



Development of a Protocol and Machine Learning Algorithms for the Derivation of Autologous Human iPSC on an Automated Platform to Enable Clinical Semi-autonomous Manufacturing

William Ansari¹, Thorsten Gorba¹, Ha Tran¹, Cullen Pivaroff¹, Sean McCann¹, Natalie Maloney¹, Caroline Johnson², Derren Barken¹, Michael Pesavento¹, Ethan Xu¹, Uyen Nguyen¹, Andres Bratt-Leal¹, Kim Raineri¹
1 – Aspen Neuroscience, Inc. 2 – Cell X Technologies, Inc.

Introduction

Aspen Neuroscience is developing autologous induced-pluripotent stem cell (iPSC) derived cell therapies to address diseases with high unmet medical need, beginning with dopaminergic neuron replacement for both sporadic and genetic forms of Parkinson's disease.

A core element of our cell therapy platform is the robust and regular derivation of high quality iPSCs. However, the process of reprogramming iPSCs from somatic cells is historically a slow, labor intensive, and subjective process due to dependence on the operator's skill. As a result, a manual process for iPSC production poses a hurdle for scalability to the commercial market, thus limiting the accessibility of iPSC derived therapies to a broader patient population. To address these issues, we are developing an automated process and control algorithms including computer vision systems to reprogram fibroblasts into iPSCs. The iPSCs produced via automation exhibit characteristics comparable to those derived manually, as assessed by our stringent quality control (QC) criteria. The results demonstrate that automated reprogramming is reproducible and scalable, capable of meeting future projected commercial demand.

Fibroblasts from 3 different donors were transduced with non-integrating reprogramming reagents. The automated platform was used to perform cleaning around emerging colonies to remove the fibroblasts and isolate transduced cells. Once colonies reached an adequate size, they were picked and transferred to new culture wells by the instrument. The iPSC clones were subsequently expanded with a combination of positive selection passaging and bulk passaging and then harvested. The iPSCs derived from the automated process met all QC criteria, including cell viability; expression of pluripotent cell markers by flow cytometry; residual reprogramming agent clearance, and were indistinguishable from the control iPSCs derived from the manual process. This study demonstrates the feasibility of automated reprogramming, which can facilitate the large-scale production of autologous iPSCs, thereby potentially providing treatments to a global population of patients with Parkinson's disease in the future.

