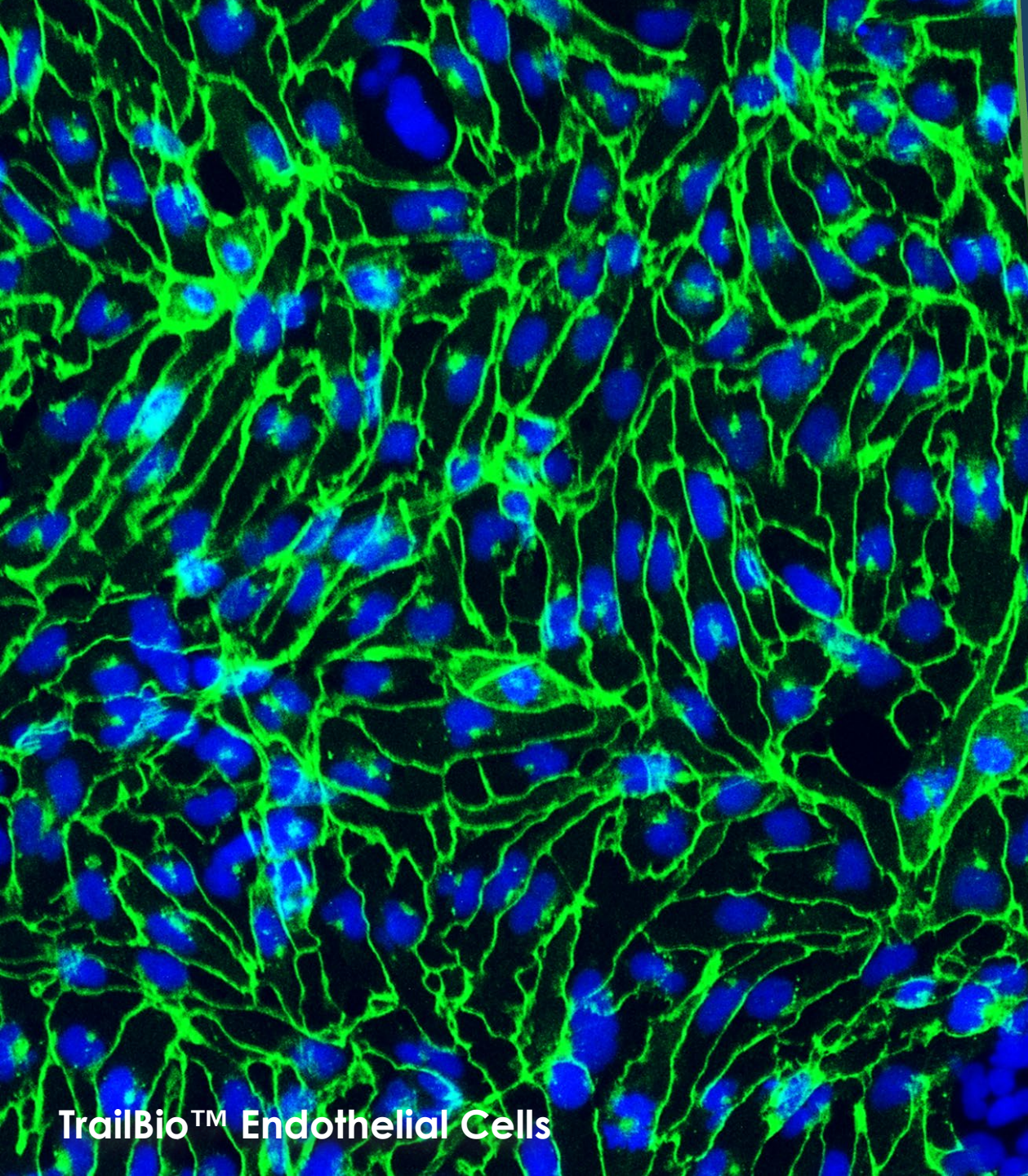



# Addressing Biology by Mathematics, rather than Hypotheses to enable Cell Manufacturing

Jan Jensen, PhD
CEO/CSO Trailhead Biosystems

National Academy of Sciences Workshop
Oct. 17th 2023

'Emerging Technologies and Innovation in Manufacturing Regenerative Medicine Therapies'

Session III (Automation and **Algorithms in Regenerative Medicine Manufacturing**

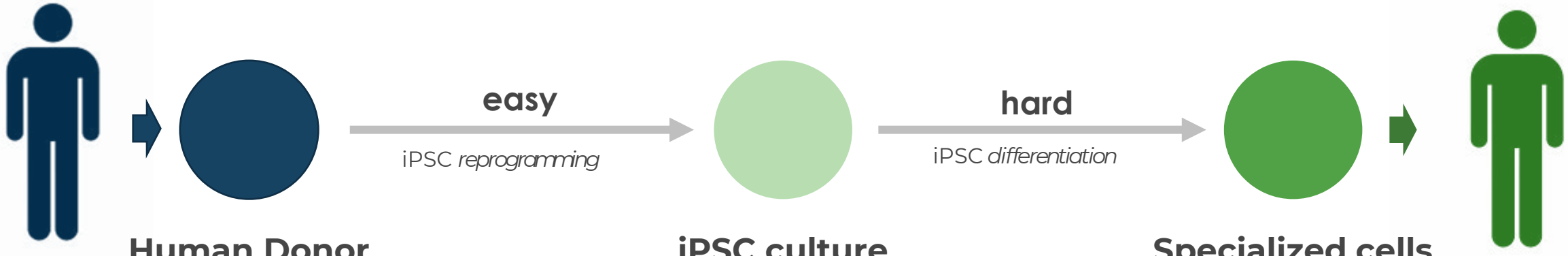
# Disclosure

- J. Jensen is founder, CEO and CSO of Trailhead Biosystems Inc. A for-profit company incorporated in Delaware, doing business in the state of Ohio.
- J. Jensen is a board member and shareholder of Trailhead Biosystems

# Why DoE/MVDA?

- DoE is mathematics but practically saves run costs!
- MVDA converts reams of data into mathematical models
- You capture interactions – not just primary effects
- You can now explore the entire space you tested freely and with statistical confidence
- You can identify the critical process parameters
- When DoE is sufficiently geared, even biology yields

# 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 iPSC robustly enter specialized fates is very difficult

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

**Manual labor and open processes combined with hypothesis-driven one factor at time testing underpins most current clinical cell therapy programs. This adds risk.**



