



Potency Assay Matrix for Dopaminergic Neuron Precursor Cell Replacement Therapy for Parkinson's Disease

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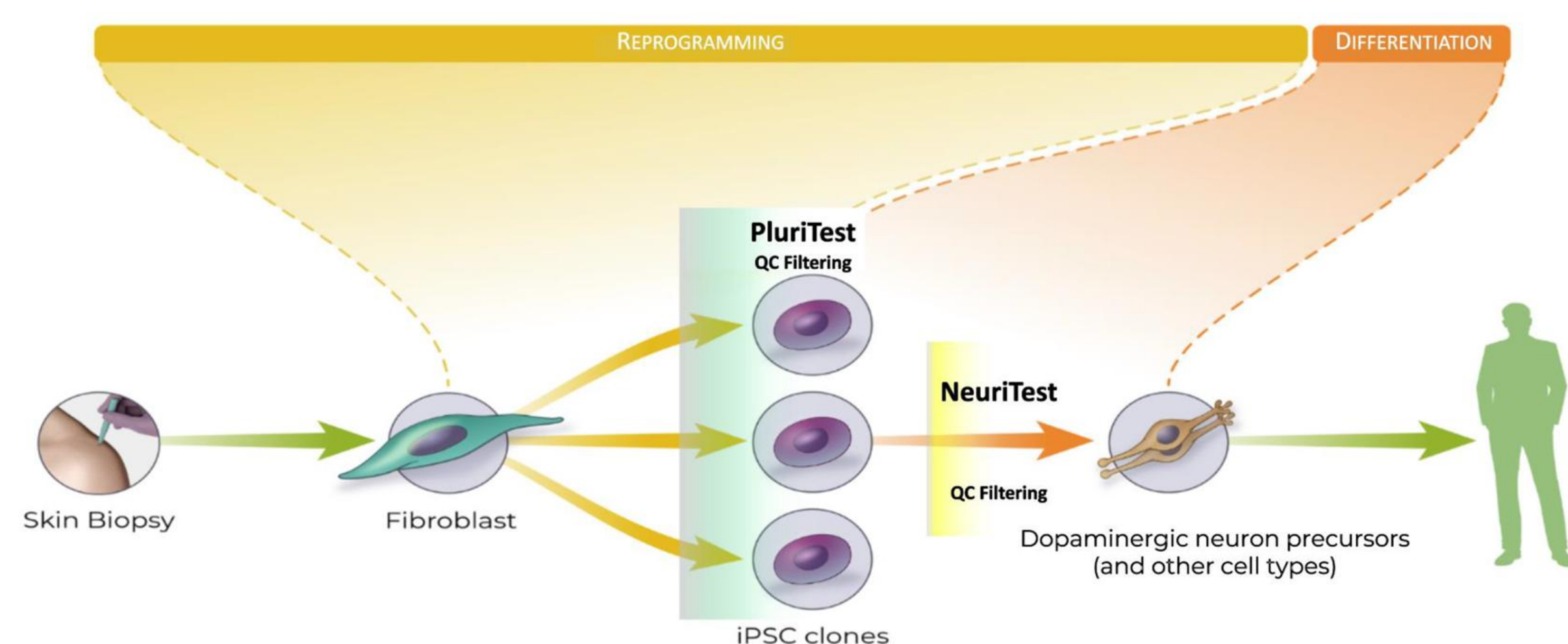
Introduction

Induced pluripotent stem cell (iPSC) derived therapies with a complex spectrum of potential mechanisms of action require functional characterization that can quantitatively and accurately characterize the various important functions of these cells following administration. As we advance an autologous iPSC-derived dopaminergic neuron precursor cell (DANPC) therapy for Parkinson's Disease into clinical trials, a novel suite of tests was developed to address this challenge.

Here, we show that a set of bioinformatics tests performed on bulk RNA sequencing (RNAseq) data of DANPCs can provide predictive analytics for the cell therapy's optimal maturity/identity, engraftment potential, and dopamine production potential. Furthermore, dopa decarboxylase (DDC) was identified as an enzyme that is critical to the production of dopamine and is highly expressed in DANPCs (1). A DDC enzymatic activity assay was developed to assess the post-thaw function of DANPCs. This stability-indicating, conventional, enzymatic bioassay of DANPC functionality in combination with the predictive bulk RNAseq-based bioinformatics tests provides a robust potency assay matrix strategy for Parkinson's Disease cell replacement therapy.

