

## **6PM**

**11PM in London (GMT), 8AM in Tokyo (GMT+9)**

## **Cell Manufacturing & Mapping**

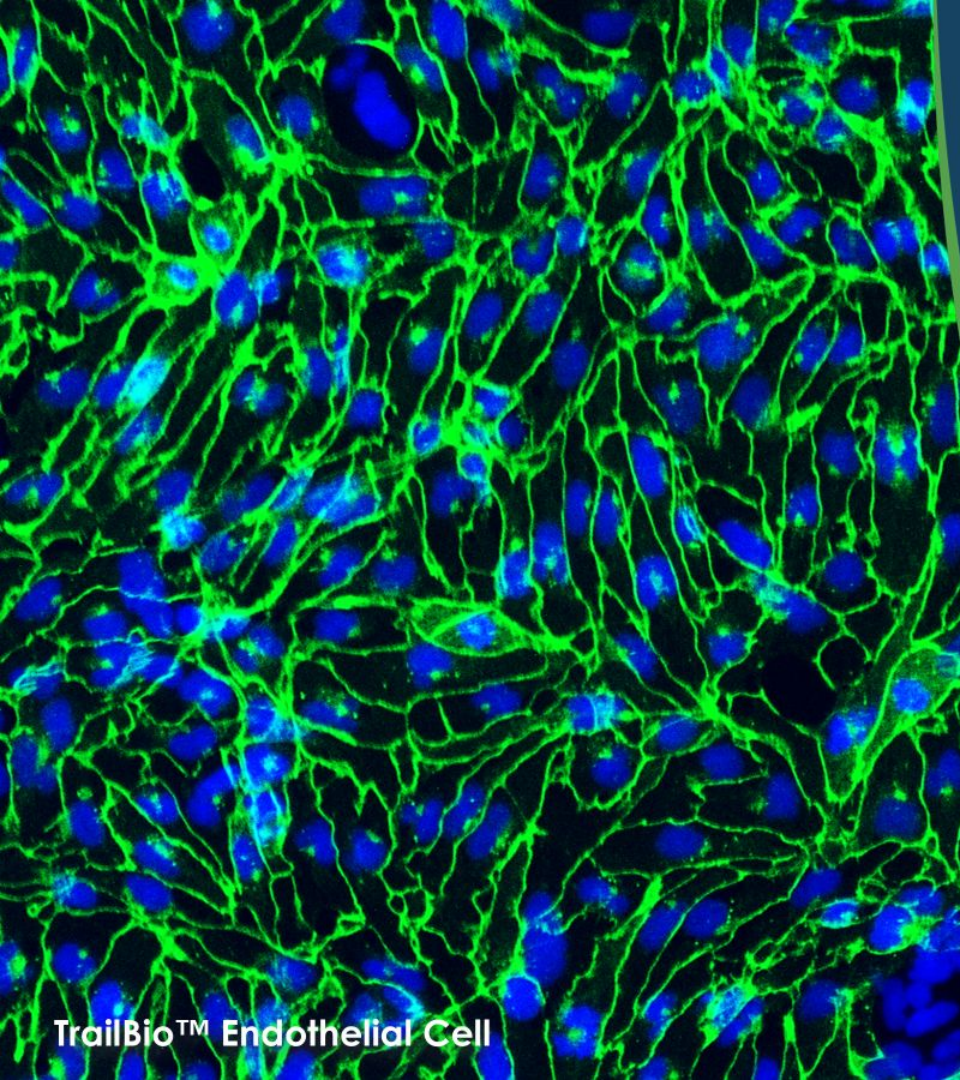
**Moderator:** Katy Börner, *Indiana University*

**Presenter:** Jan Jensen, *Trailhead Biosystems*

An abstract, artistic representation of a molecular structure or biological cell. It features several overlapping, translucent blue and green shapes that resemble organic forms or protein folds. Scattered throughout these shapes are numerous small, multi-colored dots in shades of red, green, blue, and yellow, suggesting atoms or specific functional groups. The overall composition is set against a light, neutral background.

**Jan Jensen, *Trailhead Biosystems CEO***

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# MULTI-SCALE HUMAN WORLD EVENT: Mapping and Manufacturing high quality iPSC-derived human cells for drug discovery and cell-based therapies

Jan Jensen, PhD  
CEO/CSO Trailhead Biosystems

Dec 14th,  
2024



# Disclaimer

J. Jensen is CEO/CSO, Board Member and Shareholder in Trailhead Biosystems inc.

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This document contains forward-looking statements, which include statements related to future business or financial performance, future events or future developments.

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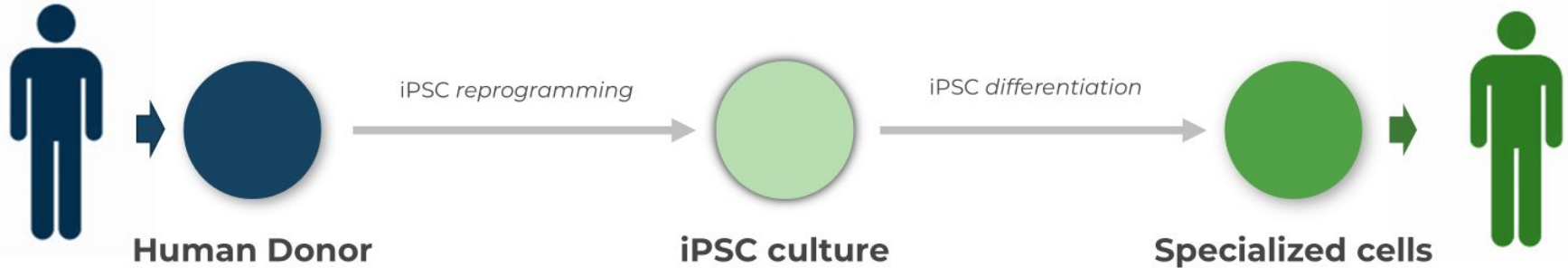


**Trailhead**<sup>®</sup>  
Biosystems

**Biology. Controlled.**



# Stem cells: The Hardest Challenge



Adult human cells are isolated from healthy or sick donors

Cells are reprogrammed to the stem cell state (pluripotency)

Specialized cells make up our body and collectively make it work

No ethical concerns related to fetal-tissue use

Yamanaka and colleagues pioneered the method (Nobel prize, 2012)

Making iPSCs robustly enter specialized fates is very difficult

Possible generation of lost or dysfunctional cells can be used to treat the patient

iPSCs are unlimited in expansion, but fail to show any specialized features

When this problem is solved, it will open a gateway to broader use of human cells





# Problem



The demand for cells is enormous, but the options are limited



Of the options available, cell purity is poor



There is a lack of consistency from batch to batch



Not available in the quantities the market demands

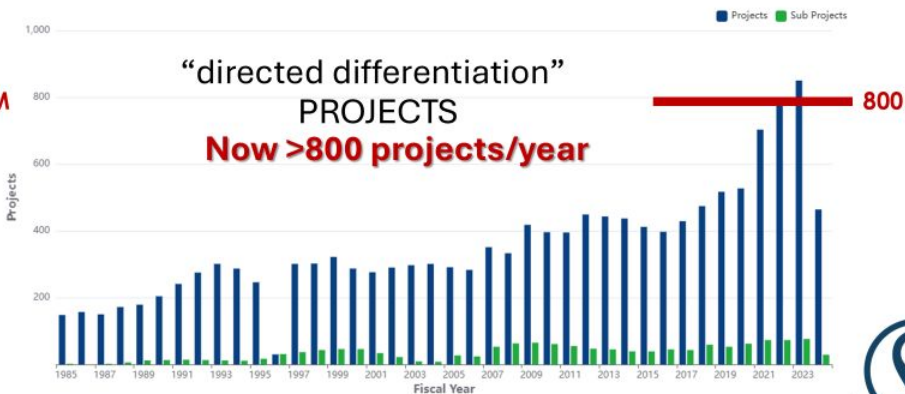
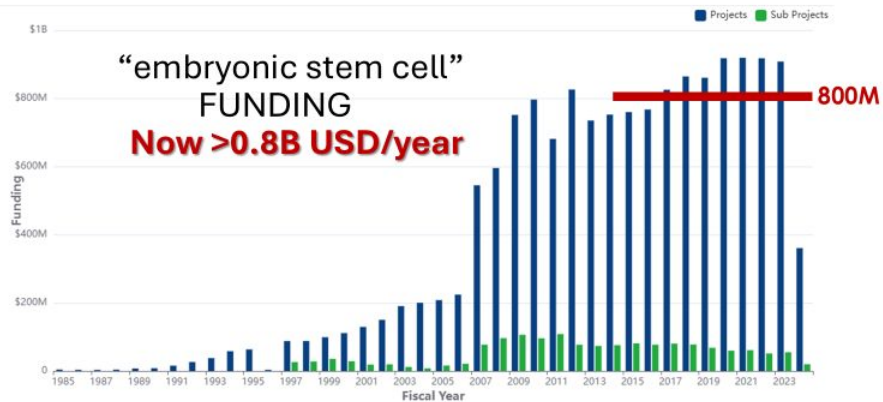


