Protocol Development for the Mass Production of hiPSC-Derived SOX6+ A9 Dopaminergic Neurons Using a High Dimensional Design of Experiment Approach

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Objective

Developing an unbiased and data-driven method to systematically approach generation of A9 dopaminergic neurons using *HD-DoE* technology in a reasonable timeframe and budget.

Why A9 Dopaminergic Neurons?

Parkinson's Disease (PD) affected over 8.5 million people in 2019 alone and has the fastest growing population of affected individuals of any neurological disorder (WHO). PD is characterized by the loss of the A9 subset of Dopaminergic Neurons (DN). While efforts have been made using mouse models to identify drugs and test cell therapies involving brain tissue grafts, one major barrier to this has been the lack of available DNs for drug discovery screening

Trailhead Pipeline/Workflow

Optimization at each stage transition to recapitulate in vivo biological transitions via HD-DoE

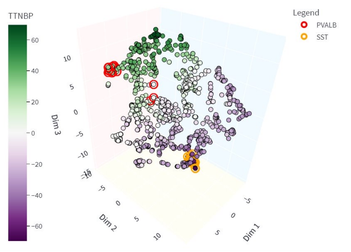
What is *HD-DoE*?

Applying mathematical models to study the developing cell space to understand the behavior of the cells and control their fate. In this approach, a large number of inputs are carefully chosen and tested at once and their individual and combinatorial effects on gene profile of differentiating cells are analyzed. (maybe take some notes from jan's reprocell presentation)

>40x factor assessment compression via mathematical modeling

High throughput, unbiased factor assessment combined with mathematical modeling via HD-DoE yields insight into both the primary effects of individual differentiation factors plus combinatorial effects that are impossible assess otherwise (see jan presentation for better description)

Better terminal cell populations by recapitulating the progression of natural developmental differentiation – only on a faster timescale

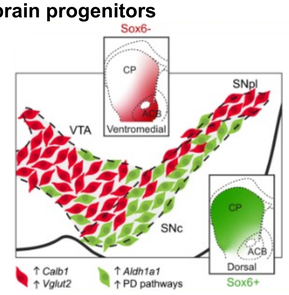


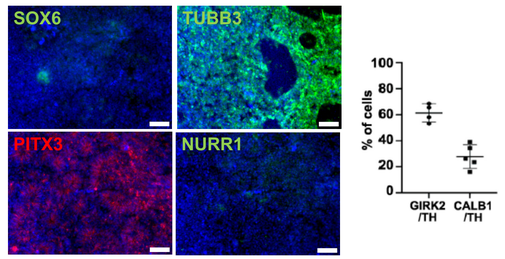
Material & Methods:

Characterization: bulk RNA-seq performed by . Flow cytometry was done on Attune … qPCR was done on QS3 and QS 12K, ThermoFisher

Imaging: Cells are images by EVOS7000 fluorescence microscope and …. Yokogawa microscope on Aurora glass bottom plates.

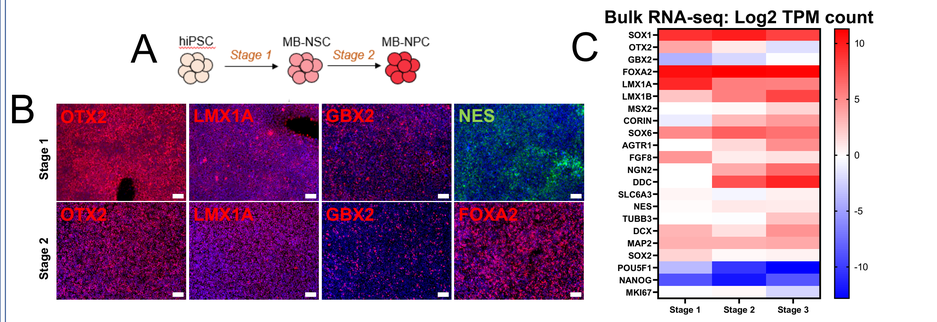
Parkinson's Disease causes degeneration of A9 dopaminergic neurons derived from SOX6+ SNc midbrain progenitors