# Better Cells – Better clinical outcomes

Current iPSC-based clinical programs suffers from 1st generation cell protocols

The protocols were developed many years prior and IND lock-in has typically occurred before production and phenotype understanding was fully understood

Lack of cell purity, lack of cell function, or lack of robust manufacturing has resulted in high clinical risk profiles

Experimental designs are mathematically calculated and executed by liquid handling robotics

# Numbers, not guesses!

We replaced the manual discovery process through machine enablement

We explore the high-dimensional space of regulatory inputs efficiently

This QbD-compliant process identifies CPPs

We then manufacture cells at scale based on critical process parameters identified

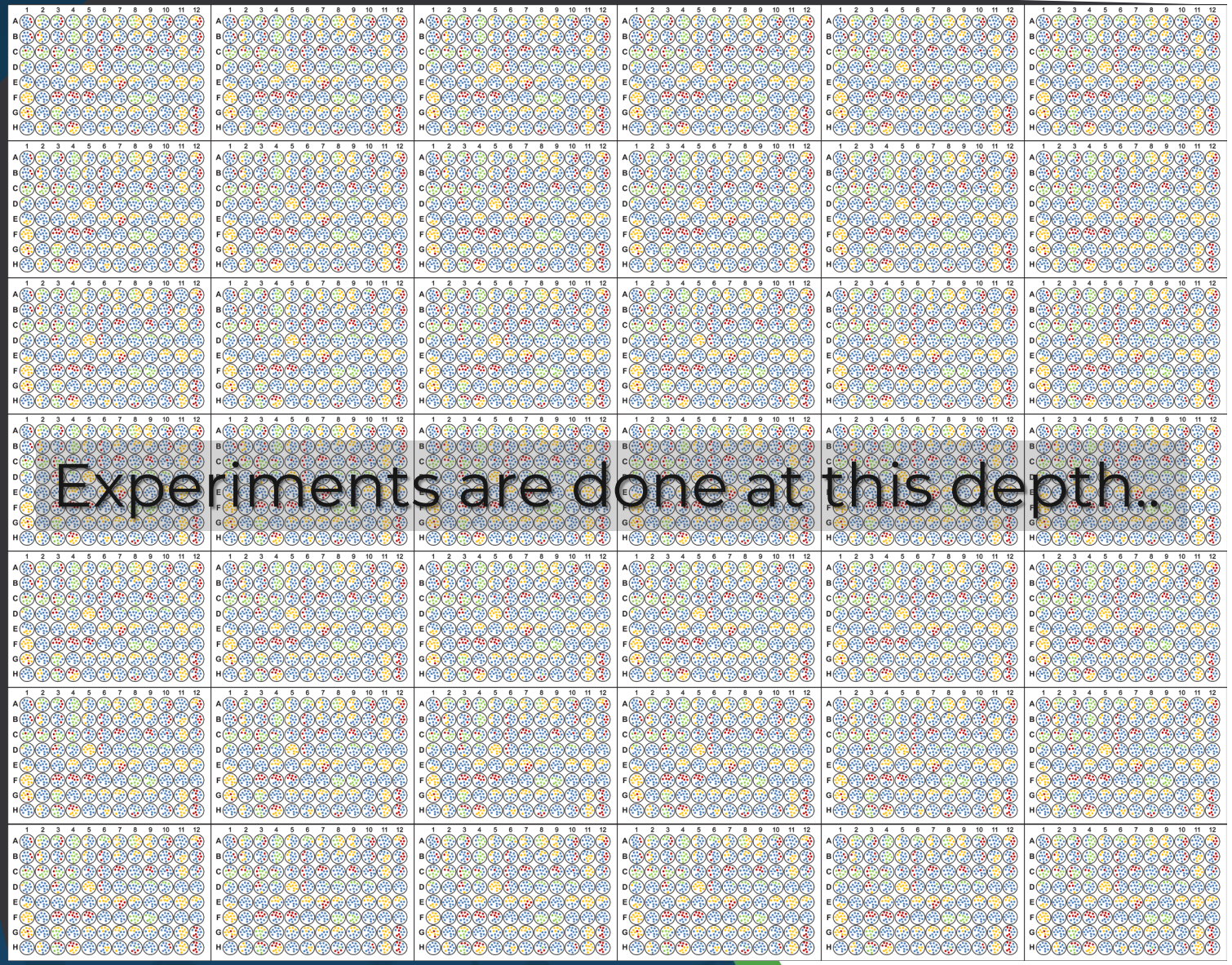
Now we are building a facility used for manufacturing of multiple cell types

## What is HD-DoE?

# Exponentially improving Cell Protocol Development

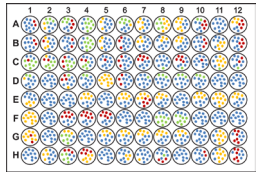
- Proprietary, internally developed software tools
- Computerized, robotically executed, experiments allow improves speed, precision, and scalability (e.g. 12 dimensions)
- Novel empirical data are created – no data mining of other people's data
- Data-driven, unbiased determination of critical process parameters (**CPPs**)

# HD-DoE technology compresses a very large testing space



Experiments are done at this depth..

