6PM

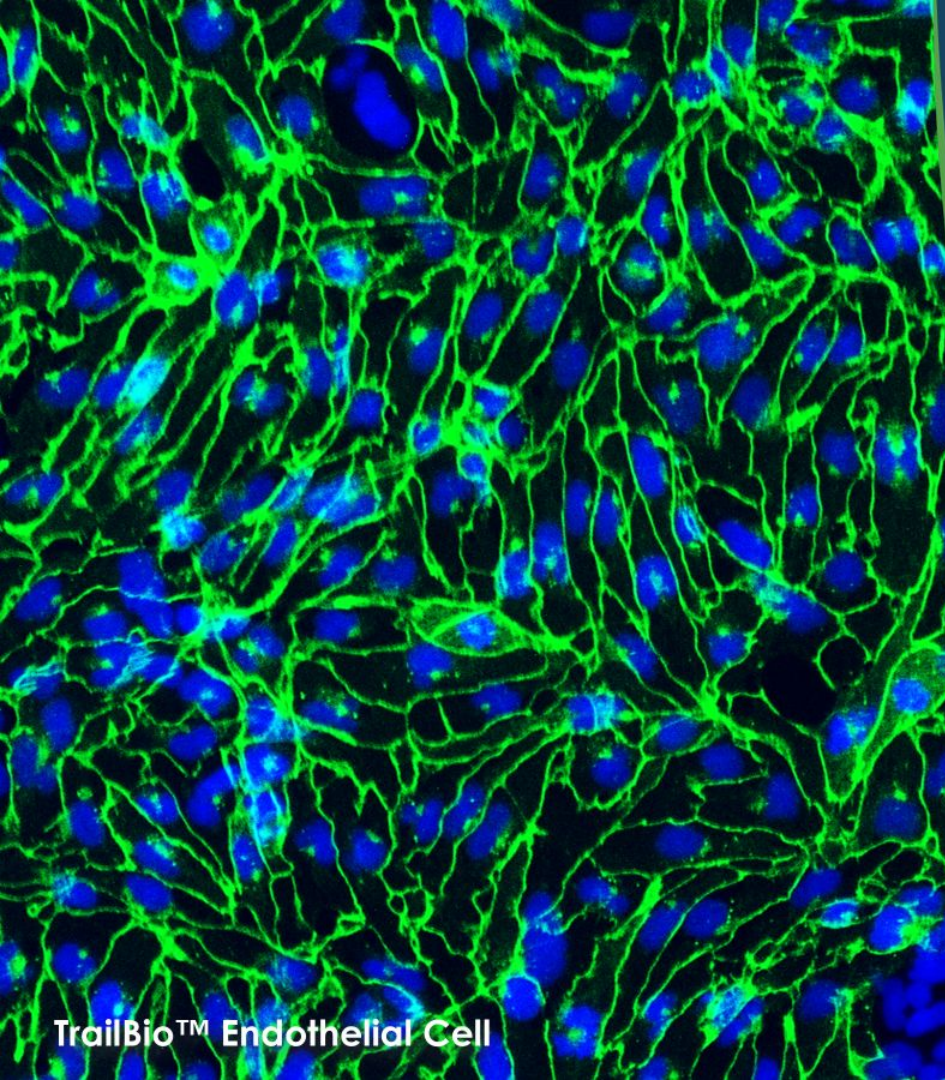
11PM in London (GMT), 8AM in Tokyo (GMT+9)

Cell Manufacturing & Mapping

Moderator: Katy Börner, *Indiana University*

Presenter: Jan Jensen, *Trailhead Biosystems*

Jan Jensen, *Trailhead Biosystems CEO*



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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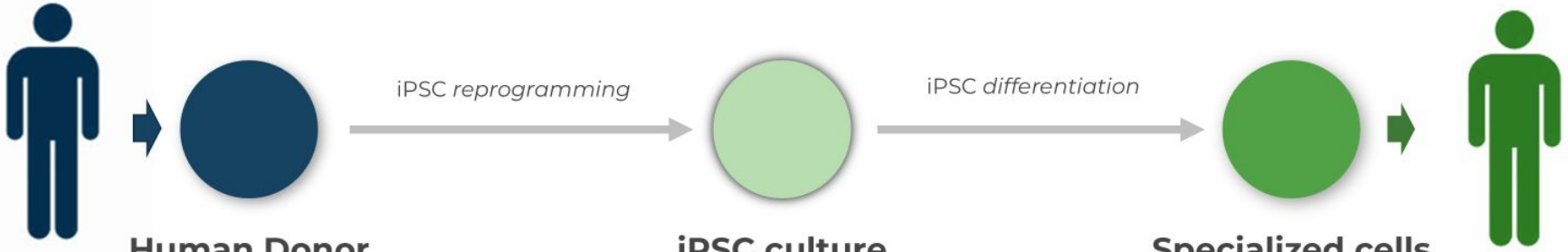
Trailhead®
Biosystems

Biology. Controlled.

Stem cells: The Hardest Challenge



Human Donor

Adult human cells are isolated from healthy or sick donors

No ethical concerns related to fetal-tissue use

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

iPSC culture

Cells are reprogrammed to the stem cell state (pluripotency)

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

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

Specialized cells

Specialized cells make up our body and collectively make it work

Making iPSCs robustly enter specialized fates is very difficult

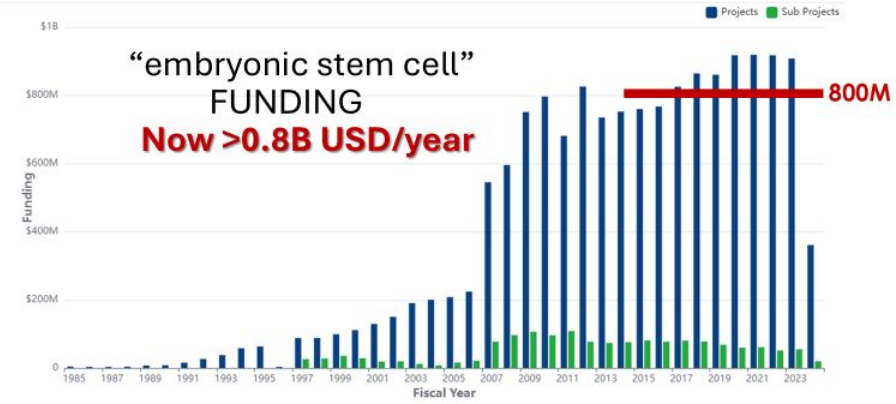
When this problem is solved, it will open a gateway to broader use of human cells

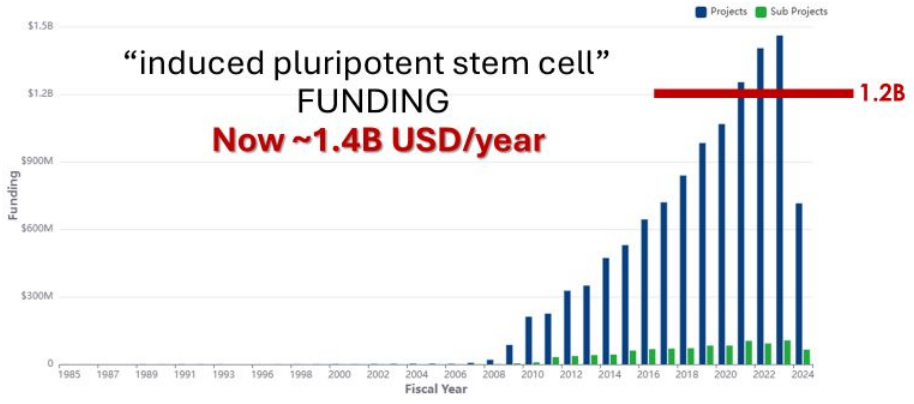
Problem

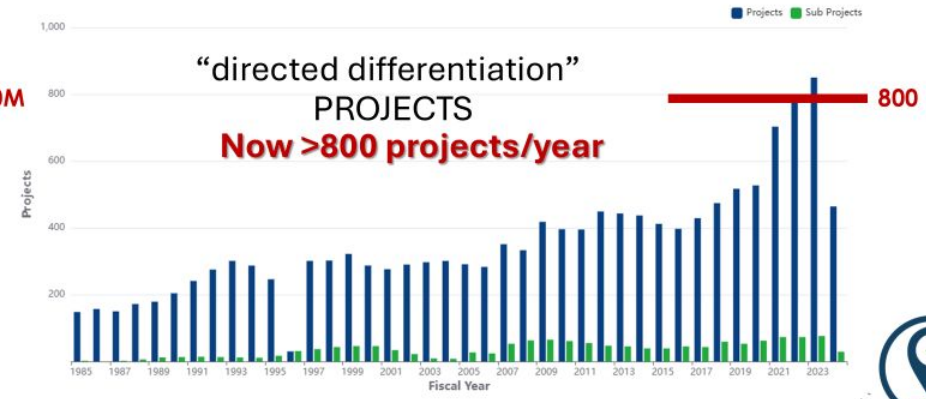
- The demand for cells is enormous, but the options are limited
- Of the options available, cell purity is poor
- There is a lack of consistency from batch to batch
- Not available in the quantities the market demands
- Development and production processes are largely done manually