Development and production processes are largely done manually

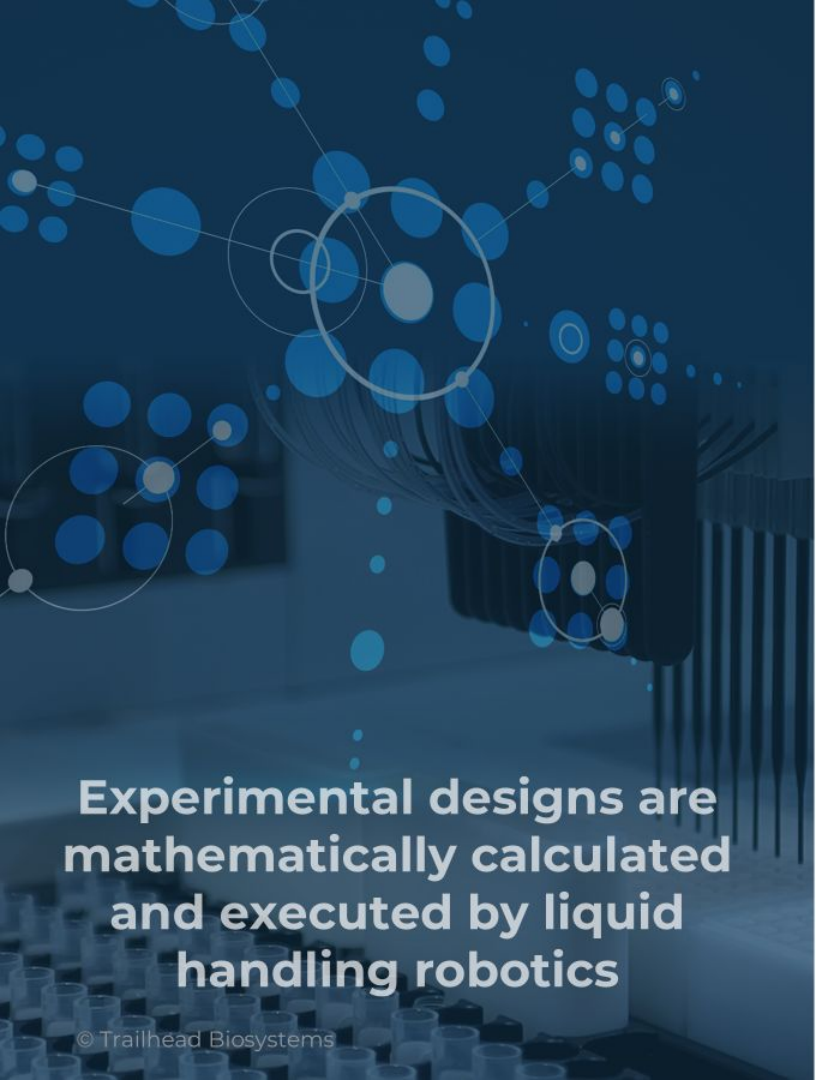


# This 'Problem' is very costly (NIH only):

Source: NIH RePORTER queries







**Experimental designs are  
mathematically calculated  
and executed by liquid  
handling robotics**

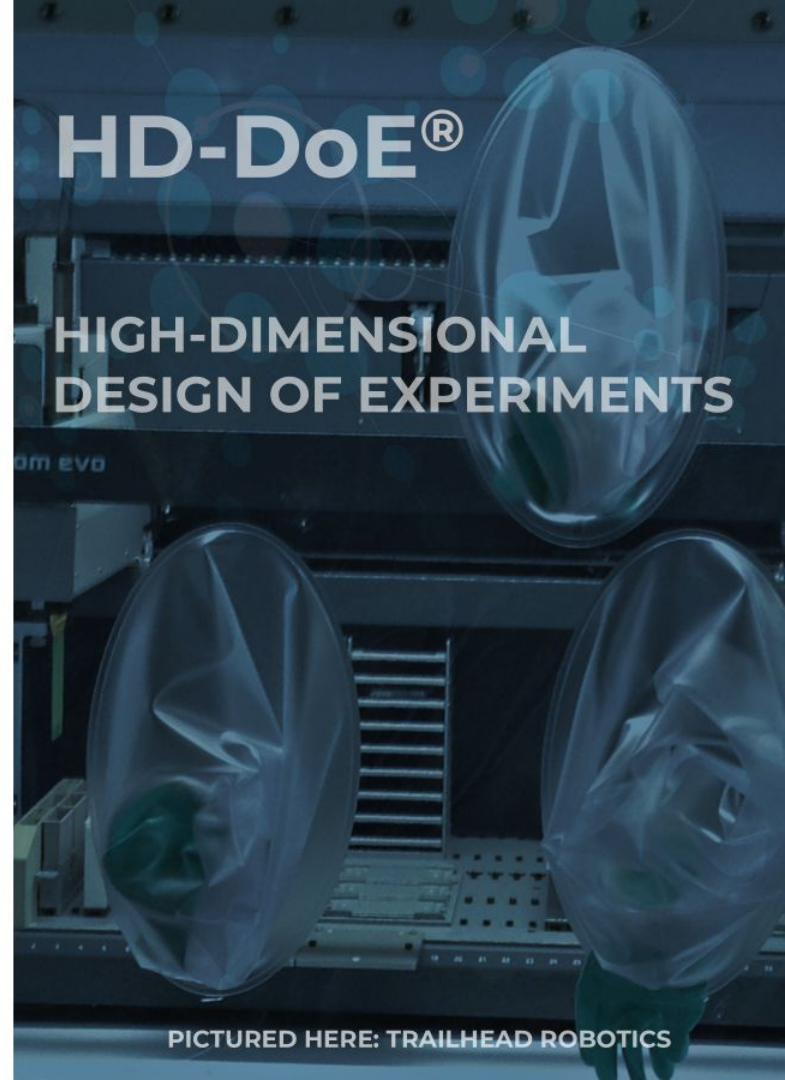
## Not Hypothesis-Driven Research...

- We replace manual discovery with robotics in a high-dimensional space of regulatory inputs
- We identify critical process parameters for each cell type
- We manufacture cells at industrial scale (billions per run) in bioreactors with high purity
- We build cost-effectiveness into each protocol by leveraging low-cost media components.

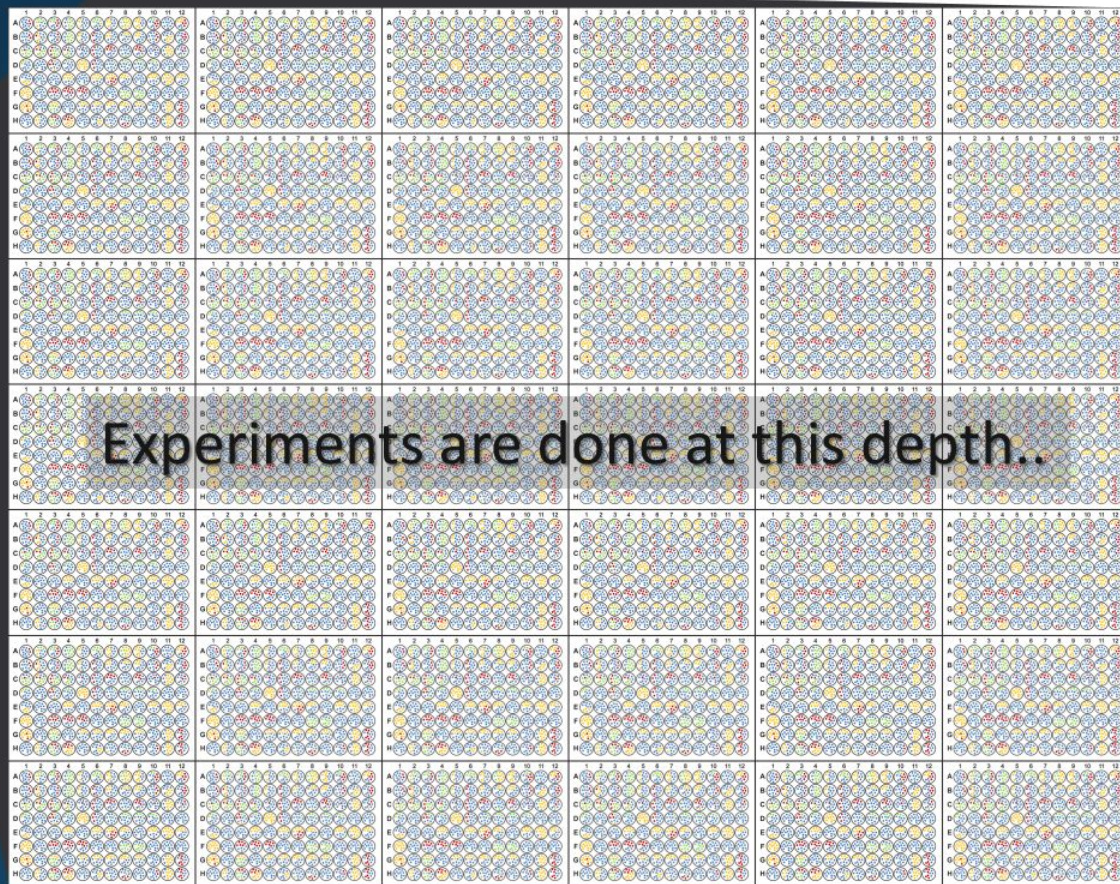


# Powered Empiricism in Developmental Biology

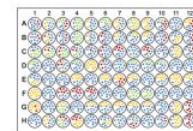
- Computerized, robotically-executed, experiments with speed, precision, and scalability
- Data-driven, unbiased determination of critical process parameters
- Proprietary, internally developed software tools
- Empirical data created and owned by Trailhead
- All protocols built from scratch



# HD-DoE<sup>®</sup> technology compresses a very large testing space



..but at a fractional cost



~\$2,000

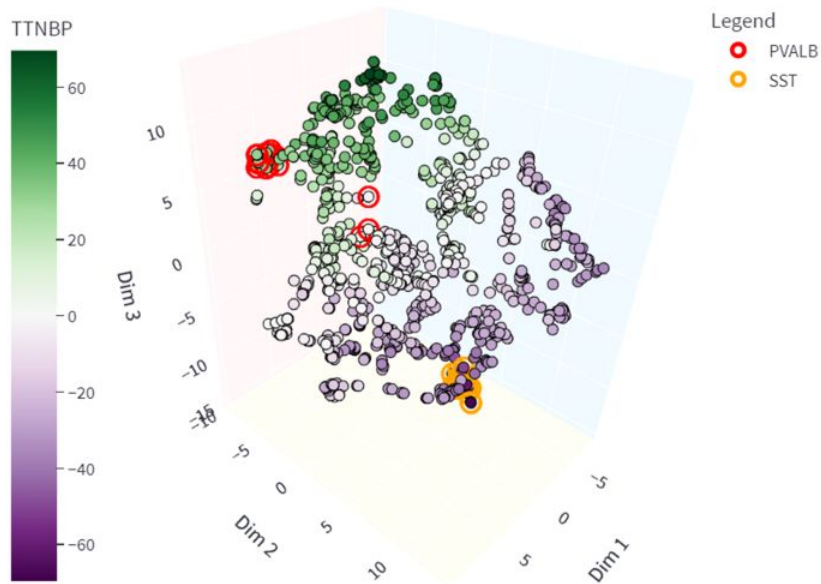
>40x compression

$2^{12} = 4096$

~\$80,000



# Accelerated Protocol Development through mathematical analysis of effector-response matrices



- We organize each response gene according to its regulatory pattern
- We dimensionally reduce the design space (8-13 factors) into 3 dimensions and visualize effects on genes by factor
- We can quickly identify **co-regulated responses** and **fate splits**, leading to more efficient recipe development



