Optimized reprogramming of human iPSCs to generate distinct neuronal subtypes

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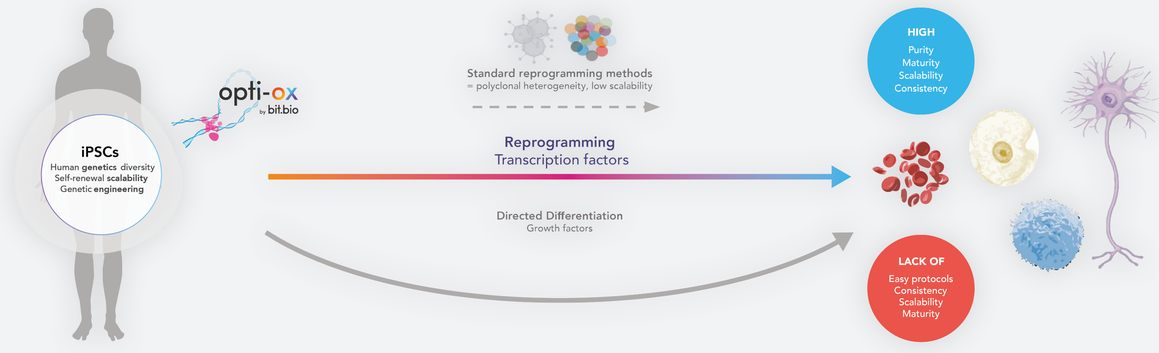
Abstract

Human brains differ remarkably in size and cellular composition from rodent models used in pre-clinical research. For example, human cortical neurons have larger dendritic trees, distinct electrophysical properties and express different protein isoforms. For neuronal indications, less than 10% of findings derived from animal models can be translated to the clinic[1]. A robust source of human iPSC-derived neurons would offer an attractive in vitro model for basic research and high content drug screens, which could reduce costs, improve screen specificity, and accelerate drug development. However, conventional iPSC differentiation protocols are often complex inconsistent, and difficult to scale. To overcome these problems, we developed a proprietary gene-targeting strategy (opti-ox) that enables highly controlled expression of transcription factors to rapidly reprogram human iPSCs (hiPSCs) into pure somatic cell types. We have manufactured consistent and homogenous cultures of glutamatergic neurons (>80% VGLUT1/2) and GABAergic neurons (VGAT1, GABA), which show homogenous molecular phenotype at single cell transcriptomics resolution. Reprogramming is highly consistent and synchronised, yielding fully functional neurons in less than 14 days. Our technology opens up novel avenues for the development of in vitro models to support research and healthcare innovations.

bit.bio's approach to cellular reprogramming

Precise control of transcription factor expression through iPSC engineering



The use of human iPSC derived cell models has been hindered by the lack of consistency and scalability of differentiation methods. Novel reprogramming technology, opti-ox, is opening new avenues by allowing controlled expression of transcription factor combinations for optimal cellular reprogramming of human cell types from hiPSCs.

1. Precise reprogramming of iPSCs into defined human cell types

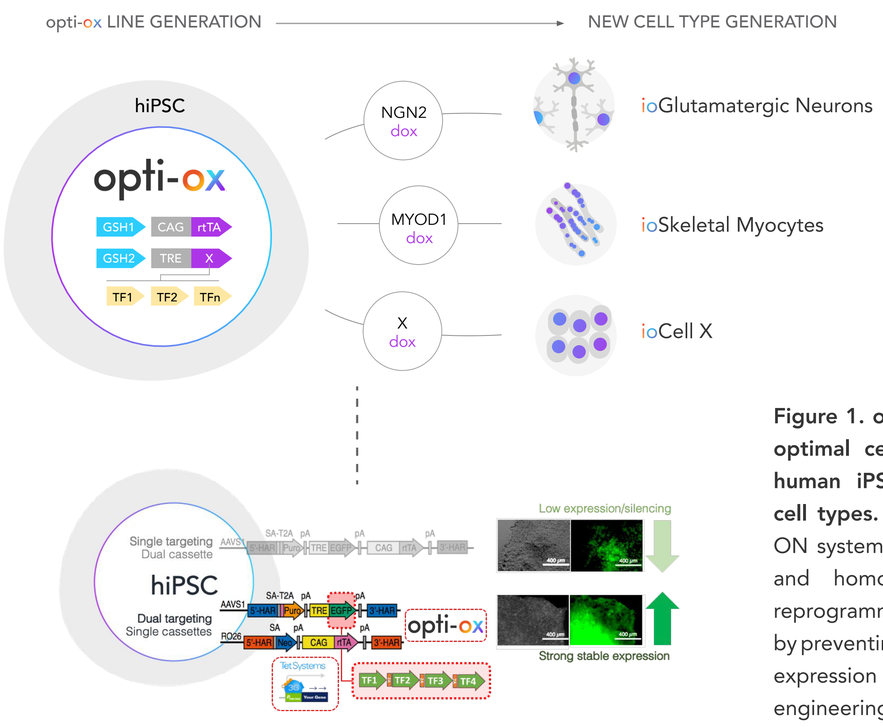
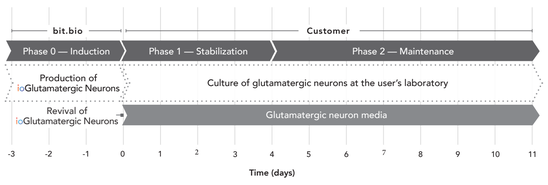