..but performed at a fractional cost

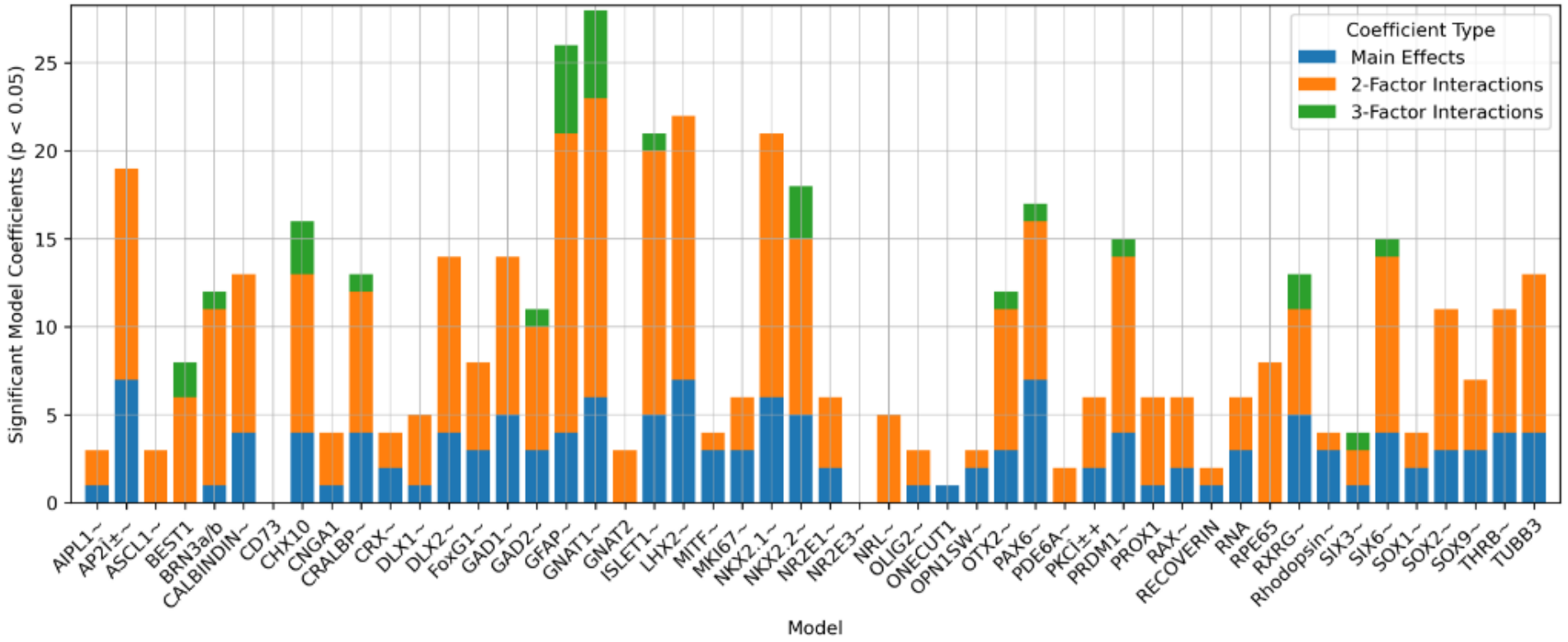


>40x compression

$2^{12} = 4096$

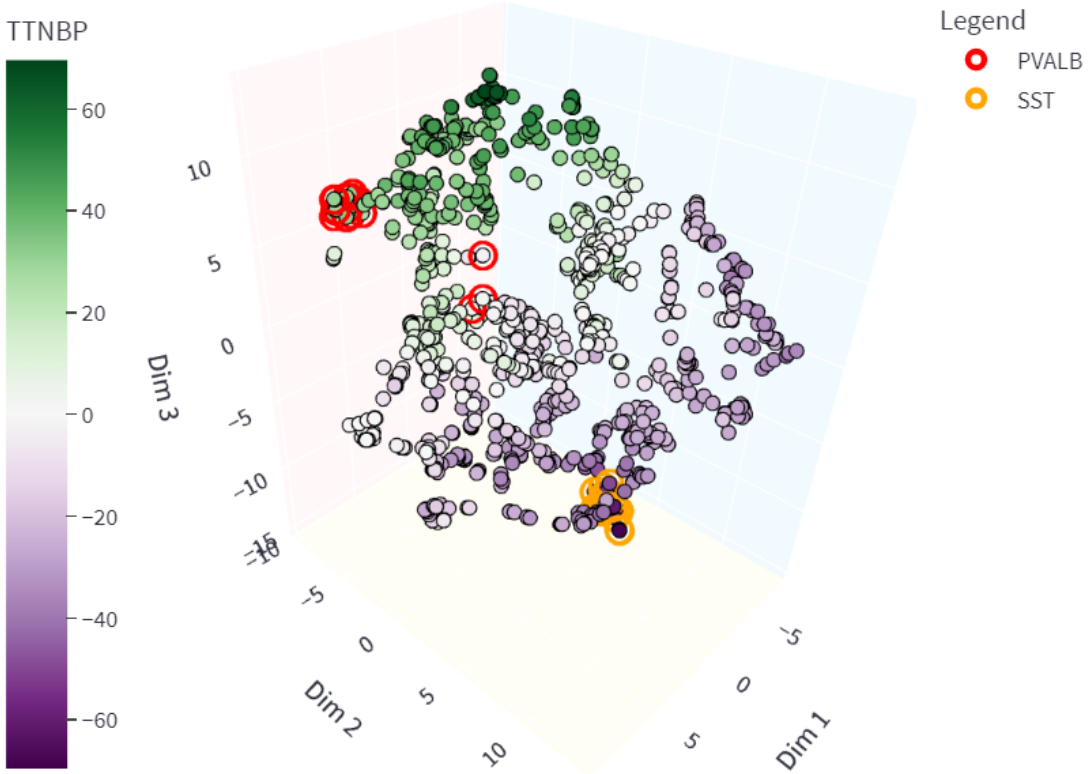
# HD-DoE extracts information within a combinatorial space **impossible to get** if not using this methodology

The plot below shows the number of significant model coefficients for each response (p-value <0.05). Main effect, 2-factor, and 3-factor interaction coefficients are counted and stacked, as shown in the legend.




We build our protocols using the BLUE, ORANGE, and GREEN SPACE of information
Hypothesis-driven, one factor at a time research can only extract the BLUE information

