Cellino: Your Cells, Your Cure Ż

A ultra-scalable biomanufacturing platform for personalized regenerative medicines

NASEM Forum on Regenerative Medicine
Washington, DC
October 17, 2023

Nabiha Saklayen, Ph.D.
CEO and Co-Founder, Cellino

Autologous iPSC -derived cell therapies can address accessibility challenges associated with donor -derived regenerative medicines

Benefits

- no immunosuppression (more accessible for aging & immuno-compromised population)
- no donor matching (more accessible for genetically diverse population)

a range of diseases

↑

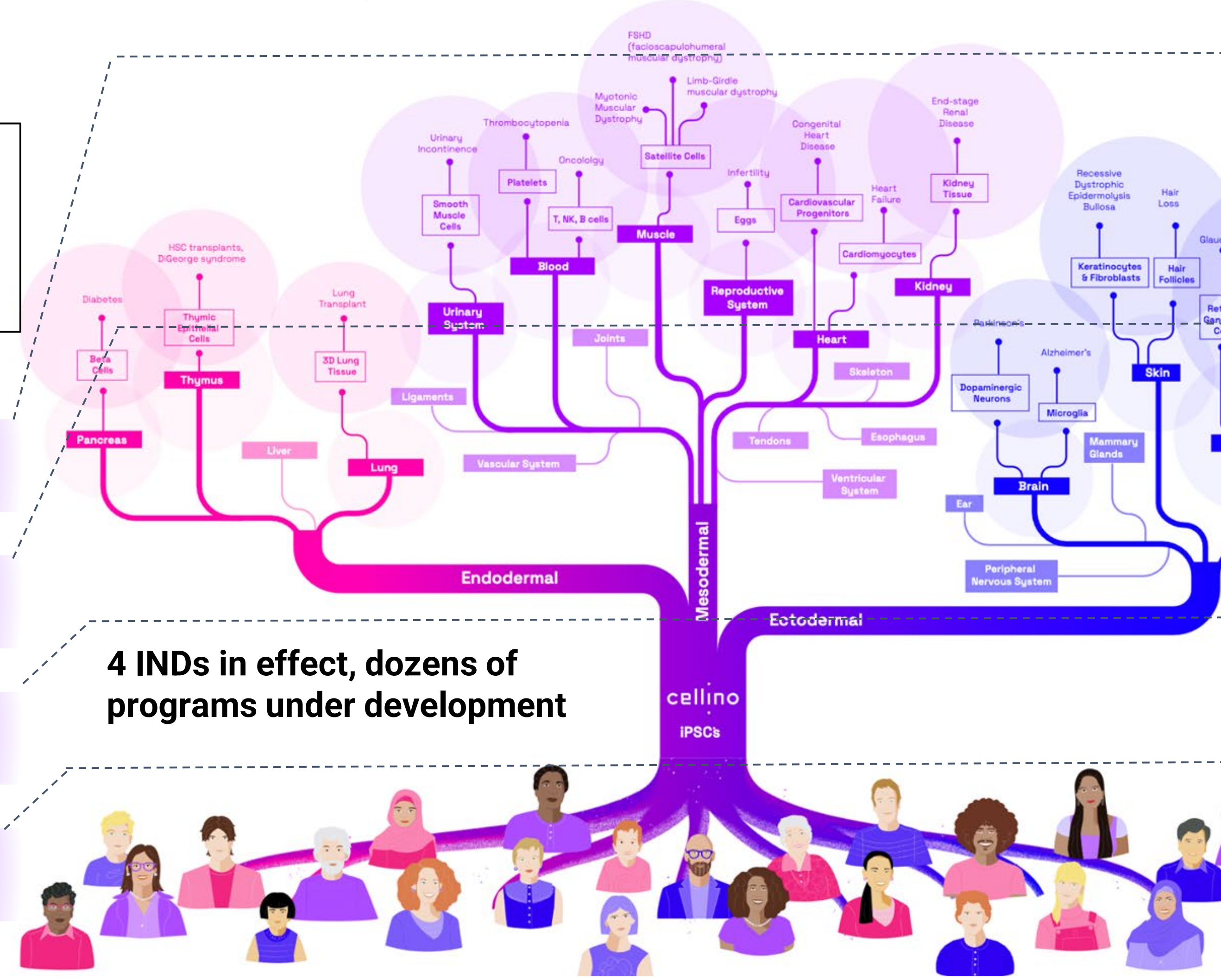
therapeutic cells and tissues

↑

induced pluripotent stem cells (iPSCs)

↑

patient blood or skin cells



4 INDs in effect, dozens of programs under development

Workshop Question

What factors limit accessibility to novel cell- and gene-based therapies and how can they be addressed?

Current manufacturing limited by expert manual operations, open bioprocesses, and high-grade cleanrooms

OPEN BIOPROCESSES IN CLEANROOMS

manual culture, scraping, and transfers by hand

Visual inspection and decisions with a microscope

REPEATED OVER 30-140 days (depending on cell type)

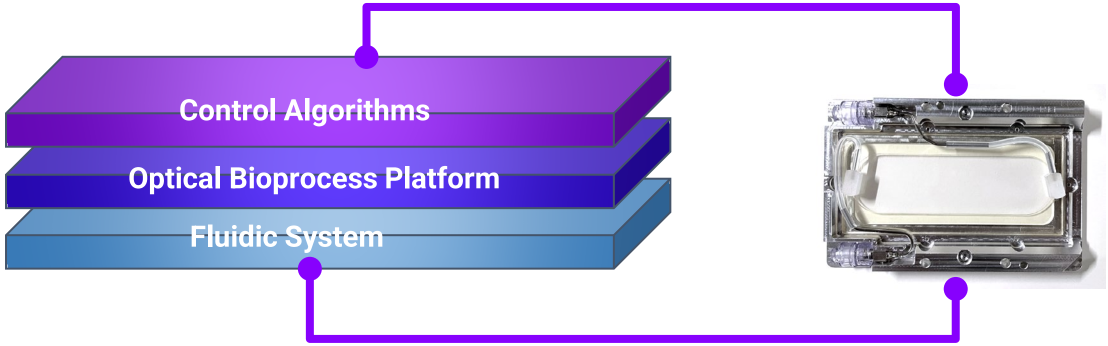
Recent advances in AI, optics, and fluidics enable the Cellino platform to be autonomous, closed, and modular

MANUAL

CLOSED

AUTONOMOUS

MODULAR



Control Algorithms

Optical Bioprocess Platform

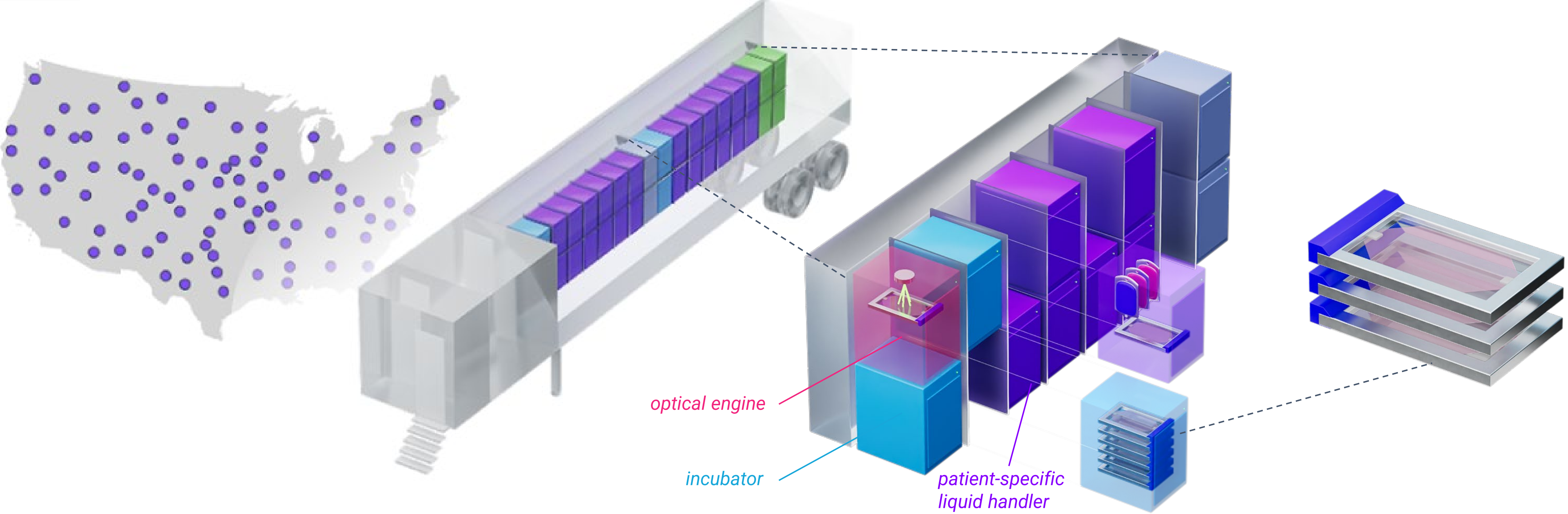
Fluidic System

Workshop Question

Describe ways in which automated technologies and artificial intelligence/ ML algorithms have evolved in recent years and the resulting impact on the field of regenerative medicine manufacturing.

Cellino vision: Point-of-care, closed, and autonomous biomanufacturing to expand access to auto iPSC-derived therapies

Patients	***Distributed*** ***~ 100,000s***	***Modular*** ***~1000s***	***Shared Clusters*** ***~100s***	***Cassettes*** ***1s***

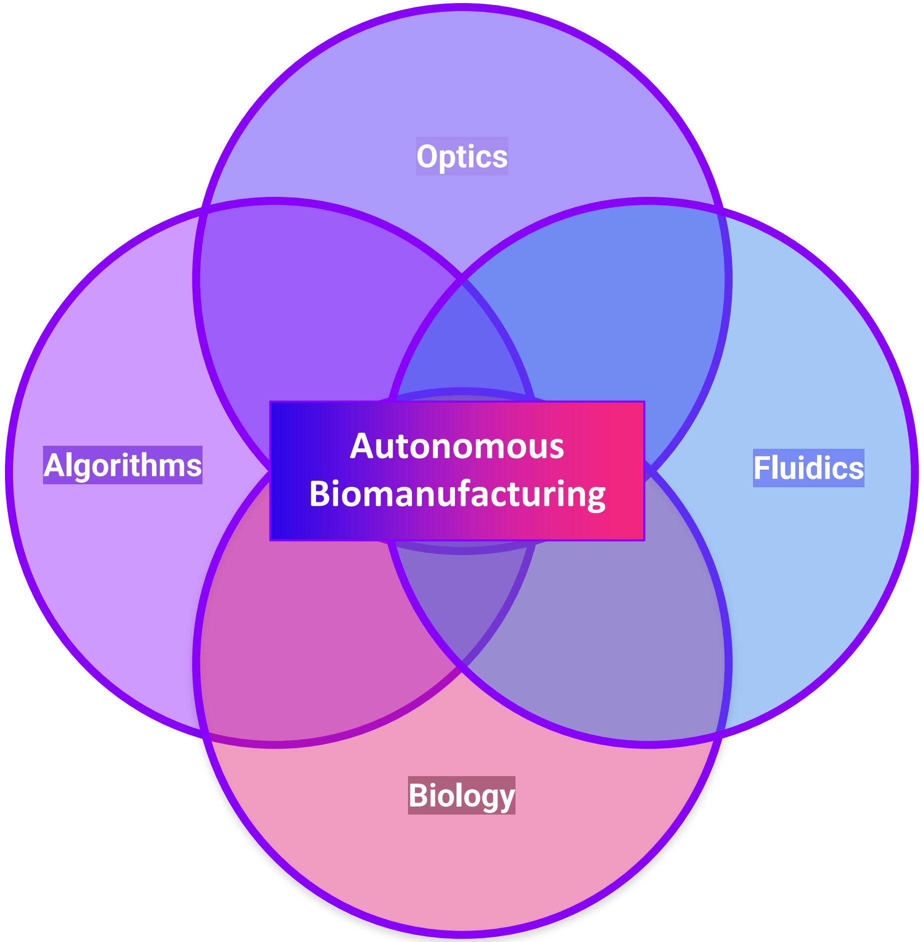


Biomanufacturing

AUTONOMOUS

What factors limit accessibility to novel cell- and gene-based therapies and how can they be addressed?