Automated Ranking, Selection, and Cleaning of Colonies

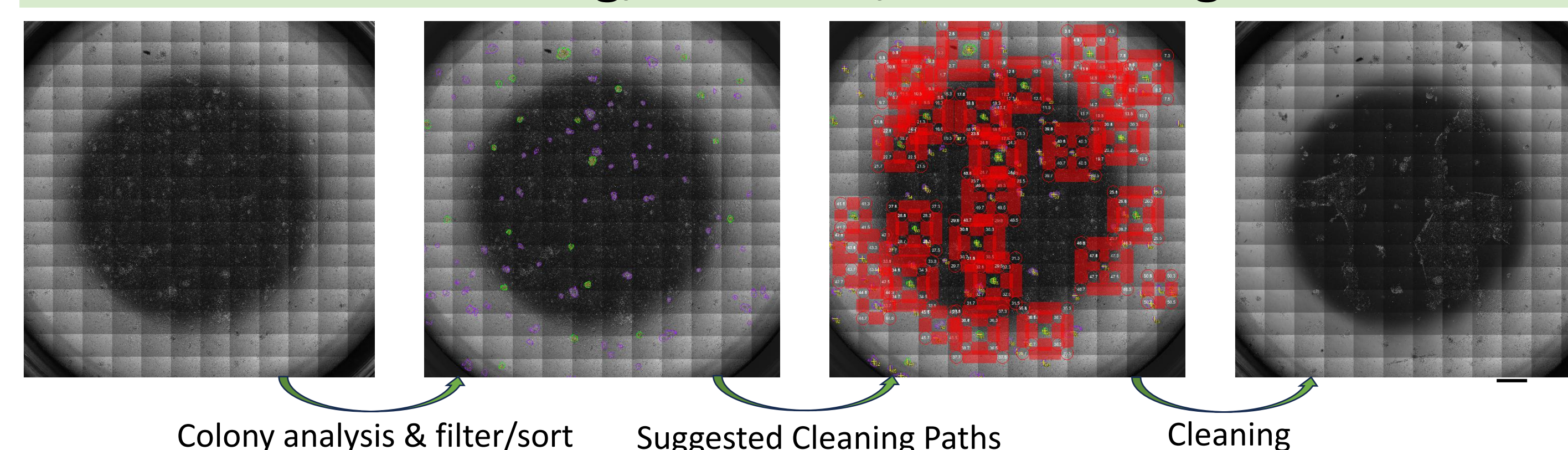


Fig 2: Colony segmentation and selection following addition of reprogramming reagent to fibroblast culture. Green outlines show colonies that pass the selection criteria, while purple have failed due to morphology or proximity to higher ranked colonies. Red boxes are computed cleaning paths to remove fibroblasts from around selected colonies.

iPSC Clone Establishment Picking

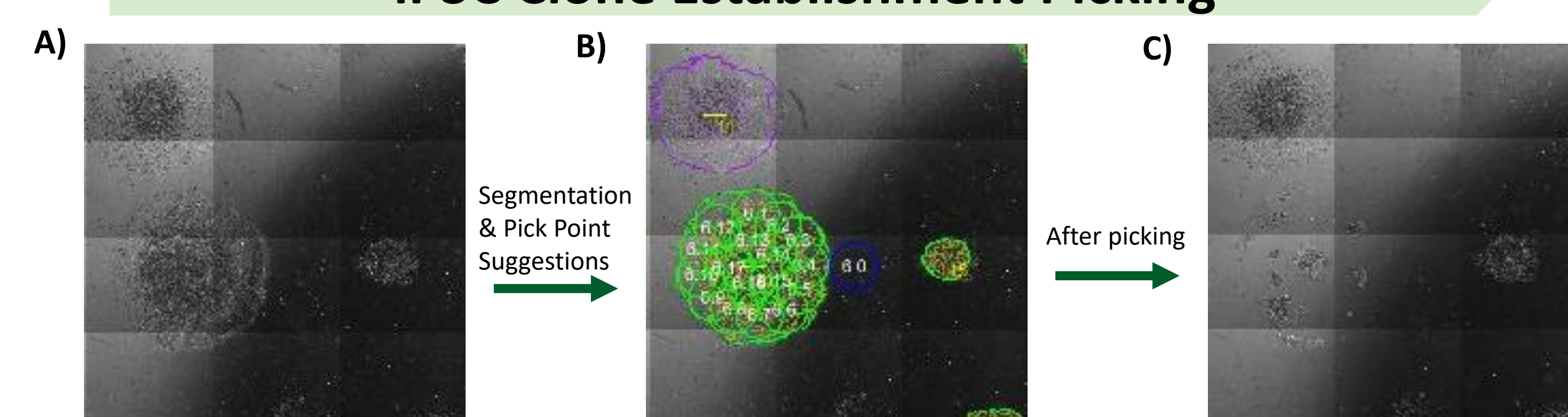


Fig 3: A) After cleaning, colonies are imaged a few days later to assess readiness for picking. B) Colony segmentation and filter/sort algorithm are employed to provide pick point suggestions on colonies meeting parameters (green circles). C) After aspiration picking, the selected colonies have been removed from the source plate and established as independent clones.

