Reprogramming cell identity: enabling the next generation of human cells

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THE CELL CODING COMPANY

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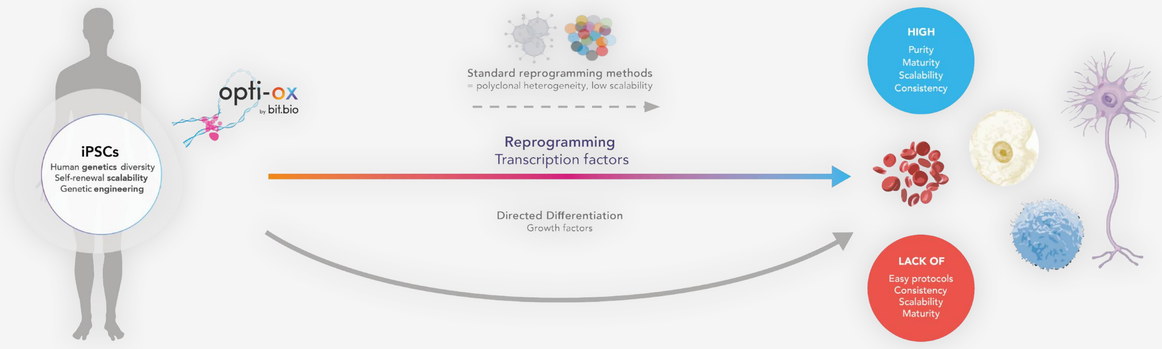
Abstract

Challenges remain for utilising induced pluripotent stem cell (hiPSC) lines in regenerative medicine and the pharmaceutical industry. Methods that enable faithful, homogeneous and scalable differentiation of hiPSCs to mature cell types are needed to replace current cell lines with limited functionality and biological significance. Coupled with modern genetic tools and the capacity of hiPSCs to reproduce human genetic diversity, hiPSC-derived cells represent a pivotal tool for improving human health by enabling disease modelling, drug discovery and the next generation of cell therapies. Based on the principle that transcription factors (TFs) control cell identity, TF-driven cellular reprogramming offers a disruptive strategy for cell differentiation, with a growing number of reprogrammed cell types described. By applying a cellular reprogramming approach supported by a proprietary genetic switch, opti-ox[1] (optimized inducible overexpression), the typical restrictions of standard hiPSC differentiation systems - length, complexity, and lack of consistency and purity - have been largely overcome. bit.bio's technology enables homogeneous and synchronous differentiation of entire hiPSC cultures through tightly controlled expression of selected TFs as demonstrated for glutamatergic neurons, skeletal myocytes, GABAergic neurons and oligodendrocytes for instance. Coupled with a unique TF combination screening platform, the increasing portfolio of cells produced by opti-ox cellular reprogramming offers human cells with high consistency and functionality, at scale, from human iPSCs, including those carrying disease-specific mutations, providing high-quality human models for enhanced research outcomes and drug discovery efficiency.

bit.bio's approach to cellular reprogramming

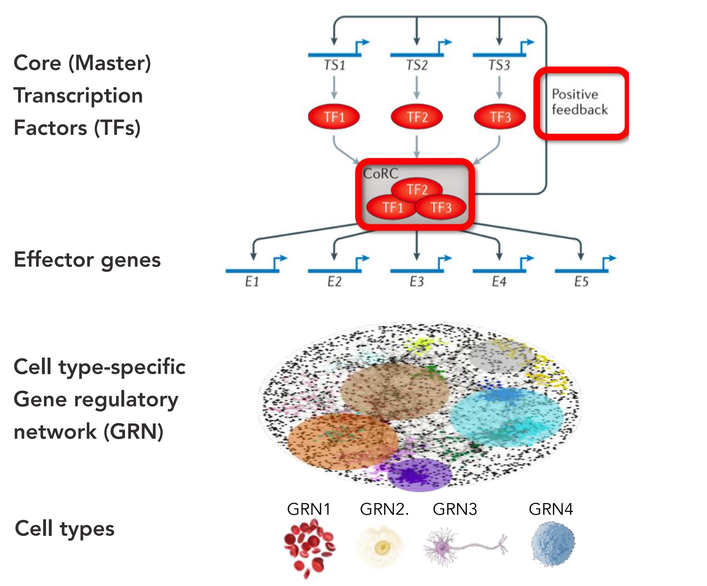
Precise control of transcription factor expression through iPSC engineering