Cellino's multidisciplinary team is building an autonomous biomanufacturing platform for patient access



Optics

Algorithms

Autonomous Biomanufacturing

Fluidics

Biology

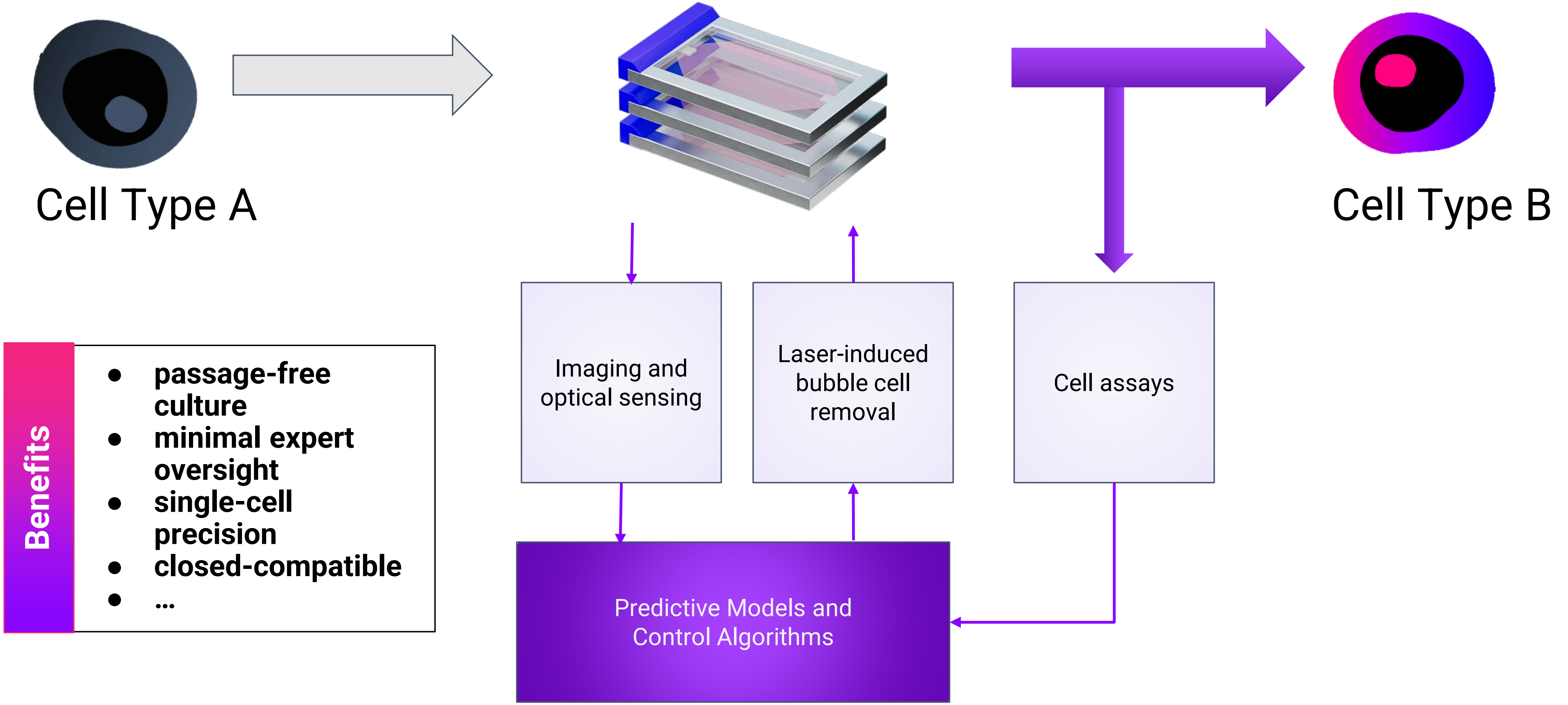
Industry Experience

Biomanufacturing: preclinical/clinical/commercial manufacturing for cell therapies
Pharma/biotech: cell therapies, molecular biology, genomics
Med devices: 25 medical devices approved + serving over 500,000 patients
Photonics/semiconductors: w/ $500MM+ products shipped
AI/ML: high-resolution, image-guided AI technologies in defense
Fluidics: oil and gas, organ-on-chip

Workshop Question

How can a multidisciplinary team be composed so that it is best equipped to tackle current manufacturing challenges and capitalize on recent advancements and technologies? What does the ideal team look like?

The platform makes in-process decisions using image-guided algorithms and removes unwanted cells using laser-induced bubbles



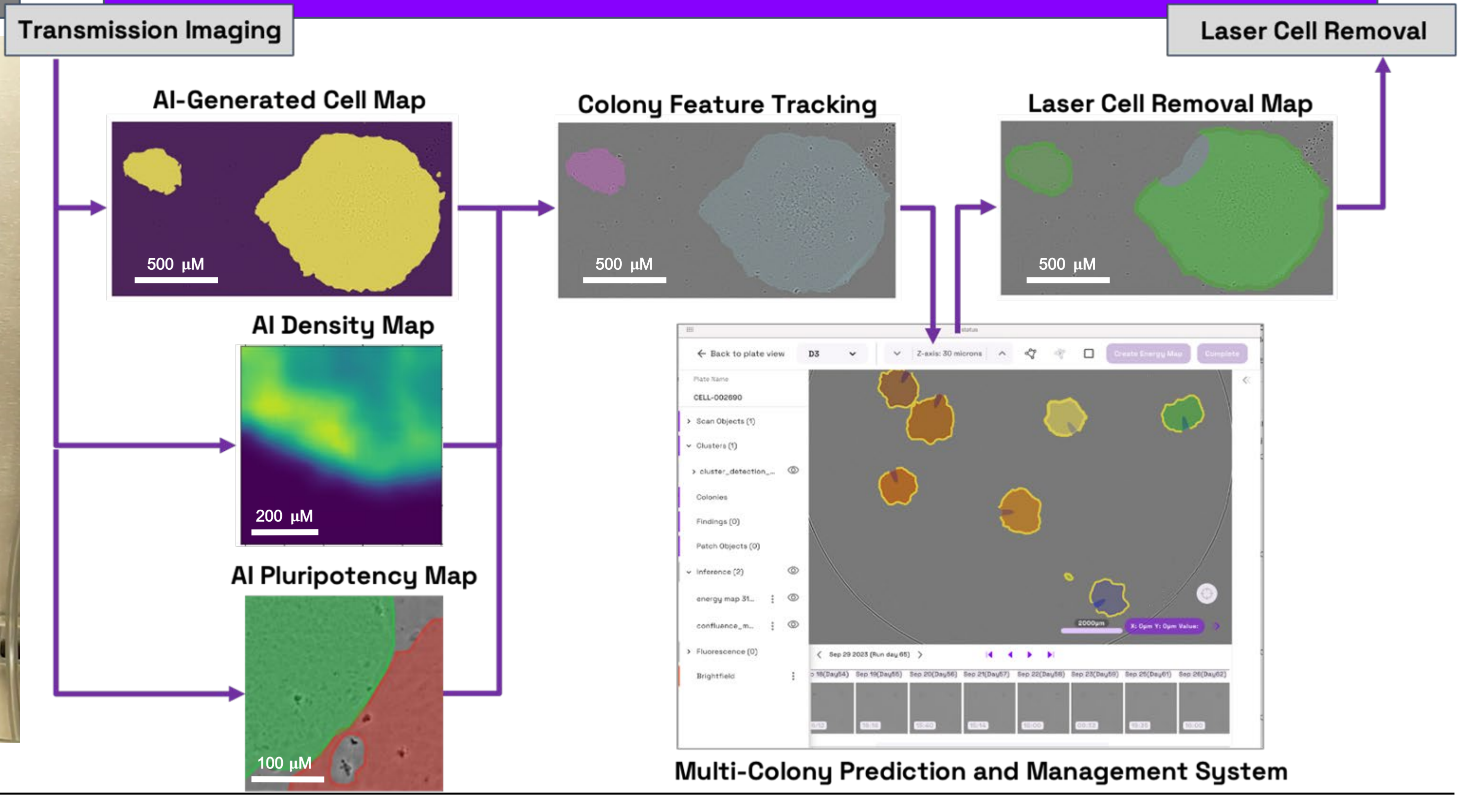
Benefits

- **passage-free culture**
- **minimal expert oversight**
- **single-cell precision**
- **closed-compatible**
- **…**

Example case #1: replacing operator-driven selection of colonies with AI-based prediction of colony quality

TRADITIONAL APPROACH
Human operator prediction of colony quality, manual scraping

CELLINO APPROACH
ML-based prediction of high-quality colony, ML-driven clonal management



Workshop Question

Describe ways in which automated technologies and artificial intelligence/ ML algorithms have evolved in recent years and the resulting impact on the field of regenerative medicine manufacturing.

cellino

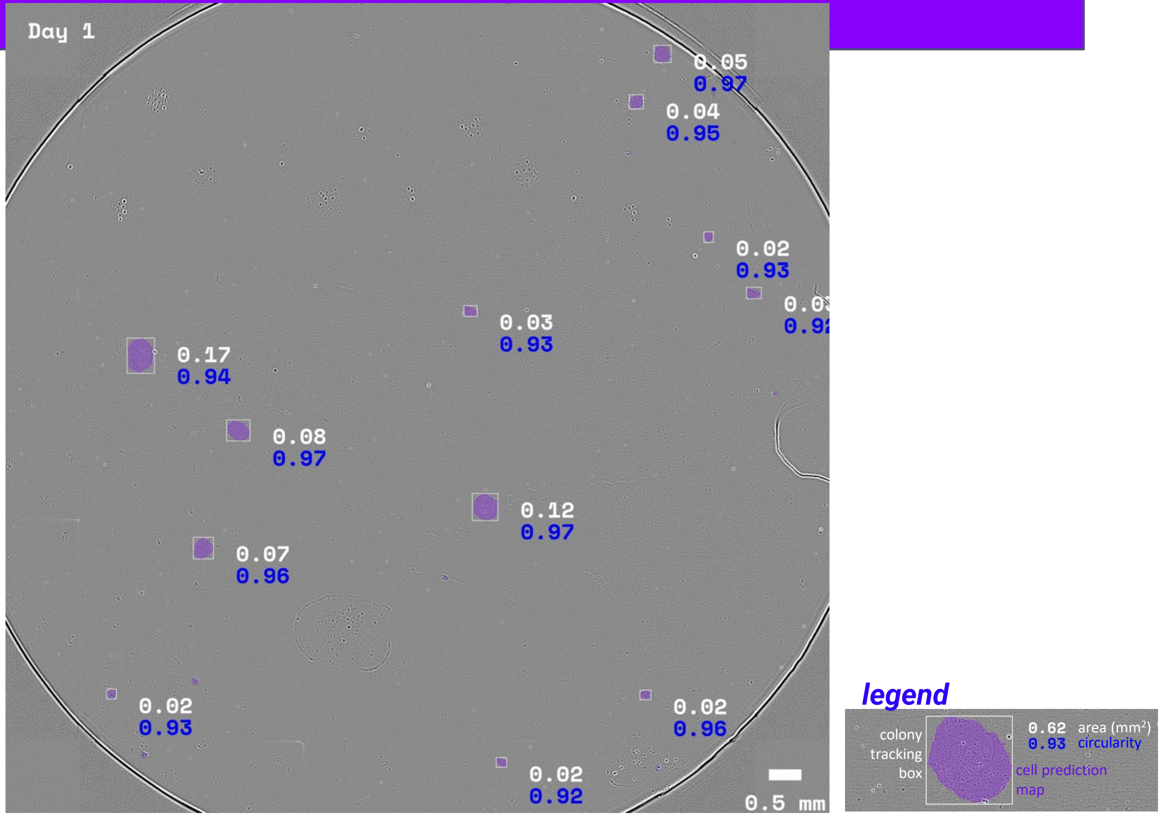
Example case #2: replacing operator-driven well-to-well passaging with ML-driven laser-based cell removal

TRADITIONAL APPROACH

Manual well-to-well passaging

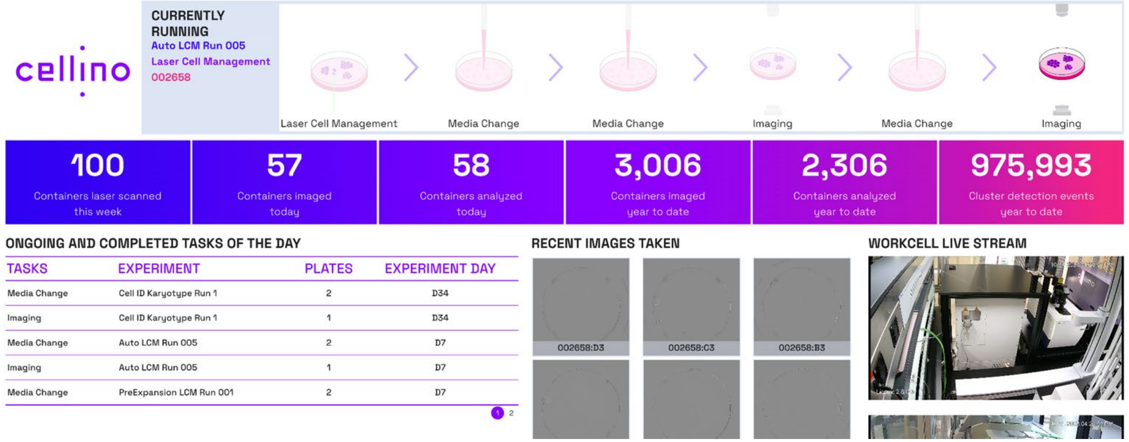
CELLINO APPROACH

Autonomous, label-free, PASSAGE-FREE bioprocess



We are advancing our foundational platform towards clinical manufacturing to support clinical programs

FOUNDATIONAL PLATFORM OPERATIONAL (Autonomous 1.0)



CURRENTLY RUNNING
Auto LCM Run 005
Laser Cell Management
002658

100 Containers laser scanned this week

57 Containers imaged today

58 Containers analyzed today

3,006 Containers imaged year to date

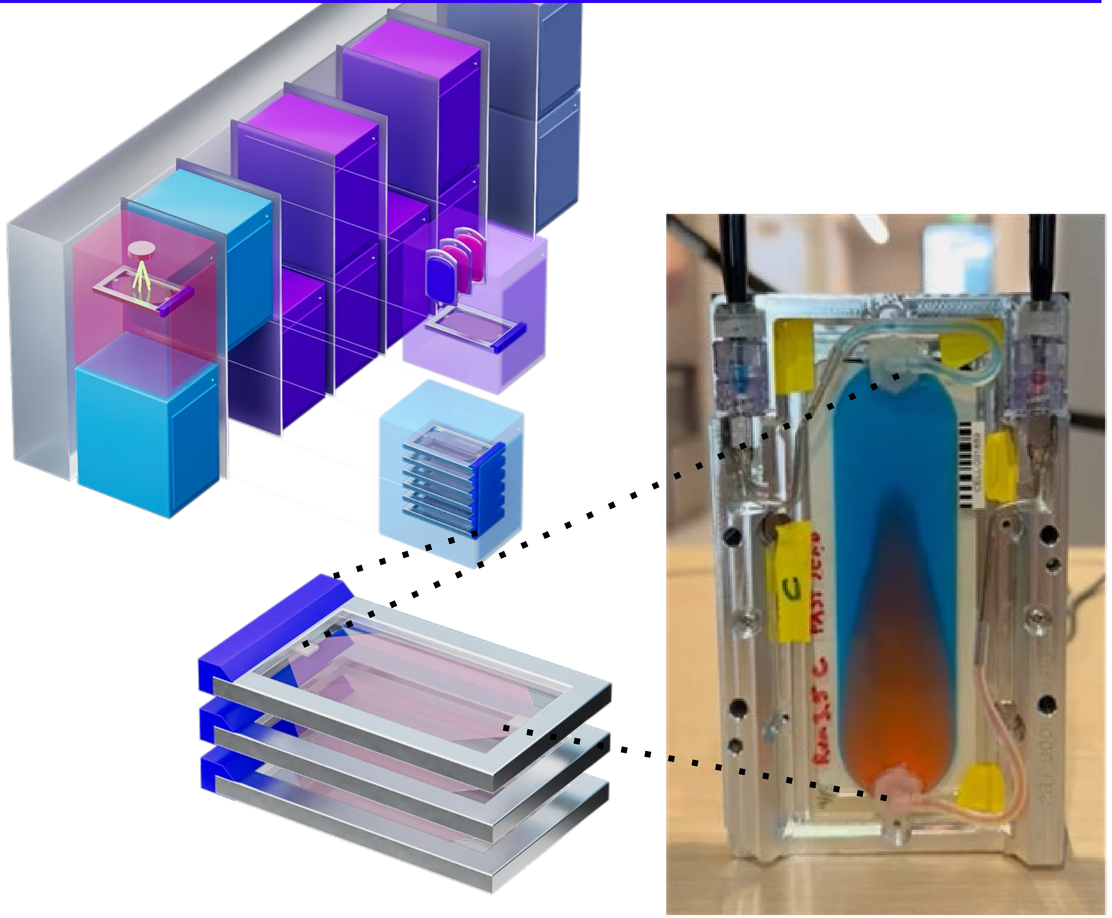
2,306 Containers analyzed year to date

975,993 Cluster detection events year to date

ONGOING AND COMPLETED TASKS OF THE DAY

TASKS	EXPERIMENT	PLATES	EXPERIMENT DAY
Media Change	Cell ID Karyotype Run 1	2	D34
Imaging	Cell ID Karyotype Run 1	1	D34
Media Change	Auto LCM Run 005	2	D7
Imaging	Auto LCM Run 005	1	D7
Media Change	PreExpansion LCM Run 001	2	D7