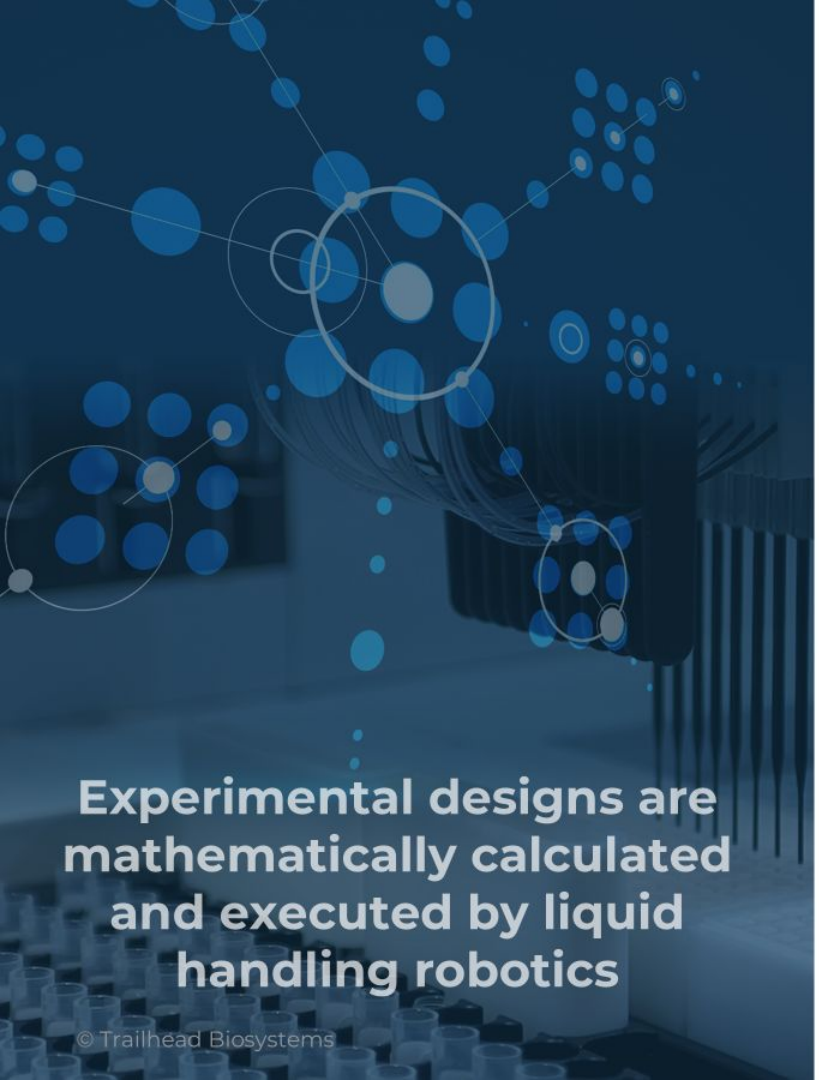
This ‘Problem’ is very costly (NIH only):

Source: NIH RePORTER queries







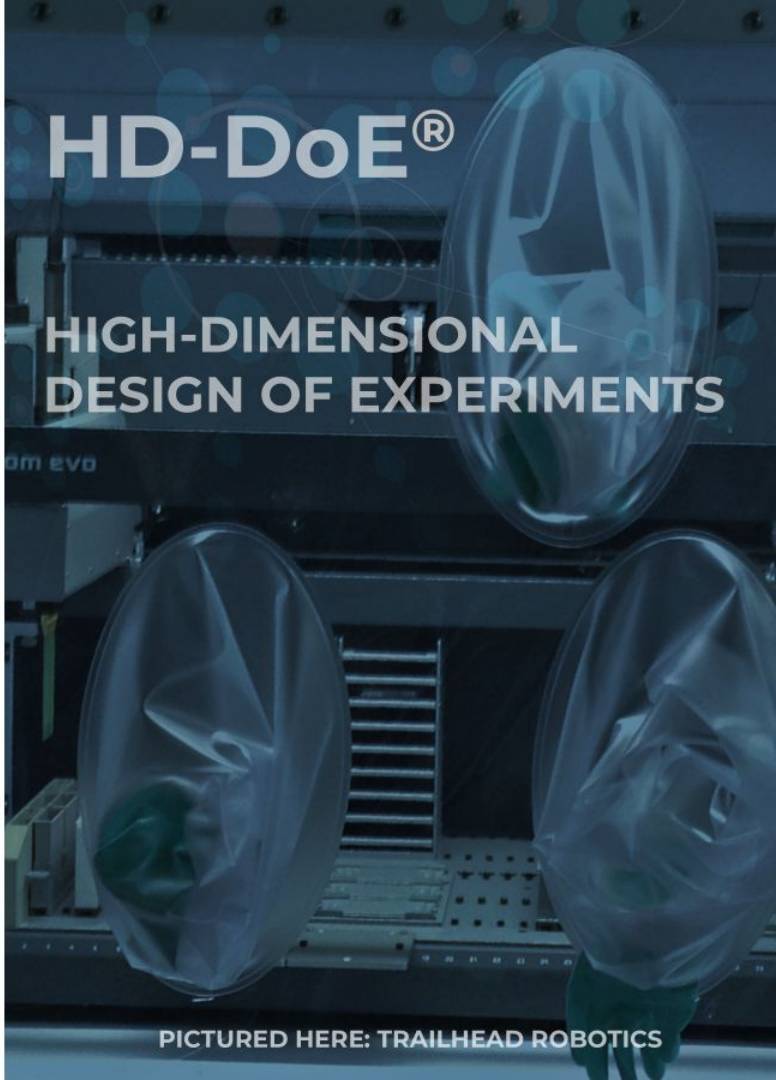


Not Hypothesis-Driven Research...

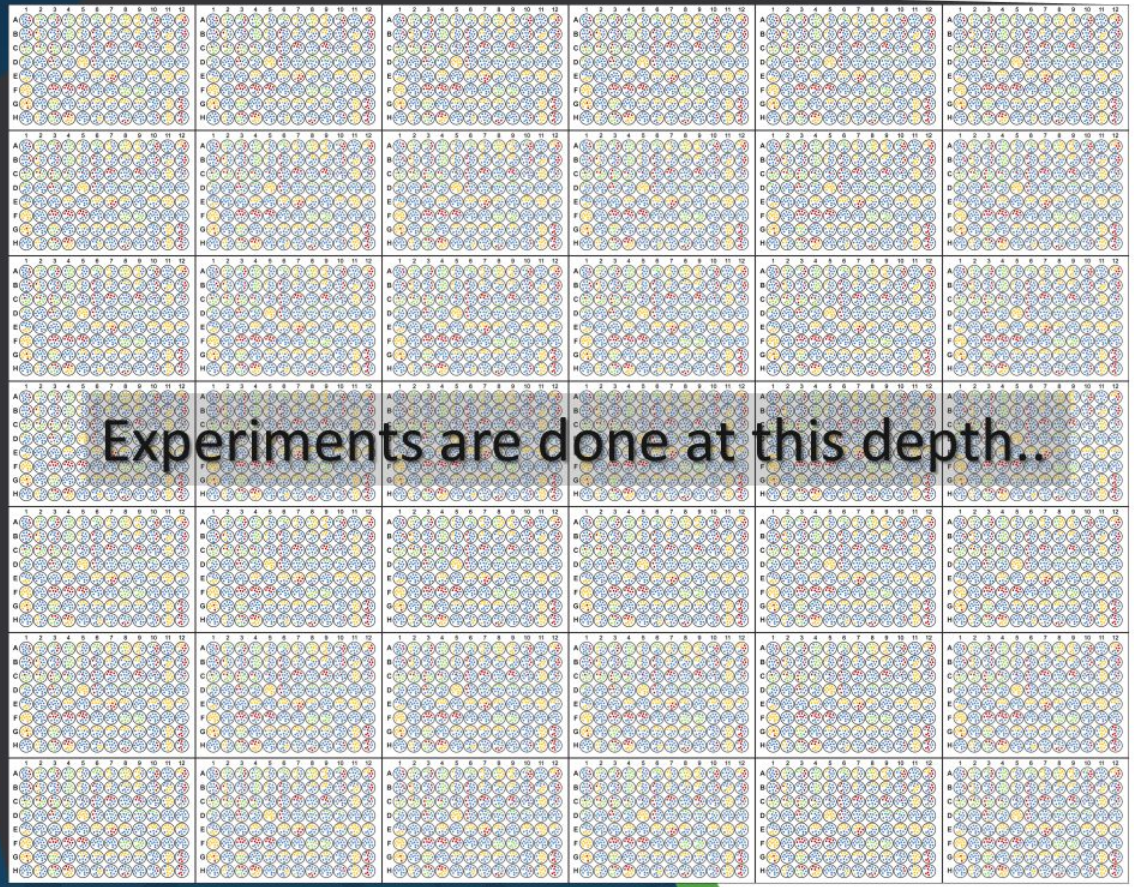
- We replace manual discovery with robotics in a high-dimensional space of regulatory inputs
- We identify critical process parameters for each cell type
- We manufacture cells at industrial scale (billions per run) in bioreactors with high purity
- We build cost-effectiveness into each protocol by leveraging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

Powered Empiricism in Developmental Biology

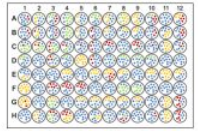
- Computerized, robotically-executed, experiments with speed, precision, and scalability
- Data-driven, unbiased determination of critical process parameters
- Proprietary, internally developed software tools
- Empirical data created and owned by Trailhead
- All protocols built from scratch



HD-DoE® technology compresses a very large testing space



..but at a fractional cost



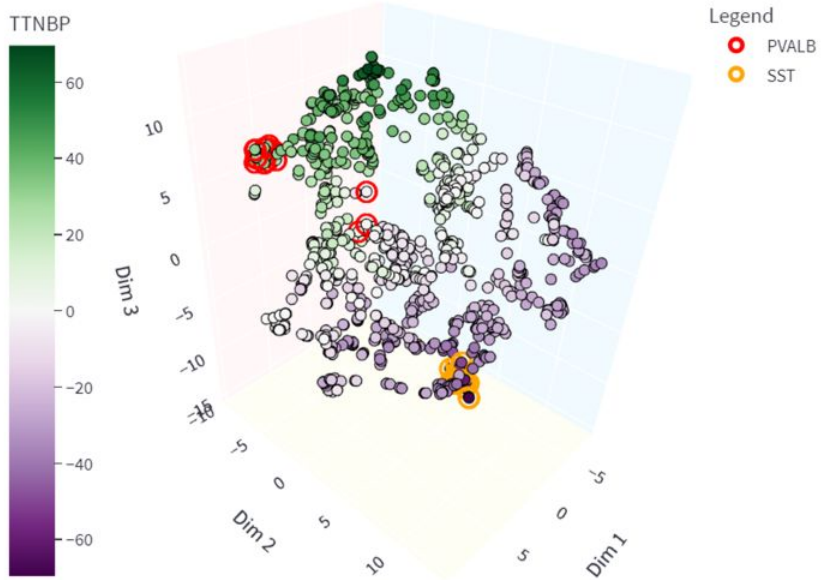
~$2,000

>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**

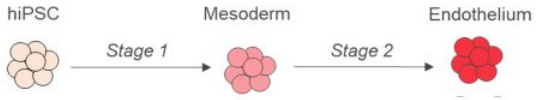
All protocols are built from beginning to end by us

