a>	Geraniol		

## Index of /pub/tox21/QC

<a href="#">Name</a>	<a href="#">Last modified</a>	<a href="#">Size</a>	<a href="#">Description</a>
----------------------	-------------------------------	----------------------	-----------------------------

 <a href="#">Parent Directory</a>			-
 <a href="#">All Publish 121014/</a>	05-Aug-2015 13:30		-
 <a href="#">GCMS/</a>	05-Aug-2015 13:14		-
 <a href="#">Index</a>	20-Feb-2015 19:27	276K	
 <a href="#">Index_old</a>	06-Feb-2015 00:25	88K	
 <a href="#">SET1/</a>	24-Oct-2014 18:59		-
 <a href="#">tox21_master_20160805.csv</a>	08-Aug-2016 10:45	712K	

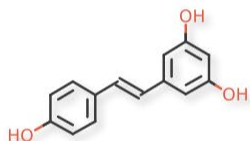
Apache/2.2.15 (Scientific Linux) Server at tripod.nih.gov Port 80

<https://tripod.nih.gov/tox21/samples>



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Translational Sciences

# Resveratrol



### QC Grade

T0	<span style="color: green;">A</span>	MW Confirmed, Purity > 90%
T4	<span style="color: green;">A</span>	MW Confirmed, Purity > 90%

### Identifiers

Tox21	Tox21_303376
NCATS	NCGC00257465-01
CAS	501-36-0
PubChem	144213690

ID Tox21\_201374 Plate Batch2-SP109801 Well P1-1-12 File SP109801\_112.D Inj Date: 10 Sep 11 6:38 am MF C14H12O3 MW 228.1 Expected Conc: 3.00 mM

NIH U.S. National Library of Medicine National Center for Biotechnology Information

PubChem OPEN CHEMISTRY DATABASE

Search Substances

[mg/mL]	Adj [ELS]	[N mM]	Adj [CLN]	#N
1.08	4.75 mM			0.0

Substance Record for SID 144213690

[Download](#) [Print](#) [Share](#) [Help](#)

PUBCHEM &gt; SUBSTANCE &gt; SID 144213690

## CAS-501-36-0

[Cite this Record](#)

Bioactivities



PubChem SID: 144213690

 PubChem CID: 445154 (resveratrol) [Related Compounds...](#)

External ID: NCGC00257465-01

Source: Tox21

Source Category: Governmental Organizations

 Version: 3 [Revision History...](#)

Available Date: 2012-10-06

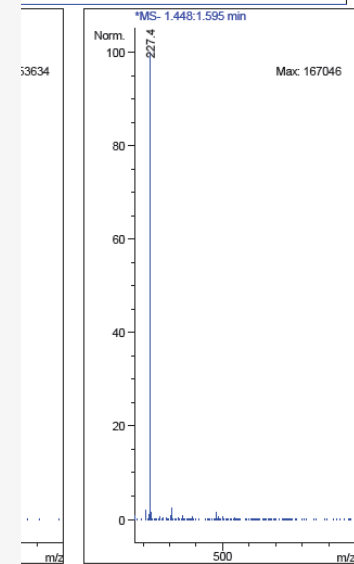
Deposit Date: 2012-10-06

Please note that the substance record below is presented as provided to PubChem by the source(depositor). For standardized chemical structure and/or annotation information, please visit the compound summary page for [resveratrol](#).