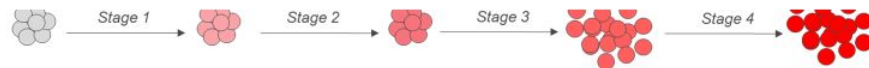
# We build **PROTOCOLS**

All protocols are built from beginning to end by us

## A9 Dopaminergic Neurons



## Red blood Cells



## Endothelial cells



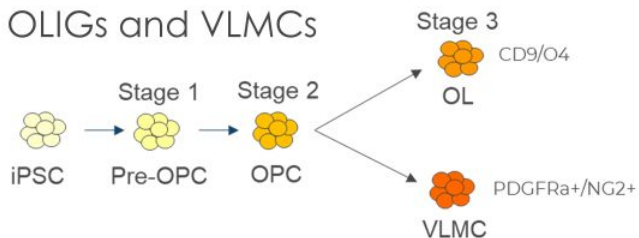
## Parvalbumin+ Interneurons



## Monocytes/Macrophages



## OLIGs and VLMCs



## SST Interneurons



## Hematopoietic Stem Cells

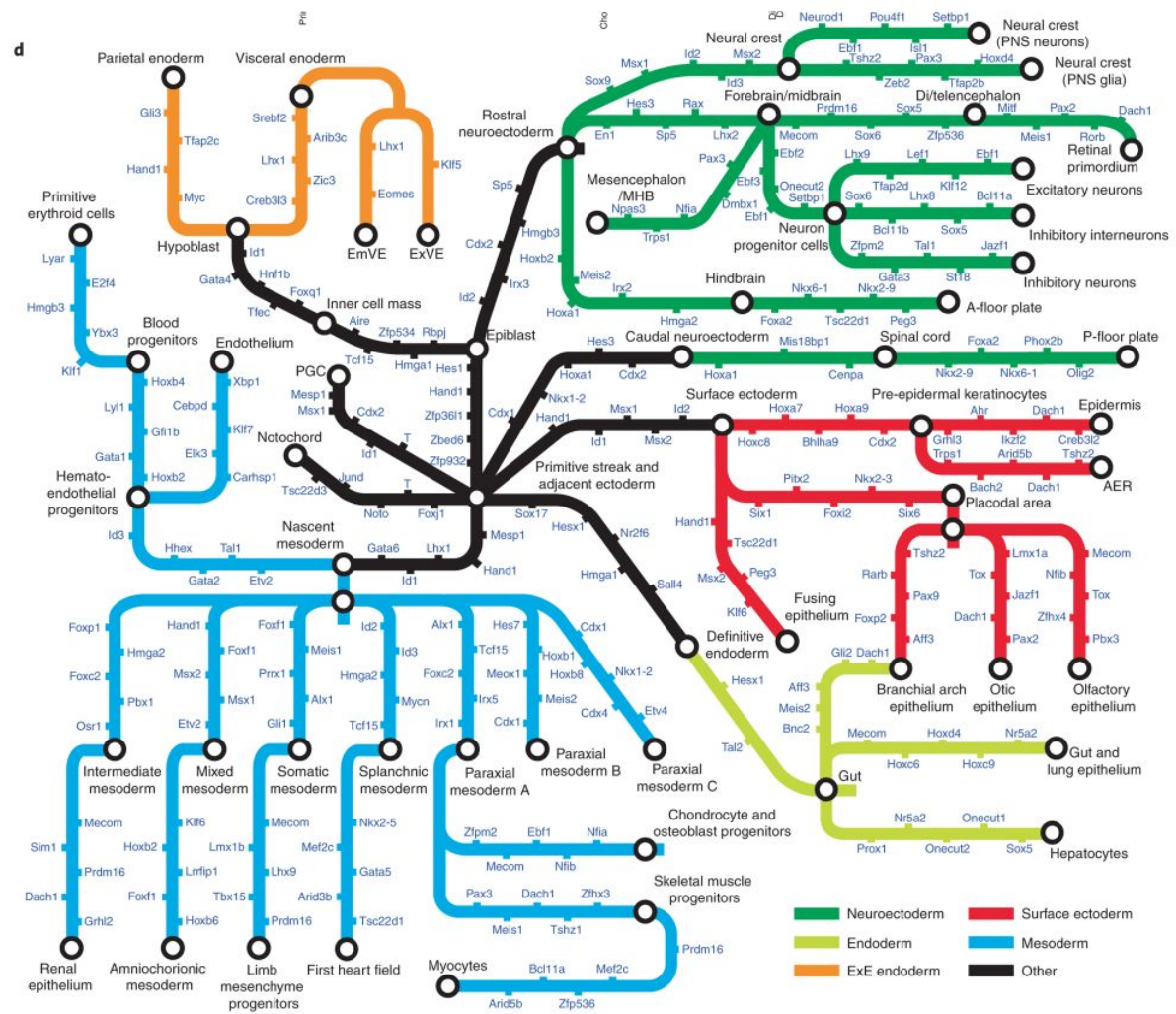


## Islet Cells



# Cell Fate 'Metro Map'

Individual TFs plotted within the lineages



ARTICLES <https://doi.org/10.1038/s41588-022-01018-x>

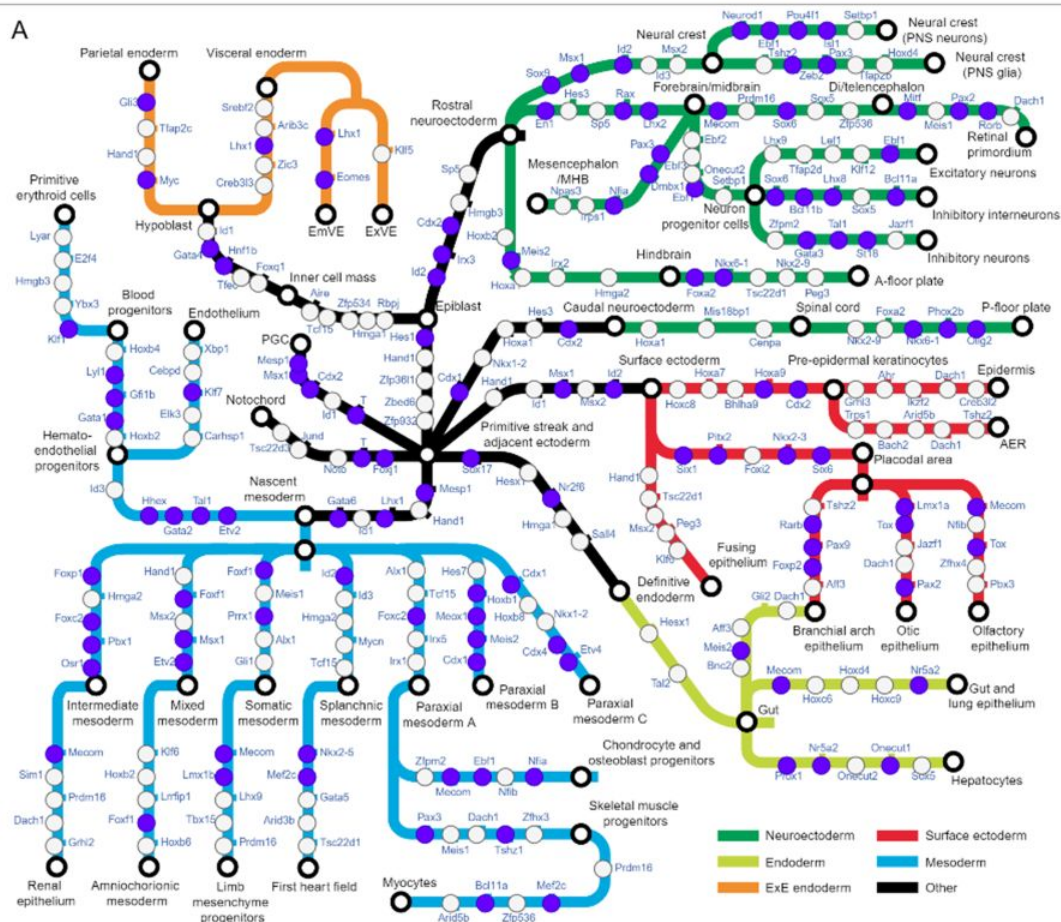
**OPEN**  
**Systematic reconstruction of cellular trajectories across mouse embryogenesis**

Chengxiang Qiu<sup>1,2,3,4,5</sup>, Junyue Cao<sup>1,2,3,4,5</sup>, Beth K. Martin<sup>1</sup>, Tony Li<sup>1,2,3,4,5</sup>, Ian C. Welsh<sup>1</sup>, Sanjay Srivatsan<sup>1,4</sup>, Xingfan Huang<sup>1,2,3,4,5</sup>, Diego Calderon<sup>1</sup>, William Stafford Noble<sup>1,2,3,4,5</sup>, Christine M. Disteche<sup>1,2,3,4,5</sup>, Stephen A. Murray<sup>1,2,3,4,5</sup>, Malte Spielmann<sup>1,2,3,4,5</sup>, Cecilia B. Moens<sup>1,2,3,4,5</sup>, Cole Trapnell<sup>1,2,3,4,5</sup> and Jay Shendure<sup>1,2,3,4,5</sup>