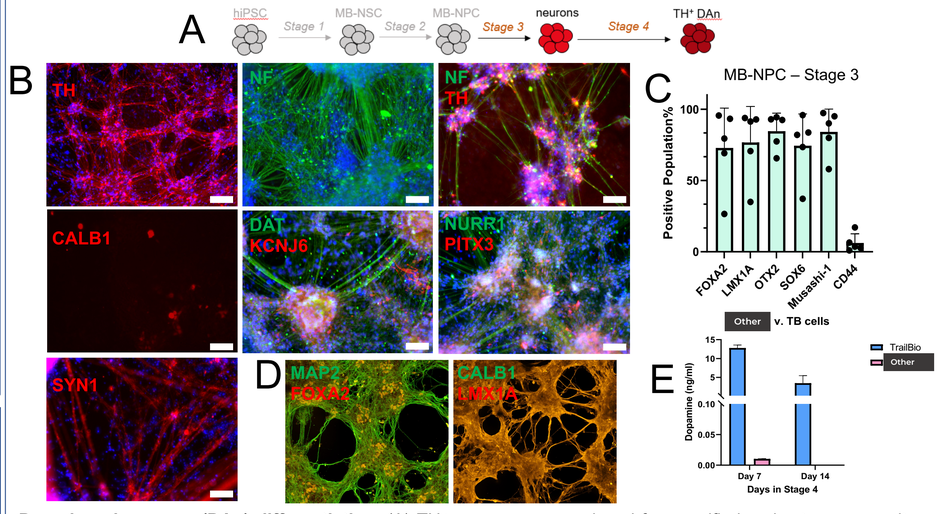


Commercially available neurons do not express the SNc/A9 marker SOX6. A substantial portion of these neurons express the VTA/A10 marker CALB

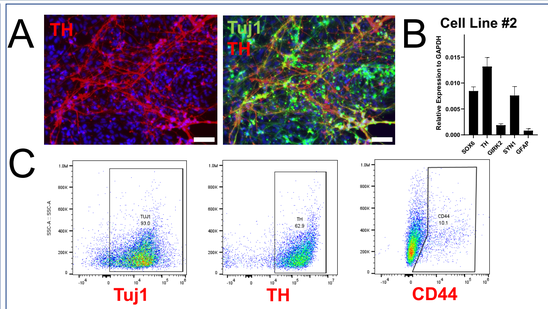
HD-DoE-guided experiments differentiates adherent cells through intermediate steps found in vivo



Early midbrain progenitor verification. (A) Initial differentiation proceeds through midbrain NSC and NPCs (B) ICC indicates expression of multiple MB-NSC and MB-NPC markers. (C) Bulk RNA-seq indicates changing gene expression patterns over differentiation stage with increasing trend in MB neuronal gene expression. Color scale represents log2 fold change of differentially expressed genes of differentiating cells compared to hiPSCs. Scale bars in B = 100um.