Celligent™ Cell Culture Automation Platform

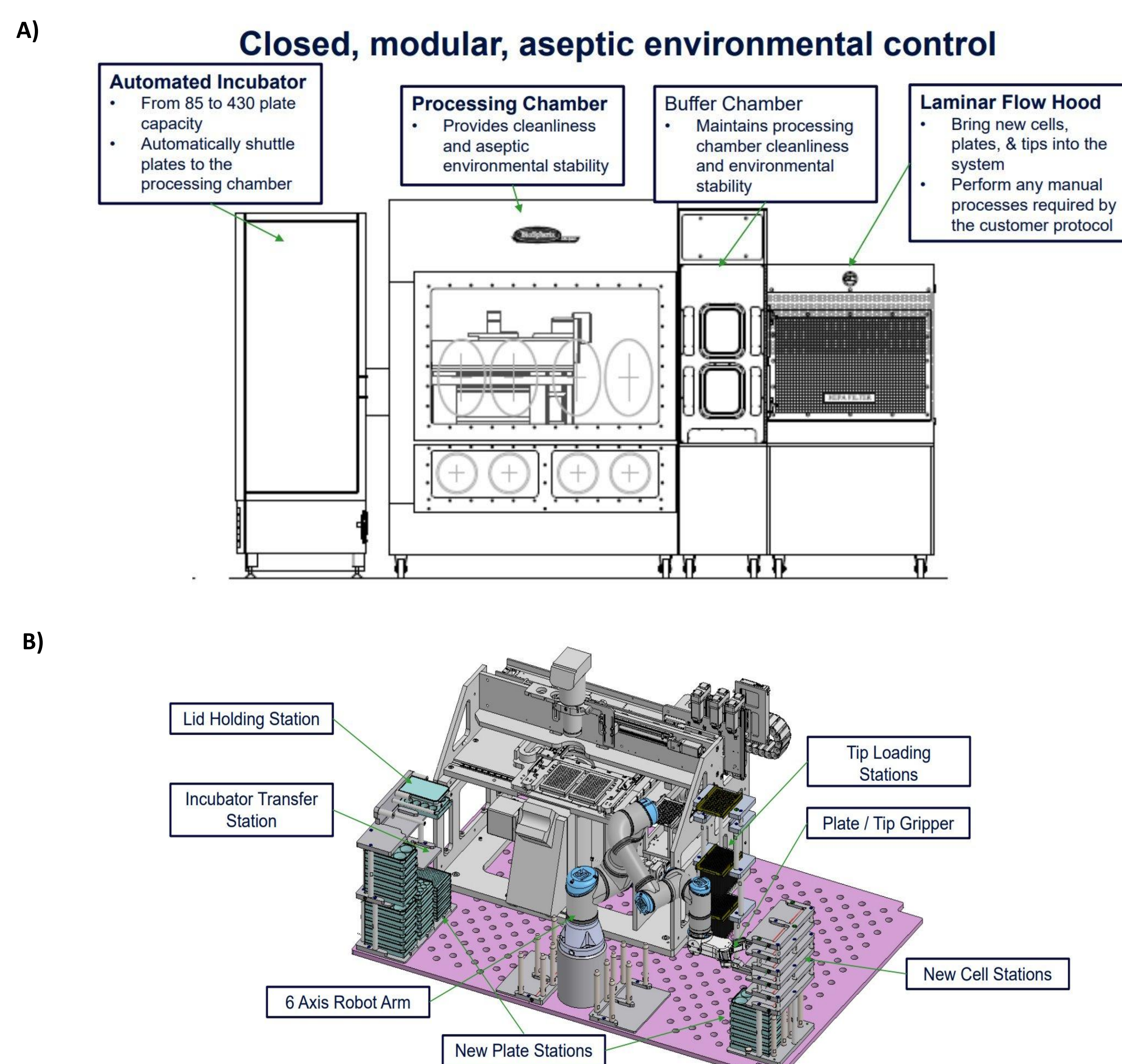


Fig 1. The Celligent™ Platform was used for automated generation of iPSCs from dermal fibroblasts. A) Robotic automated platform including laminar flow hood, passthroughs, isolation chamber, and automated incubator. B) Celligent core processing unit with robotic arm.