Product and Objectives

Parkinson's Disease leads to debilitating motor complications and currently no restorative treatments are available. By the time of diagnosis, patients have already experienced significant nigrostriatal degeneration, and cell replacement therapies aim to restore lost dopamine producing neurons. Aspen Neuroscience is advancing an autologous iPSC-derived cell replacement therapy for Parkinson's Disease; reprogramming the patient's own cells to generate iPSCs and then differentiating those iPSCs into DANPCs for transplant.



Since functional characterization of dopaminergic neurons often requires significant additional maturation time *in vitro* and *in vivo*, models predicting eventual functionality can be built utilizing bulk RNAseq data, though a significant volume of high-quality training data is required. Three predictive models were developed: NeuTest to assay cell identity and maturity, GraftTest to predict engraftment trained on *in vivo* data, and DopaTest to predict dopamine release trained on *in vitro* data. Additionally, recent guidance recommends a bioassay of a cell therapy's mechanistically relevant function, necessitating characterization beyond RNA sequencing. DDC was identified as an enzyme that is highly expressed in the drug product and converts L-dihydroxyphenylalanine (L-DOPA) to dopamine. A bioassay measuring DANPC DDC activity was developed as a mechanistically relevant, stability sensitive bioassay for use as part of a product potency characterization strategy.

Methods

Generation of DANPCs: For multiple donors with PD diagnosis, dermal fibroblasts were converted into iPSCs. Multiple clones from each donor were then successfully reprogrammed into DANPCs following Aspen's proprietary protocol. RNAseq analysis on DANPCs

RNAseq analysis on DANPCs: A sample of each DANPC lot was used for RNA extraction, cDNA synthesis, amplification, bulk RNA sequencing using T50bp PE on an Illumina platform.

Generation of Engraftment Dataset: A cell suspension of DANPCs was transplanted into the striatum of RNU rats. Brain tissues were collected following cell engraftment and stored until processing. Brain samples were sectioned and imaged. 20X images were screened for high quality resolution and were imported into analysis software. The number of surviving cells were evaluated by the total count of human nuclei in each graft using an image analysis tool developed to perform nuclear segmentation and automatic counts of nuclei for each striatal hemisphere.