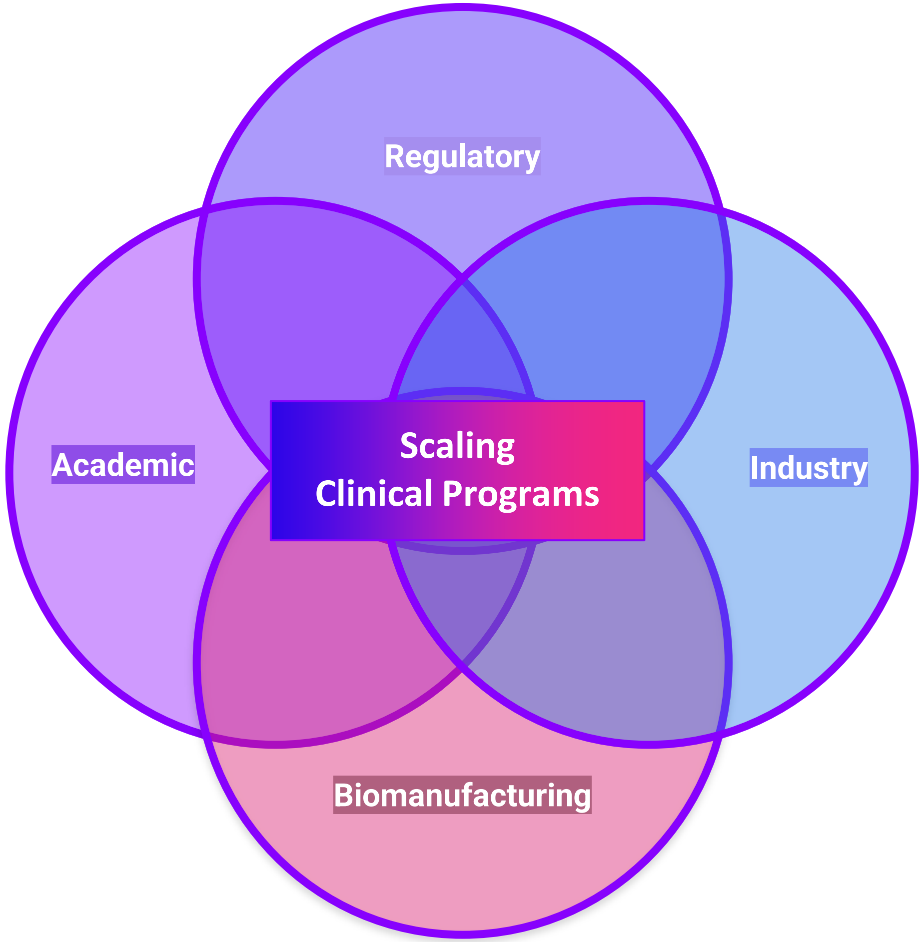
ULTRA-SCALABLE PLATFORM IN EARLY PROTOTYPE



Entity	*Cells* **Disease**	**IND**	**Ph 1/2a**	**Ph 2**	**Ph 3**	**Commercial**
National Eye Institute NCT04339764	*Retinal Pigment Epithelial Cells* **Age-related Macular Degeneration**					

Robust academic/industry/regulatory/biomanufacturing collaborations needed to support rapidly advancing programs

- How do we establish a new standard approach compared to manual biomanufacturing?
- How do we define a “good” iPSC cell?
- How do we accelerate the path of standardization to meet clinical trial timelines?
- How do we implement good machine learning practices for AI-based biomanufacturing processes?



Workshop Question

What are the most critical automation-related challenges that need to be address in regen med?
What are key considerations around data acquisition, management, validation, sharing in the context of a decentralized mfg model?

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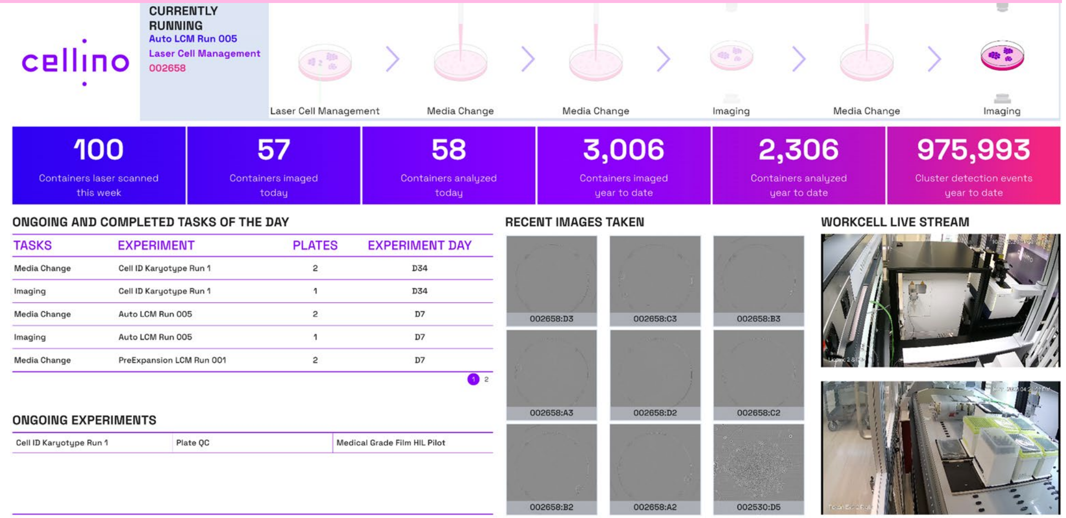
Gen1 System Running Optical Bioprocess

WORKCELL + LASER SYSTEM

CUSTOM CONSUMABLE

SOFTWARE OVERSIGHT



CURRENTLY RUNNING
Auto LCM Run 005
Laser Cell Management
002658

100	57	58	3,006	2,306	975,993
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Media Change	Auto LCM Run 005	2	D7
Imaging	Auto LCM Run 005	1	D7
Media Change	PreExpansion LCM Run 001	2	D7

1 2

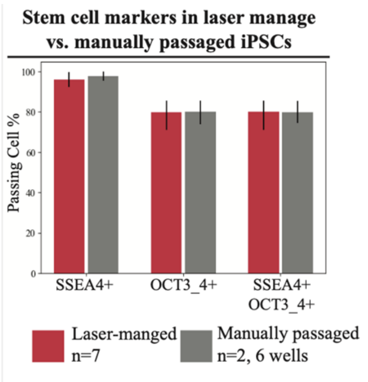
ONGOING EXPERIMENTS

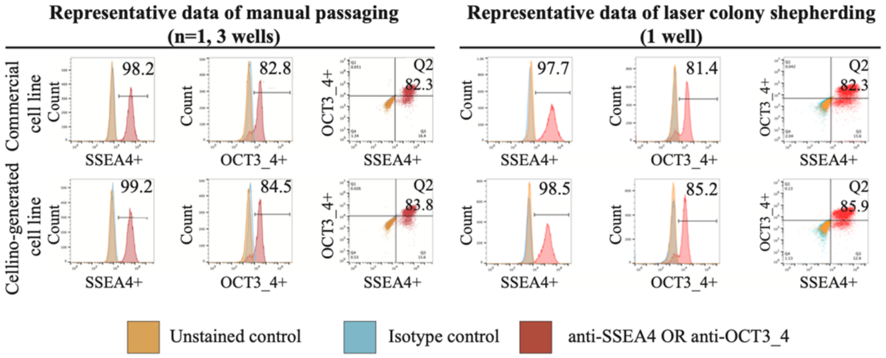
Cell ID Karyotype Run 1	Plate QC	Medical Grade Film HIL Pilot

RECENT IMAGES TAKEN

WORKCELL LIVE STREAM

iPSC DATA THUS FAR IS PROMISING

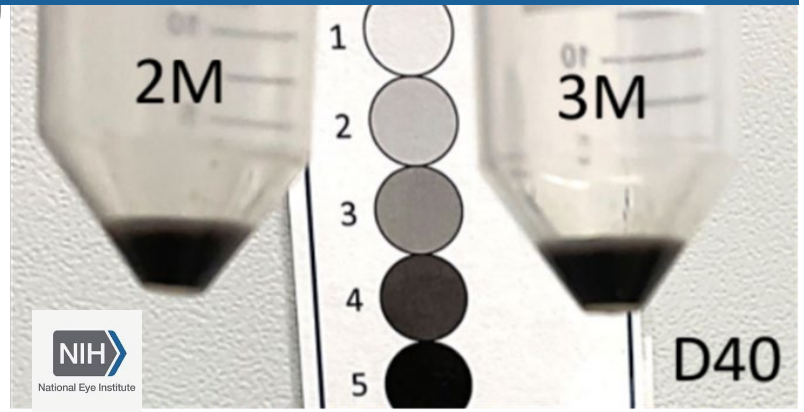

Stem cell markers in laser manage vs. manually passaged iPSCs


Representative data of manual passaging (n=1, 3 wells)

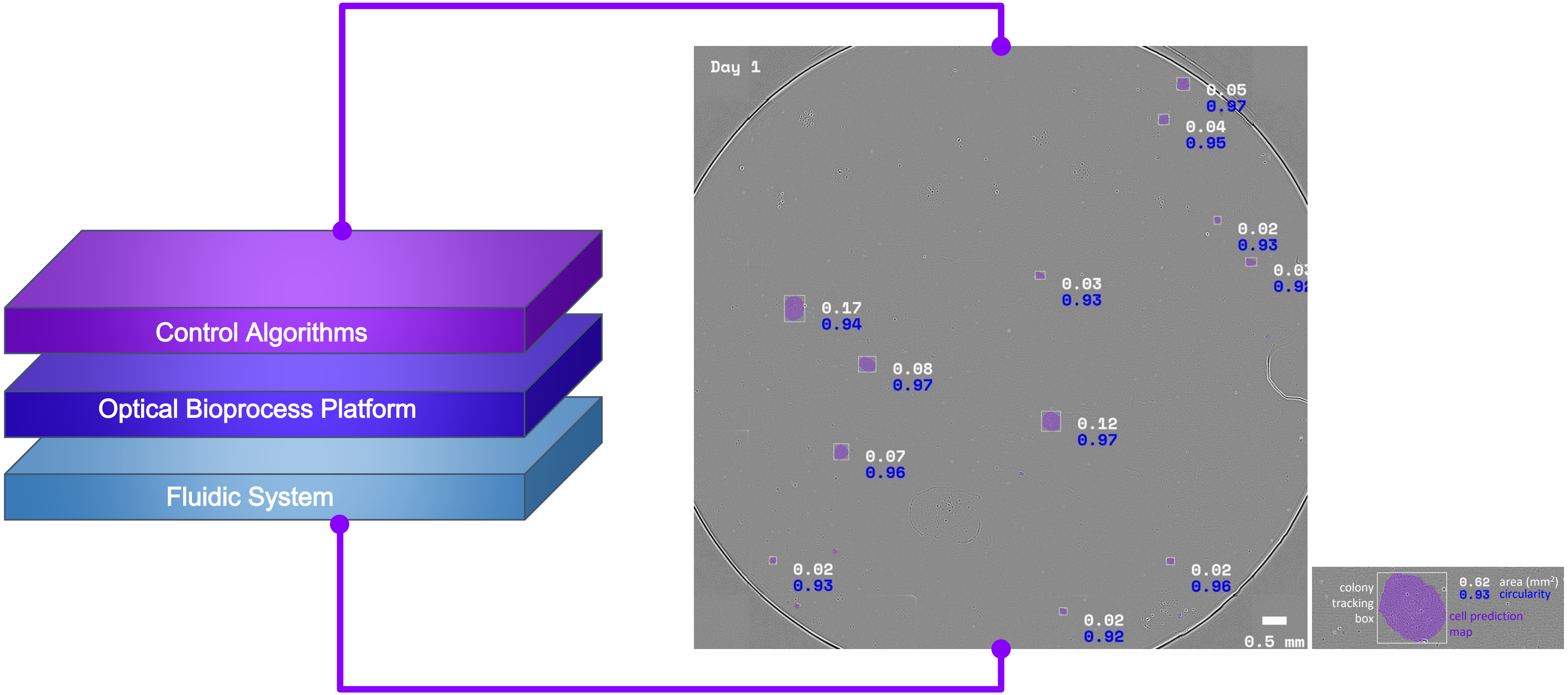
Representative data of laser colony shepherding (1 well)