# HD-DoE outputs gene regulatory information

A



B

HOXA9

Experiments that test for the expression of this gene	Programs that test for the expression of this gene	Teams that test for the expression of this gene	Number of factors that have significant coefficients for this gene
38	iPSC to HSC	Mesoderm	443

A sample of coefficients for HOXA9

Predictor	Coefficient	Standard Error	P Value	Confidence Interval	R <sup>2</sup>
redacted	2,172.56	347.3	0.0034	605.203	0.8322
	1960.06	474.236	0.0001	985.584	0.8583
	1003.1	48.175	0.0183	98.3447	0.9694
	-2,968.25	585.096	0.0002	1,137.42	0.8448
	-2,211.49	563.366	0.012	1,426.32	0.8676
	-1,168.64	493.34	0.0158	1,162.70	0.7469
	994.903	351.957	0.0045	103.56	0.9012
	-719.078	68.7768	0.049	478.56	0.6670

C

Mesoderm Ectoderm Endoderm All Teams

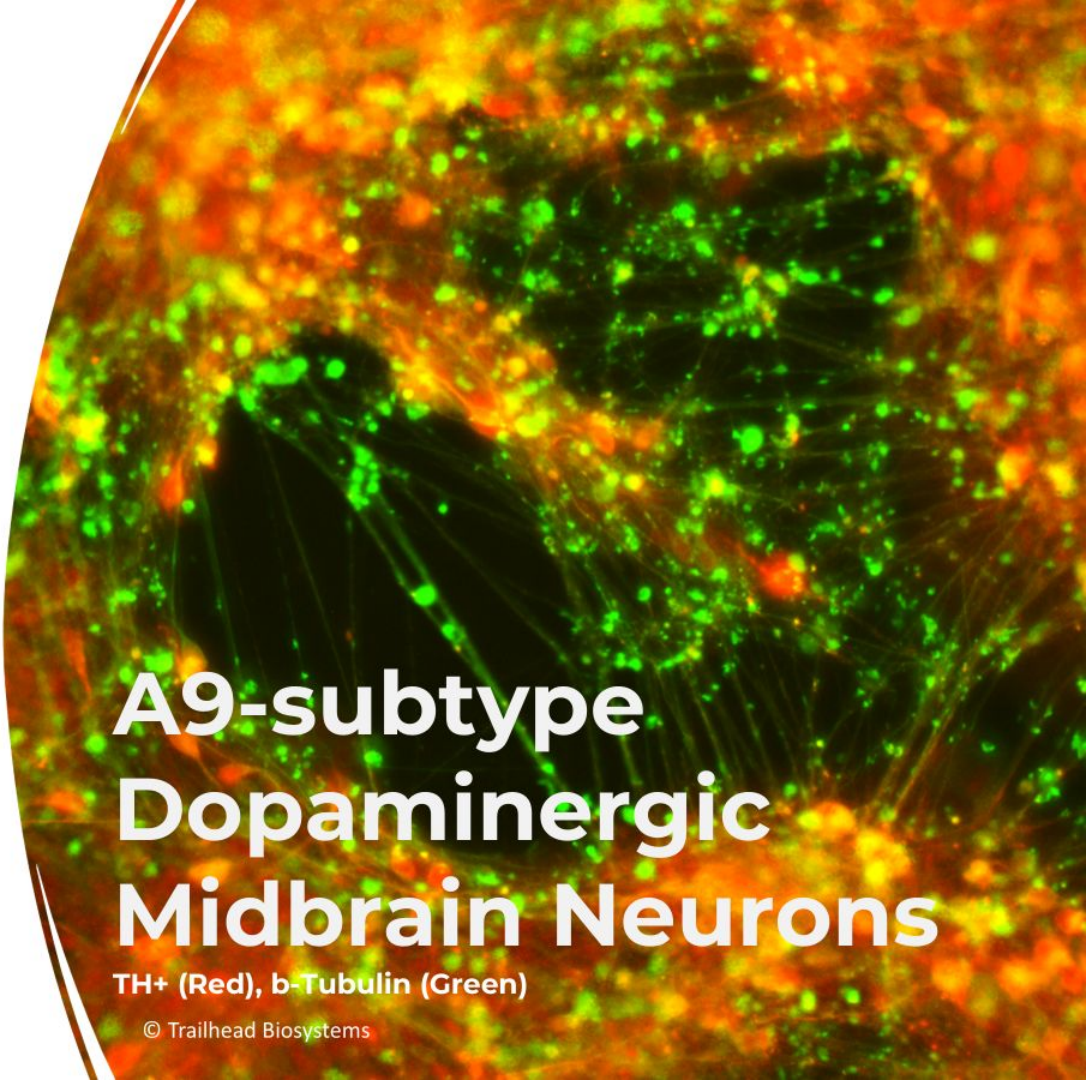
Total Unique Genes Tested	342	355	169	793
Genes with significantly predictive models (p < 0.05, R <sup>2</sup> > 0.75)	333	340	152	753
Total Coefficients	redacted			
Coefficients with significant effects on genes (p < 0.05)	4,377	5,990	3,788	13,946

Note: The values for all teams are not equal to the sum of unique genes/factors across teams, as teams may test for the same genes/factors.

# 2<sup>ND</sup> Gen Protocols

**Protocols are built to achieve purity and function**

- Each protocol is developed from scratch and starts from induced pluripotent stem cells (iPSC)
- Each protocol step aims to achieve efficient conversion to the desired fate
- Validation of critical cell determinants occurs at each stage of differentiation
- Comprehensive testing is performed using qRT-PCR, RNAseq, Immunofluorescence, flow cytometry, and multiple cell functional assays



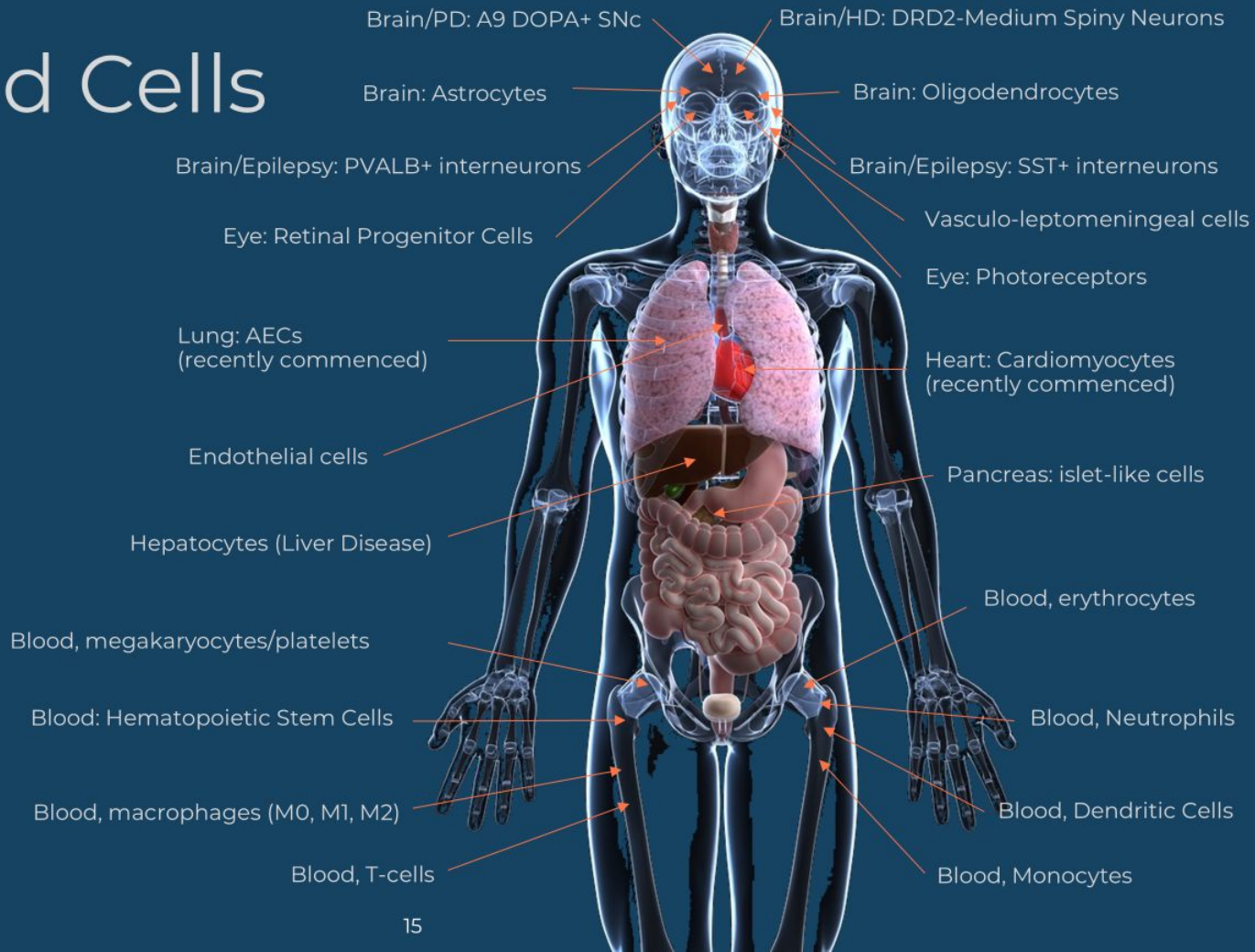
**A9-subtype  
Dopaminergic  
Midbrain Neurons**

TH+ (Red), b-Tubulin (Green)