A9 Dopaminergic Neurons

Red blood Cells

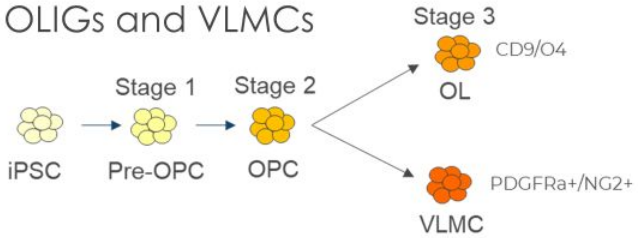
Parvalbumin+ Interneurons

Endothelial cells



Monocytes/Macrophages

OLIGs and VLMCs



SST Interneurons

Hematopoietic Stem Cells

Islet Cells

Cell Fate ‘Metro Map’

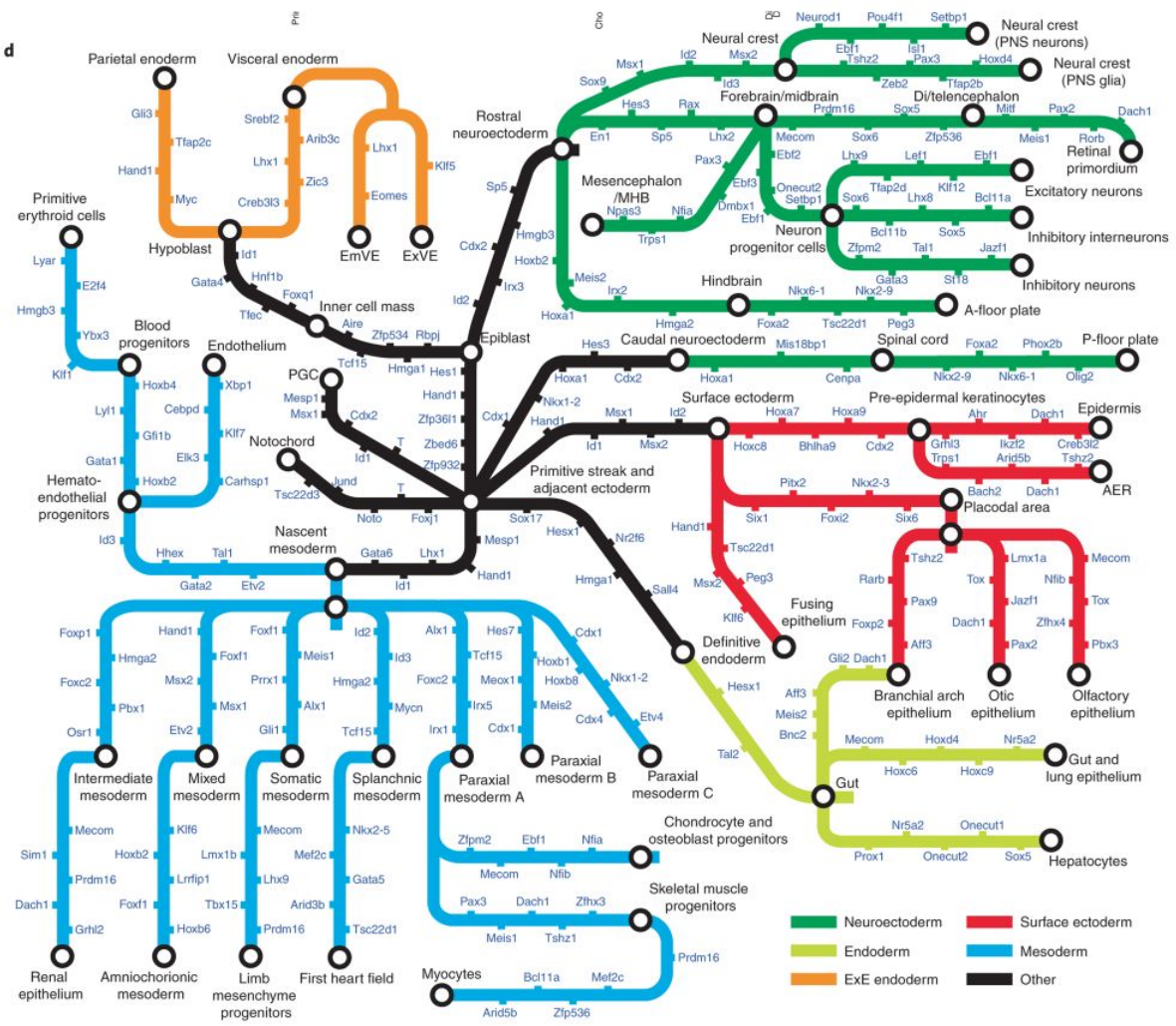
Individual TFs plotted within the lineages

ARTICLES
https://doi.org/10.1038/s41588-022-01018-x
nature genetics
Check for updates

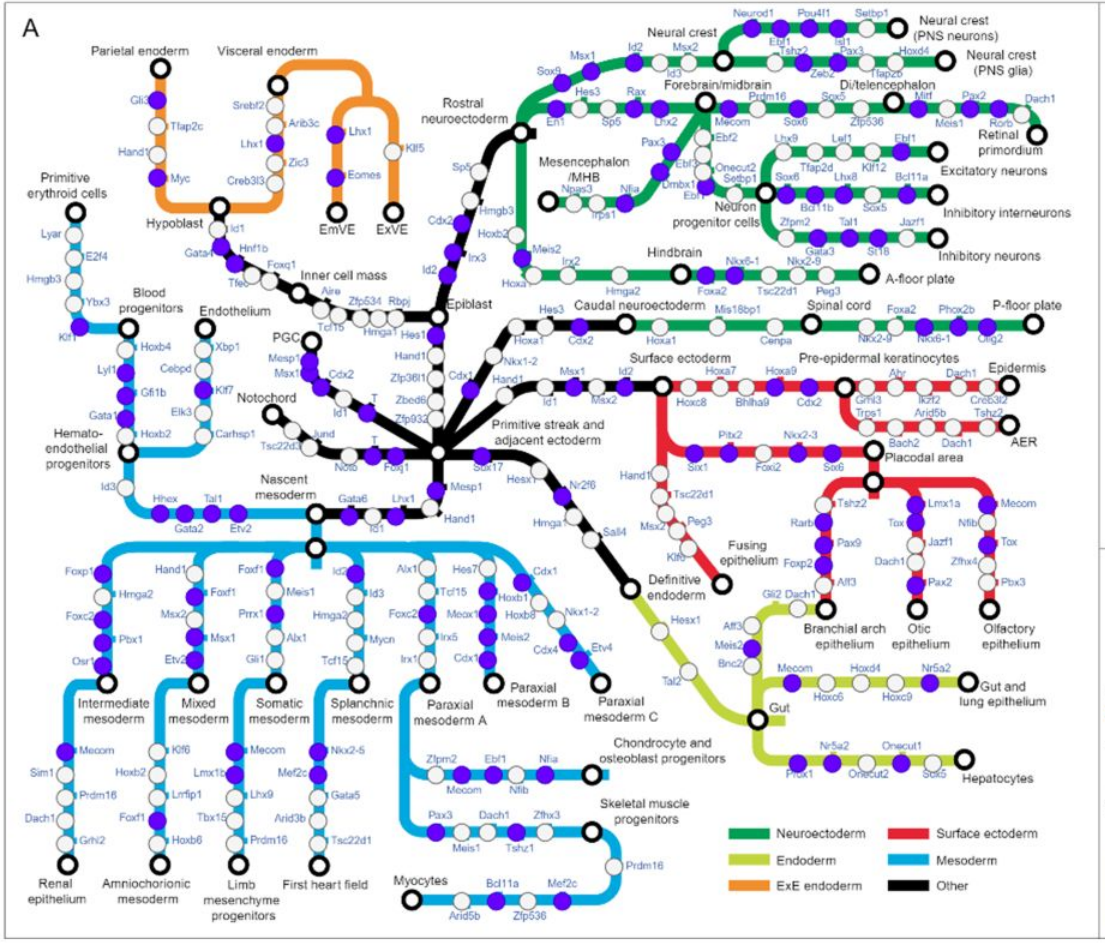
OPEN

Systematic reconstruction of cellular trajectories across mouse embryogenesis

Chengxiang Qiu[1,5], Junyue Cao[2], Beth K. Martin[1], Tony Li[1], Ian C. Welsh[1], Sanjay Srivatsan[1,4], Xingfan Huang[1,5], Diego Calderon[1], William Stafford Noble[1,5], Christine M. Disteche[6,7], Stephen A. Murray[3], Malte Spielmann[8,9], Cecilia B. Moens[10], Cole Trapnell[1,11,12] and Jay Shendure[1,11,12,13]



HD-DoE outputs gene regulatory information



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^2
redacted	2,172.56	347.3	0.0034	695.203	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	1,157.42	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
	-219.078	68.7768	0.049	478.56	0.6670