COLLABORATION WITH DR. KAPIL BHARTI'S GROUP AT NEI

Collaboration to differentiate to Retinal Pigment Epithelial cells



Cellino’s Full-stack Autonomous Optical Bioprocess



Control Algorithms

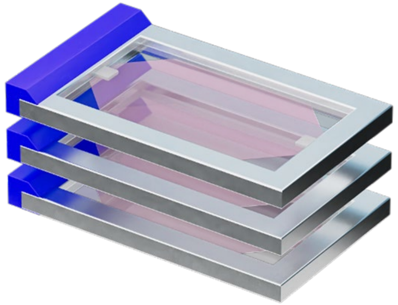
Optical Bioprocess Platform

Fluidic System

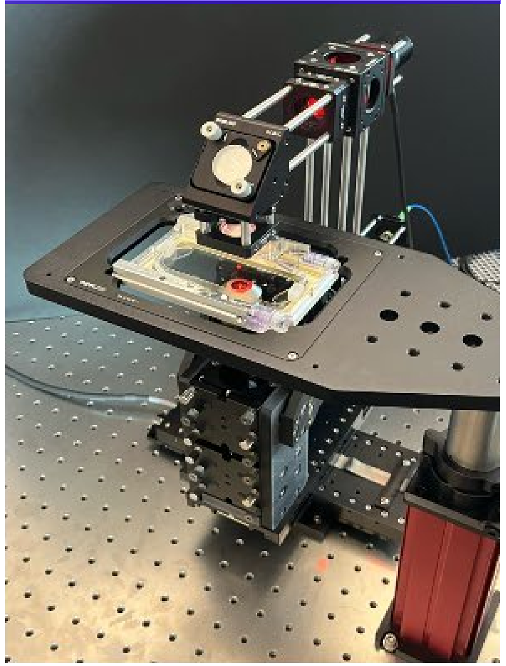
Gen2: Cassette System Development Started

Multiple Cassette Architectures for Biomanufacturing

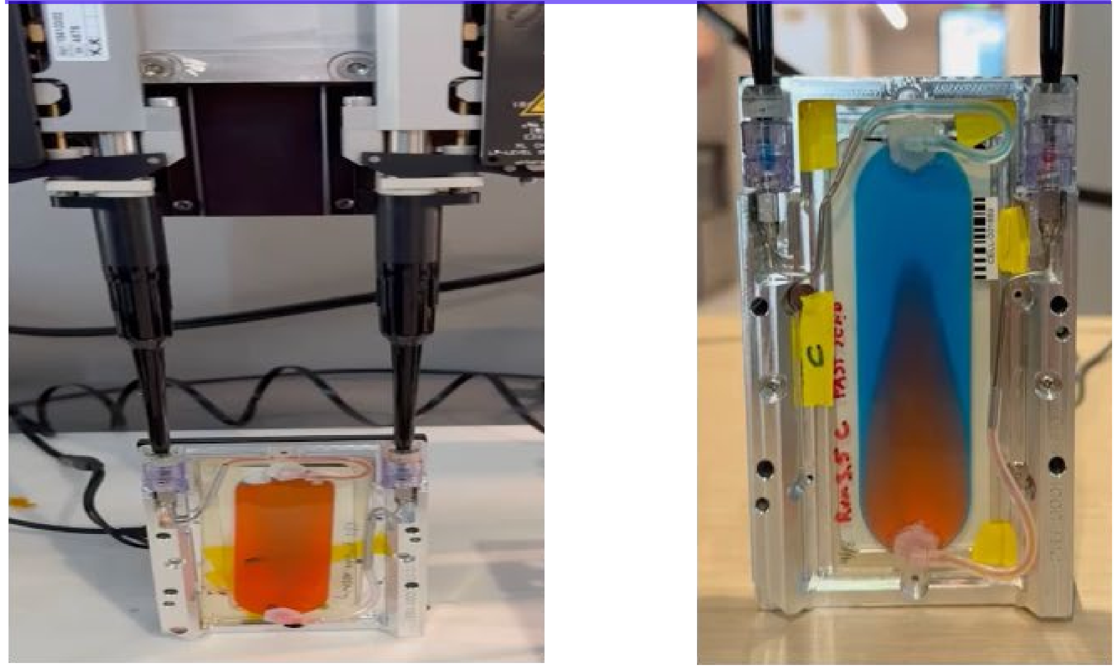
both reprogramming and differentiation capabilities



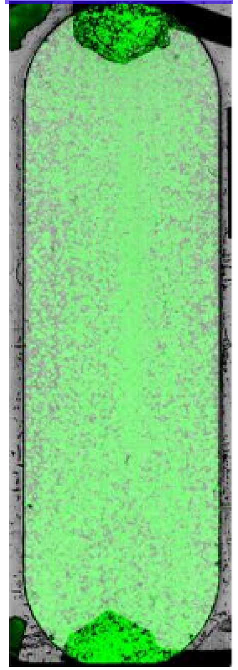
OPTICAL ENGINE



FLUIDICS



iPSCs



Cellino’s Novel Optical Bioprocess For Scale

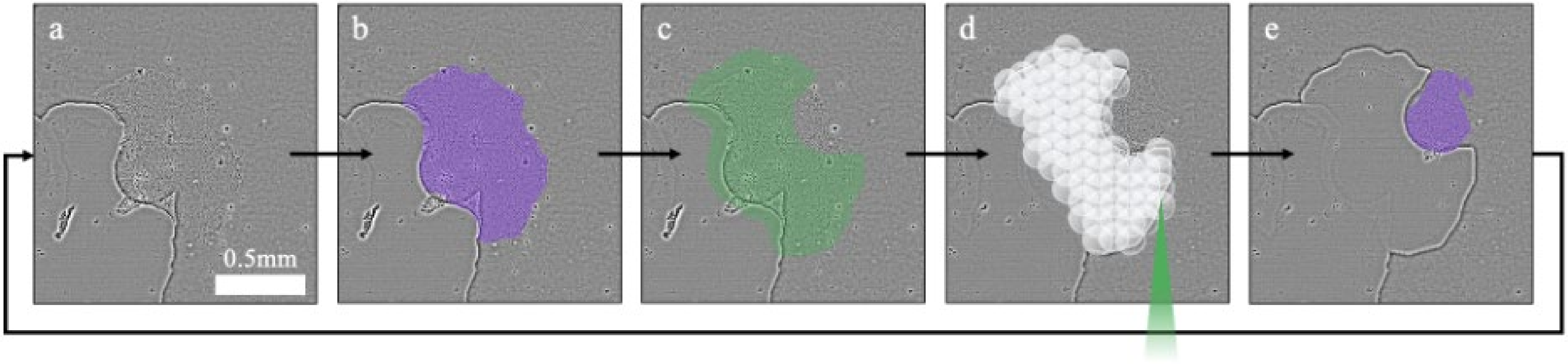
Transmission imaging

AI-driven cell and phenotype mapping

Cell removal map generation

Pulsed laser - induced microbubble cell removal

Washing → healthy reduced colony



2023 BOLD GOALS FOR U.S. BIOTECHNOLOGY AND BIOMANUFACTURING

decrease health inequities

1

- address our increasingly aging, and diversifying population
- be patient-specific to minimize risk of immune rejection
- address major public health sectors / diseases

expand access

2

- reach patients living everywhere
- be POINT-OF-CARE at hospitals

decrease cost 10-fold

4

- be affordable
  (not cost up to $3M per patient)

Cellino’s Roadmap Towards Our Biomanufacturing Moonshot

2017 - 2020

2017
Cellino founders met - laser processing of cells with custom consumables

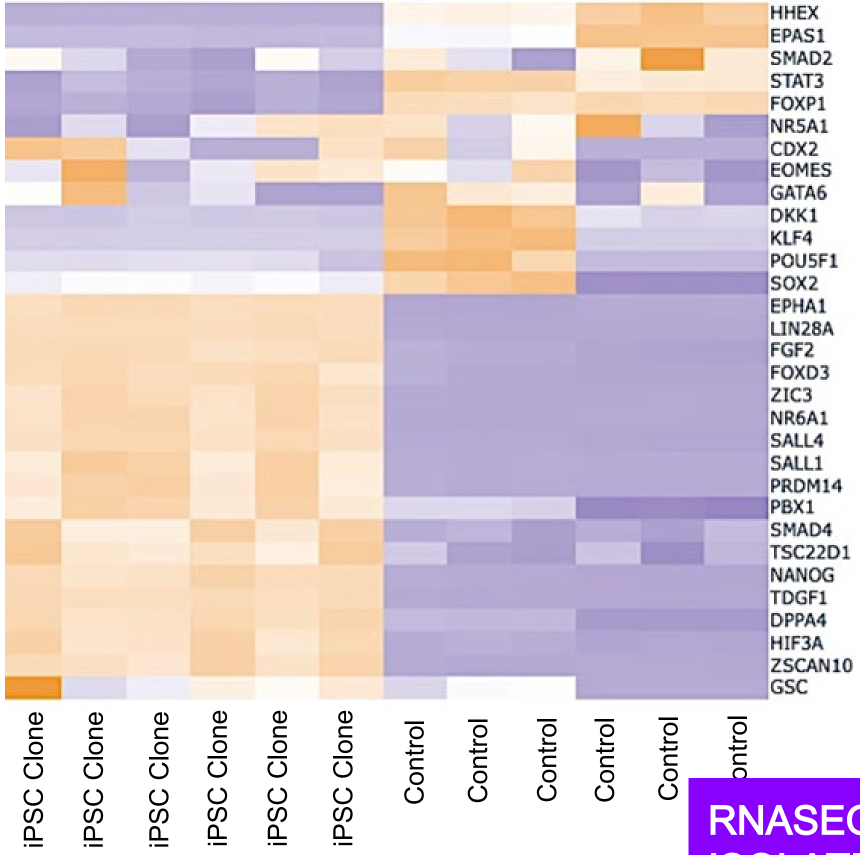
2020
Decision to focus on personalized iPSC-based cell therapies

	2021	2022	2023
AI MODELS	• Built custom AI cloud infrastructure • Incorporated AI/ML into bioprocess	• Detect cells, detect colonies, track colonies over time, calculate confluence, and automate colony selection	• Calculate cell density, identify pluripotent vs non-pluripotent cells, time-series metrics such as growth/ proliferation rates
BIOPROCESS	• Tested reprogramming techniques manually • Developed laser removal techniques (removing individual cells & entire colonies)	• iPSC101 - developed first automated Cellino iPSC process ○ Laser-based clonal isolation ○ 250 days of automated culture ○ 60 iPSC lines from 7 donors ○ Set up quality control metrics	• iPSC102 - developing prototype of passage-free clinical-grade process ○ Passage-free process ○ Human in loop→autonomous for 60 days ○ Scale quality metrics
HARDWARE	• Custom consumable plates • Laser scanner2	• Workcell #1: Automated cell culture • Laser scanner3 & high throughput consumables	• Workcell #1: Autonomous cell culture • Workcell #2: automated QC prep • Laser scanner4 & fluidic chamber prototype
DATA	• Health and viability of cells post-laser removal • POC iPSC colony generation from tested reprogramming techniques	• Characterization of IPSC101/ automatically generated iPSCs that have undergone laser-based clonal isolation: sterility, pluripotency, functionality, genomic stability, etc.	• Further characterization of iPSCs undergoing passage-free laser-based cell maintenance process: karyotyping, pluripotency, etc • Differentiation to RPEs

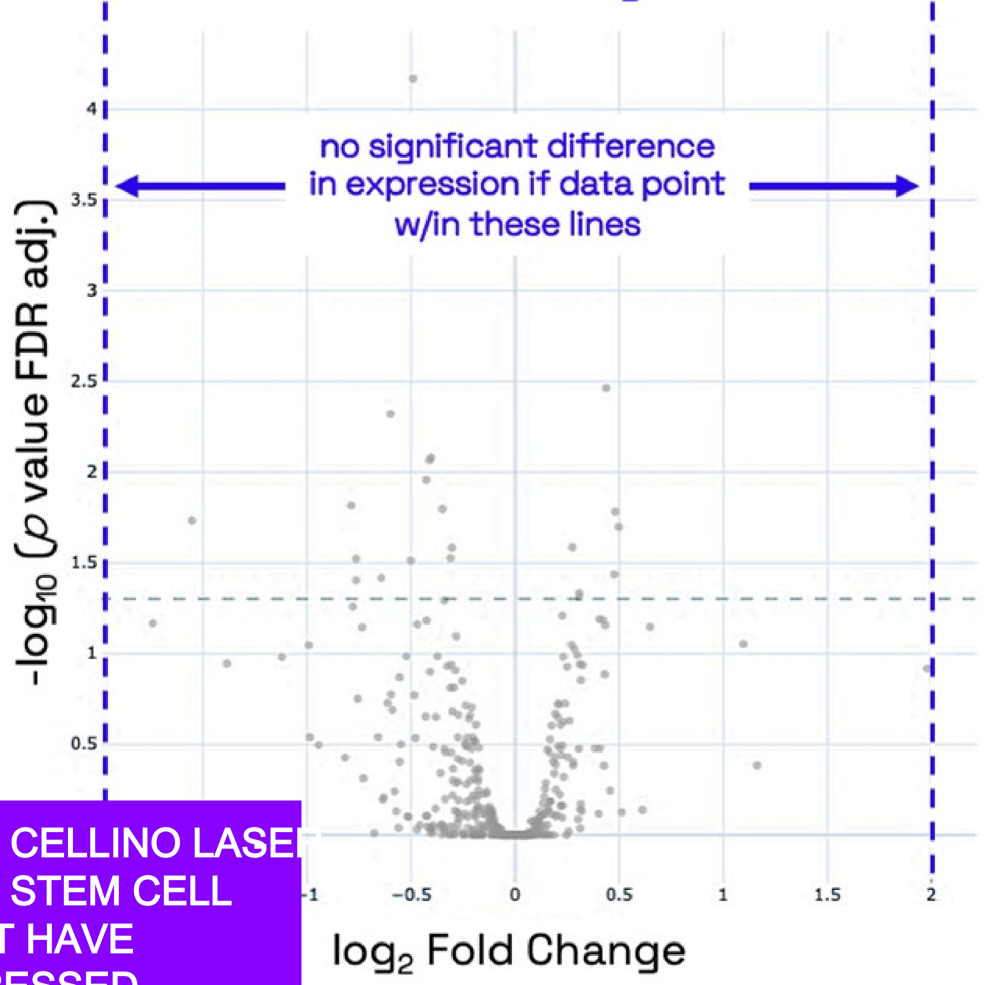
Does the laser have a negative impact on stem cell phenotype or oncogene expression of the remaining cells?

RNAseq showed laser -isolated iPSCs display stem cell signatures and do not have differentially expressed oncogenes.

Self-Renewal Gene Expression



Differential Gene Expression: Laser-Isolated & Manually -Picked iPSCs



RNASEQ DATA SHOWS CELLINO LASE ISOLATED IPSCS HAVE STEM CELL SIGNATURES & DO NOT HAVE DIFFERENTIALLY EXPRESSED ONCOGENES

Does use of the laser have negative impacts on cell health?