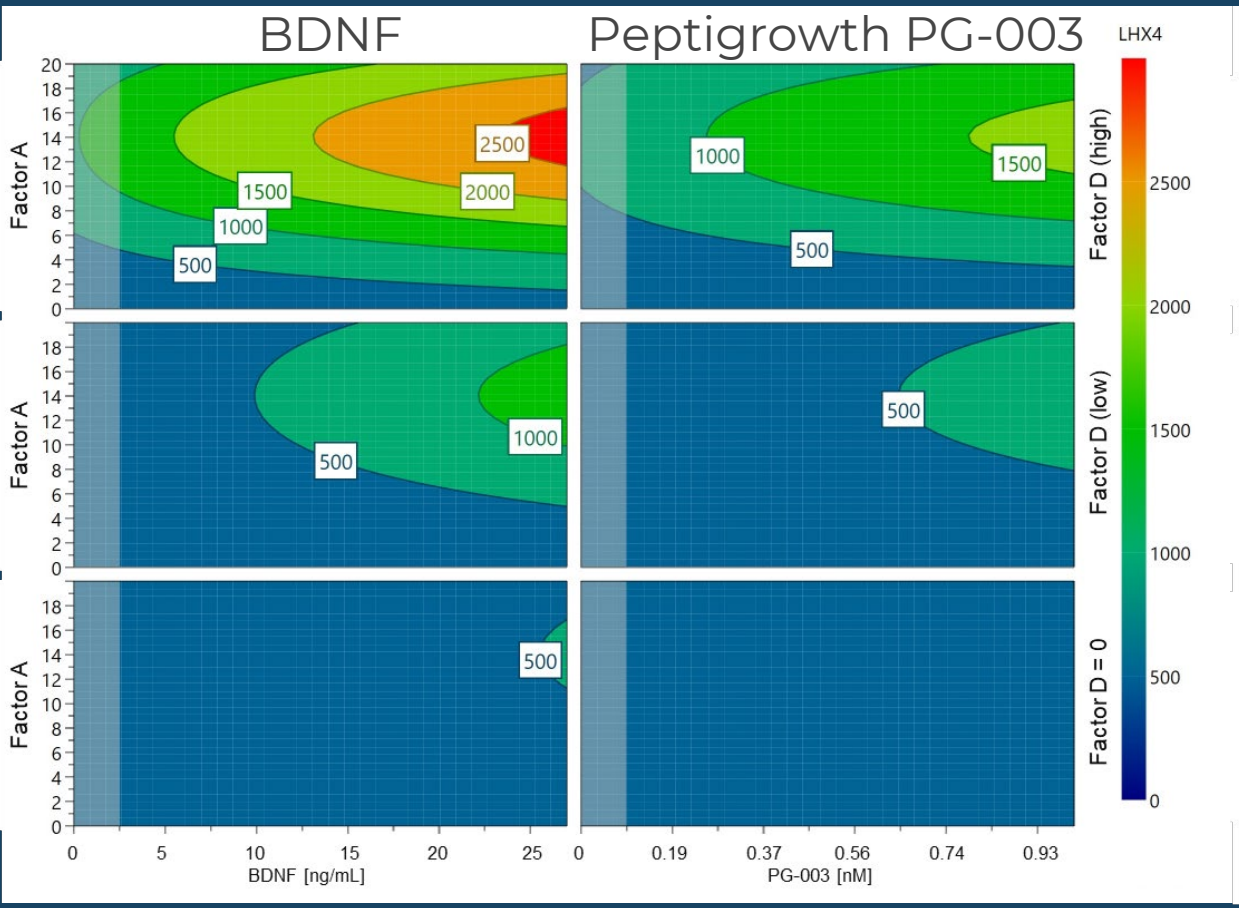
# Facilitated Protocol Development through mathematical analysis of effector-response matrices

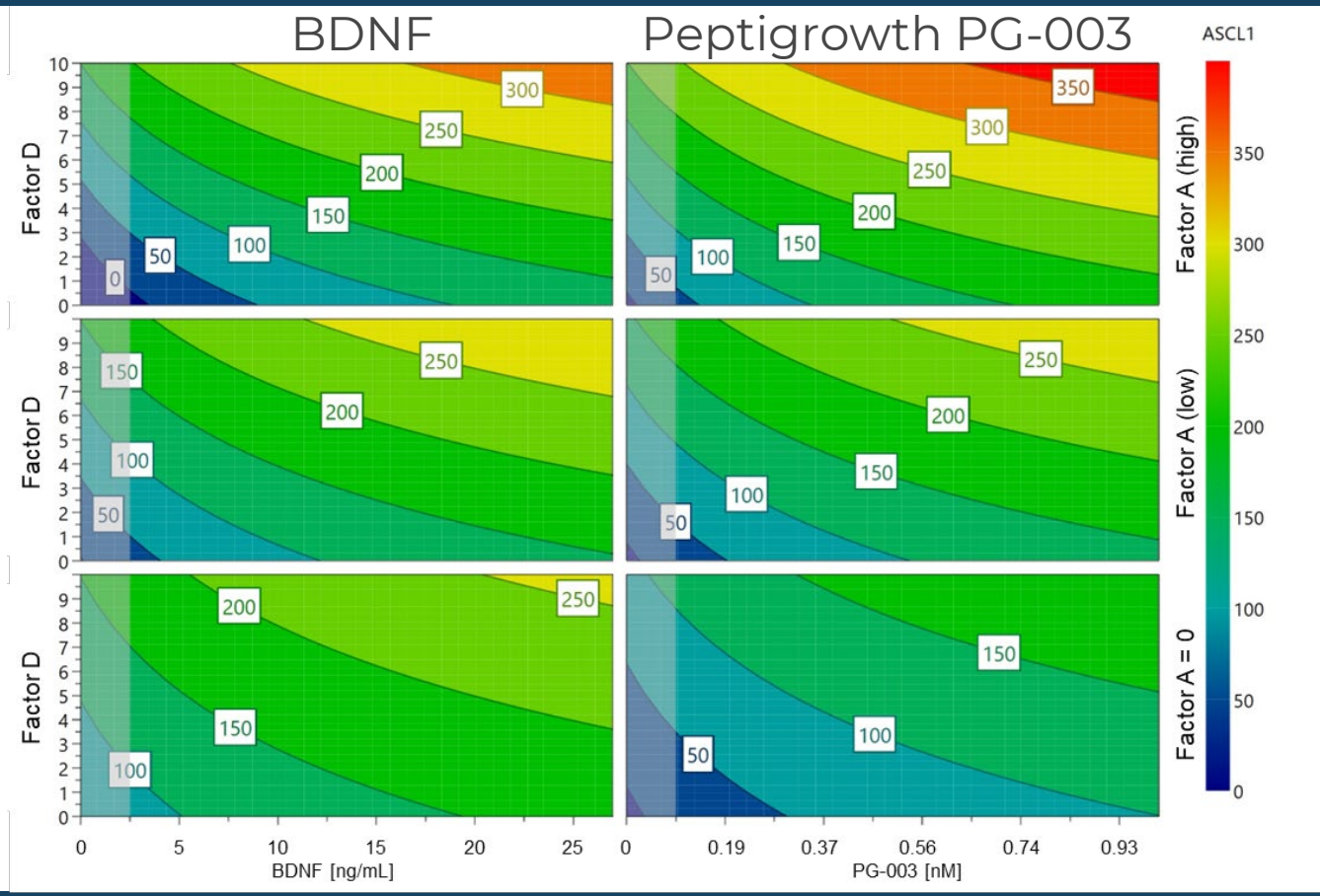



- Organize each response gene according to its regulatory pattern
- Dimensionality reduce the design space (8-13 factors) into 3 dimensions and visualize effects on genes by factor
- More quickly identify **coregulated responses** and **fate splits**, leading to more efficient recipe development