# Specialized Cells

Current Trailhead Cell Programs

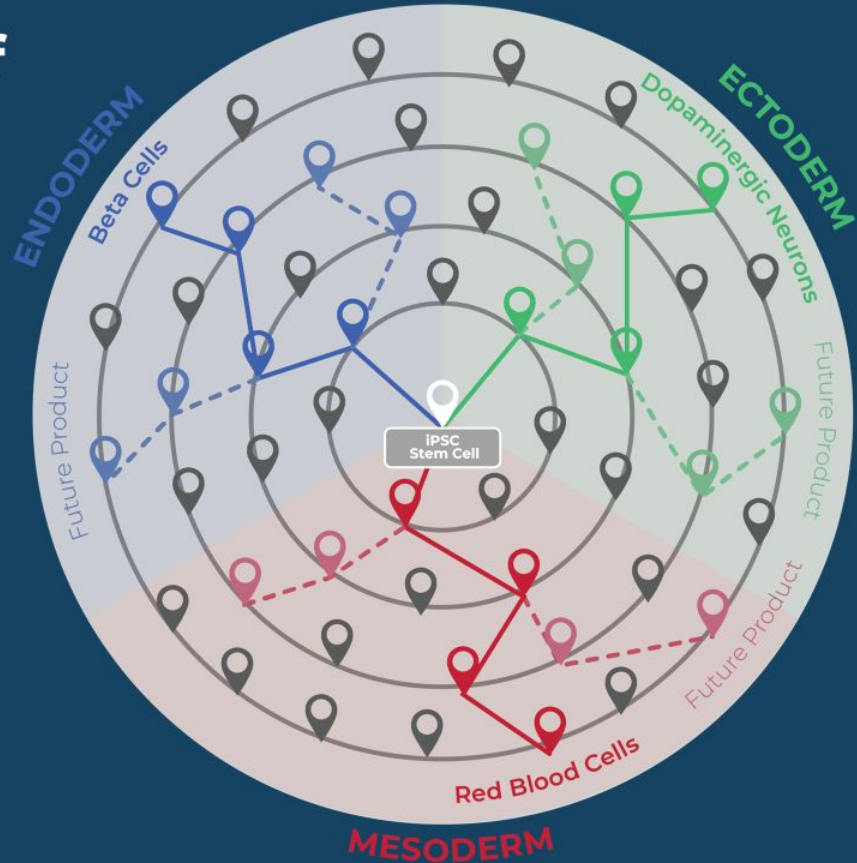


# Systematic Control of lineage selection

We iteratively apply HD-DoE® where each new experiment increases our knowledge base

The method is essentially one of **step-wise attractor jumping**

Dramatically lowers cost and time for each cell as we move forward



Human cell fate space >500 specialized cell types

Path finding  
between nodes

## Analysis Center

## JOURNEY

Start node...

End node...

Find  
Path

Clear

## STATISTICS

577

Total Nodes

576

Total Edges

21

Max Depth

## CELL LINEAGE KNOWLEDGE MAP

Lineage tree  
statsFilter cell types by  
lineage and stage

This section expands when the user clicks any node / cell type in from the radial tree visualization

Placeholders for more data

A floating tooltip to get quick information about any Node/cell type

## Dig Deep

## SELECTED NODE

Hindbrain  
E8.25

## PARENT NODE

Rostral  
neuroectoderm  
(E8)

## CHILD NODES (3)

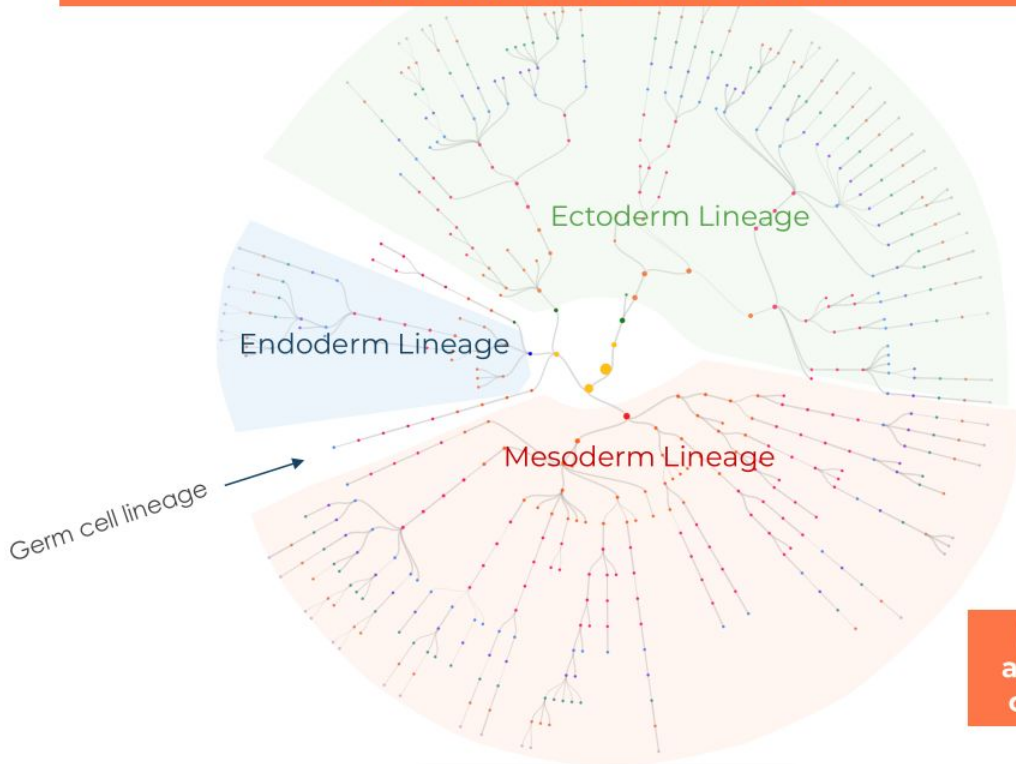
- Posterior floor plate (E8.5a)
- Anterior floor plate (E8.5a)
- Hindbrain (E8.5a)

## STUDIES

- SC-RNA SEQ STUDY
- HD-DoE STUDY



## CELL LINEAGE KNOWLEDGE MAP



The Company subdivides its protocol Development according to germ layer:

**ENDODERM**  
**MESODERM**  
**ECTODERM**

Each germ layer is arranged around the circumferential axis

● neuroectoderm ● mesoderm ● endoderm

● E6 (6) ● E7 (80) ● E8 (124) ● E9 (56) ● E10 (60) ● E11 (71) ● E12 (66) ● E13 (62)



CELL LINEAGE KNOWLEDGE MAP

Analysis Center

JOURNEY

E13.5:Hepatocytes

E3:Morula

Find Path

Clear

STATISTICS

577

Total Nodes

576

Total Edges

21

Max Depth

Journey:  
Hepatocyte fate  
Liver

Our progress can be easily  
visualized



Dig Deep

SELECTED NODE

Hepatocytes  
E13.5

PARENT NODE

Hepatocytes  
(E12.5)

CHILD NODES (0)

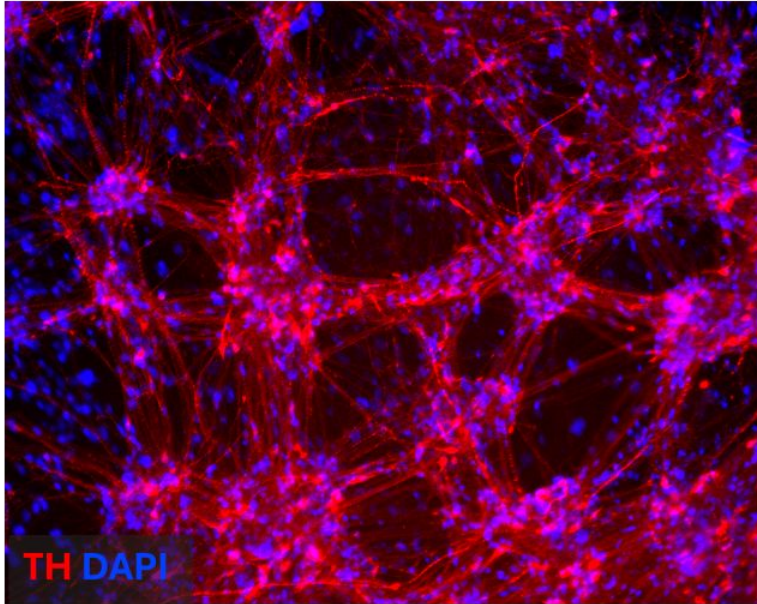
No child nodes

STUDIES

- SC-RNA SEQ STUDY
- HD-DoE STUDY



# iPSC-Derived TrailBio<sup>®</sup> A9 Dopaminergic Neurons



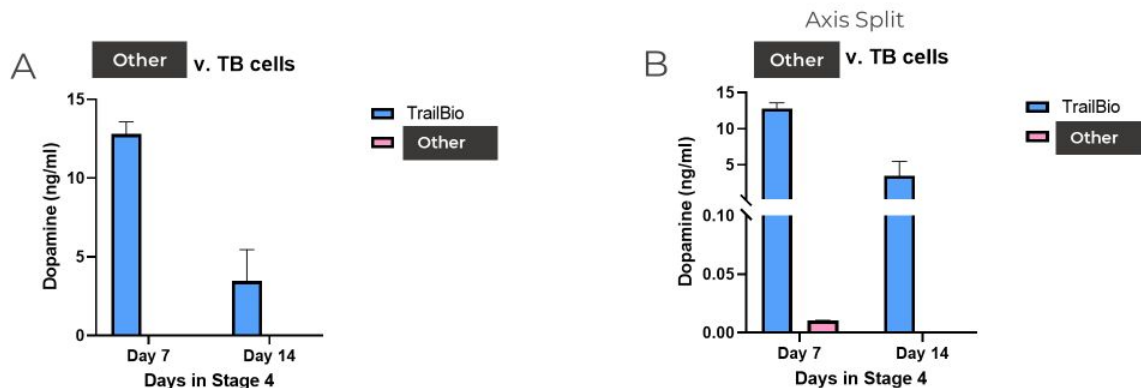
- 23-Day differentiation process leveraging low-cost raw materials
- 70% Purity measured by tyrosine hydroxylase expression
- Optimized for SOX6 expression, indicating presence of A9 subtype lost in Parkinson's and avoidance of VTA subtype prevalent in published protocols
- High Dopamine release at base level; 12 ng/ml on day 7 of the protocol compared to 0.01 ng/ml from other market product.
- Cryopreserved single cells in vials
- 80% Viability post-thaw
- Novel differentiation method built on HD-DoE<sup>®</sup> platform; no dual SMAD inhibition