A third -party vendor characterized laser -isolated iPSCs and manually -picked iPSCs and showed they were comparable for a range of assays.

Purpose

3^{rd}-party analysis by **Elixirgen Scientific** to compare laser-isolated iPSCs to traditionally-produced iPSCs

Samples Tested

- Control lines (no laser):
  - n=1 Thermo episomal iPSC ctrl
  - n=1 CIRM episomal iPSC ctrl
  - n=3 Cellino Sendai ctrl (manually picked)

- Experimental lines (laser colony isolation):
  - n=2 Thermo episomal
  - n=3 Cellino Sendai

Analyses Performed

☑ visual inspection for morphology and (absence of) spontaneous differentiation
☑ p1 viability
☑ %Oct4+ (ICC)
☑ %TRA1-60, SSEA4+ (flow cytometry)
☑ karyotype g-banding (on a subset of samples listed above)

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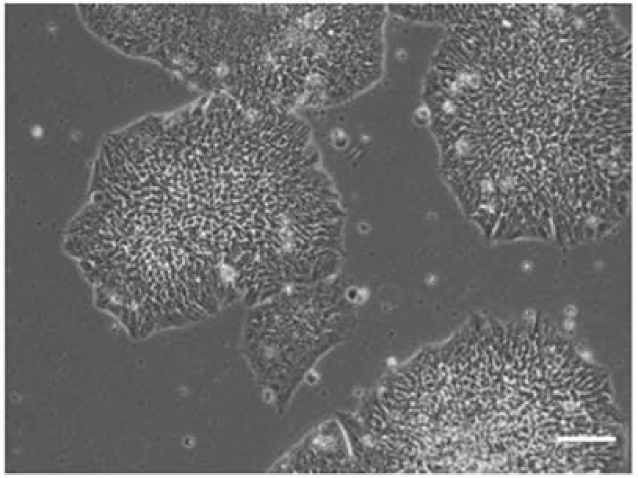
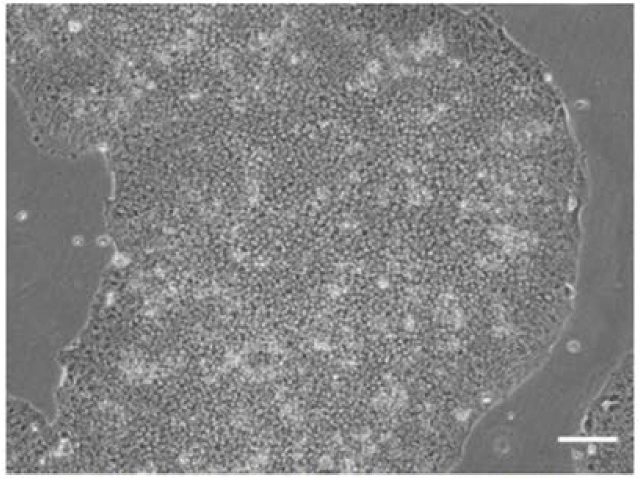
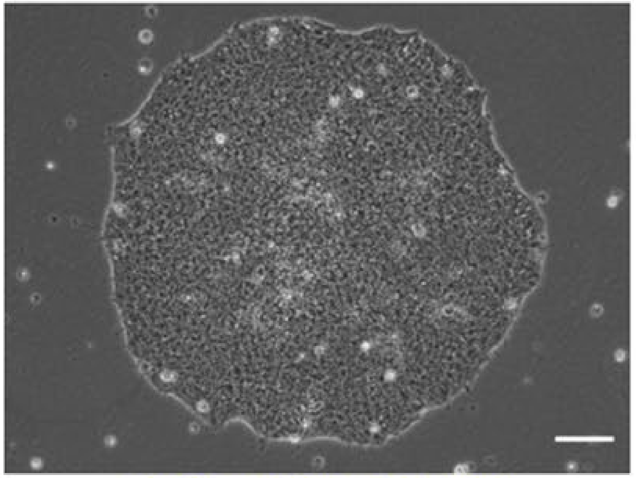


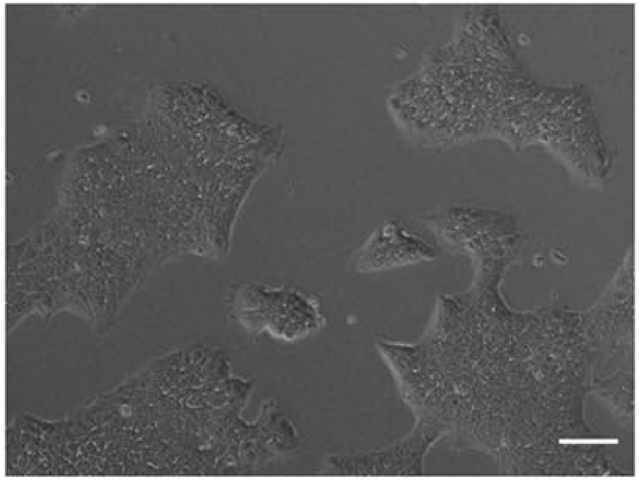
CIRM episomal ctrl



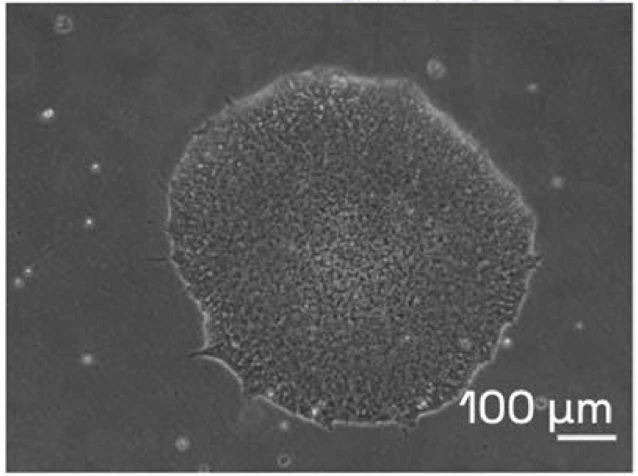
Thermo episomal ctrl



Cellino Sendai ctrl



Thermo episomal + laser



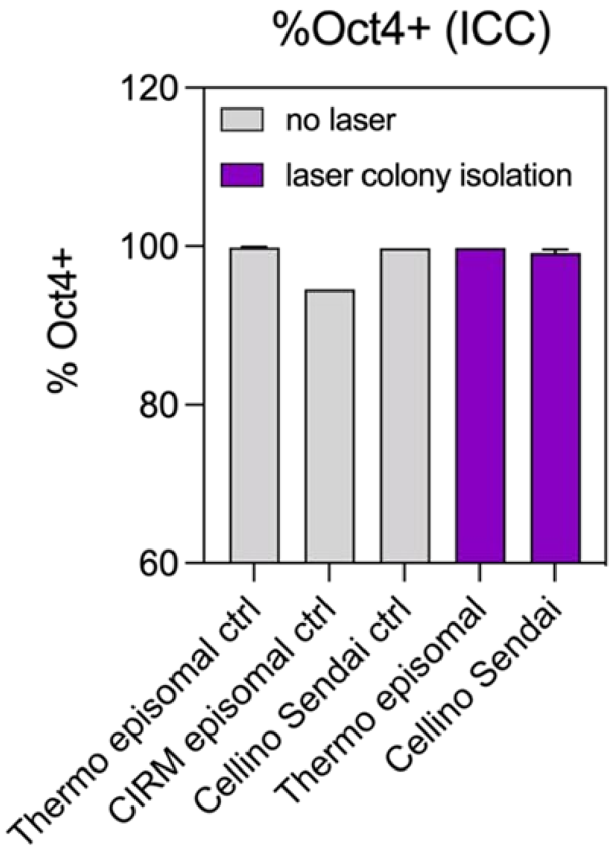
Cellino Sendai + laser

Does use of the laser have negative impacts on cell health?

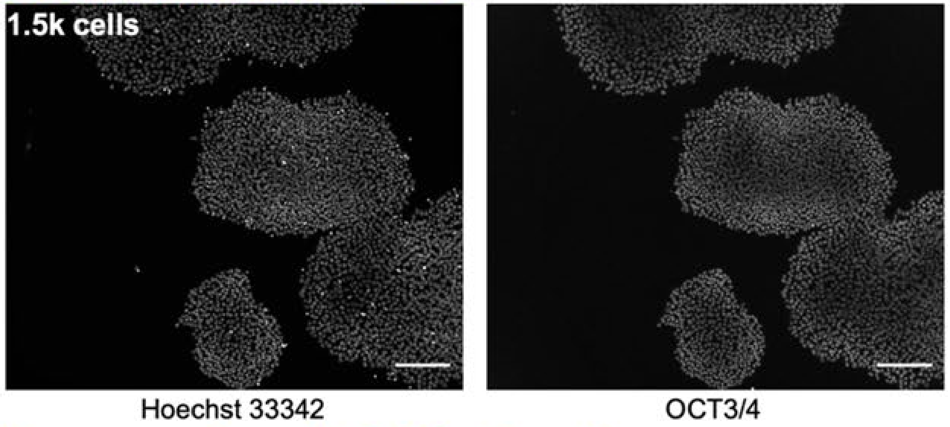
A third-party vendor characterized laser-isolated iPSCs and manually-picked iPSCs and showed they were comparable for a range of assays.

Analysis & Conclusion

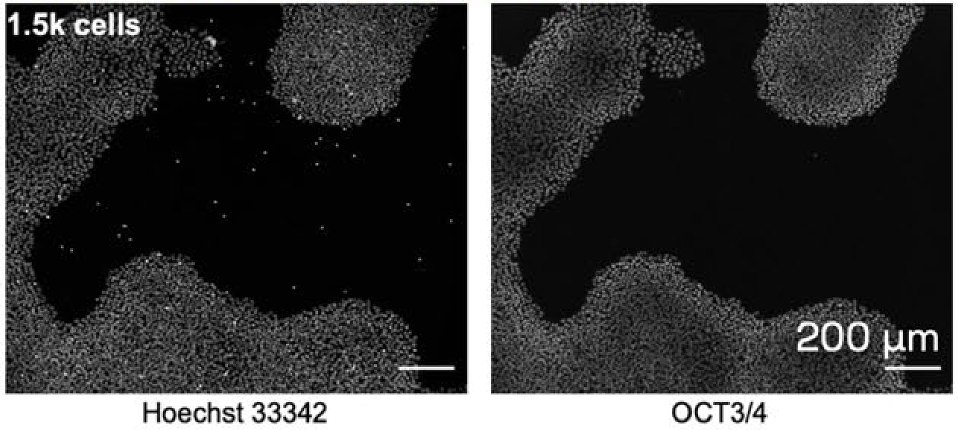
Laser-isolated iPSC lines show comparable %Oct4 expression, measured by ICC image analysis.

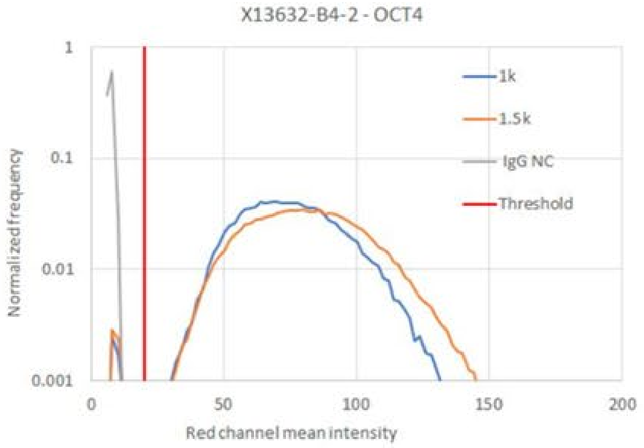


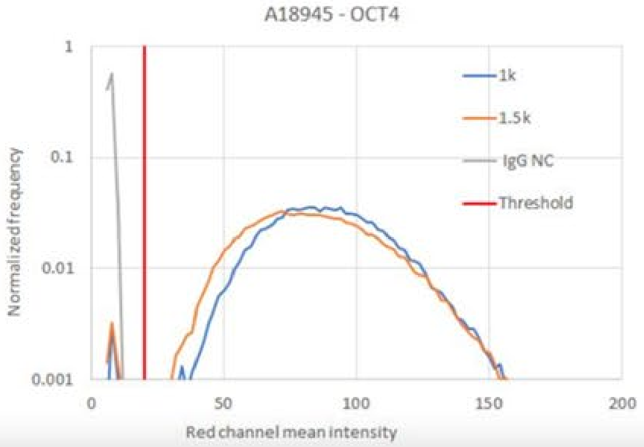
Thermo episomal + laser



Thermo episomal ctrl (no laser)