# Bio-equivalency testing (TrkB agonist)

1. We test in a known 8-dimensional response space, at known setpoint
2. We compare reference compound to new product across >50 genes
3. The system behavior of reference to unknown is inspected where pathways interact
4. We can conclude that reference and unknown operates identically

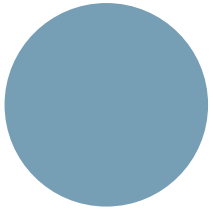


# This approach is very different from hypotheses-driven research!

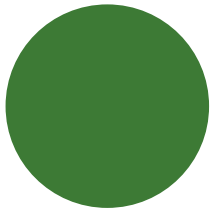
### New Protocols

Protocols are always built from scratch. All protocols are based on unique stage-inducing media, not copied from literature



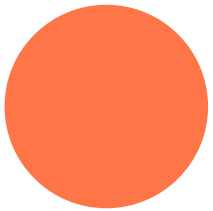
### Always using Own Data

All protocols are based on empirical data obtained using HD-DoE



### Quality-by-Design (QbD)

Our protocols identify Critical Process Parameters as they relate to Critical Quality Attributes. We calculate the process capability index (Cpk)



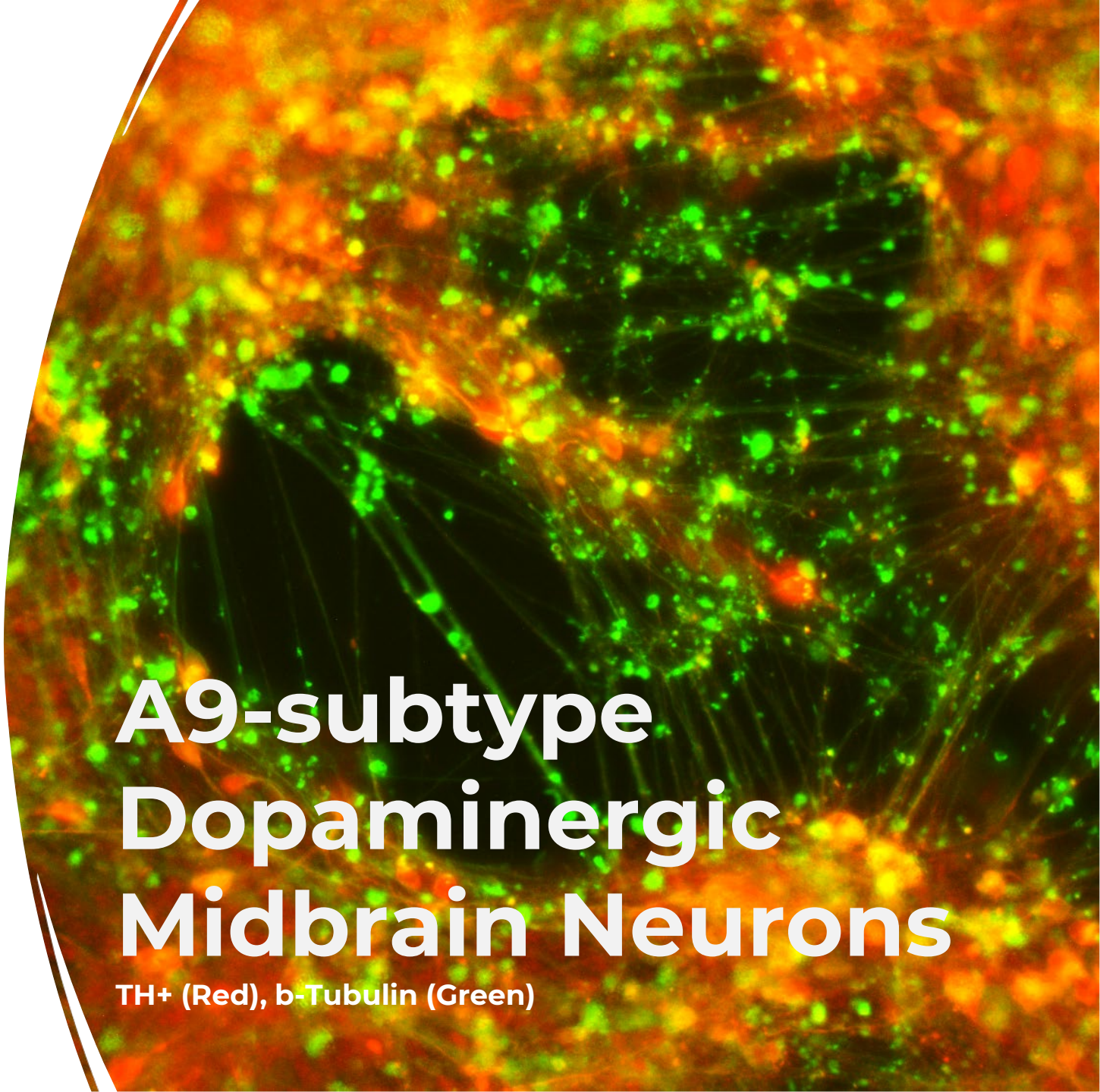
### Cost-Effective and Universal

We commonly identify small molecule replacements lowering costs during production. We observe our protocols perform well on cells from individual donors

# Quality

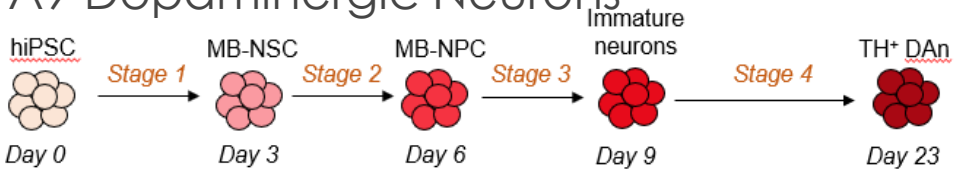
**Protocols are built to achieve Purity and Function**