# Comparative Data: Trailhead Cells vs. Other

Dopamine release of DA neurons at 2 timepoints was measured and compared to commercially available cells

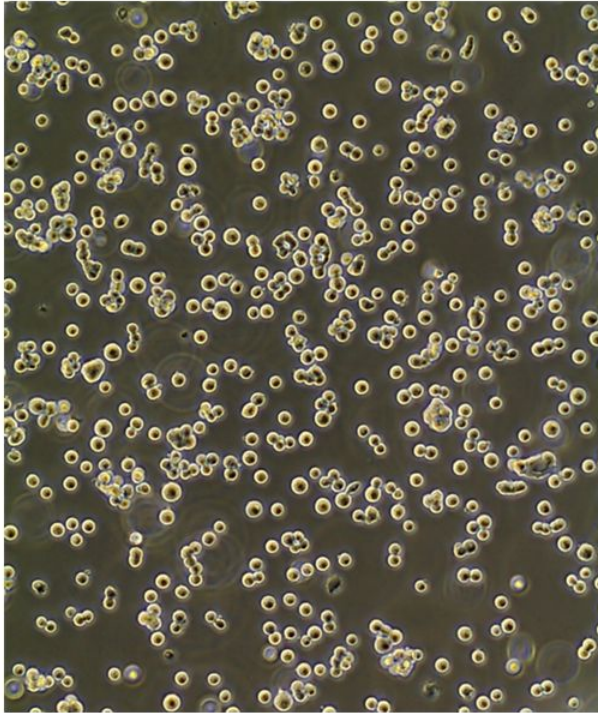
At both timepoints, 4 to 12 ng/ml dopamine was detected from Trailhead cells at base level (15 minutes at HBSS buffer). Released dopamine from Other was around 0.01 ng/ml.



Dopamine Release from Trailhead A9 Dopaminergic Neurons is significantly higher



# iPSC-Derived TrailBio<sup>®</sup> Hematopoietic Progenitors

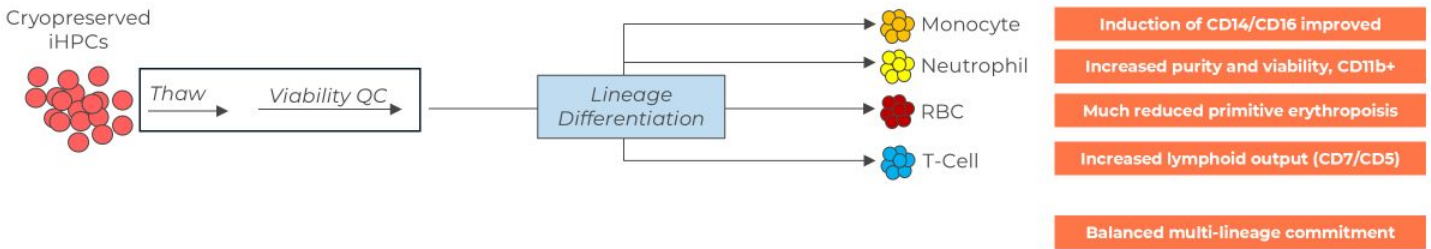


- 7-10-Day differentiation process leveraging low-cost materials/manufacturing process
- >80% CD34/CD43+ purity measured by flow cytometry assay, unpurified
- Expression of HLF, SPINK2, MECOM comparable to primary
- Full, balanced multilineage potential across all blood lineages
- Current yield: up to 300M cells/batch in 0.5 liter bioreactor; process amenable to further scale-up
- Cryopreserved as single cells
- 90% viability post-thaw
- Novel differentiation method built on HD-DoE<sup>®</sup> platform





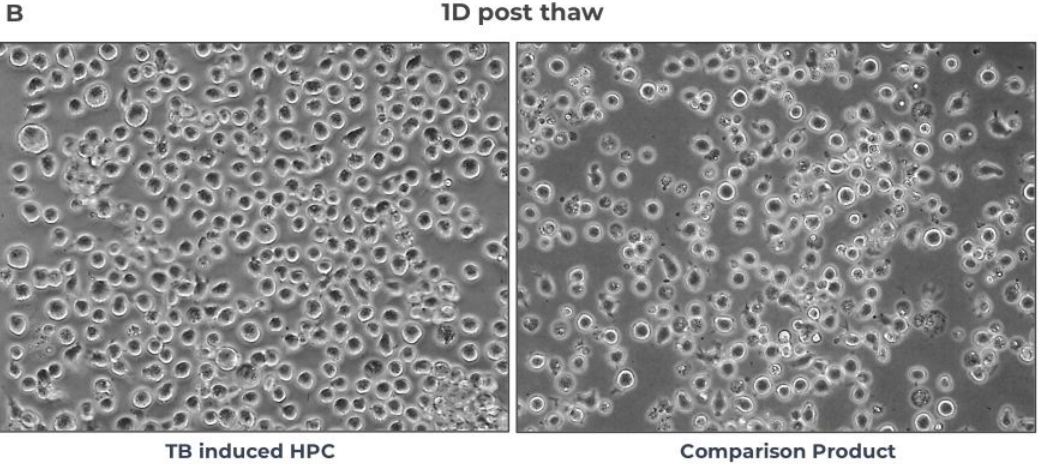
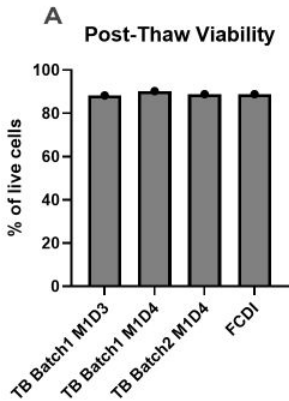
# Comparative Analysis of iHPCs: Trailhead vs. Industry Standard – Post-Thaw Viability



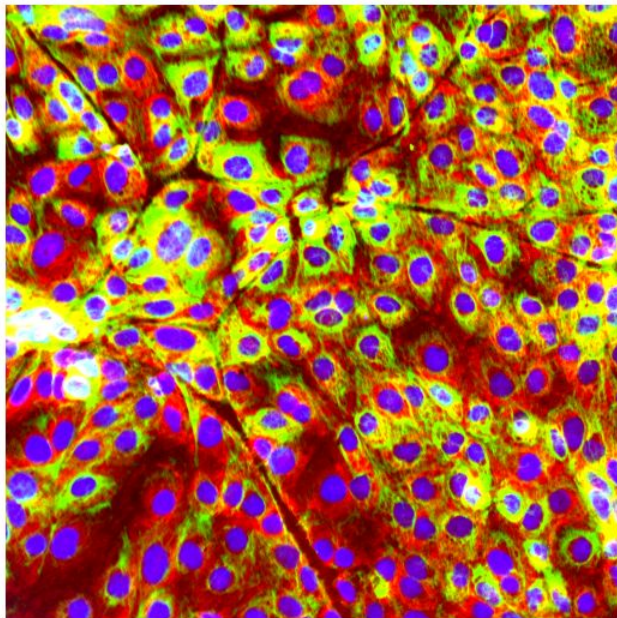
To understand whether Trailhead iHPCs are a competitive product, we compared our cells against FCDI HPCs, focusing on post-thaw viability and lineage differentiation potential. For this evaluation, FCDI cells, day 3 / 4 iHPCs from batch 1, and day 4 cells from batch 2 were thawed and test for viability via flow cytometry.

The post-thaw viability of Trailhead cells was comparable with Fuji cells (88%-90% viable - **A**) and all cells appeared morphologically healthy after 24 hours of culture (**B**).

- Trailhead iHPCs post-thaw viability comparable to competitor cells.



# iPSC-Derived TrailBio<sup>®</sup> Hepatocytes

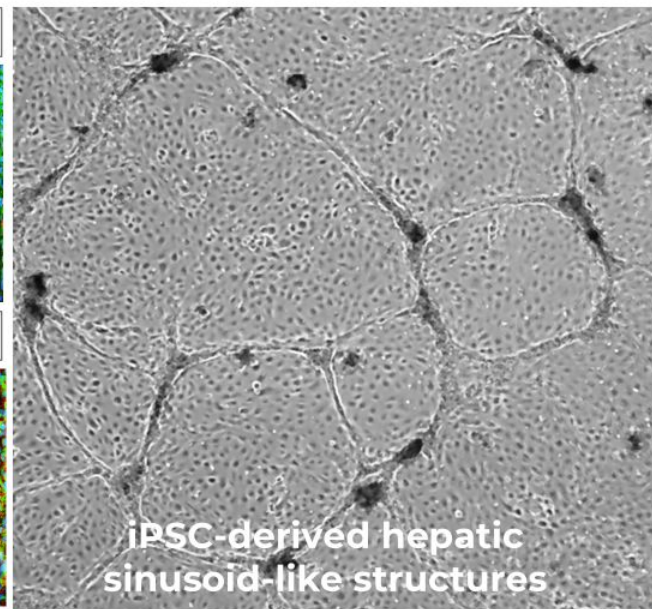
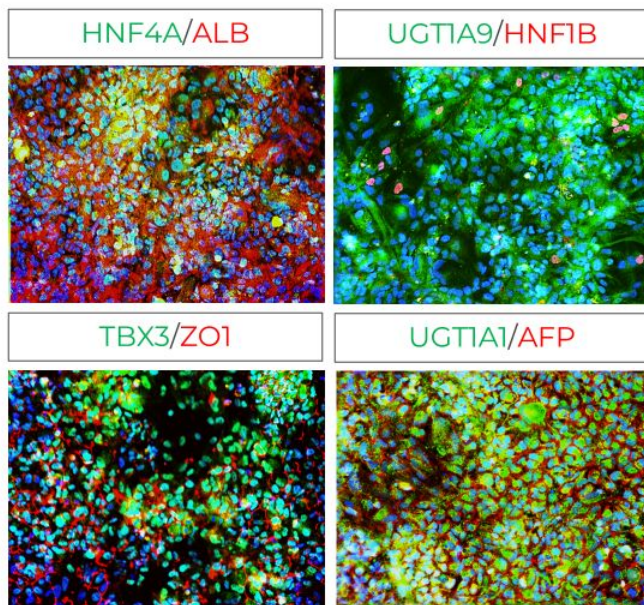
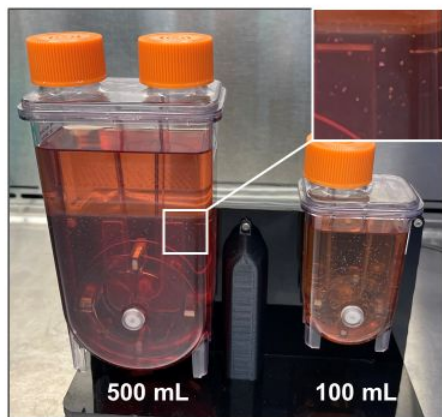