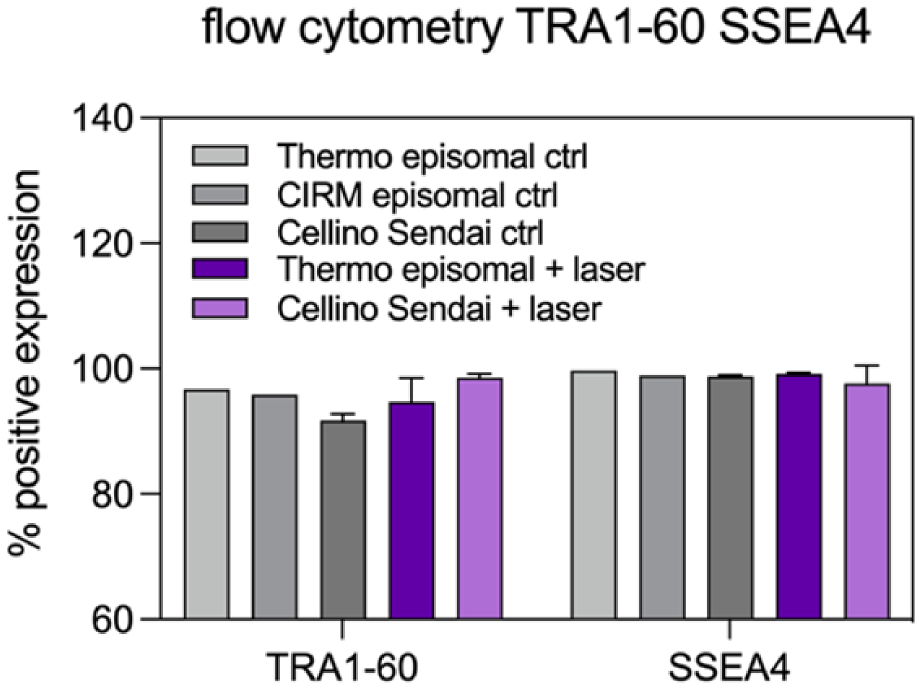


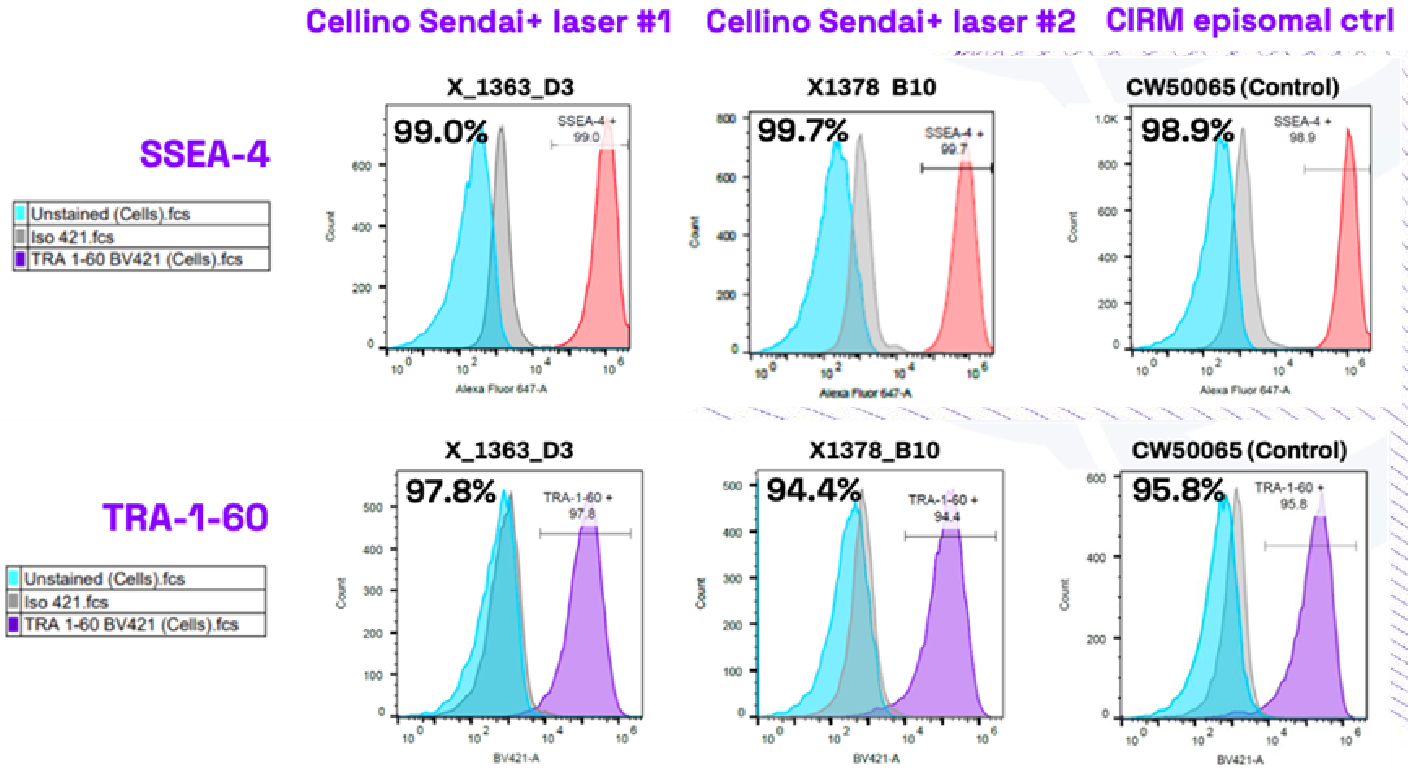
Does use of the laser have negative impacts on cell health?

A third -party vendor characterized laser -isolated iPSCs and manually -picked iPSCs and showed they were comparable for a range of assays.

Analysis & Conclusion

Laser-isolated iPSC lines show comparable TRA1-60 and SSEA4 expression, measured by flow cytometry.

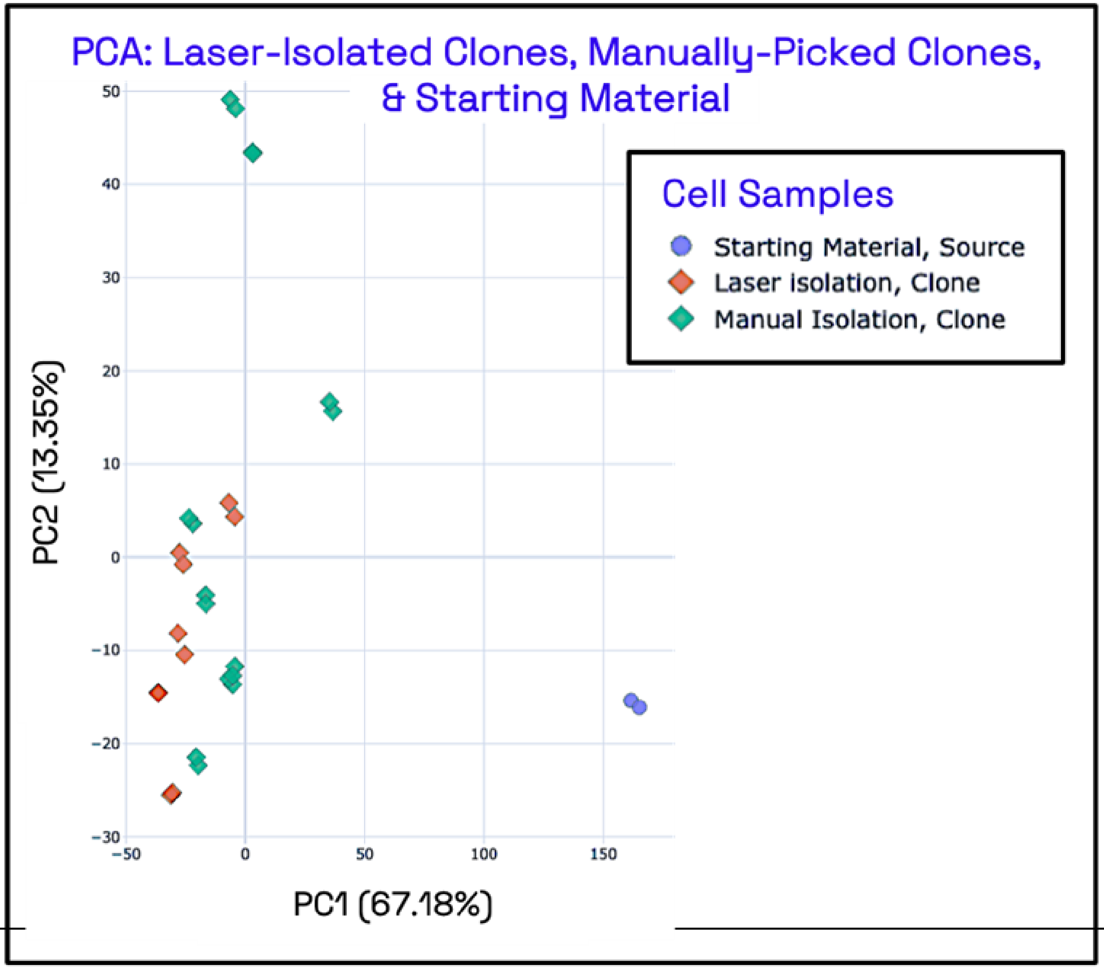




Does use of the laser have negative impacts on cell health?

A third -party vendor characterized laser -isolated iPSCs and manually -picked iPSCs and showed they were comparable for a range of assays.

Takeaway: RNAseq analysis shows similar clustering between laser-isolated iPSC lines and manually-picked iPSC lines generated from 1 donor sample.



1 donor sample:

- CD34+ cells
- iPSCs generated w/ Sendai reprogramming:
  - laser isolated

  vs. manually picked

Does repeated use of the laser have negative impacts on cell health?

LATEST DATA: Laser cell management for 30-60 days showing promising results in key assays

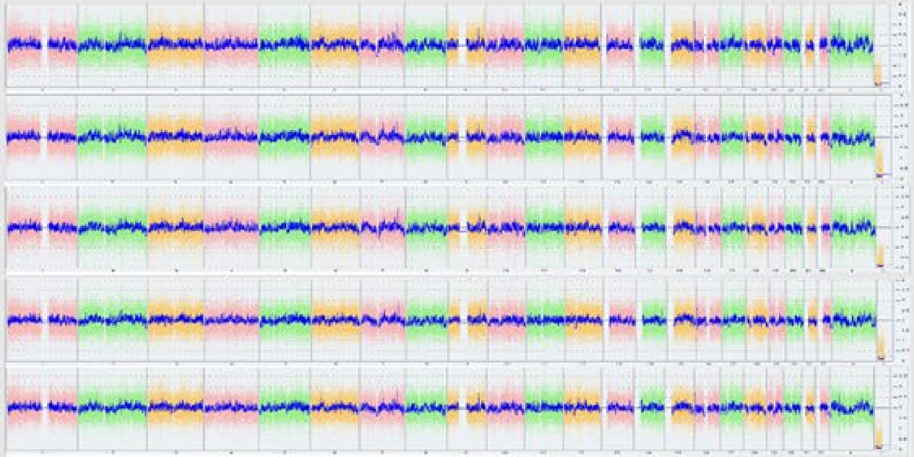
Experiments	Status	Days of LCM in single well	Wells processed	Cell lines	Results
1. **Human determined laser scanning** using laser scanner	✓	30, 60	4	Thermo line	Karyotyping 4/4 PASS Flow 3/4 PASS (¼ processing)
2. **Human determined laser scanning** using Cellino viewer	✓	30, 60	16	Thermo line Cellino line	SeV (processing)
3. **Human determined laser scanning** using Cellino viewer	✓	30, 60	16	Thermo line Cellino line	Karyotyping D58 7/8 PASS, 1/8 FAIL Flow D57 7/8 PASS, 1/8 FAIL WES (processing), RNA-Seq (processing)
4. **Limited autonomous determined scanning, human-assisted** (scan object at counterclockwise rotation) using Cellino viewer	Ongoing	30, 60 (Ongoing)	96	Thermo line Cellino line	Karyotyping (ongoing) Flow (ongoing) SeV (ongoing)
5. **Fully autonomous determined laser scanning** (scan frequency and directionality)	Future	60	TBD (> 96)	Thermo line Multiple cellino lines	Karyotyping Flow, SeV, WES, RNA-Seq

Does repeated use of the laser have negative impacts on cell health?

LATEST DATA: Laser cell management for 30 -60 days showing promising results in key assays

D58 LCM - No chromosomal aberrations in 7 / 8 wells

Thermo line A18945



PASS

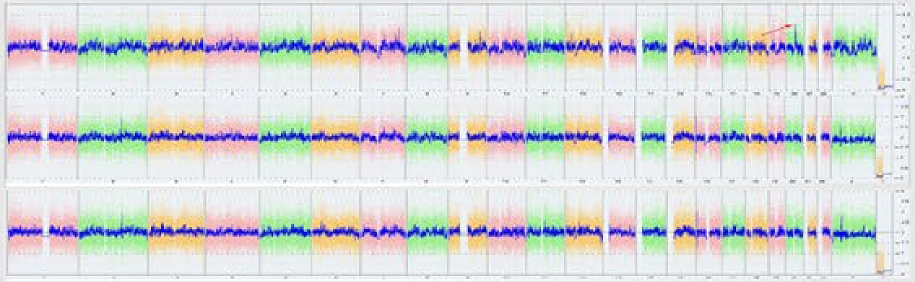
PASS

PASS

PASS

PASS

Cellino line



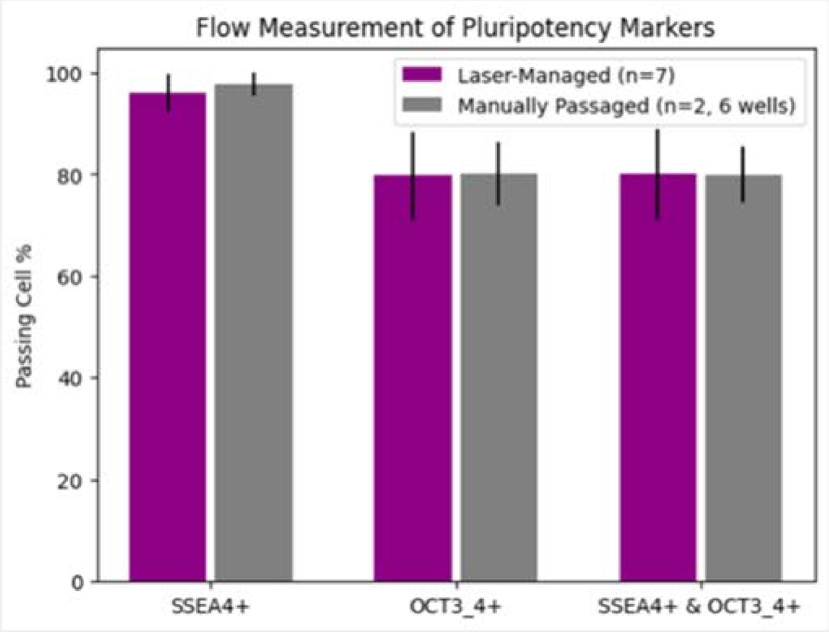
FAIL

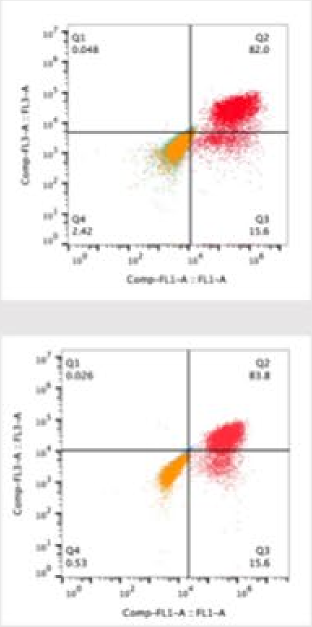
Chromosome	Type	Cytoband Start	CN State	Size (kbp)
20	Gain	q11.21	3.00	1,177