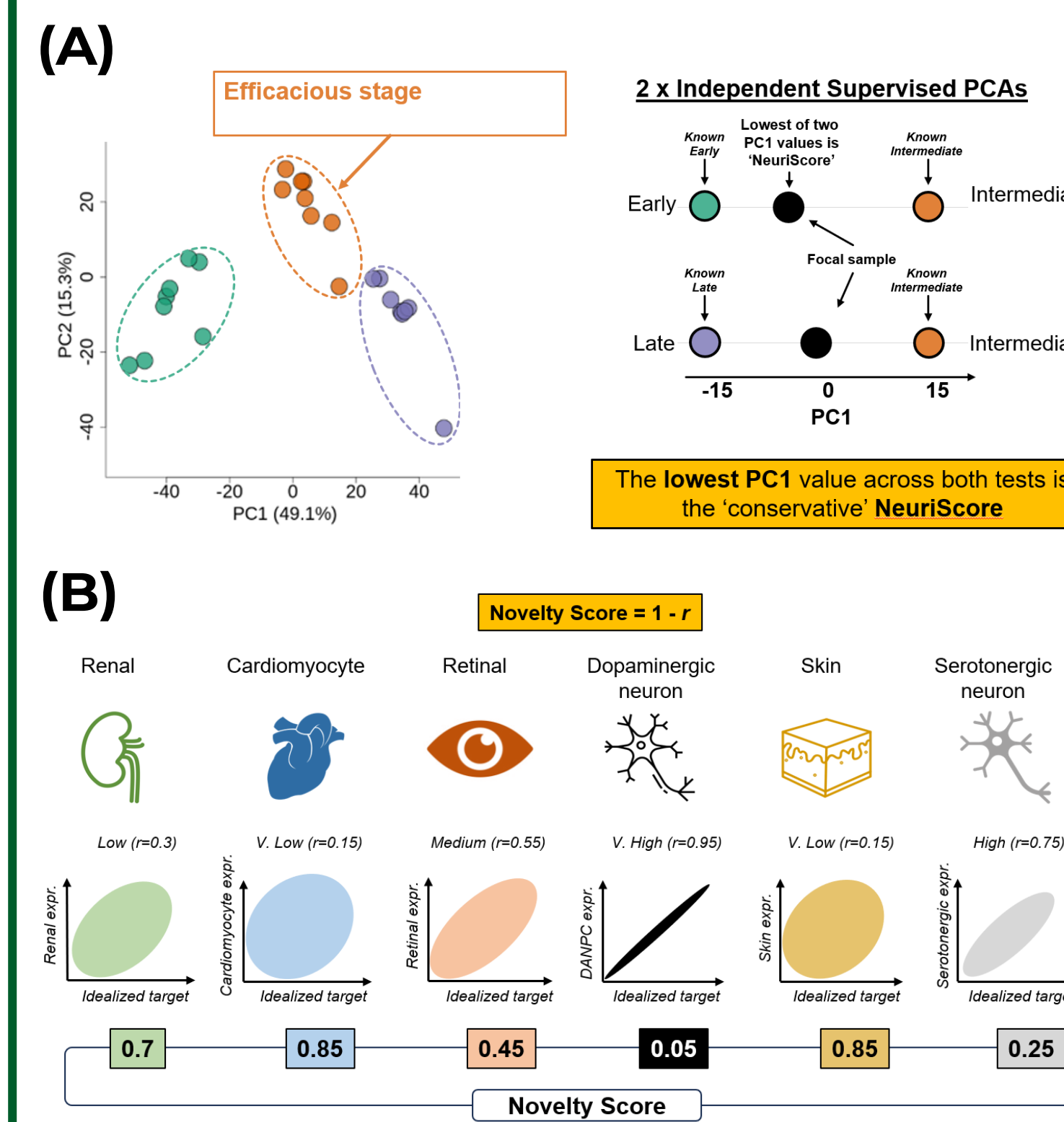
Generation of Dopamine Release Dataset: DANPCs were thawed and plated for extended in vitro maturation. After extended maturation, cell culture media was removed and buffer containing KCL or a buffer alone was applied to the cells. Supernatant was removed, placed in stability buffer, and frozen prior to analysis. Supernatant was assayed by LC-MS/MS for dopamine released in response to KCL stimulation.

Bioinformatic analysis: For each DANPC cell lot, whole transcriptome RNAseq data was used in multiple in silico-based assays. Each cell lot is confirmed to have passed NeuTestTM. Other complementary prediction tools of engraftment and dopamine production were based on RNAseq, histology data, and dopamine release data.

DDC Bioassay: DANPCs were thawed and rested to recover and adhere to the plate. On the day of the assay, cell culture media was removed and cells were washed. Buffer containing L-DOPA was added to each well and after a short incubation, incubation media was collected and added stabilization buffer. Samples analyzed by LC-MS/MS for dopamine quantification.