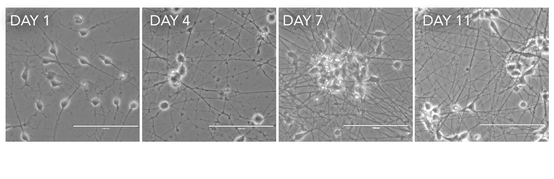
Figure 1. opti-ox technology for the optimal cellular reprogramming of human iPSCs into defined human cell types. opti-ox dual cassette Tet-ON system ensures tightly controlled and homogeneous expression of reprogramming transcription factors by preventing silencing of the inducible expression cassette after genetic engineering of hiPSCs[2].

2. Neurons express glutamatergic markers and form functional neuronal networks within 2 to 3 weeks

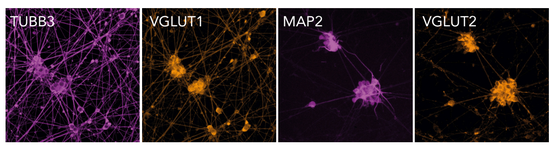
ioGlutamatergic Neurons cultures consist mainly of glutamatergic neurons (>80%) characterised by the expression of the glutamate transporter genes VGLUT1 and VGLUT2. Cells are derived from hiPSCs by Neurogenin-2 (NEUROG2) driven opti-ox reprogramming[2,3]. Four days after initiation of reprogramming, ioGlutamatergic Neurons show no expression of pluripotency markers and express pan-neural genes (data not shown).



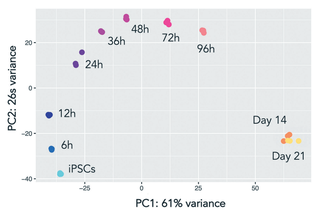
(A) Arrive ready to plate. The 3 phase protocol for generating ioGlutamatergic Neurons:
1. Induction (carried out at bit.bio)
2. Stabilization for 4 days with Doxycycline
3. Maintenance during which the neurons mature.



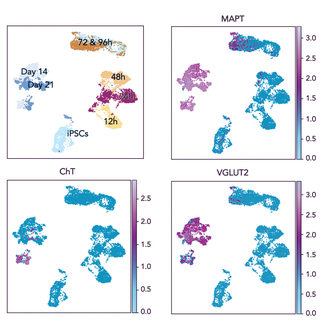
(B) Convert into mature neurons within days. ioGlutamatergic Neurons after revival over the course of the first 11 days of culture. Day 1 to 11 post-thawing; 40X magnification; scale bar: 100µm.



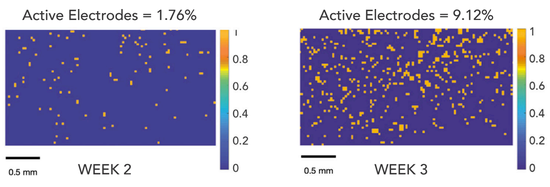
(C) Express glutamatergic neuron-specific markers & exhibit neurite outgrowth. Immunofluorescent staining 11 days post-revival demonstrates homogenous expression of pan-neuronal proteins (MAP2 and TUBB3) and glutamatergic neuron-specific transporters (VGLUT1 and VGLUT2).



(D) Consistent and synchronised reprogramming. PCA plot of bulk RNAseq transcriptomes from the iPSC stage to Day 21 post-induction, show consistent and synchronised transcriptional change between triplicates for each timepoint.



(E) Highly pure population of hiNeurons expressing glutamatergic (>80%) and cholinergic (~15%) markers. scRNA-seq analysis of different time-points during the reprogramming of iPSCs into hiNeurons. The analysis shows that the post-mitotic population of ioGlutamatergic Neurons homogenously express the panneuronal marker MAPT, and primarily consists of glutamatergic neurons (VGLUT2) and a sub-population of cholinergic neurons (ChT).