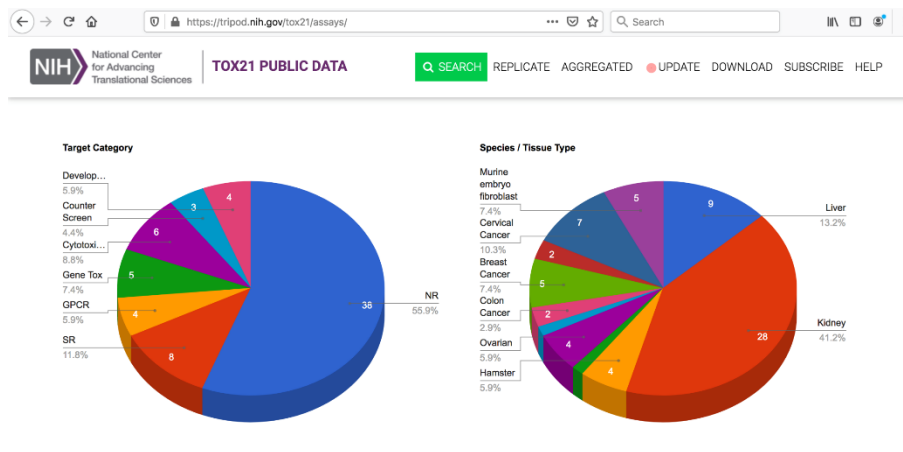
### Contents

- 1 2D Structure
- 2 Identity
- 2.1 Source
- 2.2 External ID
- 2.3 Source Category
- 2.4 Depositor-Supplied Synonyms
- 2.5 Deposit Date

### 1 2D Structure

[Search](#) [Download](#) [Get Image](#)


# Tox21 Public Data Browser - Assays



<https://tripod.nih.gov/tox21/assays>

- Tox21 data in public domain
  - 223 AIDs in PubChem
  - ~100 million data points

☆ Used in Tox21 Challenge

Protocol Name	Assay Target	Target Category	Cell Line	Cell Type
<a href="#">tox21-ahr-p1</a> ☆	Ahr	NR	HepG2	Liver
<a href="#">tox21-ap1-agonist-p1</a>	AP-1 agonist	SR	ME-180	Cervical Cancer
<a href="#">tox21-ar-bla-agonist-p1</a> ☆	AR-BLA agonist	NR	HEK293	Kidney
<a href="#">tox21-ar-bla-antagonist-p1</a>	AR-BLA antagonist	NR	HEK293	Kidney
<a href="#">tox21-are-bla-p1</a> ☆	ARE	SR	HepG2	Liver
<a href="#">tox21-ar-mda-kb2-luc-agonist-p1</a> ☆	AR-MDA agonist	NR	MDA-MB-453	Breast Cancer
<a href="#">tox21-ar-mda-kb2-luc-agonist-p3</a>	AR-MDA agonist (with antagonist)	NR	MDA-MB-453	Breast Cancer
<a href="#">tox21-ar-mda-kb2-luc-antagonist-p1</a>	AR-MDA antagonist	NR	MDA-MB-453	Breast Cancer
<a href="#">tox21-ar-mda-kb2-luc-antagonist-p2</a>	AR-MDA antagonist (lower agonist)	NR	MDA-MB-453	Breast Cancer
<a href="#">tox21-aromatase-p1</a> ☆	Aromatase	SR	MCF-7	Breast Cancer
<a href="#">tox21-car-agonist-p1</a>	CAR agonist	NR	HepG2	Liver
<a href="#">tox21-car-antagonist-p1</a>	CAR antagonist	NR	HepG2	Liver



# The NCATS BioPlanet

U.S. Department of Health & Human Services | National Institutes of Health | National Center for Advancing Translational Sciences

NIH National Center for Advancing Translational Sciences **BioPlanet** Keywords or gene IDs

Home Pathway **Category** Assay Availability- Enrichment Resource

1-5 of 1658  Collapse All Page size: 5

### 1 ▼ 2-LTR circle formation

[Pathway Map](#) [Category](#) [Assay Availability](#)

- [Genes](#)
  - DNA repair
  - Human Diseases
    - Infectious diseases
      - Viral
- [Diseases](#)
  - Infectious disease

[Assay Availability](#)

- Tox21

### 2 ▼ 4-1BB-dependent immune response

[Pathway Map](#) [Category](#) [Assay Availability](#)

- [Genes](#)
  - Cancer
  - CellActivation
  - CellSignaling
  - Immune system
  - Immunology
  - Infectious disease
  - Stress response
  - Transcription
- [Diseases](#)

[Assay Availability](#)

- Commercial
- NCATS
- PubChem
- Tox21
- ToxCast

### 3 ▼ ABC transporters

[Pathway Map](#) [Category](#) [Assay Availability](#)

- [Genes](#)
  - Digestive system
  - Environmental Information Processing
    - Membrane transport
  - Lipid metabolism
  - Small molecule metabolism
  - Transport
- [Diseases](#)