- 24-Day differentiation process leveraging low-cost materials
- Higher CYP450 activity when compared to published protocols
- CYP3A4 and A1AT expression comparable to primary human hepatocytes
- Specific data on CYP2C9 and CYP3A4 metabolism activity
- Albumin secretion as measured by ELISA; reduced expression of the fetal marker AFP.
- Current yield: 1 billion cells/batch in 1-liter bioreactors; process amenable to further scale-up
- Novel differentiation method built on HD-DoE<sup>®</sup> platform; Hepatocytes are generated from a highly regionalized foregut progenitor.

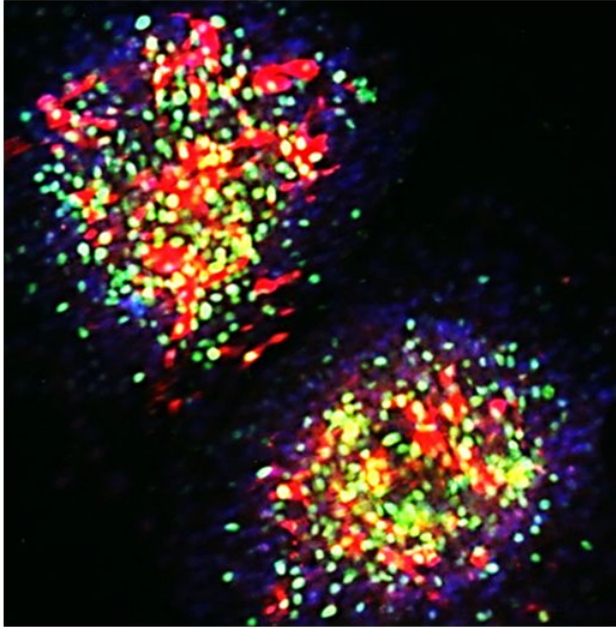


# iPSC-Derived TrailBio<sup>®</sup> Hepatocytes

- Bioreactor produced iPSC-derived hepatocytes were cryopreserved & recovered to assess phenotype stability
- Robust expression of HNF4A, ALB, TBX3, ZO1, UGT1A9 & UGT1A1 was observed.



# iPSC-Derived TrailBio<sup>®</sup> Pancreatic Beta Cells



- 17-Day differentiation process leveraging low-cost raw materials
- >20% CPEP+ purity measured by flow cytometry
- No generation of enteroendocrine cell subtype in contrast to published protocols
- Resembles primary human islets in endocrine composition: Alpha, Beta and Delta cells within iPSC derived aggregates
- High levels of insulin secretion as indicated by ELISA analysis.
- Current yield: 1 billion cells/batch in 1-liter bioreactors; process amenable to further scale-up
- Cryopreserved as aggregates
- >85% viability post-thaw
- Novel differentiation method built on HD-DoE<sup>®</sup> platform; cells are generated without the use of TGF $\beta$  or WNT agonism and are differentiated through the dorsal endoderm lineage.



# Trailhead Product Process

## R&D



- HD-DoE®
- 2D Cultures
- **Conceptual & Testing**

### Ectoderm

Forebrain MGE Somatostatin+ Interneurons  
 Forebrain MGE GABAergic interneurons (mix)  
 Forebrain MGE PVALB+ Interneurons  
 Forebrain LGE Medium Spiny Neurons (DRD1)  
 Forebrain LGE Medium Spiny Neurons (DRD2)  
 Midbrain SOX6+ progenitors  
 Midbrain A9 Dopaminergic Neurons  
 Glial: Vascular Leptomeningeal Cells

### Mesoderm

Hematopoietic Stem Cells  
 Common Myeloid Progenitor Cells  
 E-lineage Hematopoietic Cells  
 M-lineage Hematopoietic Cells  
 Monocytes (CD14+/CD16+/CD33+)  
 Macrophages (CD163+, CD11b+, CD11c)  
 Neutrophils (CD14-, CD15+/MPO+)  
 Dendritic Cells

### Endoderm

Pancreatic Insulin-Producing Cells (INS+/NKX6.1+)  
 Hepatocytes (TBX3+/ALB+)  
 Biliary cells (CK7+/CD19+)



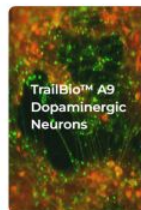
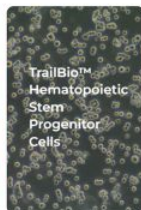
## Design Transfer (DT)



0.5-1 liter

0.25-1 billion cells

- 3D Aggregates in Bioreactor
- DT implements a “Hybrid QC” method
- **Pre-Released Products (for sale under MTA)**



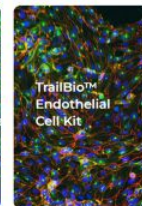
## Manufacturing



3 liter

1.5-6 billion cells

- 3D Aggregates Scaled-up
- In-depth QC
- **Launched Products (off-shelf)**



# Research-Use Products

- **Current Offerings**

- Normal (non-disease) cells/Kits released at Batch Scale +1B
- 1M to 5M cells per vial, ready to use, vial reservation option
- Kits: Cells + Media + Instructions
- Custom iPSC differentiation for key clients

**Trailhead Biosystems**  
EC-01-05  
**TrailBio™**  
Pre-Myelinating  
Oligodendrocyte  
5 x 10<sup>6</sup> cells  
Lot# EC1-0014

**Trailhead Biosystems**  
Cells Services Therapeutic Partnerships Company [Buy Cells](#)

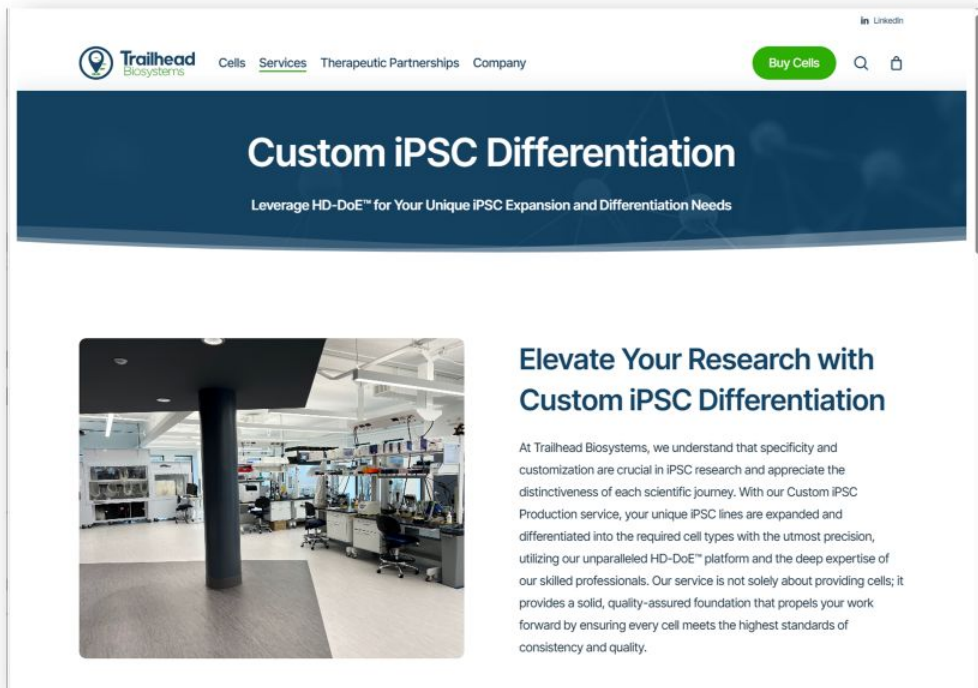
## TrailBio™ Cells

Available Now    Launching Soon    In Development    Get Notified of New C

# Trailhead Custom Cell Solutions

## Service: We differentiate and manufacture iPSCs under contract

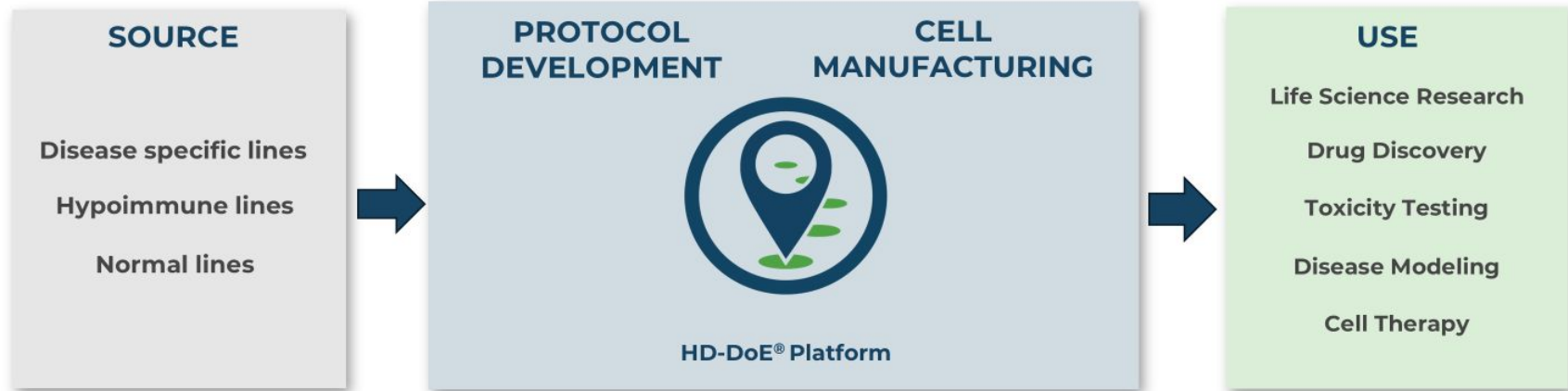
- Contract manufacturing engagement
- Your cells are used (MTA to us)
- Smaller engagement fee
- Immediate gap-fill for you
- Acceptance Criteria fulfilled by us to receive payment upon shipping
- Addresses your differentiation problems