C

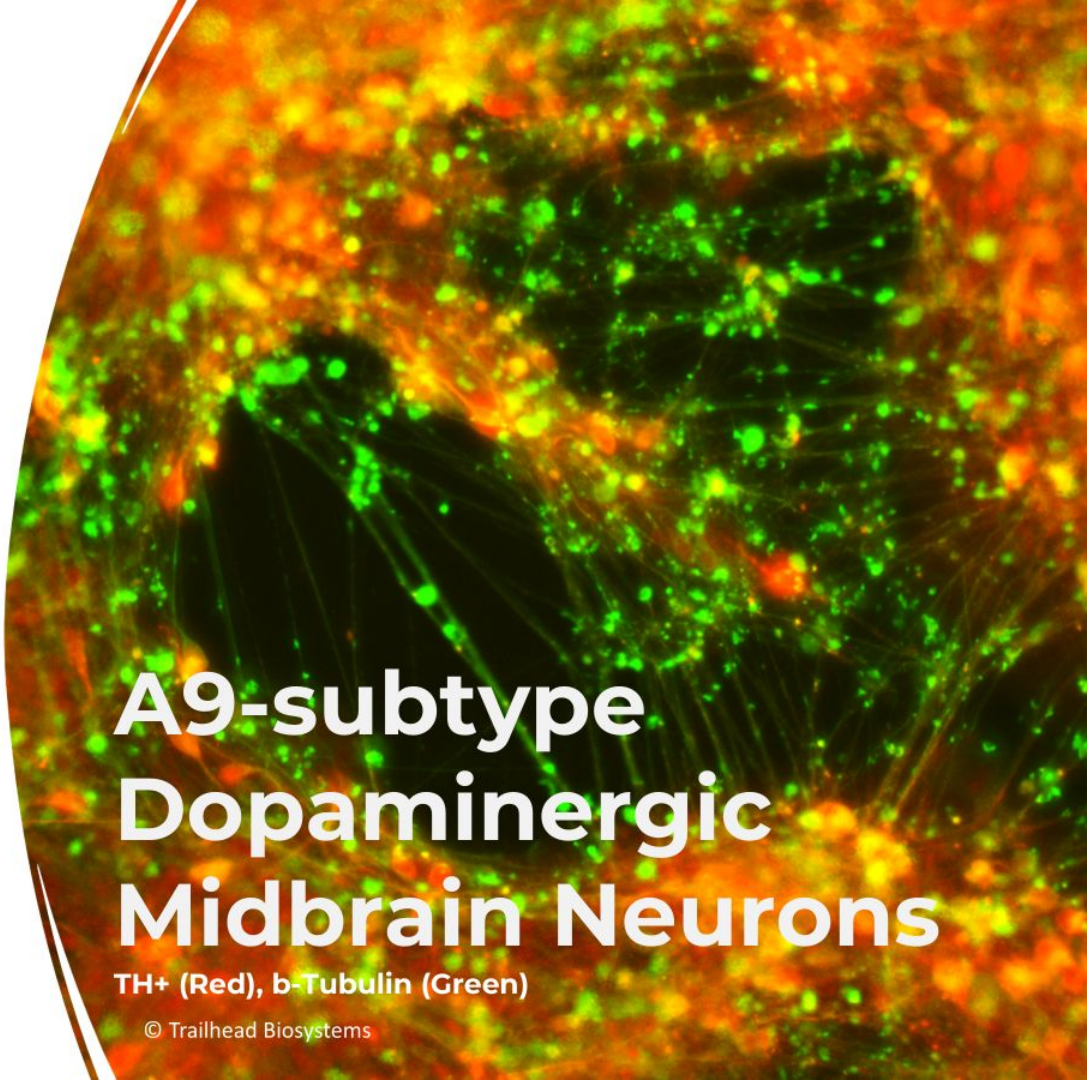
	Mesoderm	Ectoderm	Endoderm	All Teams
Total Unique Genes Tested	342	355	169	793
Genes with significantly predictive models ($p < 0.05, R^2 > 0.75$)	333	340	152	753
Total Coefficients	redacted			
Coefficients with significant effects on genes ($p < 0.05$)	4,377	5,990	3,788	13,946

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.**

2ND Gen Protocols

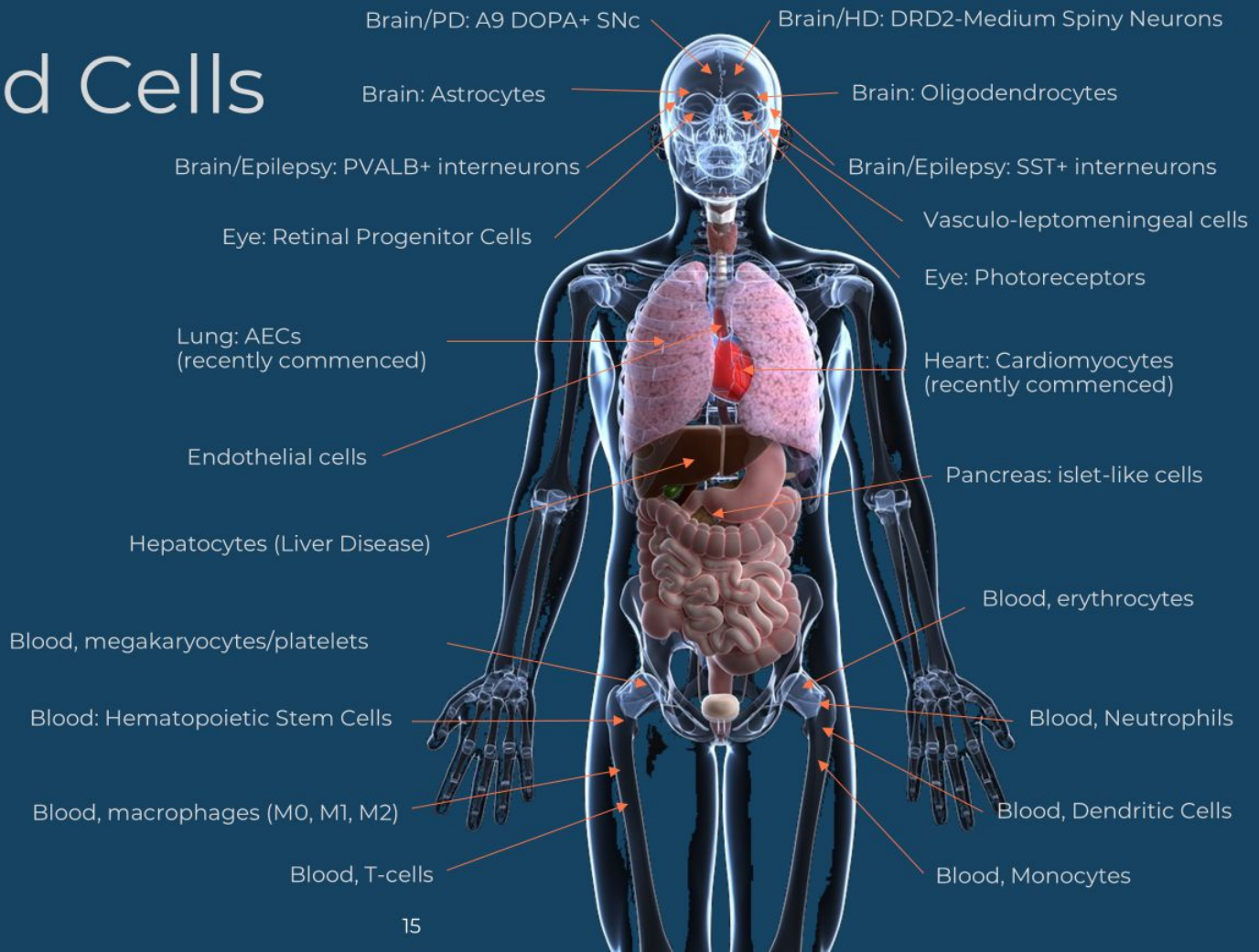
Protocols are built to achieve purity and function