[Assay Availability](#)

- Commercial
- PubChem
- ToxCast

### 4 ▼ ABCA transporters in lipid homeostasis

[Pathway Map](#) [Category](#) [Assay Availability](#)

- [Genes](#)
  - Digestive system
  - Environmental Information Processing
    - Membrane transport
  - Lipid metabolism
  - Small molecule metabolism
  - Transport
- [Diseases](#)

[Assay Availability](#)  
N/A

NIH National Center for Advancing Translational Sciences **BioPlanet** Keywords or gene IDs

Home Pathway **Category** Assay Availability- Enrichment Resource

## Comprehensive Collection of Human Pathways for Systems Toxicology and Chemical Genomics

BioPlanet offers interactive browsing and analysis of pathways, and exploration of pathway connections.

### About BioPlanet (Version 1.0)

The NCATS BioPlanet is a comprehensive, publicly accessible informatics resource that catalogues all pathways, their healthy and disease state annotations, and targets within and relationships among them. The BioPlanet integrates pathway annotations from publicly available, manually curated sources that have been subjected to thorough redundancy and consistency cross-evaluation via extensive manual curation. The browser supports interactive browsing, retrieval, and analysis of pathways, exploration of pathway connections, and pathway search by gene targets, category, and availability of bioactivity assays. We intend for such a platform to enable the rational construction of probing assays that could be used to query all of pathway space experimentally. The current version of the BioPlanet (v1.0) incorporates 1,658 distinct human pathways encompassing 9,818 human genes. In future releases, pathways for other species will be added as well as links to data from small molecule, gene expression and siRNA screens performed at NCATS and data from other researchers.

Since the BioPlanet is built on a foundation of current understanding of pathways and their interconnections, it is certainly prone to errors despite our best curation efforts. We therefore view the ongoing curation and growth of the BioPlanet as a community effort, and encourage comments, corrections, contributions, and suggestions for additional features through our feedback mechanism. All contributions will be acknowledged and attributed on this page.

If you make use of the BioPlanet in your research, please cite the BioPlanet paper to acknowledge the BioPlanet resource.

<http://tripod.nih.gov/bioplanet/>

Annotates 1,658 curated human pathways (~10,000 genes)



National Center for Advancing Translational Sciences



ORIGINAL RESEARCH ARTICLE

Front. Pharmacol., 26 April 2019 | <https://doi.org/10.3389/fphar.2019.00445>



# The NCATS BioPlanet – An Integrated Platform for Exploring the Universe of Cellular Signaling Pathways for Toxicology, Systems Biology, and Chemical Genomics

Ruilu Huang<sup>1\*</sup>, Ivan Grishagin<sup>2</sup>, Yuhong Wang<sup>1</sup>, Tongan Zhao<sup>1</sup>, Jon Greene<sup>2</sup>, John C. Obenauer<sup>2</sup>, Deborah Ngan<sup>1</sup>, Dac-Trung Nguyen<sup>1</sup>, Rajarshi Guha<sup>1</sup>, Ajit Jadhav<sup>1</sup>, Noel Southall<sup>1</sup>, Anton Simeonov<sup>1</sup> and Christopher P. Austin<sup>1</sup>

<sup>1</sup>Division of Pre-Clinical Innovation, National Center for Advancing Translational Sciences, National Institutes of Health, Rockville, MD, United States

<sup>2</sup>Rancho BioSciences, San Diego, CA, United States

Chemical genomics aims to comprehensively define, and ultimately predict, the effects of small molecule compounds on biological systems. Chemical activity profiling approaches must consider chemical effects on all pathways operative in mammalian cells. To enable a strategic

Adopted by BMDEExpress (NTP), Enrichr (Mount Sinai) for pathway analysis

## Home

Scott S Auerbach edited this page on Dec 19, 2019 · 53 revisions

## Introduction

Download BMDEExpress 2 Software

New Features in BMDEExpress 2 Build 3

BMDEExpress 2 is a desktop application for Windows, Mac or Linux (See **IMPORTANT warning**) that enables analysis of dose-response data produced in differential gene expression experiments. It provides stepwise workflows that combine benchmark dose (BMD) calculations with functional classification analysis based on the combined probesets for individual genes, Gene Ontology (GO), Signaling Pathways (Reactome, BioPlanet), or custom categories provided by the user. The end results are estimates of doses at which cellular processes are altered, based on an increase or decrease in response in expression levels compared to untreated controls. These estimates depend on fitting curves through