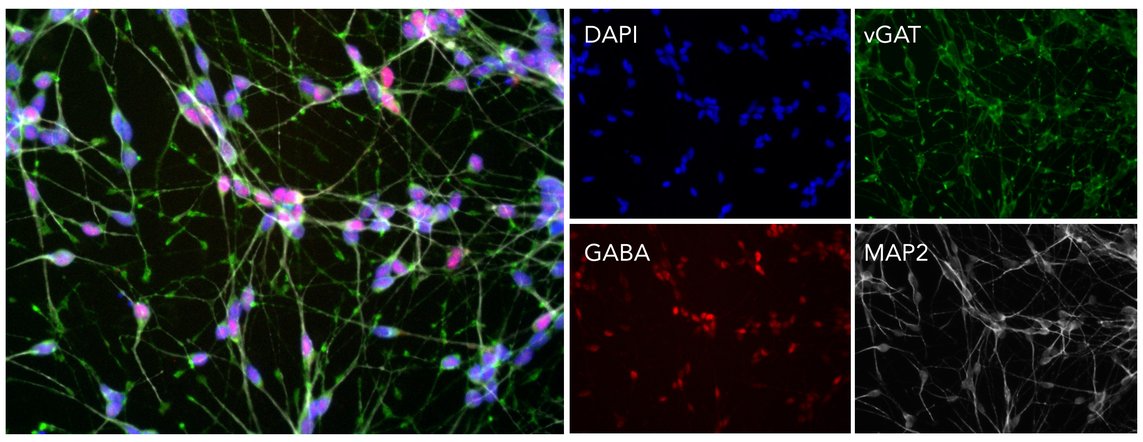


(F) Functional activity demonstrated by MEA. MaxOne high-resolution multi electrode array (MEA) recordings of ioGlutamatergic Neurons in BrainPhys™ media. The activity maps show % of active electrodes and demonstrate a time-dependent increase of spontaneous activity during neuronal maturation from 2 to 3 weeks post plating[5].

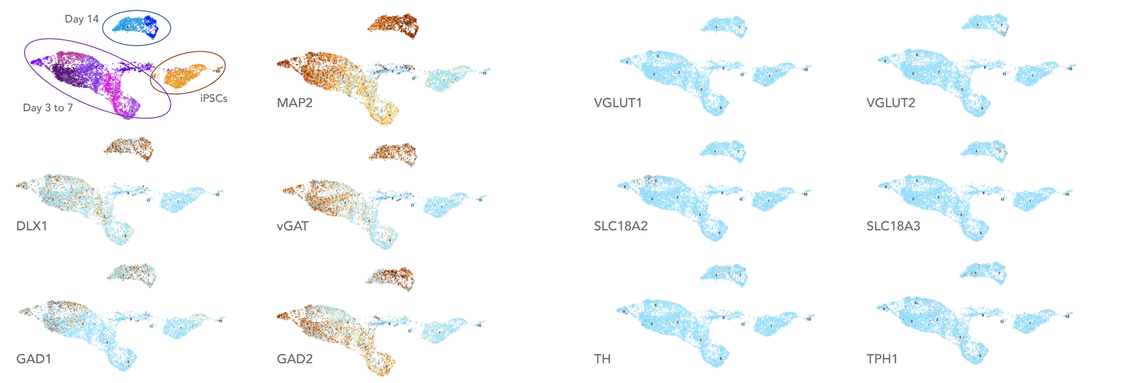
3. Expression of ASCL1 and DLX2 efficently convert iPSCs into GABAergic neurons

Figure 3. ioGABAergic Neurons are derived from hiPSCs by ASCL1 and DLX2[4] - driven opti-ox reprogramming. (**A**) Uniform expression of GABAergic neuronal markers. Immunofluorescence staining as early as 7 days post-induction demonstrates robust expression of the pan-neuronal marker MAP2 and the GABAergic markers GABA and vGAT. 20X magnification. (**B**) Expression of key marker genes of GABAergic neurons. Gene expression assessed by 10x Genomics scRNA-seq shows expression of key GABAergic interneuron-specific markers. Markers indicative of other neuronal lineages (glutamatergic, dopaminergic, cholinergic or serotonergic) are largely absent. (Product in development.)

A



B



Summary

- NGN2 driven opti-ox reprogramming converts hiPSCs to ioGlutamatergic Neurons in a highly synchronised and consisted manner.
- Reprogrammed iPSCs give rise to mature neurons that form functional neuronal network within 2 to 3 weeks.
- ASCL1 & DLX2 driven opti-ox converts hiPSCs to GABAergic neurons, which show uniform expression of key GABAergic markers.
- Single cell transcriptomics data show highly pure populations of human GABAergic and glutamatergic neuronal subtypes.
- Clinically relevant mutations can be introduced into human iPSC derived neuronal subtypes to model for specific neurodegenerative diseases, which could facilitate drug development.

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1. Vargas-Caballero M, et al., Expert Opin Drug Dis, 2016; **2.** Pawlowski M, et al., Stem Cell Reports, 2017; **3.** Zhang Y, et al., Neuron, 2013; 4. Yang N, et al., Nature Methods, 2017; **5.** Iovino et al., Charles River Laboratories, 2019.