PASS

PASS

D57 LCM - Equivalent OCT4 and SSEA4 expression to manually passaged wells (Thermo & Cellino lines, n=7)





cellino

3. What is new about your approach? Why do you think you can be successful at this time?

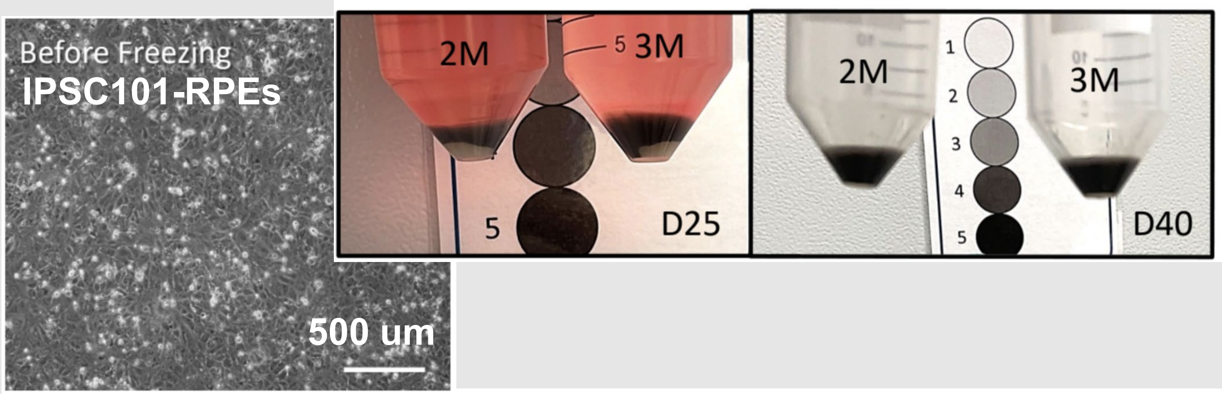
We collected extensive data on iPSCs generated with Cellino bioprocess (clonal selection)

2022 IPSC LINE GENERATION

60 clonal iPSC lines generated & cryopreserved

7 donors: 2 African-American, 1 Latino, 4 Caucasian

COLLABORATION WITH NEI (DR. KAPIL BHARTI)



Quality Control by FACS, TEER and POS

	Day25			Day 40				
Day-2	Pigm.	TYRP1, %	MITF1, %	Pigm.	TYRP1, %	PMEL17, %	CRALBP, %	MACS, %
2M	4	57.75	93.85	5	98.95		78.75	65.8
3M	4	69.50	97.10	5	97.75	94.75		64.4

MODEL TO AUTOMATE COLONY SELECTION

86% of time, model's #1 choice matched SME trained on 3284 images of colonies

AUTOMATION STATS

250 days automated operations

520 wells underwent laser colony isolation

Imaging capacity: 3456 96-wells/day

laser colony isolation capacity: 576 96-wells/day

MANUFACTURING READINESS

3 QC vendor relationships established

1 expansion partner relationship established

9 internal QC assays developed

2 blood cell vendor relationships established

3. What is new about your approach? Why do you think you can be successful at this time?

We collected extensive data on iPSCs generated with Cellino bioprocess (clonal selection)

RELEASE CRITERIA

ATTRIBUTE	TEST	METHOD	TESTED MATERIAL	RELEASE CRITERIA
Safety	Mycoplasma	WiCell (SOP-83.01)	Medium pre-cryopreservation	negative
	Bacterial & Fungal sterility	USP<71> and <61>, Ph.Eur.2.6.27 and 2.6.1, JP17<4.05> and <4.06>	Medium post-thaw	negative
	Endotoxin	CRL (GP-C555)	Medium post-thaw	negative
	Residual Sendai vector testing	qPCR	Cells pre-cryopreservation	< 0.01 copies/ cell
	Chromosome analysis	G Band karyotyping	Cells pre-cryopreservation	Identical to patient/ donor karyotype
	Oncogene integrity	WES, RNA-Sequencing	Cells pre-cryopreservation	Identical to patient/ donor oncogene sequence
Identity	Patient/ donor dentity	STR	Source material & iPSCs post-thaw	identical to patient/ donor
	Cell identity	Flow cytometry	Cells post-thaw	>90% SSEA4 NANOG +/+
Cell Health	Viability	Dye exclusion test	Cells post-thaw	>60% post-thaw viability

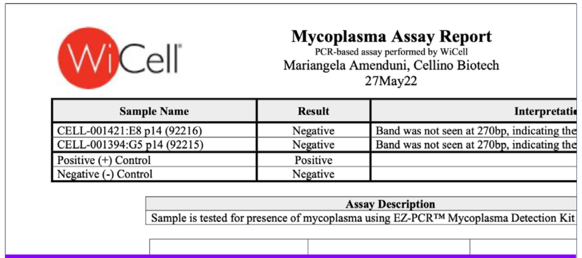
FOR INFO. ONLY

ATTRIBUTE	TEST	METHOD	TESTED MATERIAL
Colony information	Time-series colony growth tracking	Time-series image collection of colony growth pre-laser isolation	Cells in-process during reprogramming
	Single timepoint colony morphology	Image-based characterization to assess circularity, area, & edge smoothness, at time of laser colony isolation	Cells in-process during reprogramming
Potency	Pluripotency	Directed differentiation towards endoderm, mesoderm and ectoderm (trilineage)	Cells post-thaw
Expression profiling	Transcriptome analysis	RNA-Sequencing	Cells pre-cyropreservation

CELLINO only

3. What is new about your approach? Why do you think you can be successful at this time?

We collected extensive data on iPSCs generated with Cellino bioprocess (clonal selection)

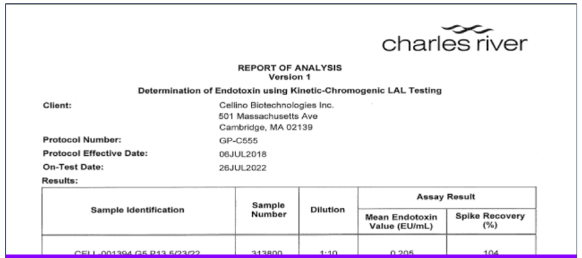


Mycoplasma Assay Report
PCR-based assay performed by WiCell
Mariangela Amenduni, Cellino Biotech
27May22

Sample Name	Result	Interpretati
CELL-001421:E8 p14 (92216)	Negative	Band was not seen at 270bp, indicating the
CELL-001394:G5 p14 (92215)	Negative	Band was not seen at 270bp, indicating the
Positive (+) Control	Positive	
Negative (-) Control	Negative	

Assay Description
Sample is tested for presence of mycoplasma using EZ-PCR™ Mycoplasma Detection Kit

mycoplasma



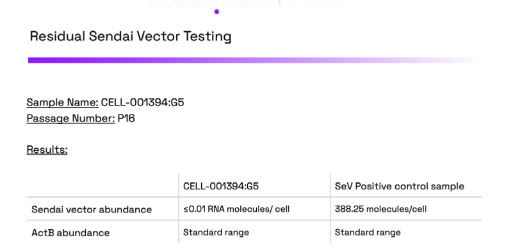
charles river

REPORT OF ANALYSIS
Version 1
Determination of Endotoxin using Kinetic-Chromogenic LAL Testing

Client: Cellino Biotechnologies Inc.
501 Massachusetts Ave
Cambridge, MA 02139
Protocol Number: GP-C555
Protocol Effective Date: 06JUL2018
On-Test Date: 26JUL2022
Results:

Sample Identification	Sample Number	Dilution	Assay Result	
			Mean Endotoxin Value (EU/mL)	Spike Recovery (%)
CELL-001394 G5 P13 5/23/22	313800	1:10	0.205	104

endotoxin



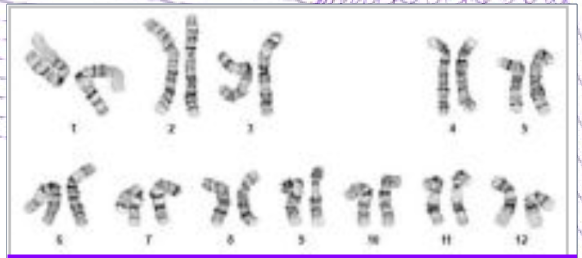
Residual Sendai Vector Testing

Sample Name: CELL-001394:G5
Passage Number: P16

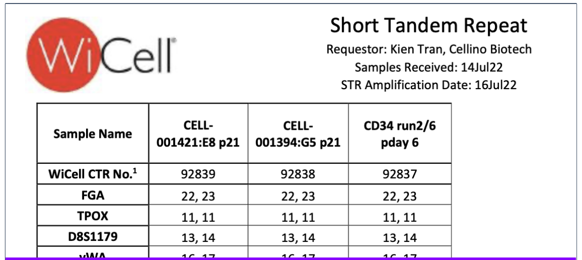
Results:

	CELL-001394:G5	SeV Positive control sample
Sendai vector abundance	≤0.01 RNA molecules/ cell	388.25 molecules/cell
ActB abundance	Standard range	Standard range

residual vector testing



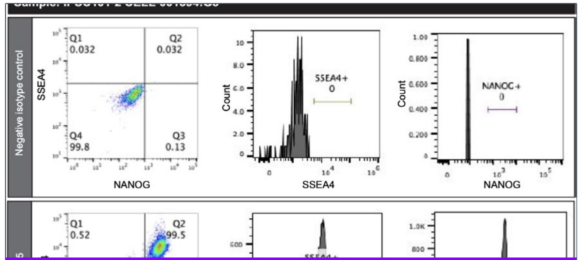
karyotyping g -banding



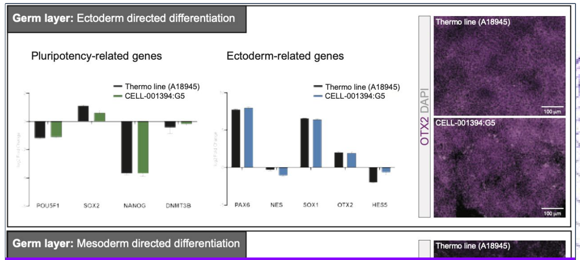
Short Tandem Repeat
Requestor: Kien Tran, Cellino Biotech
Samples Received: 14Jul22
STR Amplification Date: 16Jul22

Sample Name	CELL-001421:E8 p21	CELL-001394:G5 p21	CD34 run2/6 pday 6
WiCell CTR No.[1]	92839	92838	92837
FGA	22, 23	22, 23	22, 23
TPOX	11, 11	11, 11	11, 11
D8S1179	13, 14	13, 14	13, 14
vWA	16, 17	16, 17	16, 17

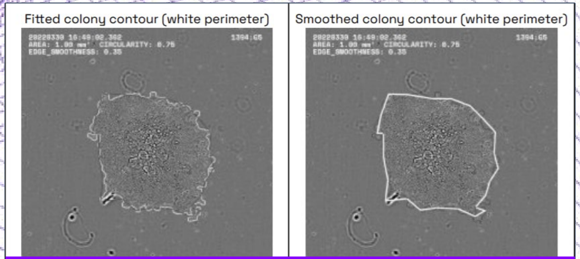
short tandem repeats (STR)



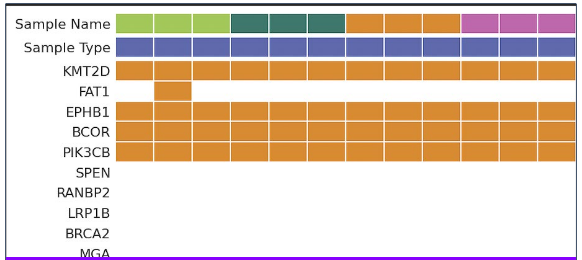
flow cytometry



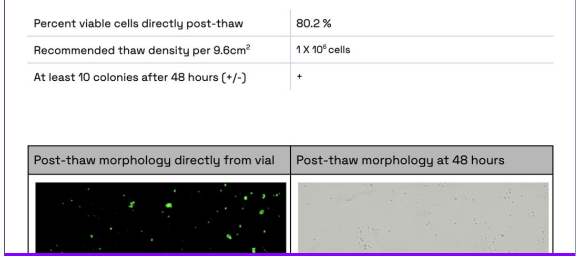
trilineage differentiation



colony morphology metrics



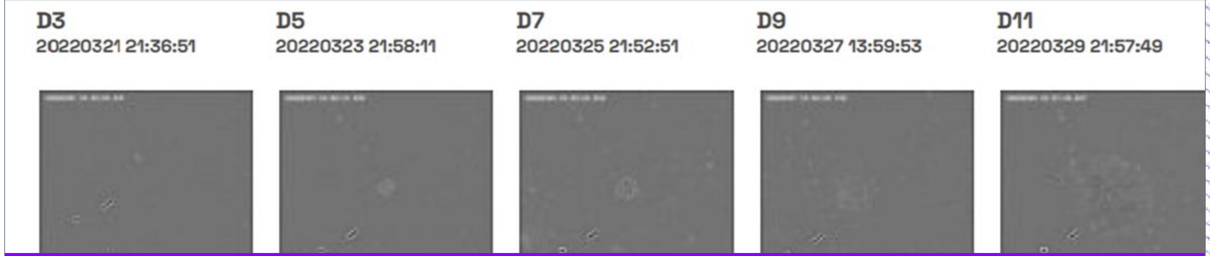
whole exome sequencing



Percent viable cells directly post-thaw	80.2 %
Recommended thaw density per $9.6 cm^2$	1×10^6 cells
At least 10 colonies after 48 hours (+/-)	+