Pages 10

- Home
  - Basic Workflow
  - Tutorial Videos
- Overview of the Main View
  - Main Toolbar
    - File
    - Tools
    - Help
  - Data Selection Area
    - Additional Functions



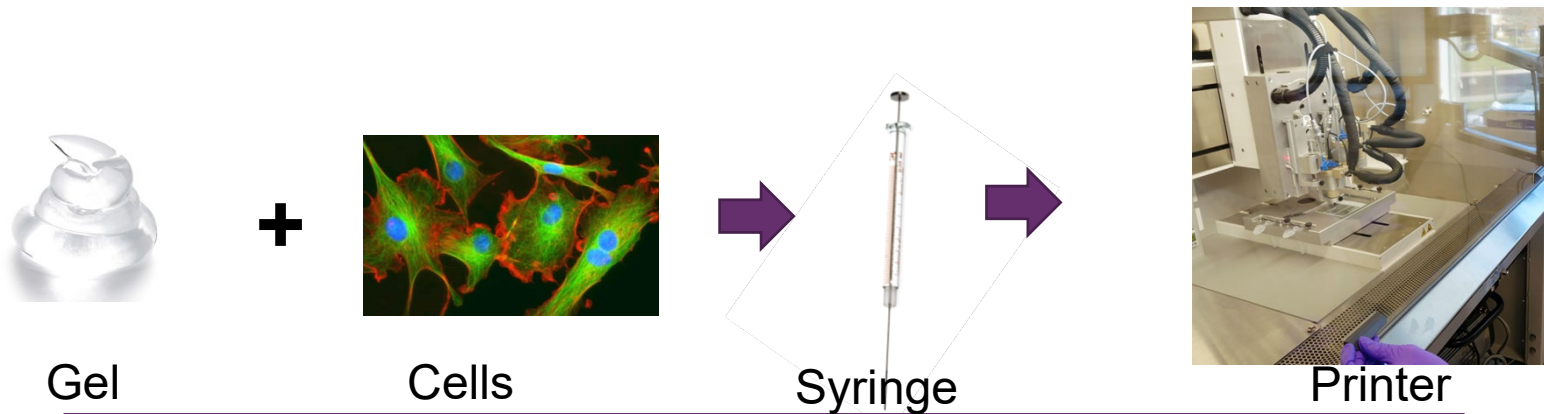
Login | Register

Transcription Pathways Ontologies Diseases/Drugs Cell Types Misc Legacy Crowd

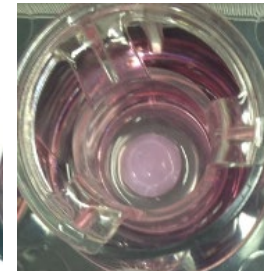
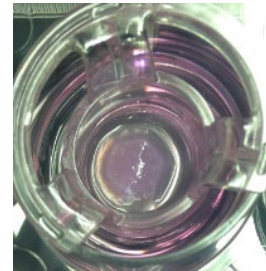
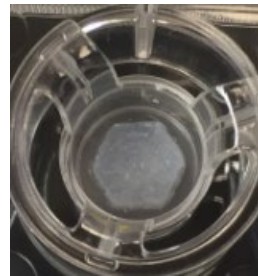
Description No description available (515 genes)

<p><b>BioPlanet 2019</b></p>	<p><b>WikiPathways 2019 Human</b></p>	<p><b>WikiPathways 2019 Mouse</b></p> <p>MAPK signaling pathway WP493</p> <p>Insulin Signaling WP65</p> <p>EGFR1 Signaling Pathway WP572</p> <p>Regulation of Actin Cytoskeleton WP523</p> <p>Focal Adhesion WP85</p>
<p><b>KEGG 2019 Human</b></p>	<p><b>ARCHS4 Kinases Coexp</b></p> <p>TGFBR2 human kinase ARCHS4 coexpression</p> <p>IKBKE human kinase ARCHS4 coexpression</p> <p>PLK3 human kinase ARCHS4 coexpression</p> <p>PIK3CG human kinase ARCHS4 coexpression</p> <p>STK38 human kinase ARCHS4 coexpression</p>	<p><b>KEGG 2019 Mouse</b></p>

# Increasing the predictivity of *in vitro* assays: 3D tissue bioprinting



Hydrogel polymer is mixed with cells and loaded into syringe.