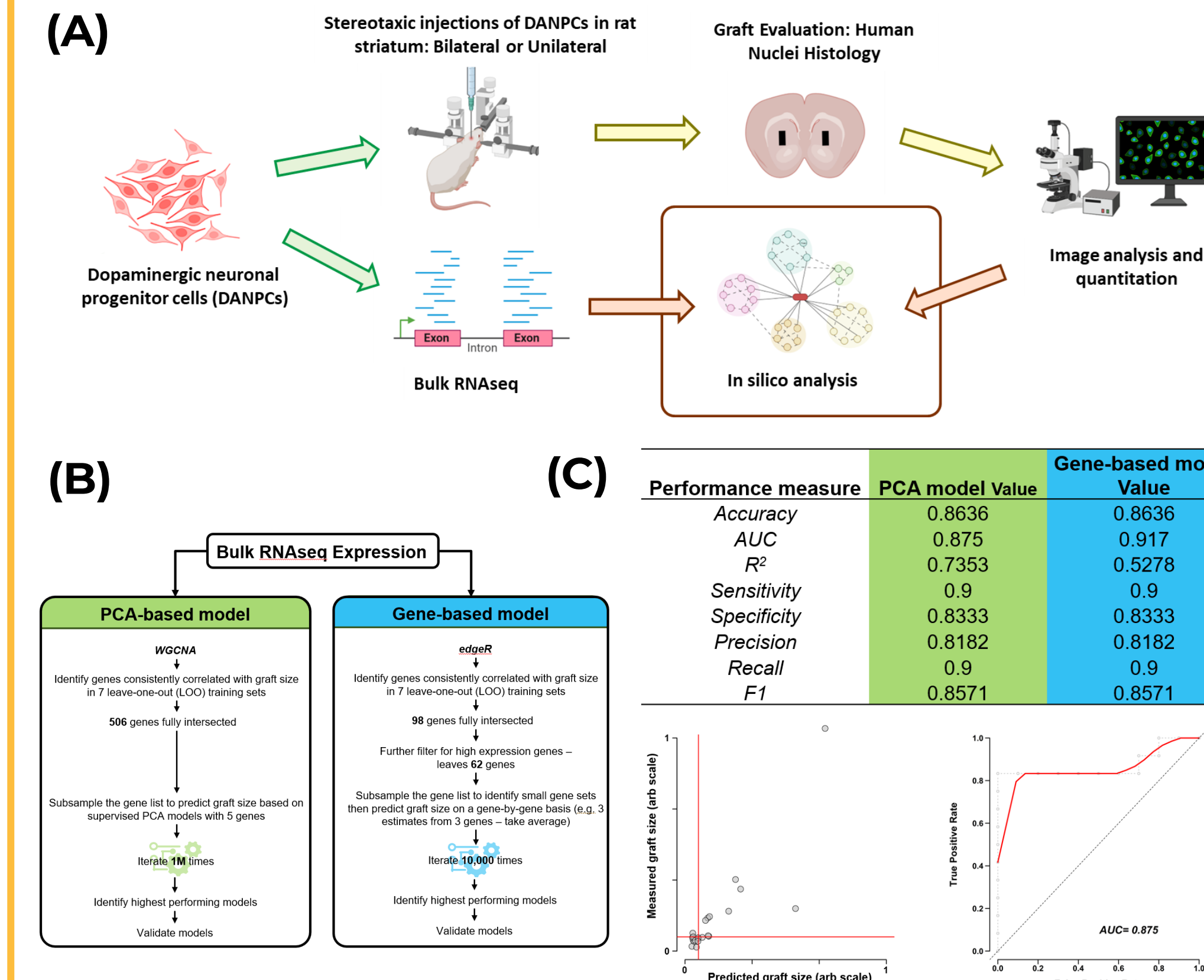
Predictive Models of Identity, Maturity, Engraftment, and Dopamine Release