- 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



Specialized Cells

Current Trailhead Cell Programs

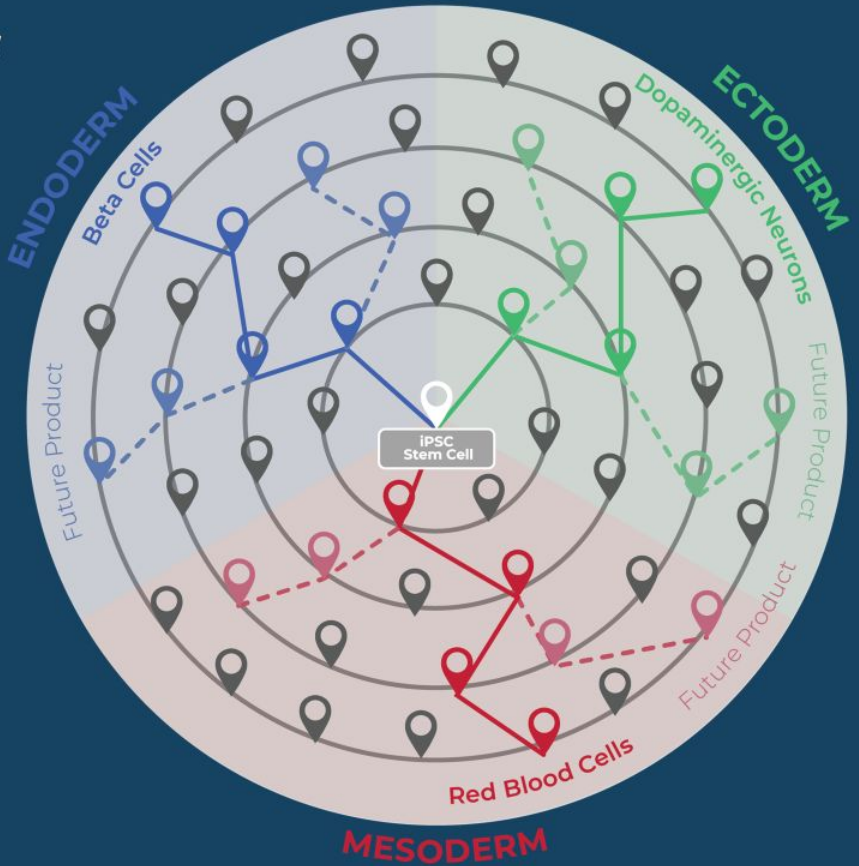


Systematic Control of lineage selection

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

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

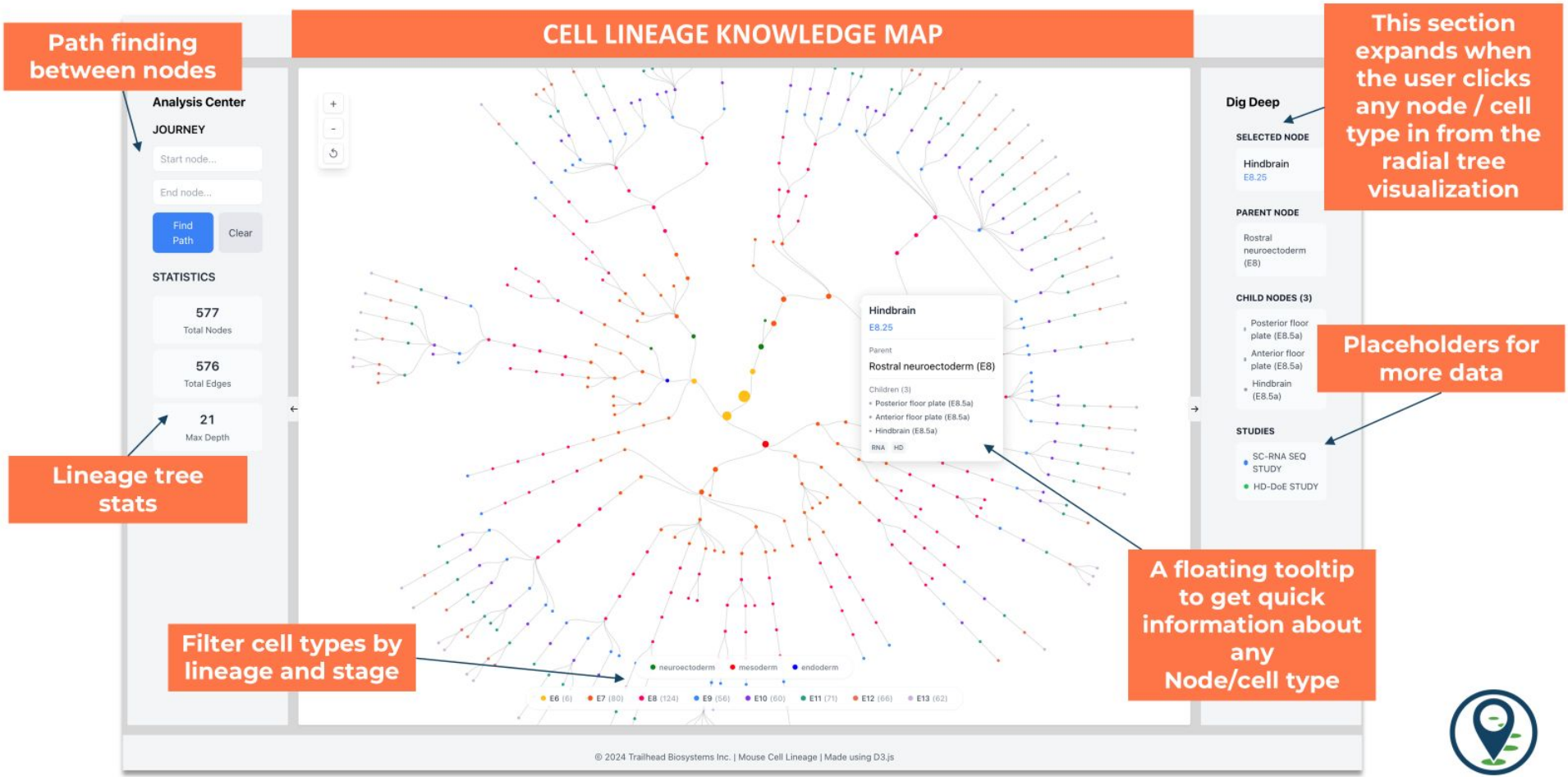
Dramatically lowers cost and time for each cell as we move forward