Printed construct

1 day

1 week

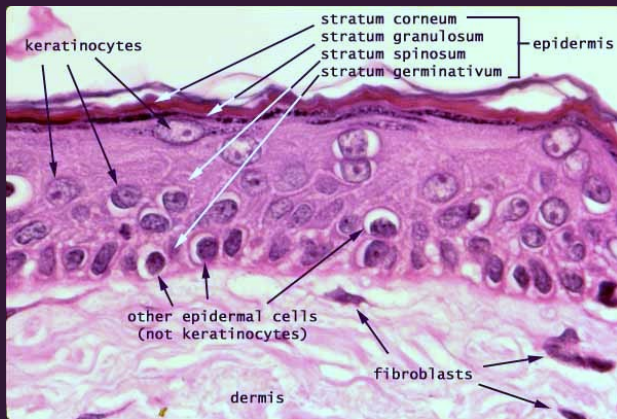
2 weeks

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.

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

## Native Skin



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

## 3D-Bioprinted Skin

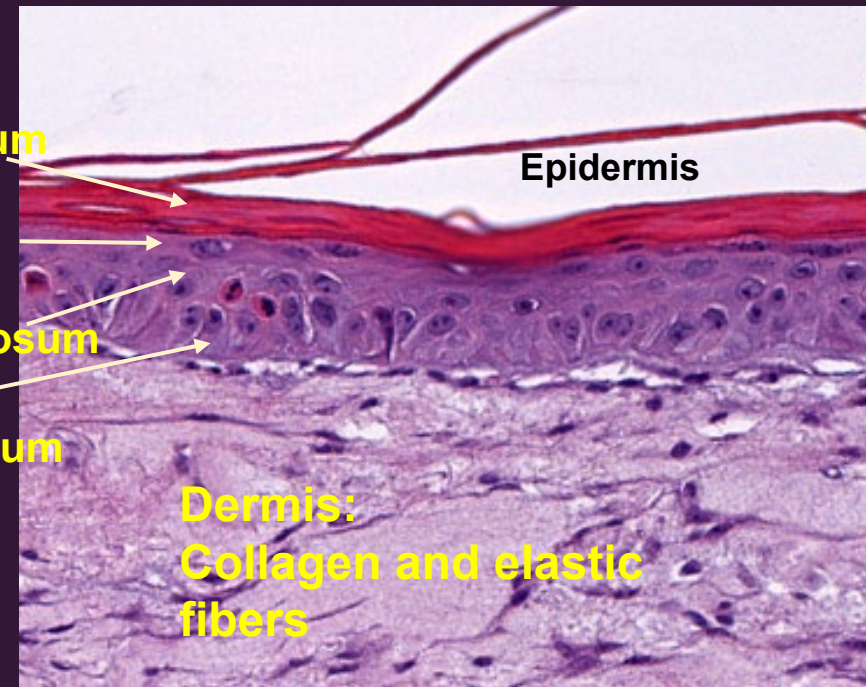
Stratum corneum

Stratum granulosum

Stratum spinosum

Stratum germinativum

Dermis:  
Collagen and elastic  
fibers

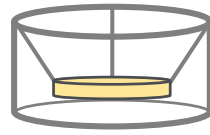


# Generation of bioprinted skin tissues

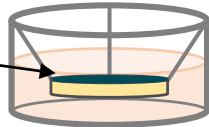
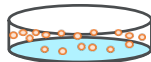
## Full thickness skin tissue (FTS)

### Reconstructed human epidermis (RhE)

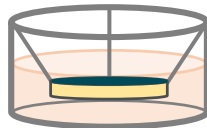
1. Coat the 96-well transwell insert membrane with collagen