Post-thaw morphology directly from vial	Post-thaw morphology at 48 hours

viability

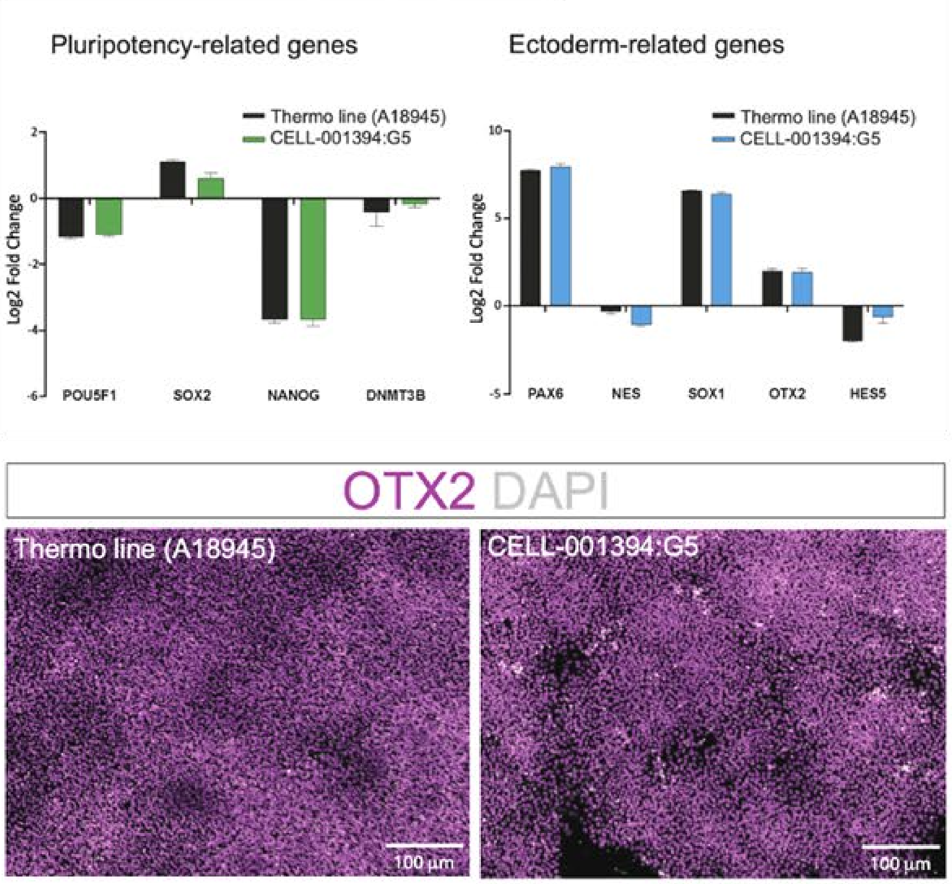


time -series image of colony growth

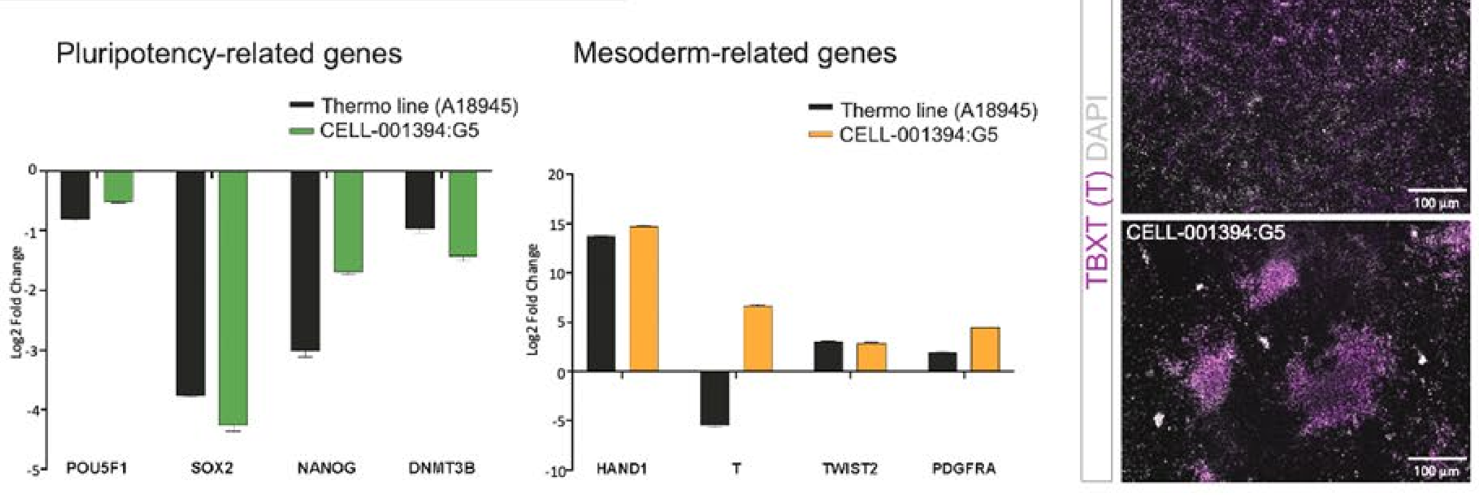
3. What is new about your approach? Why do you think you can be successful at this time?

We collected extensive data on iPSCs generated with Cellino bioprocess (clonal selection)

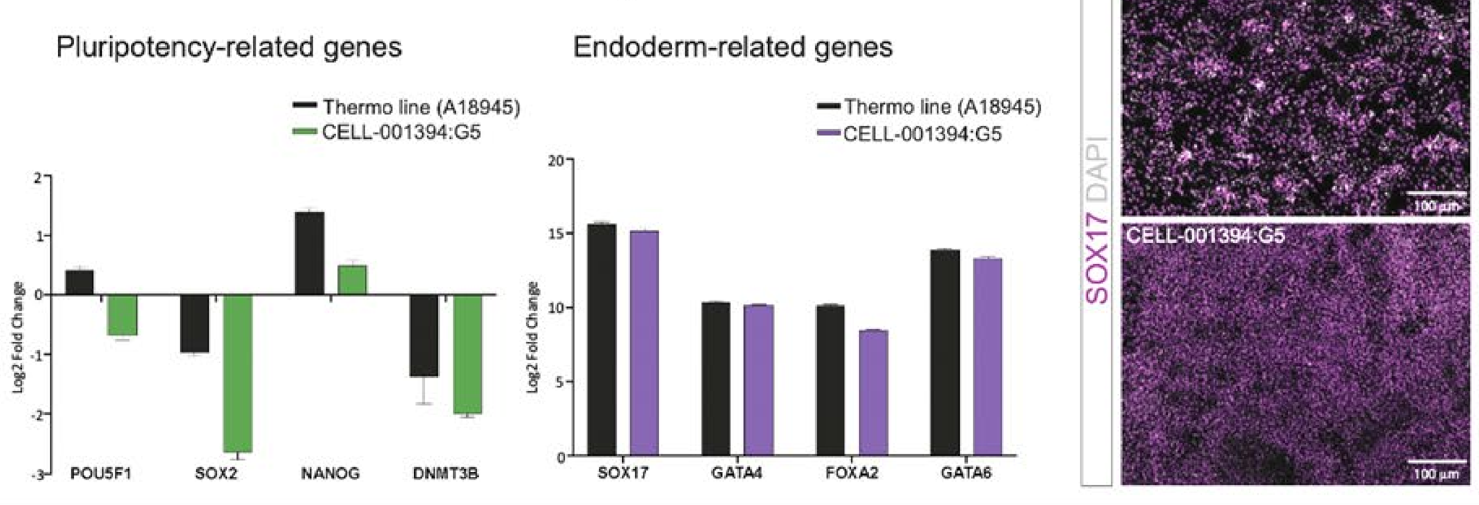
Germ layer: Ectoderm directed differentiation



Germ layer: Mesoderm directed differentiation



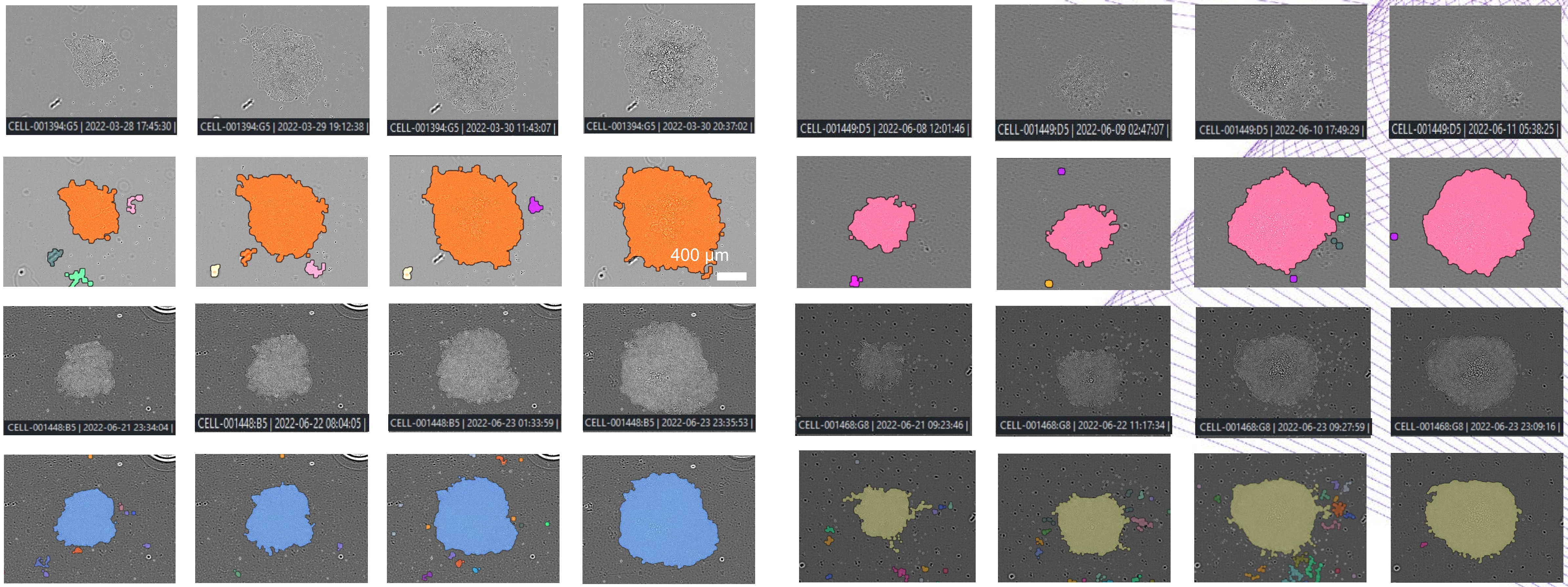
Germ layer: Endoderm directed differentiation



3. What is new about your approach? Why do you think you can be successful at this time?

AI infrastructure in the cloud to track cells and run processes autonomously

Time-series tracking images of 4 iPSC colonies from automated reprogramming process



cellino

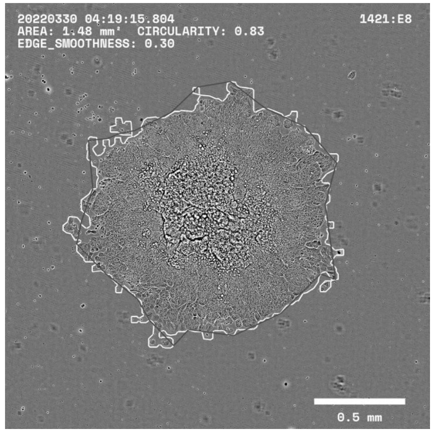
How did you develop the algorithm to predict colony selection?

We developed a cluster ranking algorithm to autonomize the selection of iPSC colonies (typically done by an SME)

What we did: We build an initial model that can, based on images of a colony, predict whether a colony would have been selected by a subject matter expert (SME) for downstream processing. **How we did this:**

1 Collect image training data

- 112 96-wells
- 3284 colonies total; 57 colonies selected by SMEs
- metrics: area, edge smoothness, density, circularity, perimeter, position w/in well, etc.



2 Determine correlation between image-able features and whether a colony is selected by an SME

$$r_{pb} = \frac{(\bar{y}_1 - \bar{y}_2).\sqrt{pq}}{s_y}$$

Point-biserial correlation

Used to correlate continuous features (eg area) w/ binary outcome (eg selected or not)

3 Build probability model to predict, based on imageable features, which colony is likely to be selected by an SME

$$P(selected|features) = \frac{P(features|selected)P(selected)}{P(features)}$$

Bayes rule

Used to calculate probability of colony being selected based on morphological features

4 Test model on images of colonies

Testing: tested on a different batch run, w/ different donor material:

86% of time, **model's #1 choice matched SME**

Cellino ML Models - Dataset Scales

Model name	4X Confluence Model	4X Density Model	4X Pluripotency Model	Cluster Ranking Model
Signal used for extraction of "Ground Truth"	Calcein AM Live Stain	Genetically encoded nuclear fluorescence (EGFP tag on endogenous HIST1H2BJ protein)	Calcein Red-Orange AM Live Stain + Alkaline Phosphatase (AP) Live Stain	Colony object predictions
Contents of 1 datapoint	1 4X BRT image + 1 4X Confluence Map	1 4X BRT image + 1 4X Density Map (Derived from Nuc Segmentation)	1 4X BRT image + 1 4X Pluripotent cell map	Colony features (density, edge smoothness) + SME-based selection labels
Number of available raw (full well) datapoints	59	137	75	171
Number of (full well) datapoints used for training current version	44	27	N/A (training in progress)	112
Number of cell lines covered in dataset	3	1	3	2
Names of cell lines used	A18945 (ThermoFisher Episomal line); AICS61 (Allen Institute Sendai Line w/ Nuc. EGFP); PBMC-derived iPSC Line (Cellino In-House, new)	AICS61 (Allen Institute Sendai Line w/ Nuc. EGFP)	A18945 (ThermoFisher Episomal line); AICS61 (Allen Institute Sendai Line w/ Nuc. EGFP); PBMC-derived iPSC Line (Cellino In-House)	2X CD34+ Cell-derived iPSC clones (Cellino In-House)

Cellino ML Models - Performance Metrics

Model Name	**4X Confluence Model**		**4X Cell Density Model (In Progress)**		**Colony Ranking Model**	
Evaluation Metric	**Precision**	**Recall**	**Pearson's r**	**Classification Accuracy**	**Mean Reciprocal Rank**	**% Match to Expert Selection**
Explanation	Fraction of true positives over predicted positives (i.e. how good is the model in only detecting true cells?)	Fraction of true positives over all positives (i.e. how good is the model in detecting all true cells?)	Strength of linear relationship between true and predicted local cell density (between -1 to 1)	Fraction of correct region classifications as empty, normal density, or high density	Average of reciprocal of predicted rank of SME-selected colony (1 for perfect model, lower for worse performance)	Percentage of cases where model also selects SME-selected colony
Average value for test dataset	%98.98	%95.24	0.9-0.95	70-87%	0.9	%83.3