Human cell fate space >500 specialized cell types

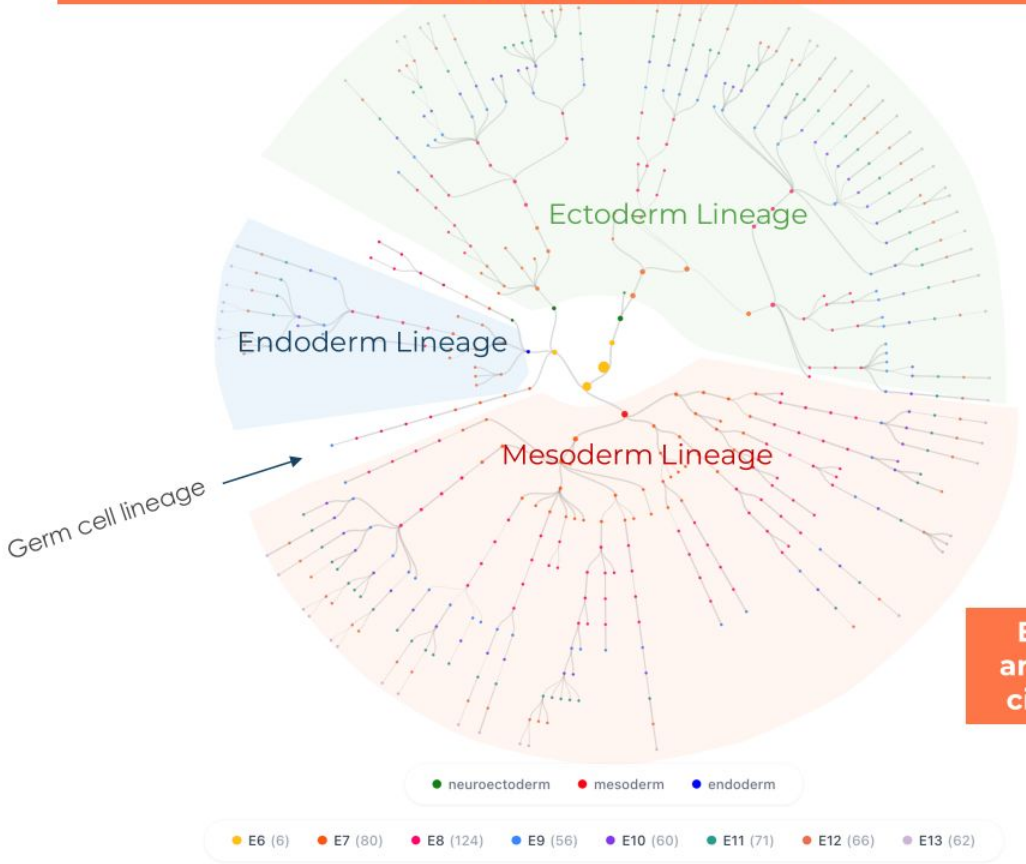
Trailhead Biosystems

Cell lineage visualization Prototype



Cell lineages – Scoping and Coverage

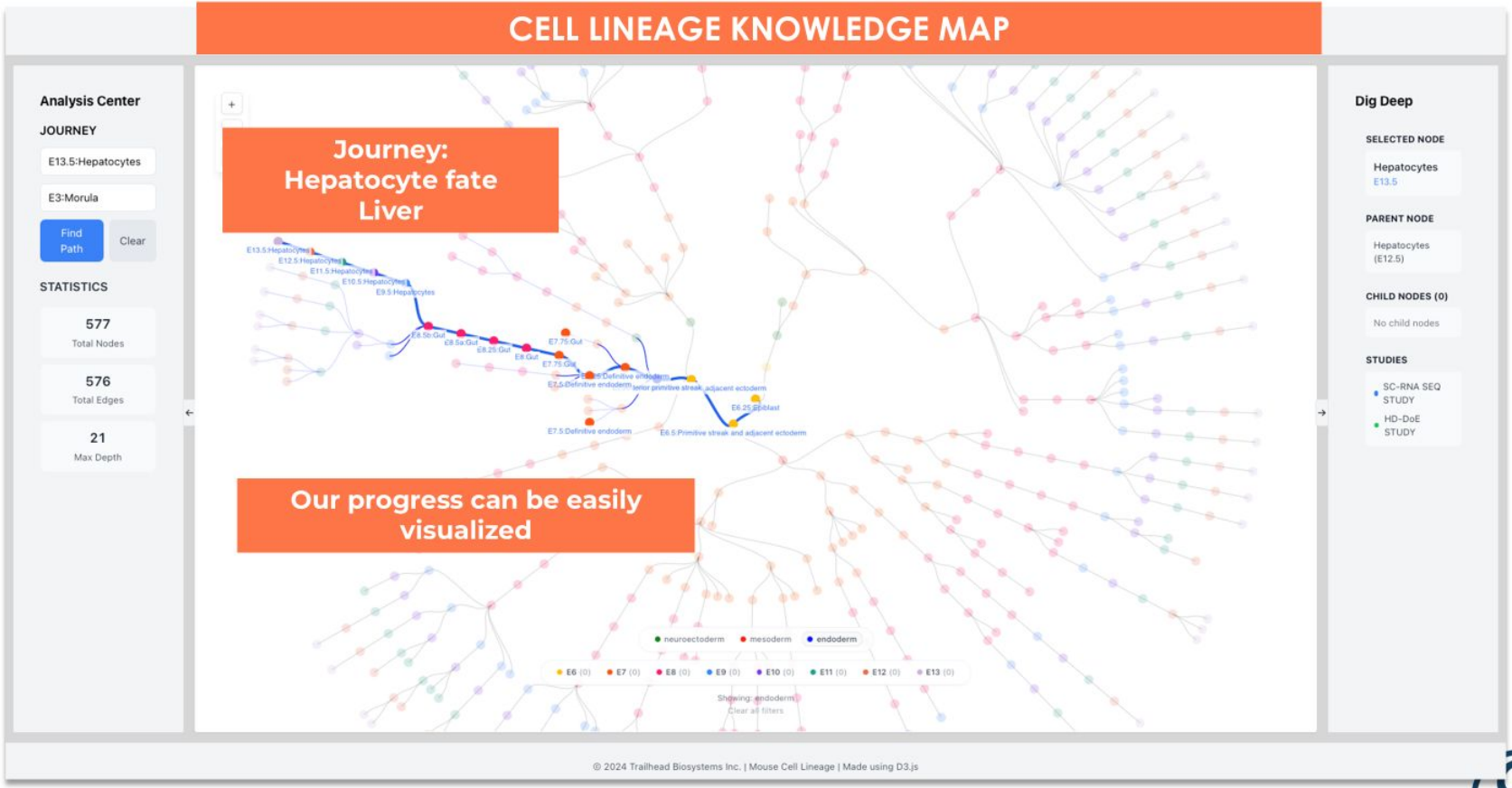
CELL LINEAGE KNOWLEDGE MAP



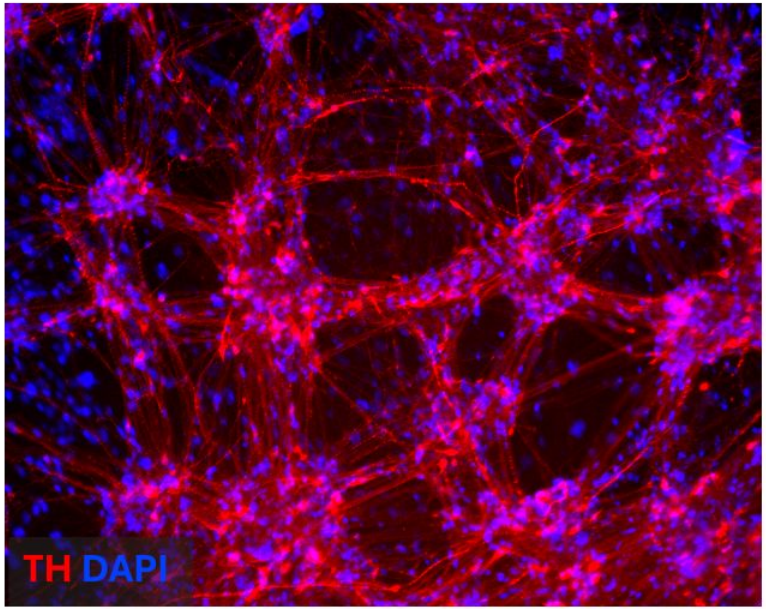
The Company subdivides its protocol Development according to germ layer:
ENDODERM
MESODERM
ECTODERM

Each germ layer is arranged around the circumferential axis

Cell lineages – Scoping and Coverage



iPSC-Derived TrailBio® A9 Dopaminergic Neurons

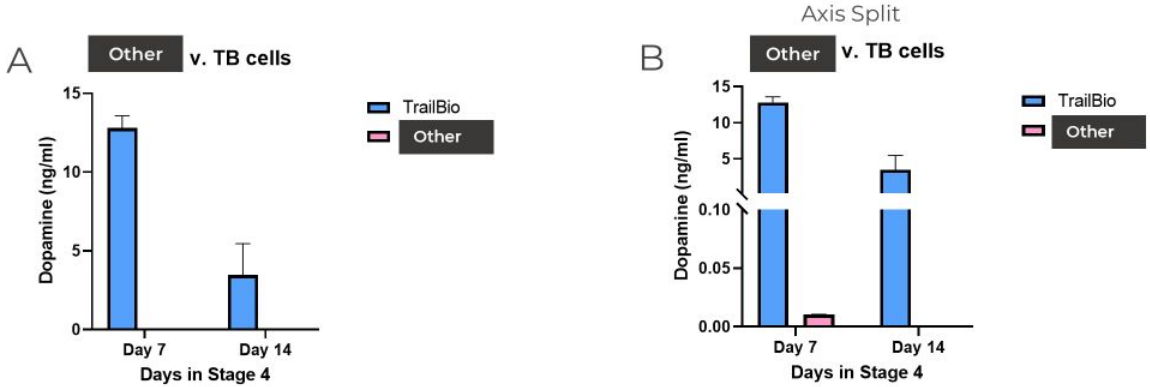


- 23-Day differentiation process leveraging low-cost raw materials
- 70% Purity measured by tyrosine hydroxylase expression
- Optimized for SOX6 expression, indicating presence of A9 subtype lost in Parkinson's and avoidance of VTA subtype prevalent in published protocols
- High Dopamine release at base 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® platform; no dual SMAD inhibition

Comparative Data: Trailhead Cells vs. Other

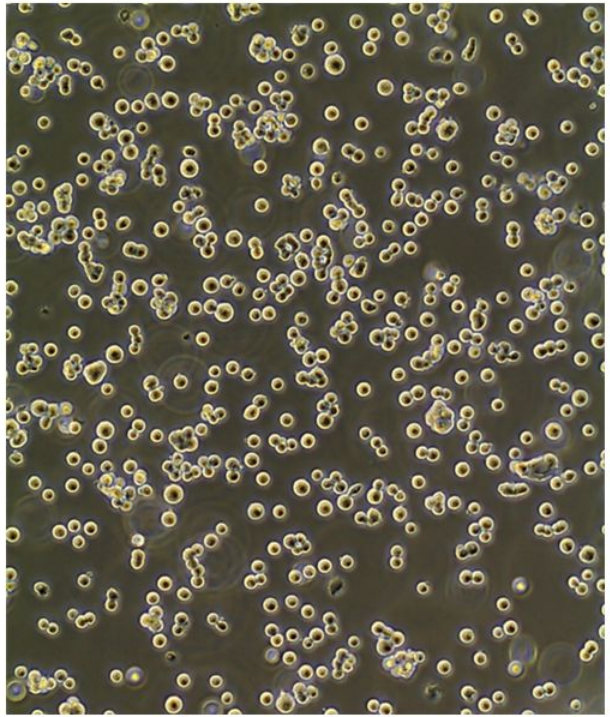
Dopamine release of DA neurons at 2 timepoints was measured and compared to commercially available cells

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.



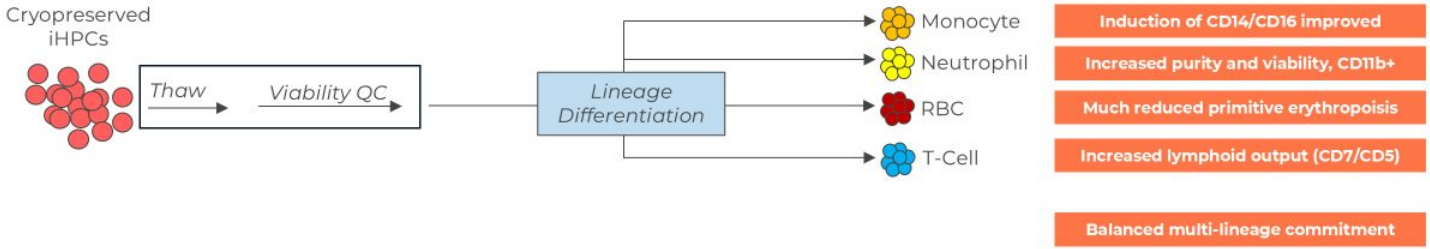
Dopamine Release from Trailhead A9 Dopaminergic Neurons is significantly higher

iPSC-Derived TrailBio® 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® platform

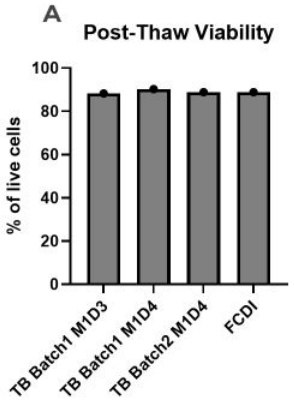
Comparative Analysis of iHPCs: Trailhead vs. Industry Standard – Post-Thaw Viability

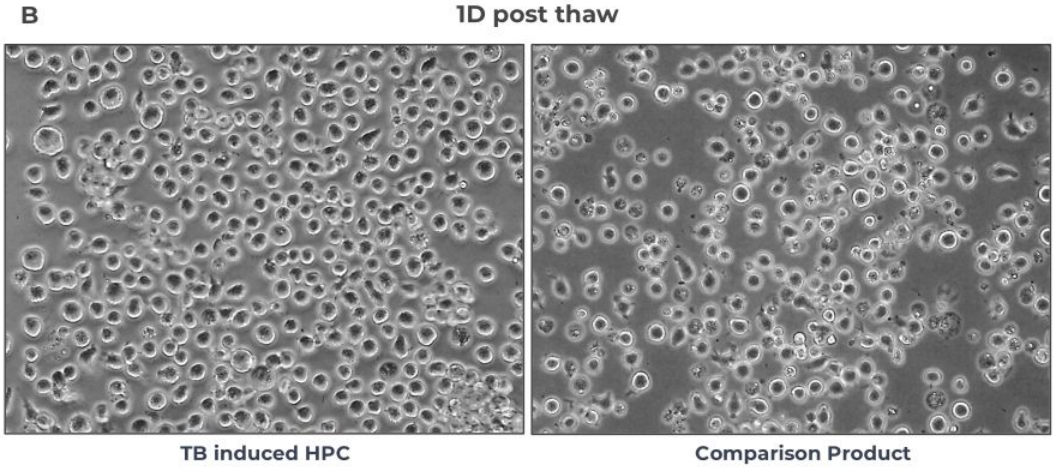


To understand weather Trailhead iHPCs are a competitive product, we compared our cells against FCDI HPCs, focusing on post-thaw viability and lineage differentiation potential. For this evaluation, FCDI cells, day 3 /4 iHPCs from batch 1, and day 4 cells from batch 2 were thawed and test for viability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

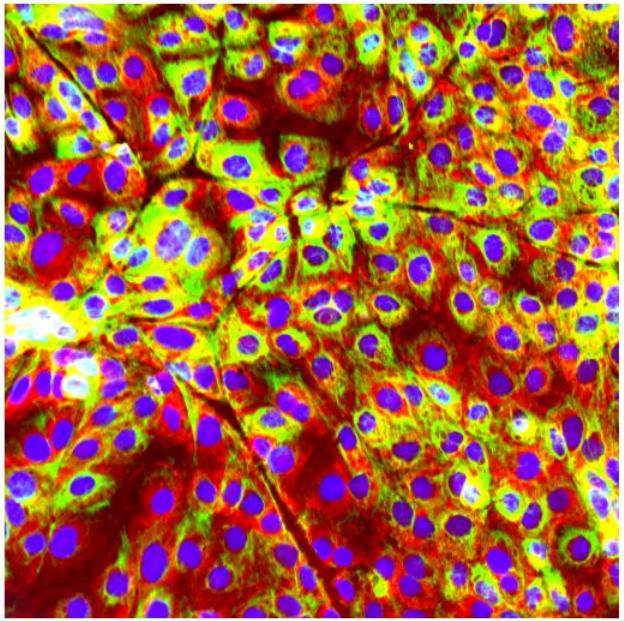
The post-thaw viability of Trailhead cells was comparable with Fuji cells (88%-90% viable - **A**) and all cells appeared morphologically healthy after 24 hours of culture (**B**).