- 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

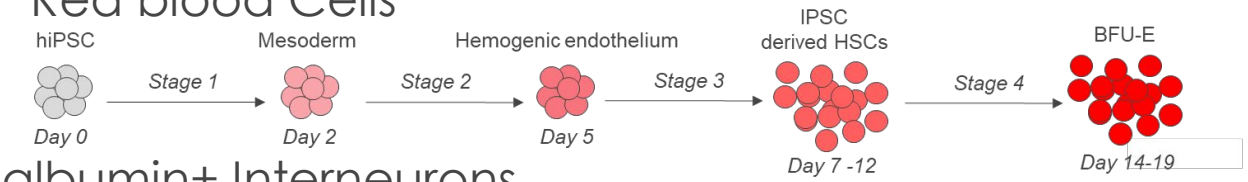


# We build new **PROTOCOLS**

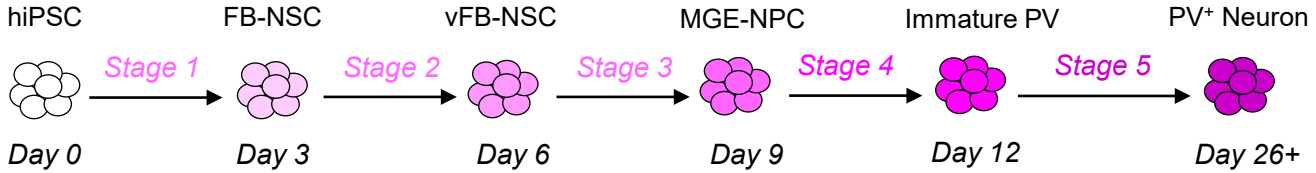
## A9 Dopaminergic Neurons



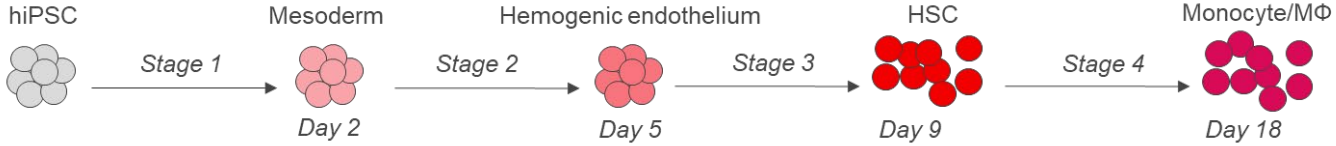

## Red blood Cells



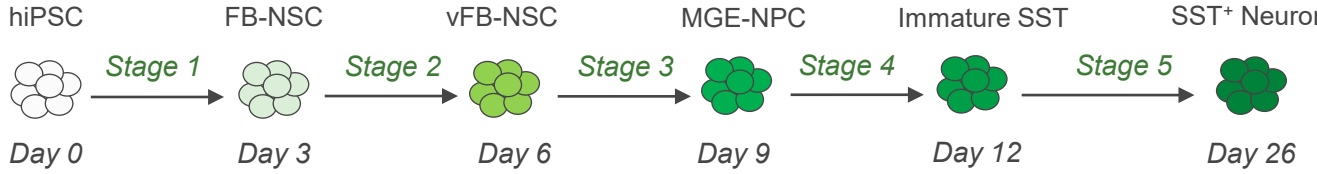

## Parvalbumin+ Interneurons



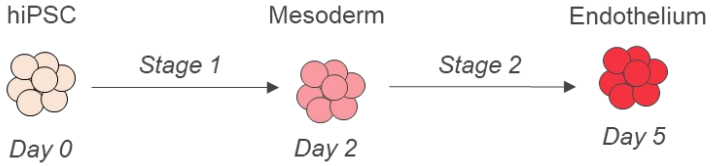

## Monocytes/Macrophages



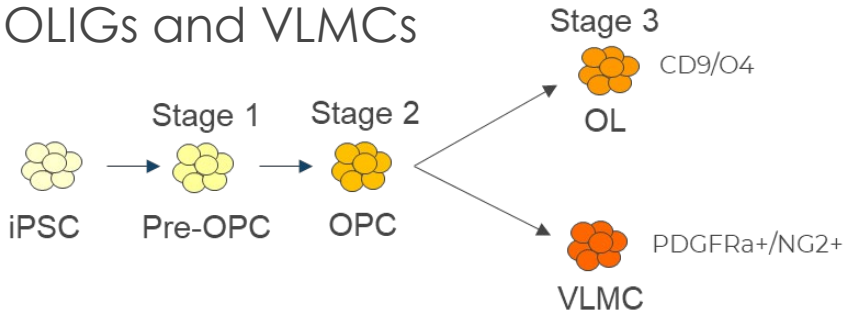

## SST Interneurons



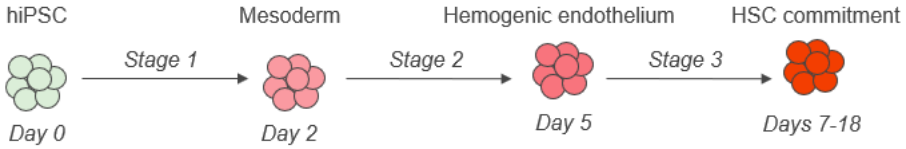

## Endothelial cells



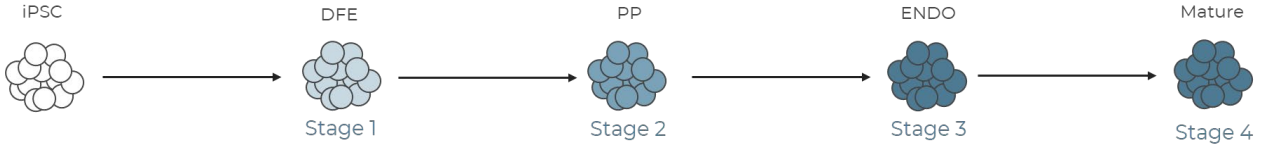