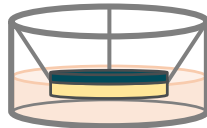
2. Add keratinocytes



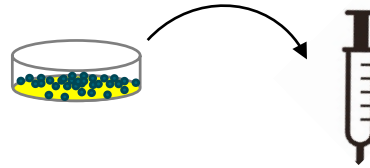
3. Submerge culture for 3 days



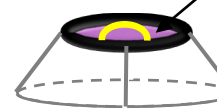
4. Air-liquid interface culture for 8 days



2. Bioprint fibroblast bioink to a 3-layer U shape on bottom side of 96-well transwell insert membrane



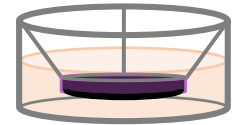
1. Suspend fibroblasts in bioprinting gel



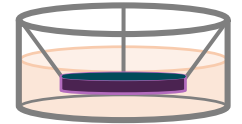
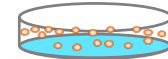
3. Add bioprinting gel to cover the U shape



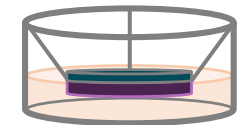
4. Submerge bioprinted tissue in medium for 7 days



5. Add keratinocytes and submerge culture for 3 days

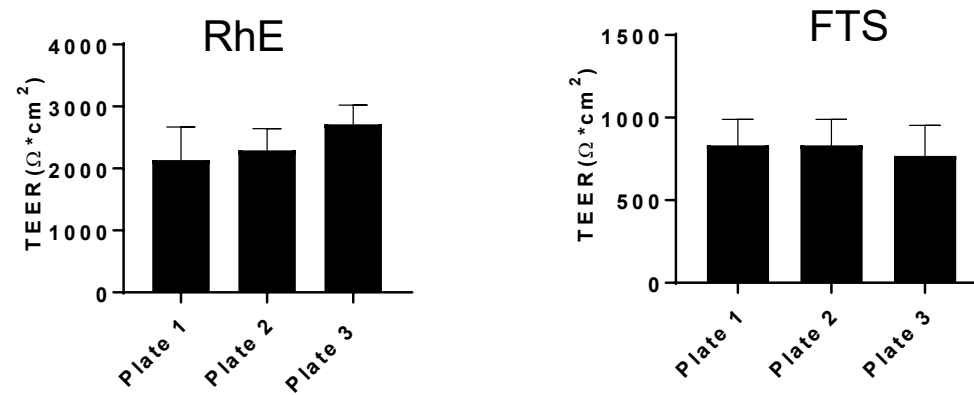


6. Air-liquid interface culture for 8 days

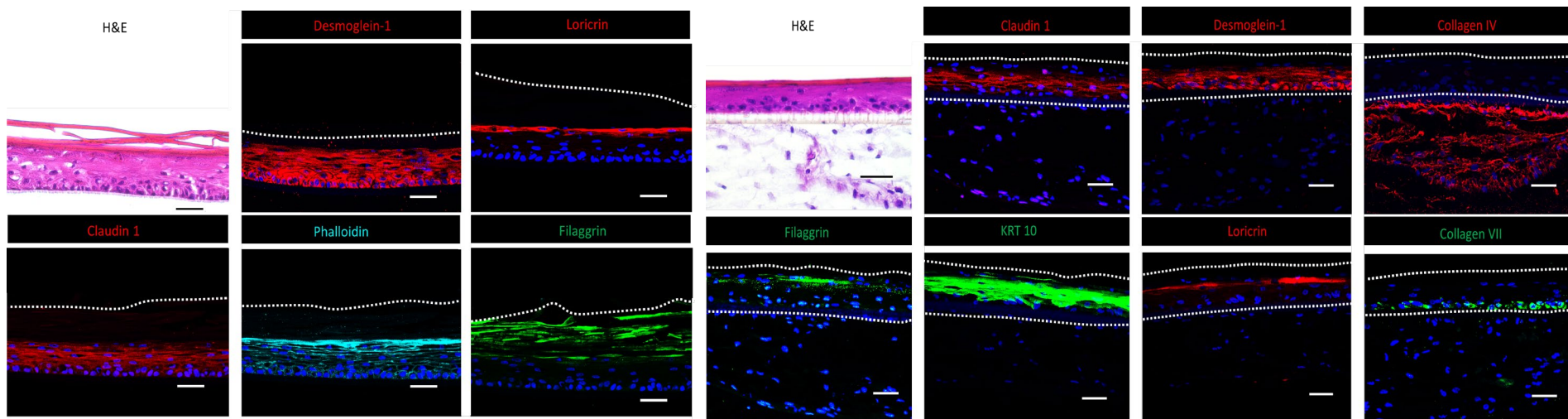


# Validation of bioprinted skin tissues for irritants

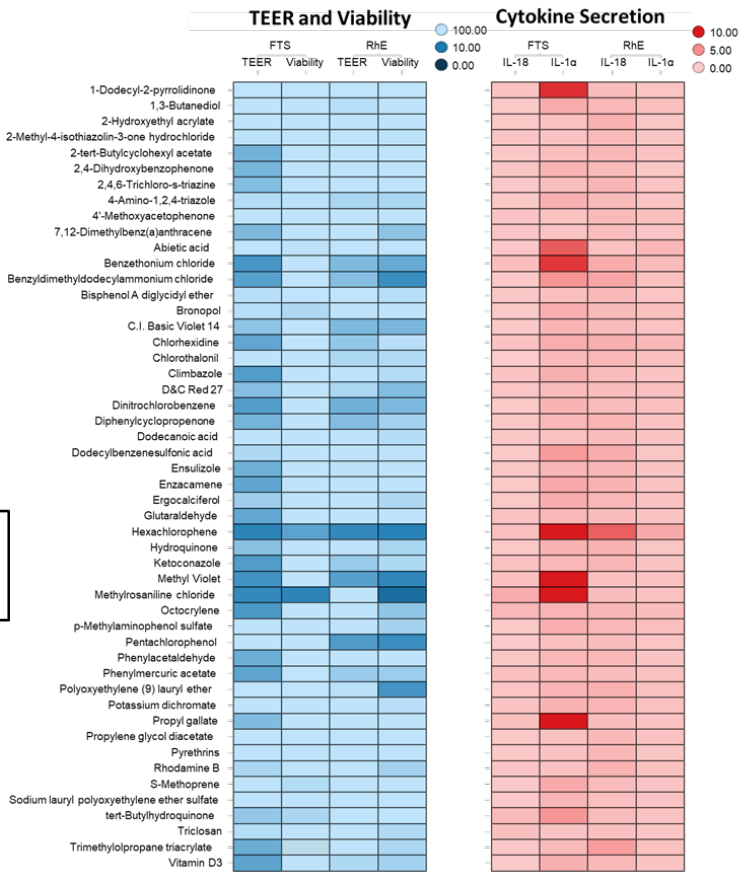
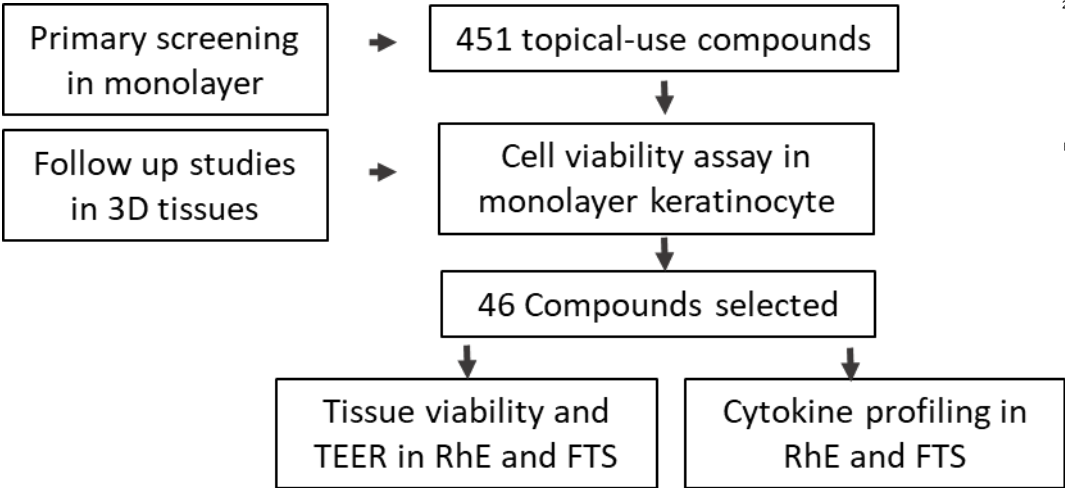
- Trans-epithelial electrical resistance (TEER): values above 500 indicated integral barrier function in Reconstructed human epidermis (RhE) and full thickness skin (FTS)