Ranking of Established iPSC Clones with Aspen Vision

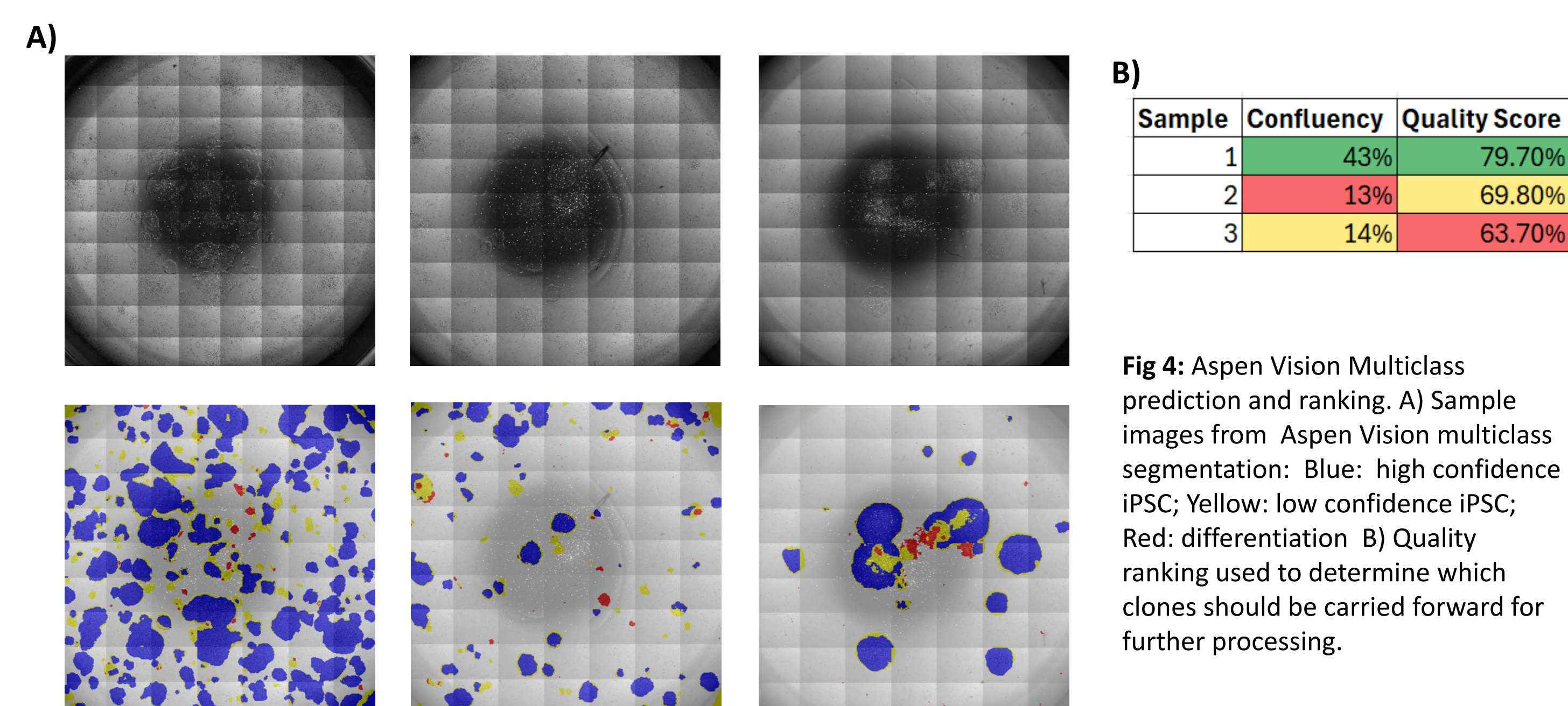


Fig 4: Aspen Vision Multiclass prediction and ranking. A) Sample images from Aspen Vision multiclass segmentation: Blue: high confidence iPSC; Yellow: low confidence iPSC; Red: differentiation. B) Quality ranking used to determine which clones should be carried forward for further processing.