The screenshot displays the Trailhead Biosystems website. At the top, the logo and navigation menu (Cells, Services, Therapeutic Partnerships, Company) are visible, along with a 'Buy Cells' button and social media icons. The main heading is 'Custom iPSC Differentiation', with a sub-headline: 'Leverage HD-DoE™ for Your Unique iPSC Expansion and Differentiation Needs'. Below this is a photograph of a modern laboratory interior. To the right of the photo, the text reads: 'Elevate Your Research with Custom iPSC Differentiation'. The text continues: 'At Trailhead Biosystems, we understand that specificity and customization are crucial in iPSC research and appreciate the distinctiveness of each scientific journey. With our Custom iPSC Production service, your unique iPSC lines are expanded and differentiated into the required cell types with the utmost precision, utilizing our unparalleled HD-DoE™ platform and the deep expertise of our skilled professionals. Our service is not solely about providing cells; it provides a solid, quality-assured foundation that propels your work forward by ensuring every cell meets the highest standards of consistency and quality.'



# What can we do for you?



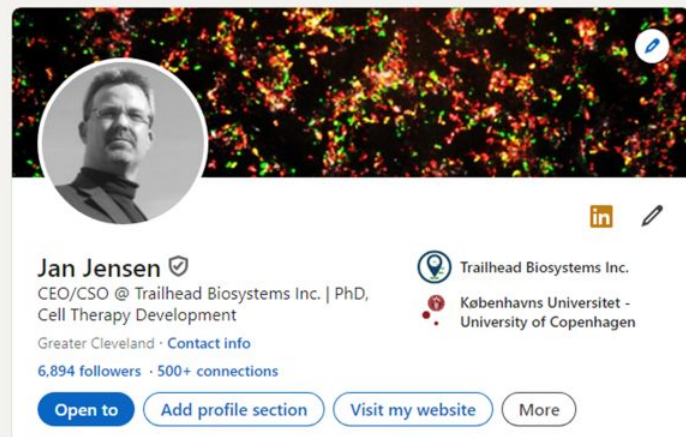



# Take-Home Items

- HD-DoE® is mathematics but practically saves run costs!
- MVDA converts reams of data into mathematical models
- We capture interactions – not just primary effects
- When HD-DoE® is sufficiently geared, even biology yields



# Stay Tuned to Cells and Lets Connect!



Jan Jensen 

CEO/CSO @ Trailhead Biosystems Inc. | PhD,  
Cell Therapy Development

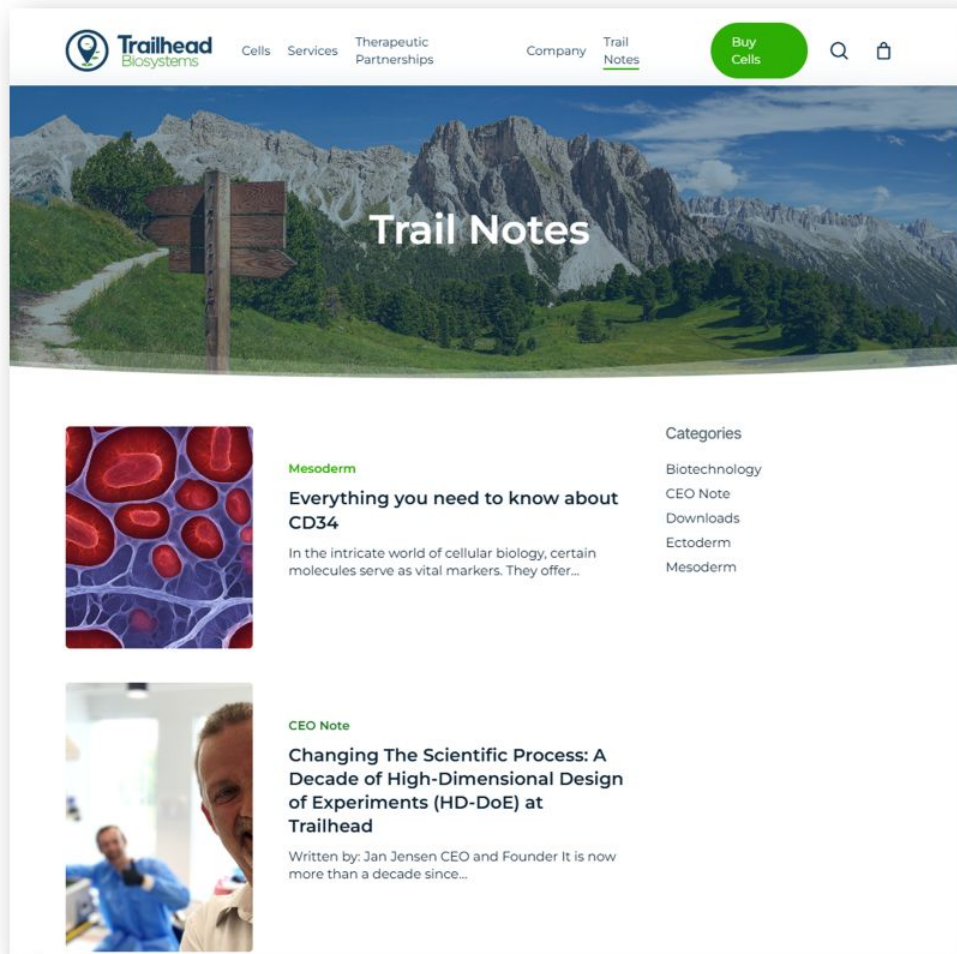
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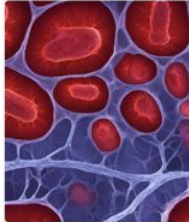
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[www.linkedin.com/in/janjensen1/](http://www.linkedin.com/in/janjensen1/)



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## Trail Notes



**Mesoderm**

### Everything you need to know about CD34

In the intricate world of cellular biology, certain molecules serve as vital markers. They offer...



**CEO Note**

### Changing The Scientific Process: A Decade of High-Dimensional Design of Experiments (HD-DoE) at Trailhead

Written by: Jan Jensen CEO and Founder It is now more than a decade since...

Categories

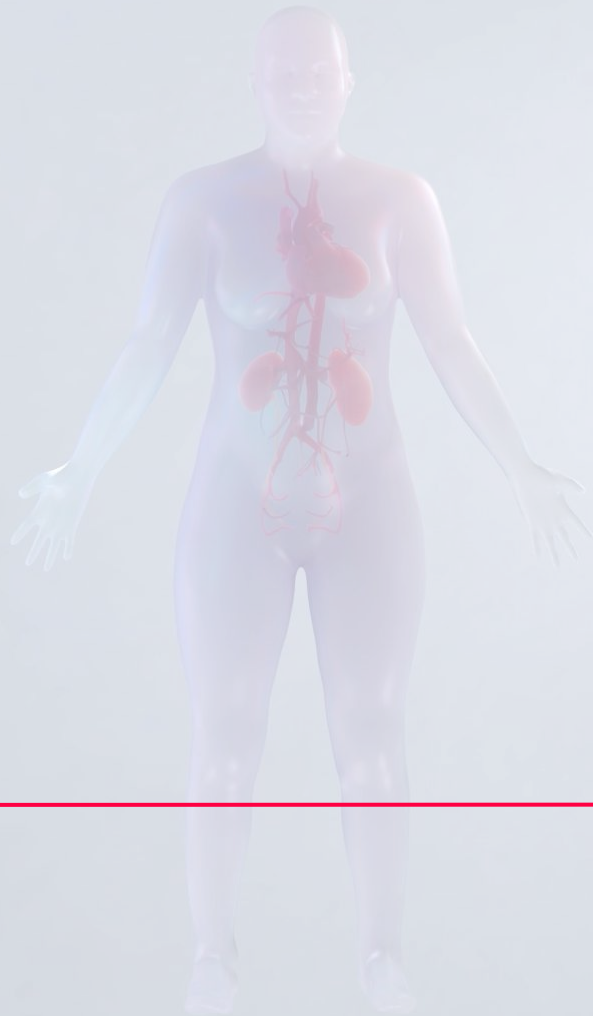
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# Q&A

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<https://humanatlas.io/events/2024-24h>

# Questions

How do we define a Multiscale Human?

How do we map a Multiscale Human?

How do we model a Multiscale Human?

What is the potential impact of availability of human cells as unlimited material?

How can iPSC-derived human cells help create better models for human biology modeling, at anatomical and functional levels

How long would it take to make all the cell types?

What is the future of biological science going to look like?

# Thank You!

Find out more about  
Trailhead Cells  
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**Trailhead**  
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