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





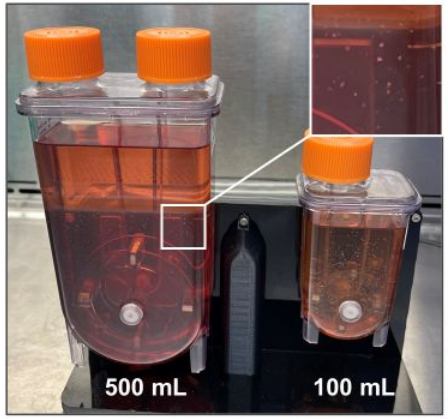
iPSC-Derived TrailBio® Hepatocytes

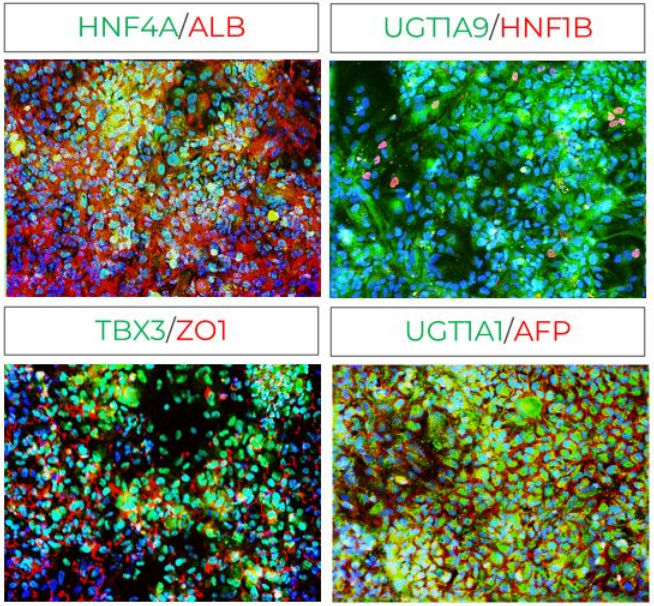


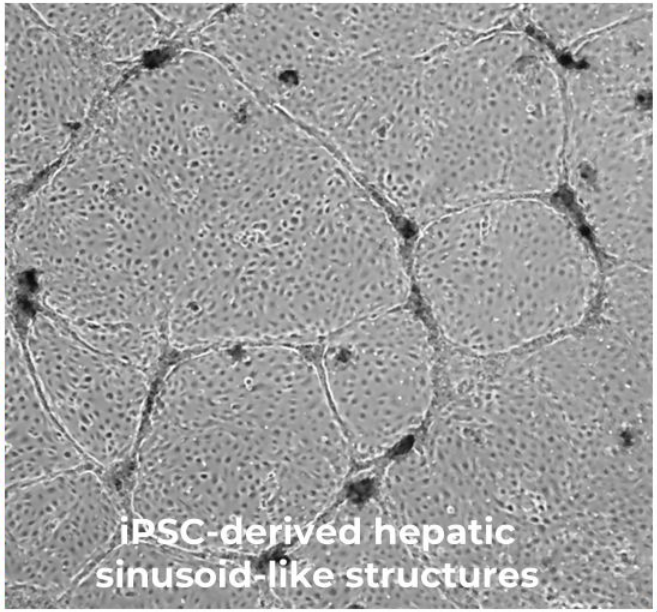
- 24-Day differentiation process leveraging low-cost materials
- Higher CYP450 activity when compared to published protocols
- CYP3A4 and A1AT expression comparable to primary human hepatocytes
- Specific data on CYP2C9 and CYP3A4 metabolism activity
- Albumin secretion as measured by ELISA; reduced 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® platform; Hepatocytes are generated from a highly regionalized foregut progenitor.

iPSC-Derived TrailBio® Hepatocytes

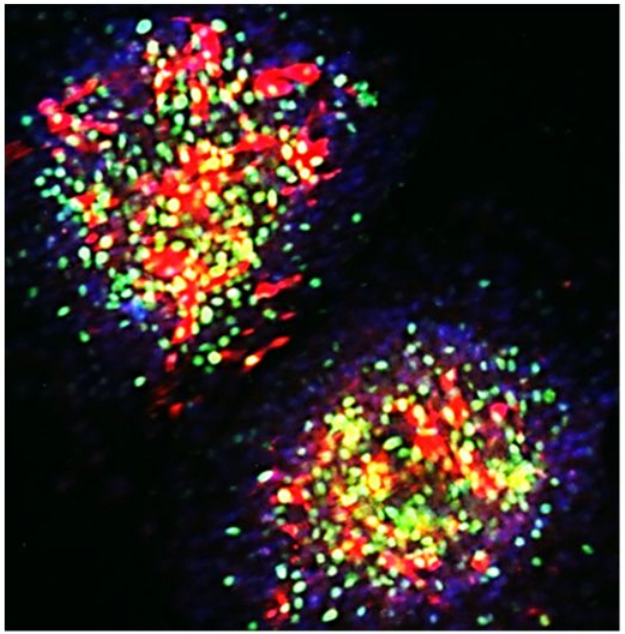
- Bioreactor produced iPSC-derived hepatocytes were cryopreserved & recovered to assess phenotype stability
- Robust expression of HNF4A, ALB, TBX3, ZO1, UGT1A9 & UGT1A1 was observed.







iPSC-Derived TrailBio® 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® platform; cells are generated without the use of TGFβ 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

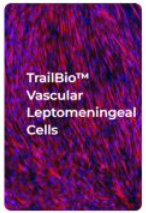
Pancreatic Insulin-Producing Cells (INS+/NKX6.1+)
Hepatocytes (TBX3+/ALB+)
Biliary cells (CK7+/CD19+)

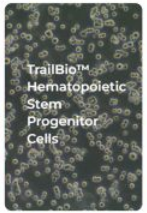
Design Transfer (DT)

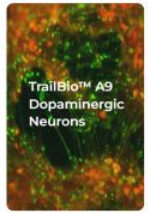
0.5-1 liter

0.25-1 billion cells

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





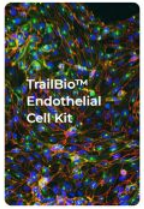


Manufacturing

3 liter

1.5-6 billion cells

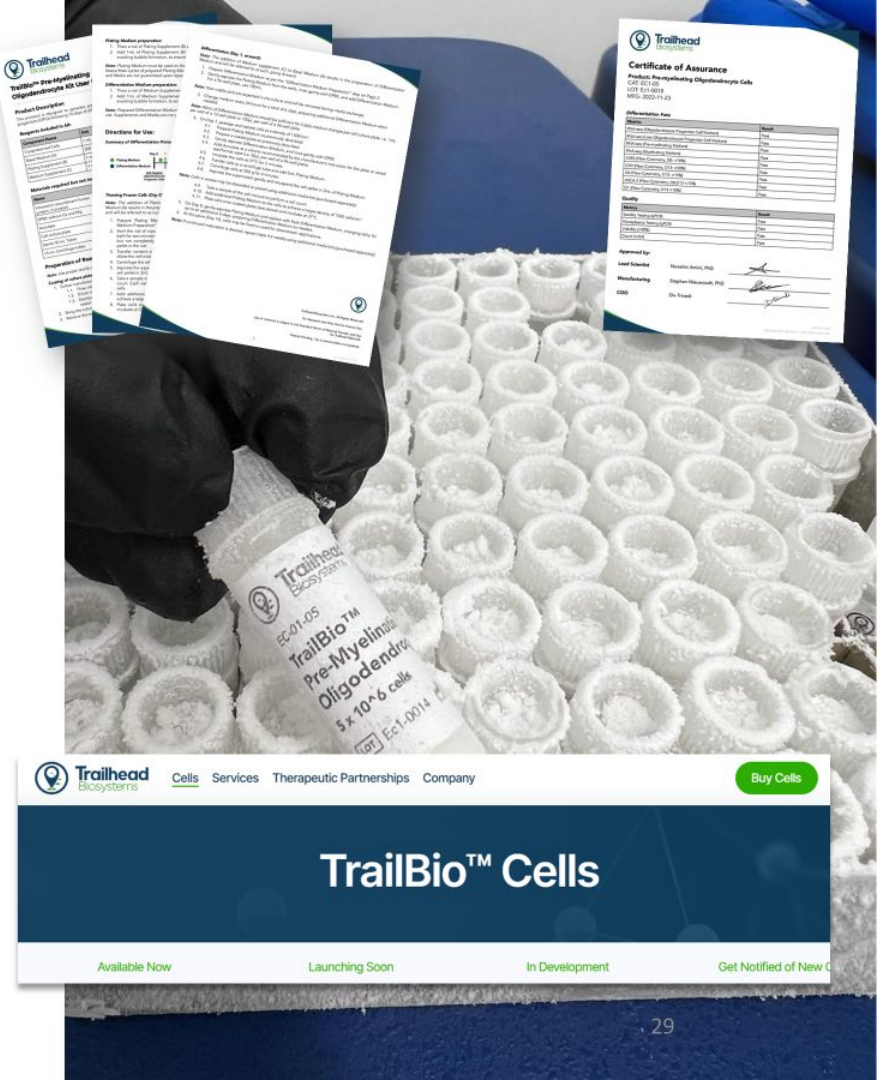
- 3D Aggregates Scaled-up
- In-depth QC
- **Launched Products (off-shelf)**