Selective iPSC Enrichment with Aspen Vision

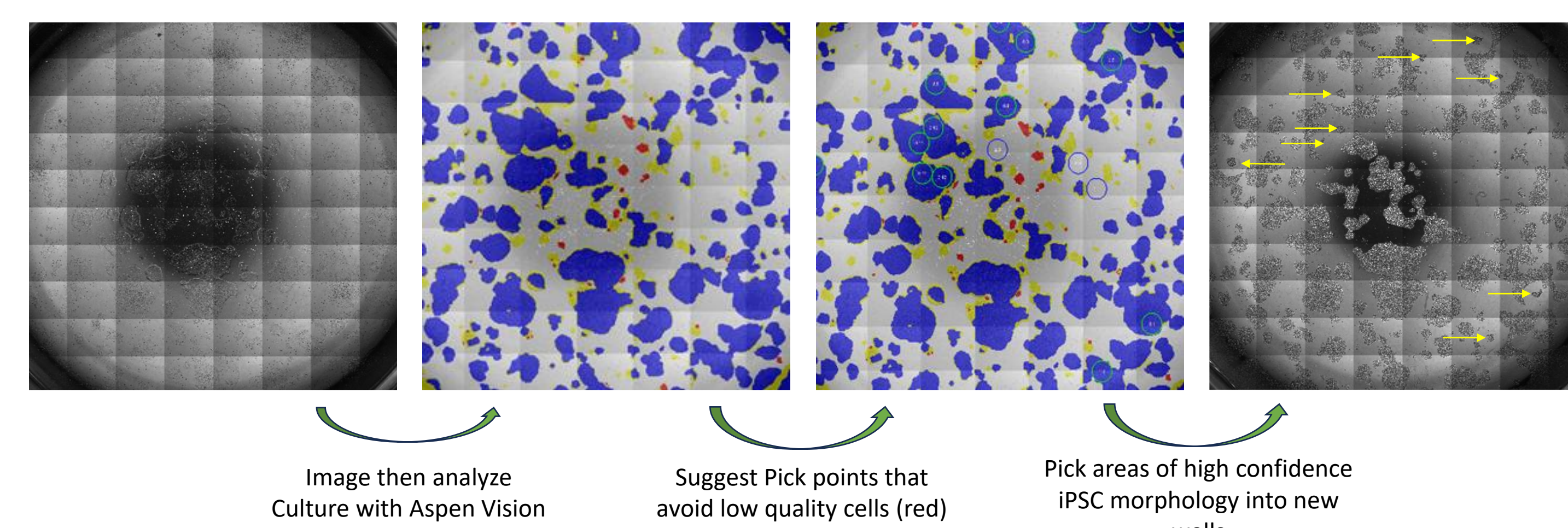


Fig 5. Positive Selection Picking with Aspen Vision. Aspen Vision used in conjunction with Celligent™ platform software to automate placement of pick points in areas of high confidence iPSCs. Yellow arrows show regions where cells have been removed after aspiration picking has completed.