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.Cell type-specific gene regulatory networks

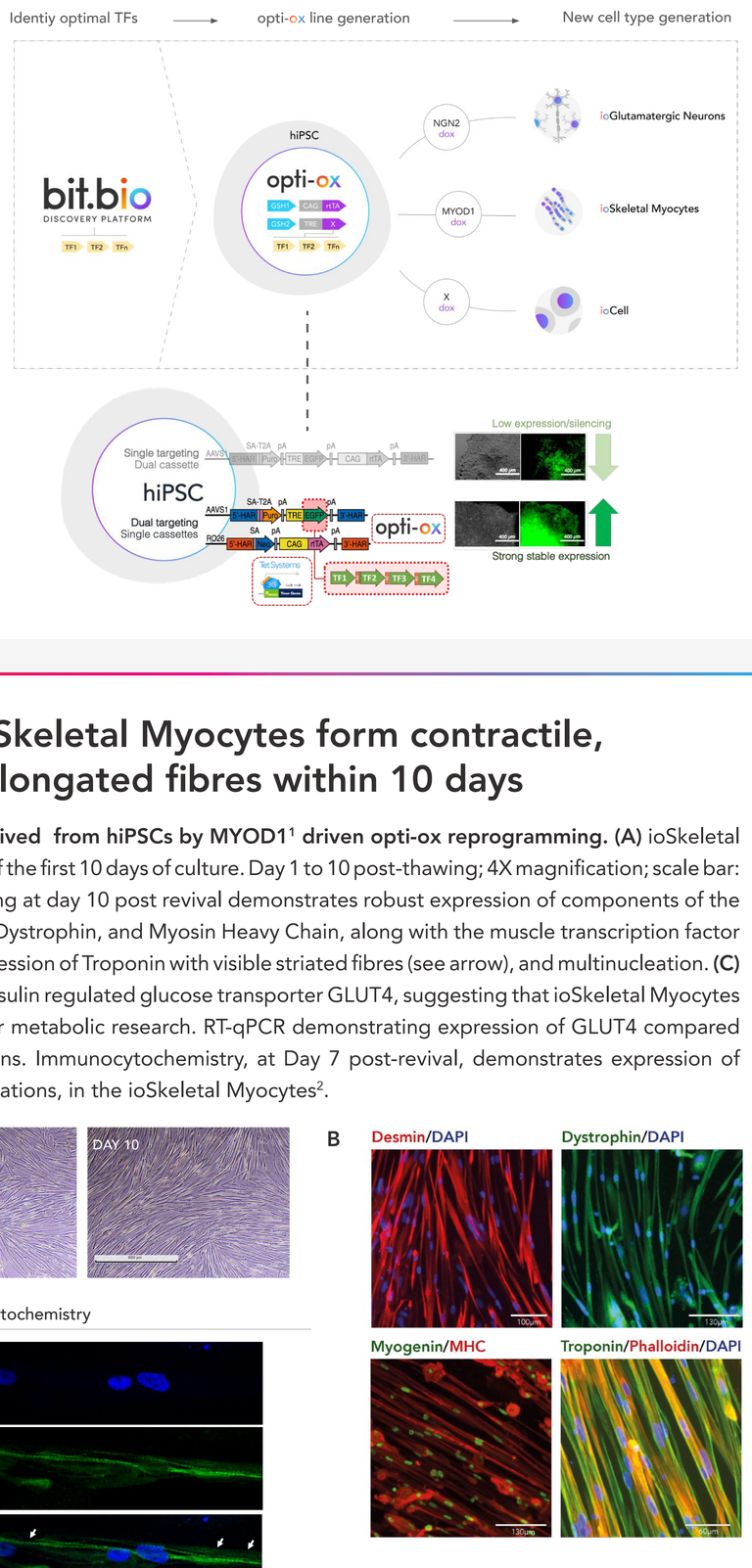
Figure 1. A small number of cell type specific core (master) transcription factors control expression of downstream (effector) genes, which in turn regulate expression of larger sets of genes (modules) associated with cell type specific structure and functions.