NeuTest



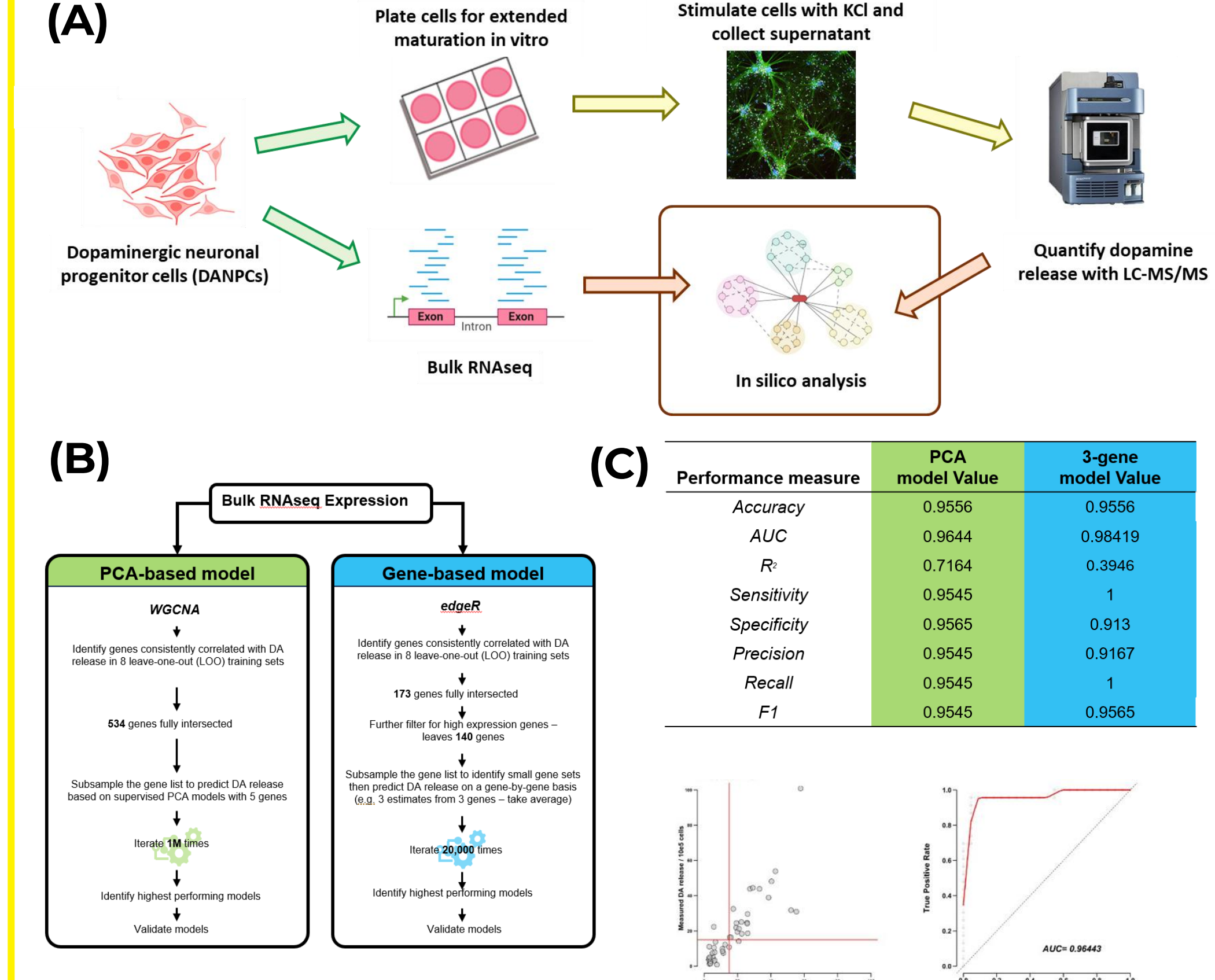
NeuTest™ is a predictive 2-score model trained on bulk RNAseq data from efficacious samples in animal experiments to target optimal DANPC identity and maturity. (A) The first score is to assess the maturity of the DANPC sample (NeuScore), in which we identified genes with differential expression between DANPCs of different maturity. The lowest PC1 value, indicating the greatest similarity to either the "Early" or "Late" state, across both tests is used as the NeuScore, ensuring the most conservative result is reported. (B) The second is the global, transcriptome wide similarity of the sample to an idealized DANPC sample (Novelty Score). To illustrate the Novelty Score, non-DANPC example cell identities are also shown.