- Maturation of stratum corneum in both RhE and FTS

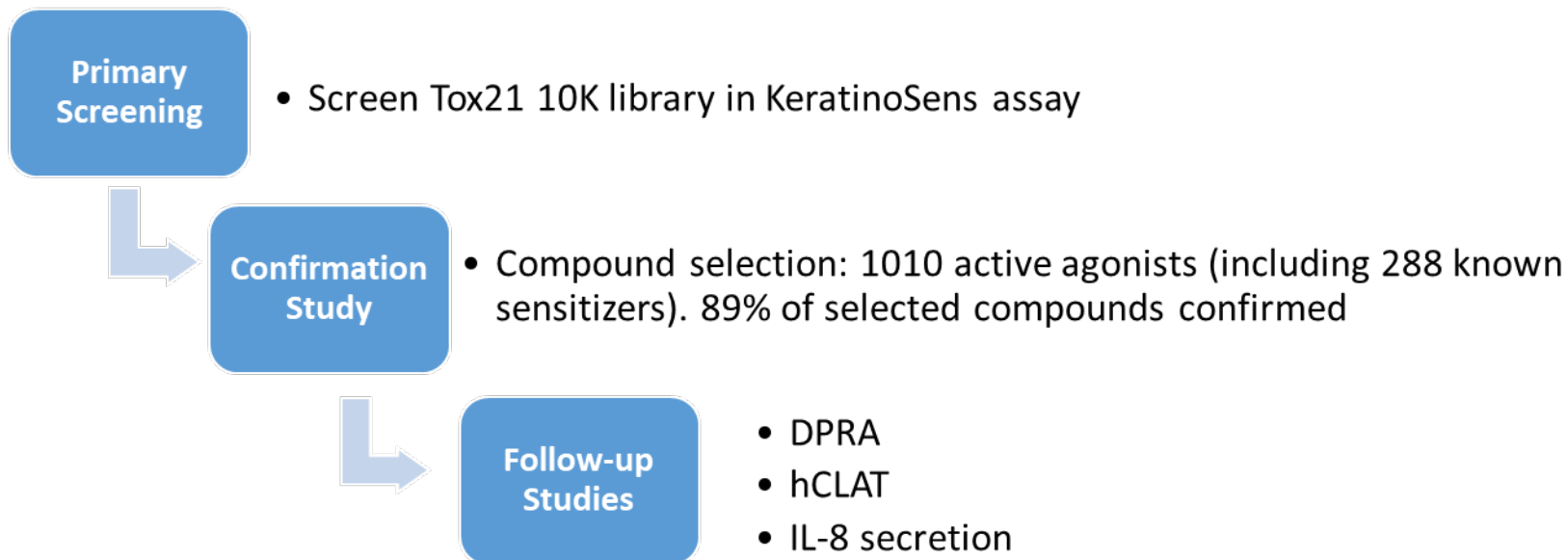


# Functional studies in 3D tissues for potential irritants



Wei, *et al.*, *Frontiers in Bioengineering and Biotechnology* 8:109, 2020

# Ongoing CPP: profiling the Tox21 chemicals for sensitizers



Based on sensitization adverse outcome pathway (AOP), the following assays have been used/planned to profile the Tox21 chemicals for their sensitization potential:

- KeratinoSens assay: induction of Nrf2/ARE in keratinocytes
- Direct peptide reactivity assay (DPRA) : covalent binding with cysteine/lysine
- hCLAT: activation of dendritic cells (surface marker CD86 CD54 expression in THP1 cells)
- Cytokine: IL-8 secretion



# Learn More About NCATS



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