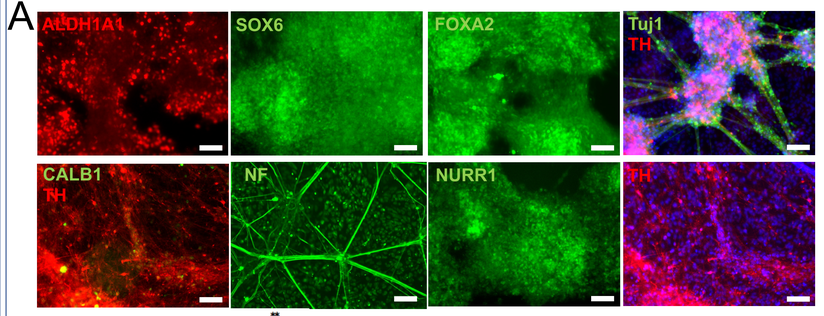


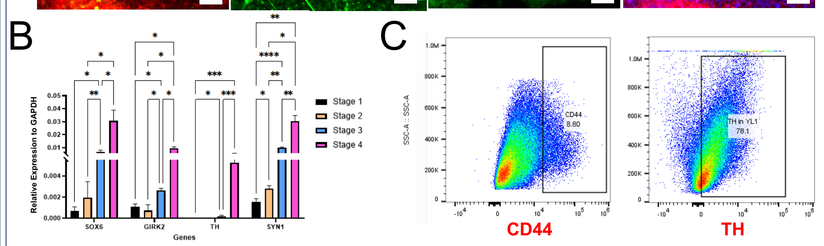
Dopminergic neuron (DAn) differentiation. (A) TH+ neurons are produced from verified early stage progenitors. Expression of DAn and general neuronal and synaptic markers of Stage 3/4 cells are verified via ICC (B, D, Stage 4) and flow cytometry (C, Stage 3). Images in D were taken on Yokagowa scope … (more data). (E) ELISA detection of dopamine is enhanced compared to commercially available neurons. Scale bars in B = 100um, D = um



Protocol robustness: DAn differentiation in additional cell line. HD-DoE-developed protocol produces cells expressing TH and SOX6 with little astrocyte differentiation from a second cell line as indicated via ICC (A), RT-PCR (B), and flow cytometry (C). Scale bars in A = 100um

HD-DoE-developed adherent protocols produce A9 dopaminergic neurons at scale via bioreactor





Adherent-cell-validated HD-DoE protocol successfully produces SOX6+ DAn neurons from bioreactors. iPSCs were differentiated in bioreactors using stage-specific morphogens validated in adherent culture. Cells were then dissociated and used for RT-PCR and flow cytometry. For ICC, cells were plated briefly before labeling. (A) A9-specific DAn markers indicated IHC. (B) RT-PCR of bioreactor grown cells indicates changing gene exspression patterns over differentiation stage with increasing trend to MB neuronal gene expression. (C) Flow cytometry indicates high proportion of TH+ cells (right) with few CD44+ astrocytes. Scale bars in A = 100um

Future Directions

A9 Dopaminergic Neurons

- Functional validation by electrophysiology
- Large scale production in larger bioreactors

HD-DoE

- Basic strategy applicable to all cell types
- Neuronal and non-neuronal cell differentiation
- Large scale production

References

Mb progenitors image source: Pereira Luppi M, Azcorra M, Caronia-Brown G, Poulin JF, Gaertner Z, Gatica S, Moreno-Ramos OA, Nouri N, Dubois M, Ma YC, Ramakrishnan C, Fenno L, Kim YS, Deisseroth K, Cicchetti F, Dombeck DA, Awatramani R. Sox6 expression distinguishes dorsally and ventrally biased dopamine neurons in the substantia nigra with distinctive properties and embryonic origins. Cell Rep. 2021 Nov 9;37(6):109975. doi: 10.1016/j.celrep.2021.109975. PMID: 34758317; PMCID: PMC8607753. *source: Data from Kim et al. Cell Stem Cell (2021)*

GIRK2/CALB1 quantification source: Kim TW, Piao J, Koo SY, Kriks S, Chung SY, Betel D, Socci ND, Choi SJ, Zabierowski S, Dubose BN, Hill EJ, Mosharov EV, Irion S, Tomishima MJ, Tabar V, Studer L. Biphasic Activation of WNT Signaling Facilitates the Derivation of Midbrain Dopamine Neurons from hESCs for Translational Use. Cell Stem Cell. 2021 Feb 4;28(2):343-355.e5. doi: 10.1016/j.stem.2021.01.005. PMID: 33545081; PMCID: PMC8006469.