GraftTest



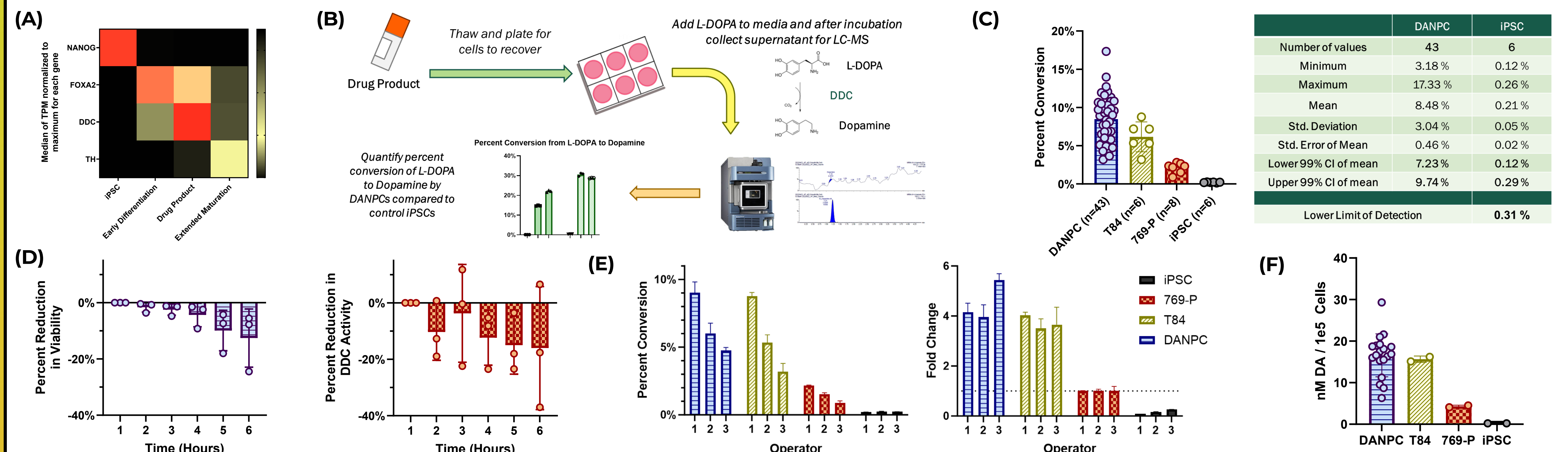
(A) To generate a predictive model of engraftment, DANPCs were transplanted into the striatum of RNU rats, and following engraftment, brains were collected and sectioned for histological analysis of human nuclei content. Graft quantifications were paired with bulk DANPC RNAseq data for in silico analysis and model building. (B) Two main model types were explored, including Supervised Principal Components Analysis (SPCA) and multiple gene-based estimates. (C) In both approaches, genes with inherent correlations with graft size were the features of interest, and the outcome variables were human nuclei counts provided by image analysis. The top ranked models (by Accuracy, then AUC) showed similar performance with a small trade-off between the R2 and AUC measures based on validation on in-house data.

DopaTest



(A) To generate a predictive model of dopamine release, DANPCs were thawed and plated to quantify neurotransmitter release. Dopamine measurements were paired with bulk DANPC RNAseq for in silico analysis and model building. (B) Two main model types were explored, including SPCA and multiple gene-based estimates. In both approaches, genes with inherent correlations with dopamine production were the features of interest, and the outcome variables were in vitro dopamine release. The top ranked models (by Accuracy, then AUC) showed similar performance with a trade-off between the R2 and AUC measures based on validation on in-house data.

DDC Bioassay to Characterize Mechanistically Relevant Function and Cell Stability



(A) Relative gene expression of critical genes at various stages of DANPC maturity, where DDC is significantly elevated in drug product DANPCs. (B) Overview of DDC bioassay; where DDC function is quantified by percent conversion of L-DOPA to dopamine. (C) Data from DANPCs and iPSC negative controls was compiled to characterize the range and sensitivity of the assay. (D) DANPCs were thawed and left in cryoprotectant for up to 6 hours post-thaw. Each hour DANPCs were assayed for viability by propidium iodide staining and plated to assay DDC activity. Reduction in viability over time correlated with reduction in DDC activity (R2 = 0.70 p = 0.04). (E) Bankable, reference control cells (T84 and 769-P) can be used to normalize for inter-operator and inter-assay variability. (F) Dopamine release can also be normalized to cell count, following dissociation and counting of cells after collection of supernatant.

Conclusions

- In silico predictive analytics of bulk RNAseq data can make accurate predictions of DANPC maturity, identity, engraftment potential, and dopamine production potential. These tests can be utilized for robust characterization of DANPC function.
- DDC activity of freshly thawed DANPCs can be assayed as a traditional enzymatic test of functional activity. DANPCs release dopamine at a significant level above iPSC negative controls, DDC activity is a stability sensitive measurement, and can be controlled and normalized to assure assay quality.
- Together, in silico assays and a DDC bioassay provide a robust analytical potency strategy for DANPC functional characterization. By removing the need for extended maturation of cells in vitro prior to characterization, assays can be performed on the drug product itself and still assure a consistent and functional cell therapy product.

Acknowledgements

The authors thank other contributors from Aspen Neuroscience including the R&D, Bioinformatics, Translational, Analytical Development and Process Development departments. Additional support from CIRM, Summit For Stem Cell, Dr. Jeanne Loring and the Loring Lab, and Cardiff University: Mariah Lelos and Rachel Hills.

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