## OLIGs and VLMCs



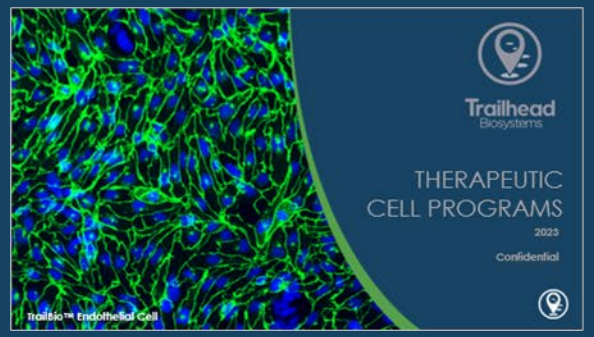

## Hematopoietic Stem Cells



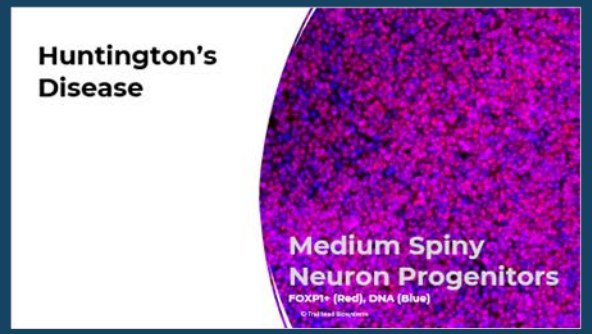

## Islet Cells

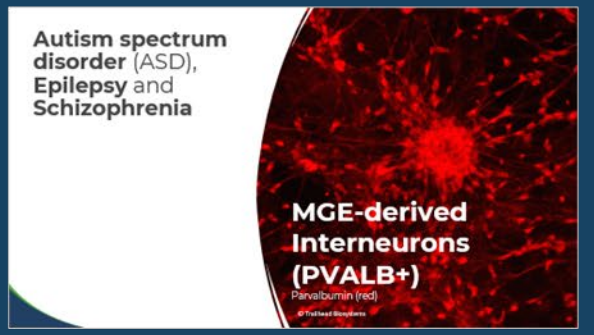


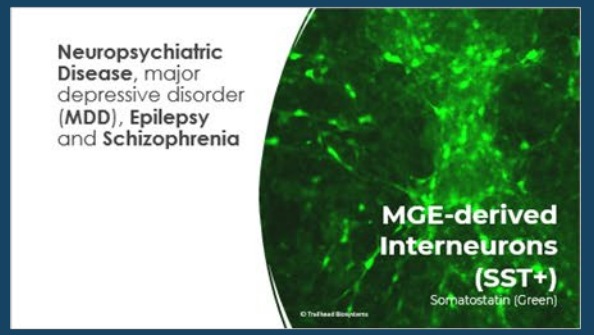
# Therapeutic Programs

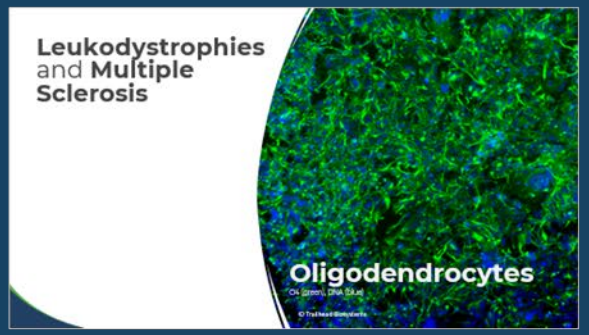


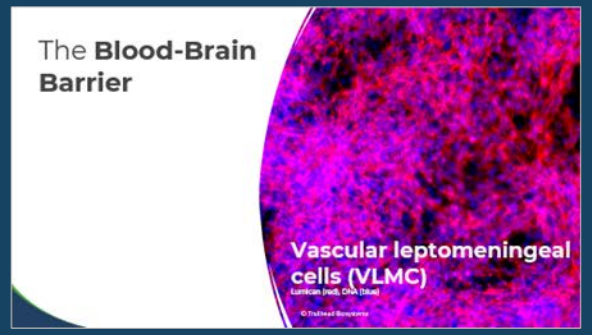


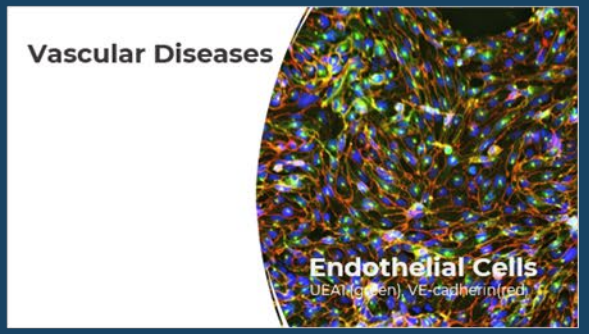


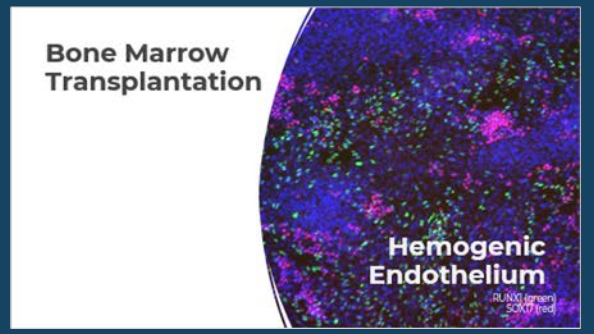



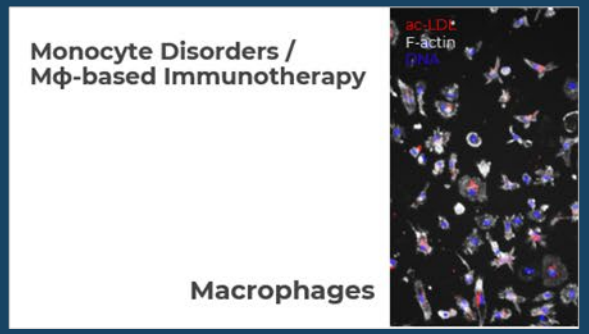



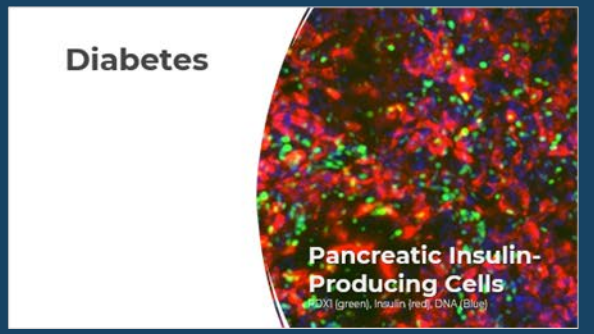