2. bit.bio's technologies

Discovery Platform and opti-ox

Figure 2. bit.bio have developed two key proprietary technologies for the optimal differentiation of human iPSCs by cellular reprogramming. The high throughput Discovery Platform allows the screening of millions of TF combinations in parallel and the rapid identification of the optimal TFs for cellular reprogramming. opti-ox dual cassette Tet-ON system ensures tightly controlled and homogeneous expression of reprogramming TFs by preventing silencing of the inducible expression cassette after genetic engineering of hiPSCs[1].

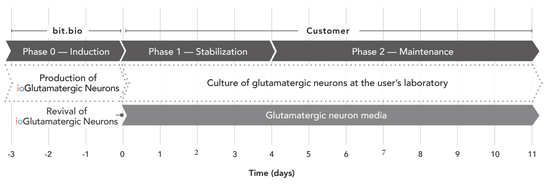


3. Human induced ioSkeletal Myocytes form contractile, multinucleated and elongated fibres within 10 days

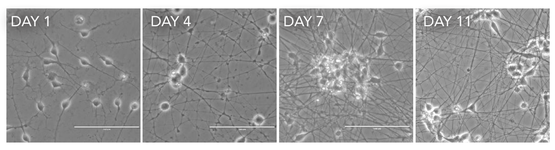
Figure 3. ioSkeletal Myocyte are derived from hiPSCs by MYOD1[1] driven opti-ox reprogramming. (A) ioSkeletal Myocytes after revival over the course of the first 10 days of culture. Day 1 to 10 post-thawing; 4X magnification; scale bar: 800µm. (**B**) Immunofluorescence staining at day 10 post revival demonstrates robust expression of components of the contractile apparatus such as Desmin, Dystrophin, and Myosin Heavy Chain, along with the muscle transcription factor Myogenin. Cells also demonstrate expression of Troponin with visible striated fibres (see arrow), and multinucleation. **(C)** Data demonstrates expression of the insulin regulated glucose transporter GLUT4, suggesting that ioSkeletal Myocytes provide a unique human cell model for metabolic research. RT-qPCR demonstrating expression of GLUT4 compared to hiPSCs and ioGlutamatergic Neurons. Immunocytochemistry, at Day 7 post-revival, demonstrates expression of GLUT4 in peri-nuclear regions, and striations, in the ioSkeletal Myocytes[2].

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

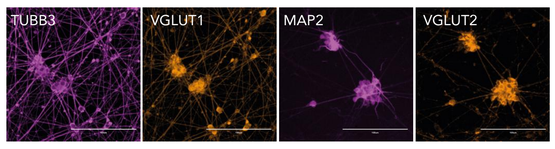
ioGlutamatergic Neurons cultures consist mainly of glutamatergic neurons (>80%) characterised by the expression of the glutamate transporter genes VGLUT1 and VGLUT2. Cells are derived from hiPSCs by Neurogenin-2 (NGN2)[4]driven opti-ox reprogramming[1,3]. Four days after initiation of reprogramming, ioGlutamatergic Neurons show no expression of pluripotency markers, and express pan-neural and glutamatergic neuron-specific genes (data not shown). Physiologically relevant and phenotypically characterised neurons are consistent at scale, easy to use and ready for experimentation within days.



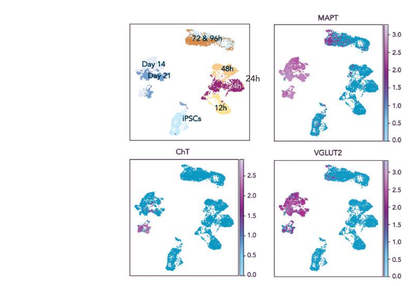
(A) Arrive ready to plate. The 3 phase protocol for generating ioGlutamatergic Neurons:
1. Induction (carried out at bit.bio)
2. Stabilization for 4 days with Doxycycline
3. Maintenance during which the neurons mature.