Aspen Vision Post Hoc Analysis

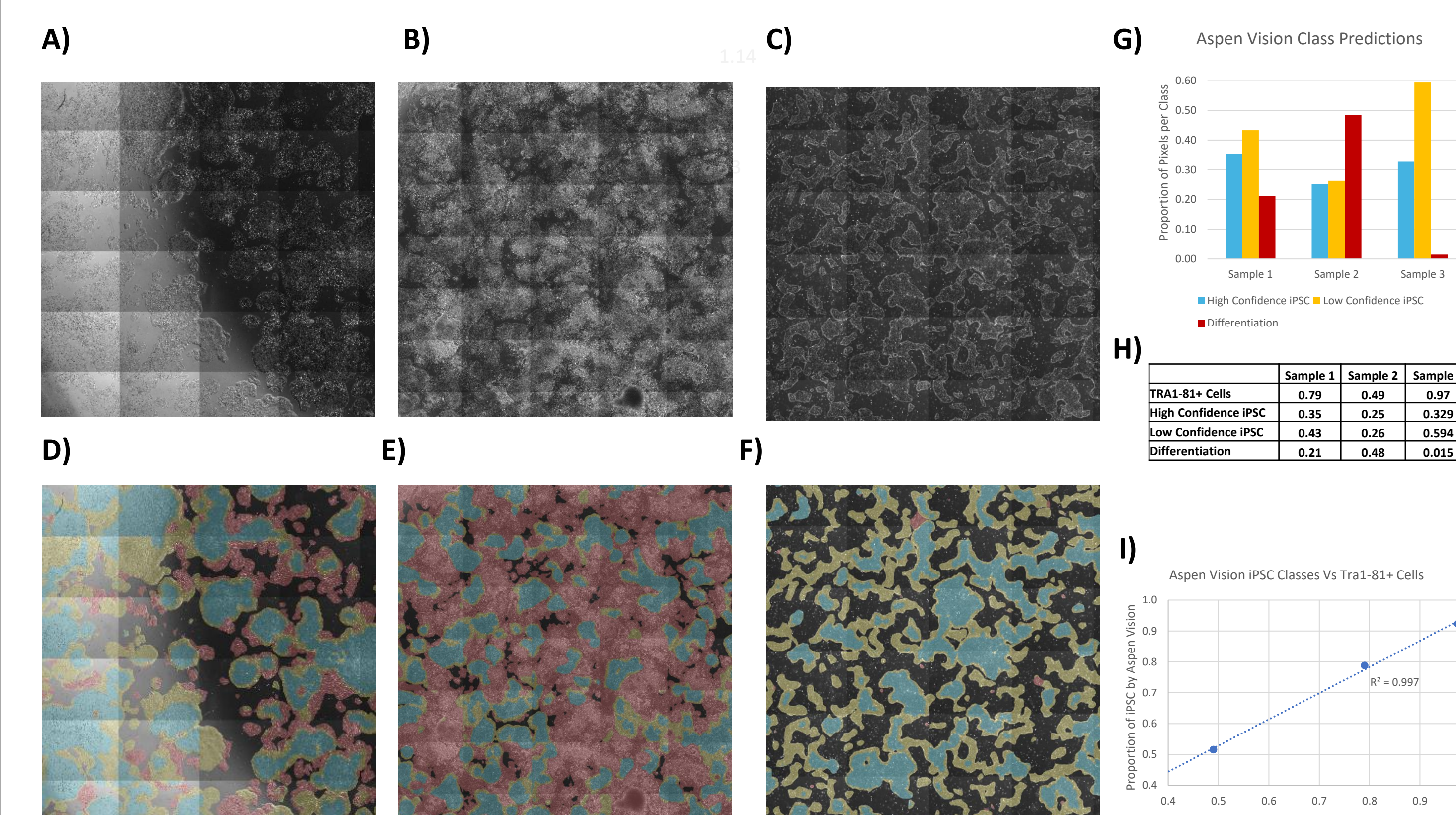


Fig 6: A-C) Example images from multiclass segmentation model: Blue: high confidence iPSCs; Yellow: low confidence iPSCs; Red: differentiation. D-F) Corresponding Aspen Vision segmentation maps. G) Proportion of various classes in images at left (A, B and C correspond to Samples 1-3, respectively). H) Proportion of Tra1-81+ cells alongside class proportion. I) Plot of proportion of Tra1-81+ cells by flow cytometry compared to proportion of iPSC classes by Aspen Vision segmentation (sum of high and low confidence iPSC classes)

iPSCs from 3 Donors Passed Quality Control Assays

- 43 of 44 Clones assayed from 3 donor cell lines passed QC Flow Cytometry and residual reprogramming reagent release specifications
- 8 of the 43 passing clones were selected for further bioinformatic analysis. 8 of 8 device-derived clones passed PluriTest and whole genome sequencing specifications at the cryopreservation stage

Cell line	Condition	Viability	OCT3/4+	TRA1-81+	Residual Reprogramming Reagents	Pluritest
PD1-1	Automated	Pass	Pass	Pass	Pass	Pass
PD1-2	Automated	Pass	Pass	Pass	Pass	Pass
PD2-1	Automated	Pass	Pass	Pass	Pass	Pass
PD2-2	Automated	Pass	Pass	Pass	Pass	Pass
PD2-3	Automated	Pass	Pass	Pass	Pass	Pass
PD3-1	Automated	Pass	Pass	Pass	Pass	Pass
PD3-2	Automated	Pass	Pass	Pass	Pass	Pass
PD3-3	Automated	Pass	Pass	Pass	Pass	Pass
PD2-10	Manual	Pass	Pass	Pass	Pass	Not run
PD2-11	Manual	Pass	Pass	Pass	Pass	Not run
PD2-12	Manual	Pass	Pass	Pass	Pass	Not run

Conclusions

- We have adapted cell culture techniques to a robotic platform that is capable of isolating emergent iPSC clones as well as passaging, enriching pluripotency, and expanding newly derived cell lines.
- Development of computer vision systems to automate the process has succeeded and correlates well to flow cytometry data, showing promise for automating the production of high quality iPSCs in autologous settings.
- Computer vision algorithms can drive the process autonomously and rank the quality of robotically derived cell lines to ensure that the best cells are used for downstream manufacturing.

Methods

Fibroblasts from three different donors were reprogrammed using a non-integrating method and cultured on the Celligent™ platform. Automated weeding of residual fibroblasts around emerging iPSC colonies was performed, followed by automated positive selection and transfer to a new culture vessel. The platform also conducted automated feeding and passaging of the clonally derived iPSC lines. Images from initial reprogramming runs were used to train computer vision models and later the trained models deployed as shown here to drive the process semi-autonomously.

Acknowledgements

- Aspen Neuroscience's Technology Development, R&D, Process Development, Bioinformatics, QC, and Assay Development teams
- Cell X Technologies, Inc.