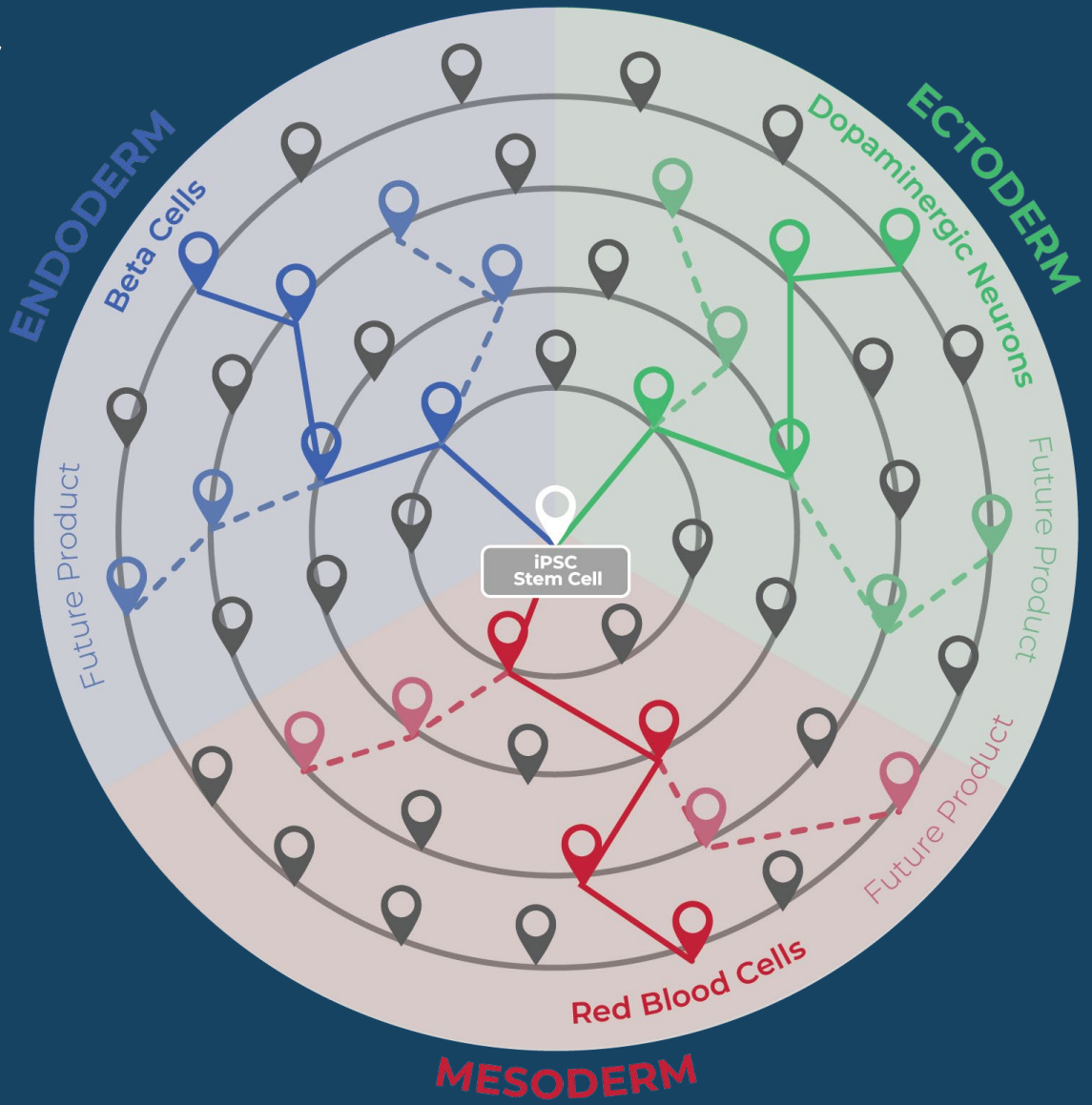


# Systematic Control of lineage selection

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

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

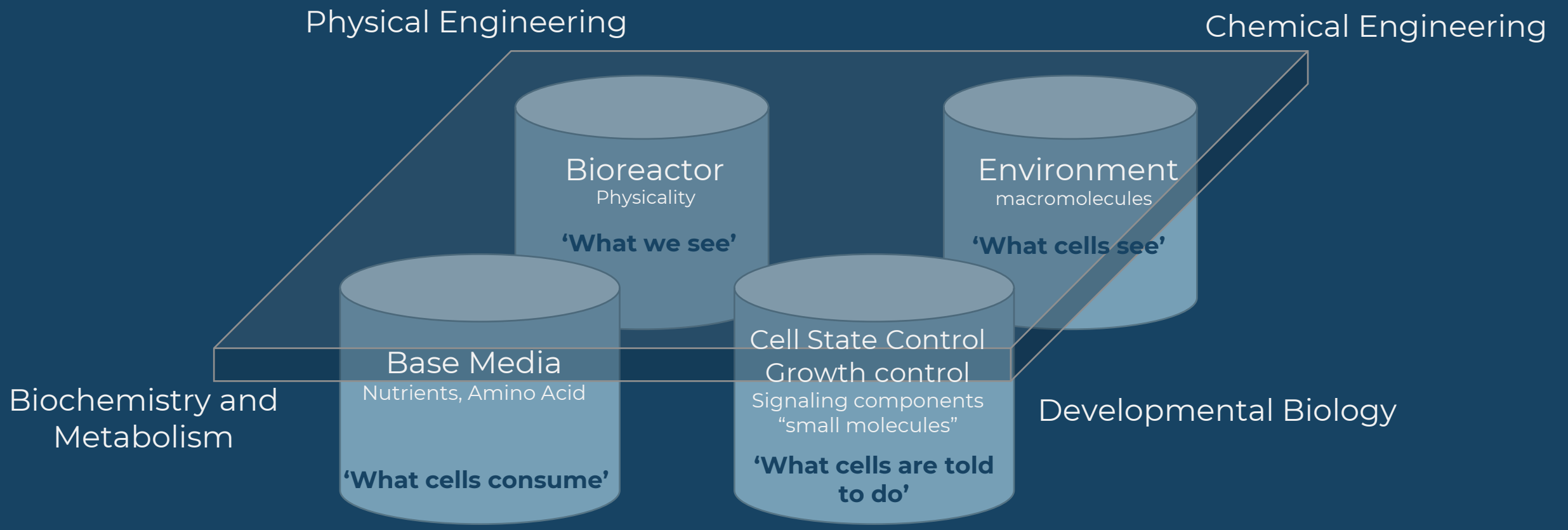
Dramatically lowers cost and time for each cell as we move forward




Human cell fate space >500 specialized cell types

# The four Pillars of controlling iPSC Growth/Differentiation - A truly interdisciplinary problem

**CPPs** are MATHEMATICALLY defined and found in all corners



Thank you!

Trailhead Biosystems

Biology. Controlled.