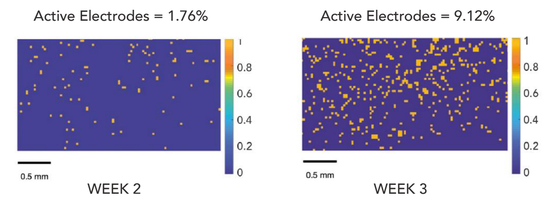
(B) Convert into mature neurons within days. ioGlutamatergic Neurons after revival over the course of the first 11 days of culture. Day 1 to 11 post-thawing; 40X magnification; scale bar: 100µm.



(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).



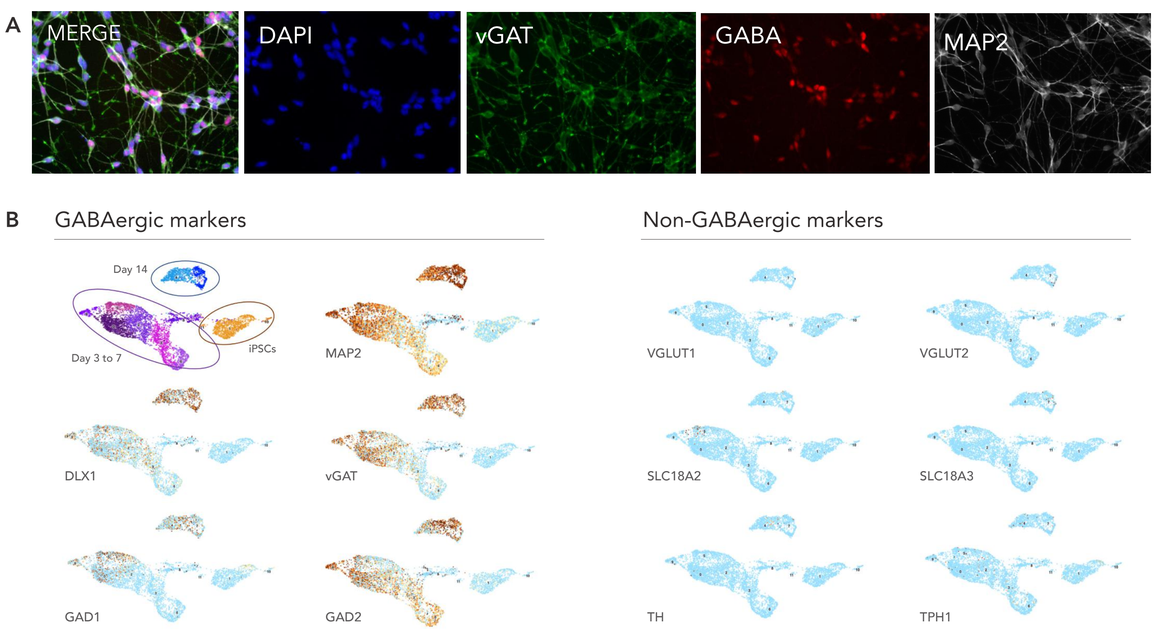
(E) Highly pure population of hiNeurons expressing glutamatergic (>80%) and cholinergic (~15%) markers. scRNA-seq analysis of different time-points during the reprogramming of iPSCs into hiNeurons. The analysis shows that the post-mitotic population of ioGlutamatergic Neurons homogenously express the 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[4].

5. In depth characterisation of ioGABAergic Neurons

Figure 5. ioGABAergic Neurons are highly defined and characterised by single cell RNA sequencing. (**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.)



1. Pawlowski M, et al., Stem Cell Reports, 2017; 2017. **2.** Dougall Norris & Daniel Fazakerley, Wellcome-MRC Institute of Metabolic Science.
3. Zhang Y, et al., Neuron, 2013; **4.** Iovino et al., Charles River Laboratories, 2019. **5.** Yang N, et al., Nature Methods