Instruments & plates not to scale

Research-Use Products

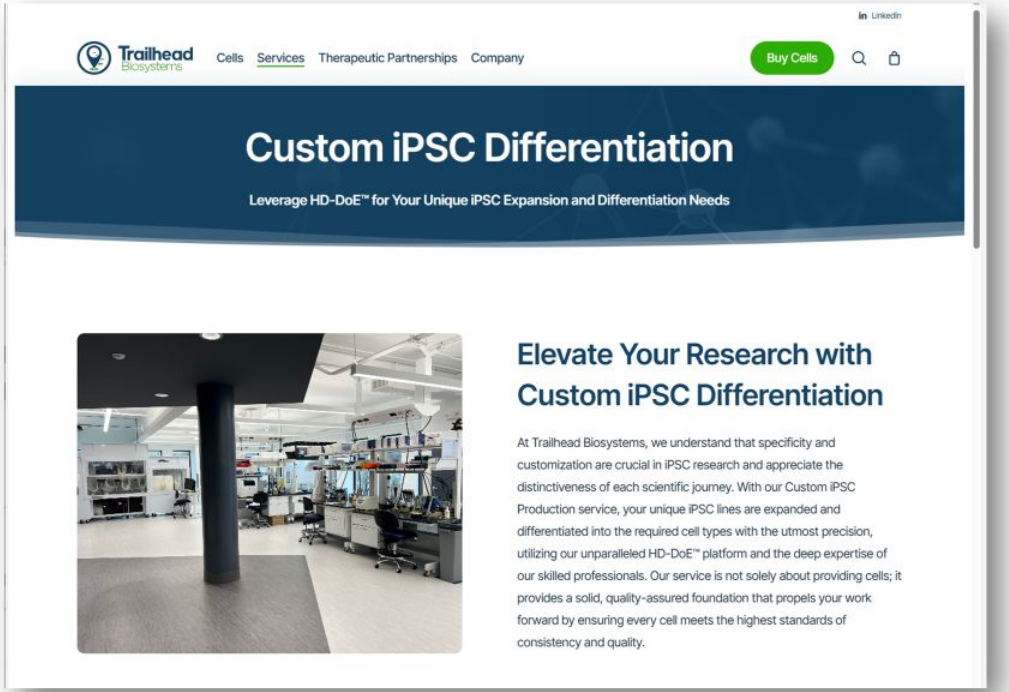
- **Current Offerings**
  - Normal (non-disease) cells/Kits released at Batch Scale +1B
  - 1M to 5M cells per vial, ready to use, vial reservation option
  - Kits: Cells + Media + Instructions
  - Custom iPSC differentiation for key clients



Trailhead 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



What can we do for you?

SOURCE

Disease specific lines

Hypoimmune lines

Normal lines

PROTOCOL DEVELOPMENT

CELL MANUFACTURING

HD-DoE® Platform

USE

Life Science Research

Drug Discovery

Toxicity Testing

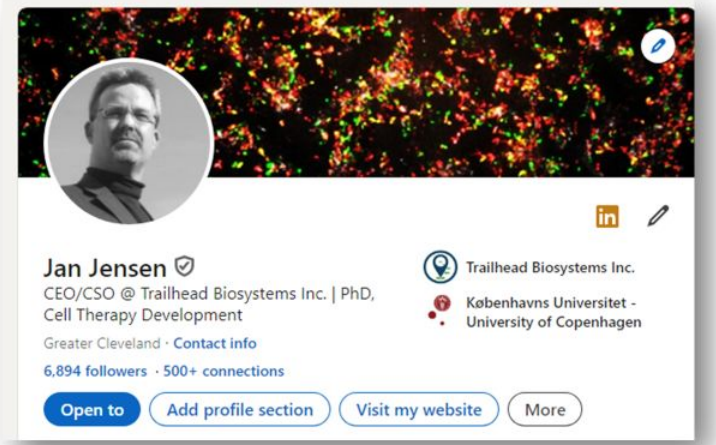
Disease Modeling

Cell Therapy

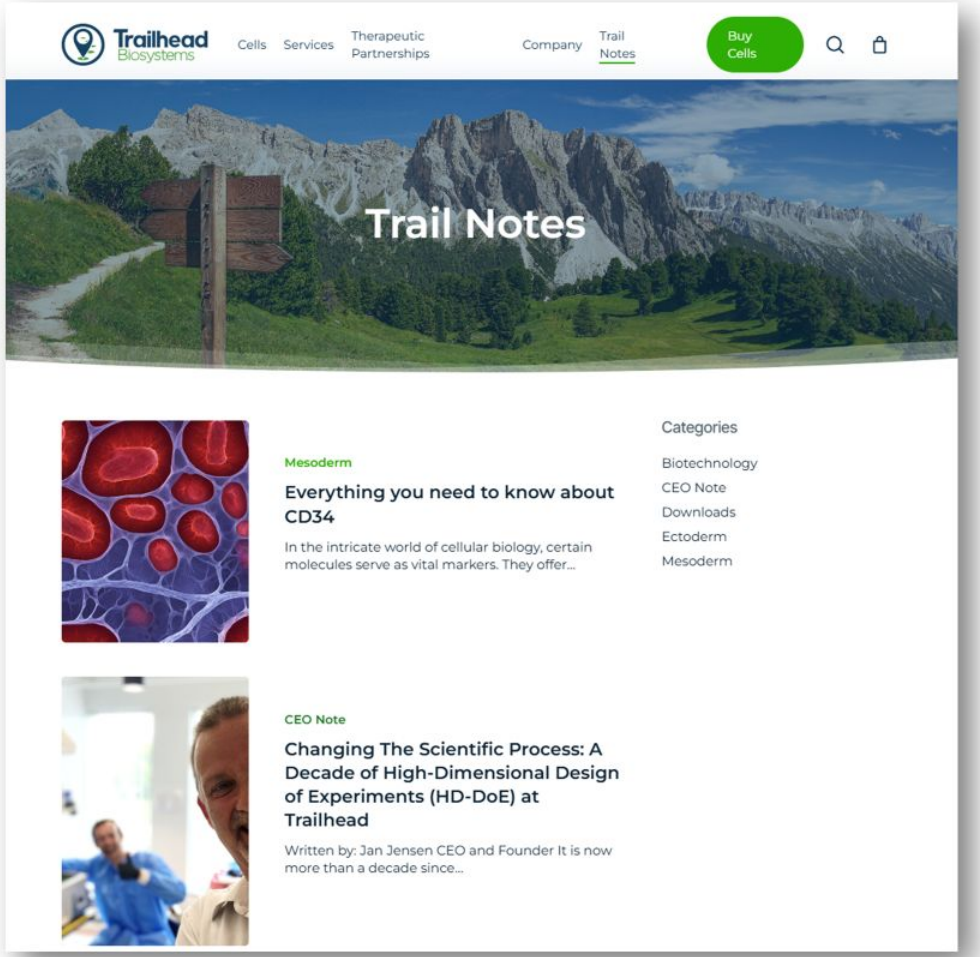
Take-Home Items

- 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!



www.linkedin.com/in/janjensen1/



www.trailbio.com

Q&A

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

Questions

How do we define a Multiscale Human?

How do we map a Multiscale Human?

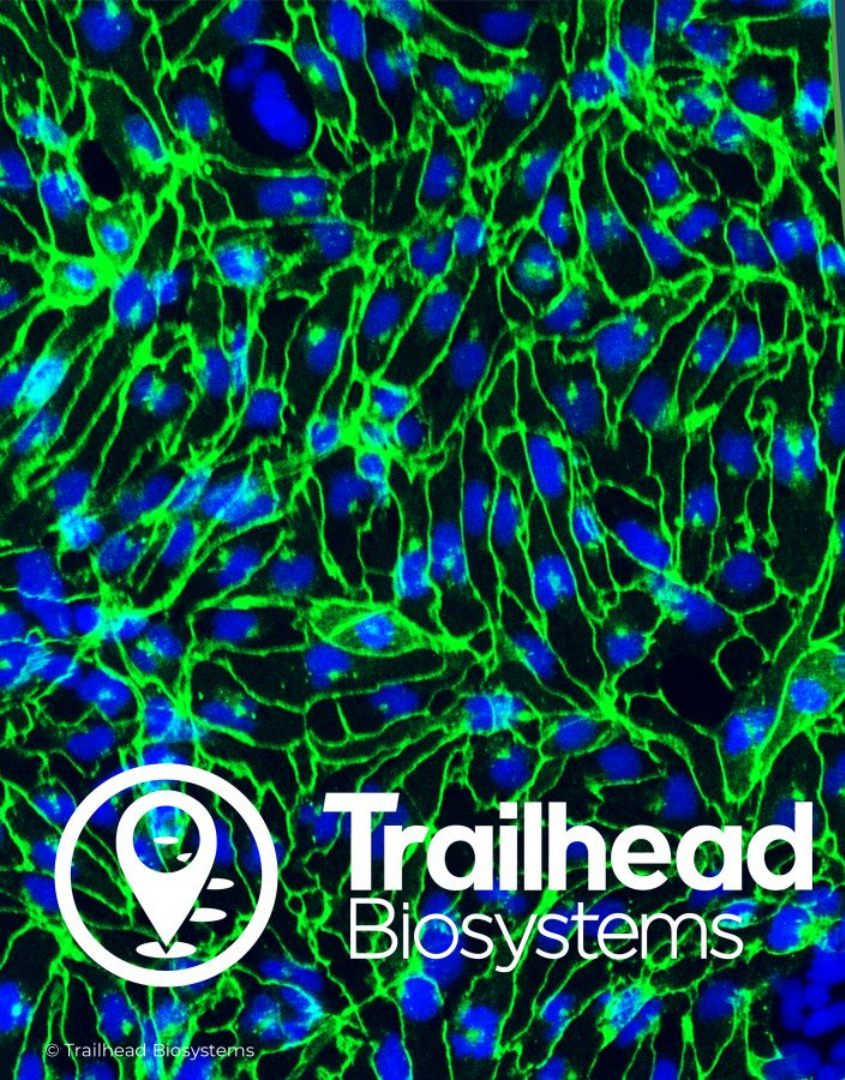
How do we model a Multiscale Human?

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

info@trailbio.com

Trailhead
Biosystems

Dec. 14th, 2024