Generation of highly pure, consistent and functional inhibitory GABAergic neurons from human iPSCs using opti-ox™ technology

Authors

*G. Mastrogiovanni
T. Oosterveen
M. Ortiz
R. Hickman
P. Parac
B. Klapholz
S. Milde
R. O'Reilly
H. Garnett
M. Raman Srivastava
F. Patell-Socha
T. Moreau
W. Bernard
M. Metzakopian
M. Kotter

*Poster presenter (gianmarco.mastrogiovanni@bit.bio)

bit.bio

The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
info@bit.bio | www.bit.bio

Abstract

Neuronal circuits in the cortex consist of two main neuronal types, glutamatergic excitatory neurons and GABAergic inhibitory neurons (IN). The inputs of IN provide cortical networks with the ability to balance spontaneous and evoked excitatory activities, preventing runaway excitation. Abnormal IN function is associated with a variety of neurological diseases such as autism, epilepsy and schizophrenia.

It has proven to be challenging to develop drugs to treat neurological diseases as less than 10% of findings derived from conventional animal models can be translated to the clinic. Scalable approaches are needed to generate human *in vitro* models suitable for high-content drug screening that consist of well-defined and pure populations of specific neurons, such as GABAergic neurons.

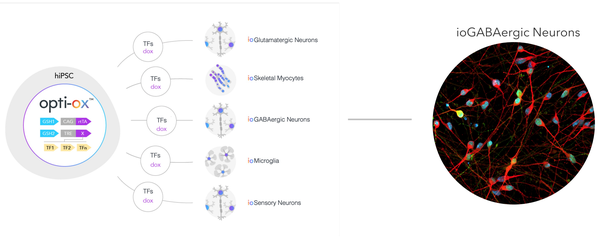
We have used our precision cellular reprogramming technology opti-ox™ (optimised inducible overexpression), to tightly control the expression of a unique combination of transcription factors to generate a highly pure (>95%) population of GABAergic neurons, named ioGABAergic Neurons, from human iPSCs, at scale, within 12 days post-revival.

A deep molecular characterisation of these human iPSC-derived GABAergic neurons by immunocytochemistry, RT-qPCR and single-cell RNA-sequencing revealed that the cultures consist of over 95% pure GABAergic neurons expressing the classical marker genes GAD1, GAD2, VGAT, DLX1, as well as DLX2 and are positive for GABA.

Remarkably, somatostatin (SST) was the only main GABAergic subtype specific marker that was detected in the transcriptomes of the single cells, further highlighting the purity of our ioGABAergic Neurons. Moreover, the transcriptomic profile of ioGABAergic Neurons was highly equivalent across three independently manufactured lots, showing that cells can be produced in a consistent manner, at scale.

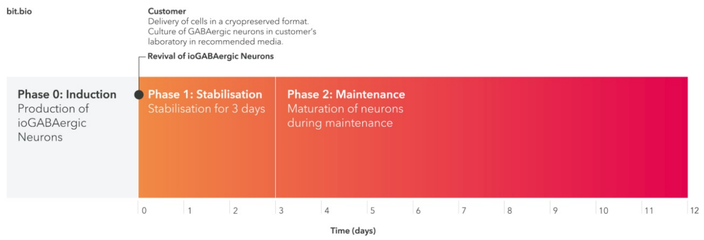
The ioGABAergic Neurons are functional as they display spontaneous neuronal activity and can easily be co-cultured with our ioGlutamatergic Neurons in the presence of astrocytes. In summary, opti-ox™ precision cellular reprogramming enables the manufacturing of highly pure (>95%), consistent, and functional GABAergic neurons that can serve as a high-quality human model to study both neurodevelopment and neurological disorders.

1. Precise opti-ox™ cellular reprogramming of hiPSCs into ioGABAergic Neurons



opti-ox™ technology for the optimal cellular reprogramming of human iPSCs into defined human cell types, including ioGABAergic Neurons. opti-ox™ dual cassette Tet-ON system ensures tightly controlled and homogeneous expression of reprogramming transcription factors (TFs) by preventing silencing of the inducible expression cassette after genetic engineering of hiPSCs .

2. ioGABAergic Neurons arrive ready to plate

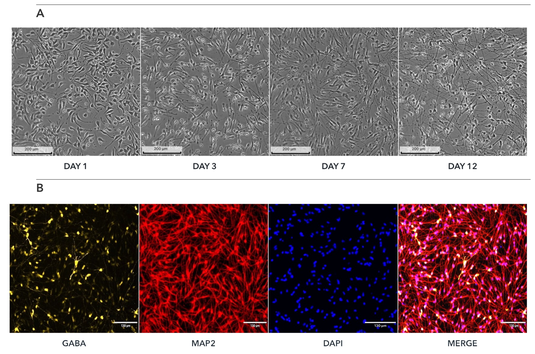


ioGABAergic Neurons are delivered in a cryopreserved format and are programmed to rapidly mature upon revival in the recommended media. The protocol for the generation of these cells is a three-phase process: Induction, which is carried out at bit.bio (Phase 0), Stabilisation for 3 days (Phase 1), and Maintenance (Phase 2) during which the ioGABAergic Neurons mature. Phases 1 and 2 after revival of cells are carried out at the customer site.

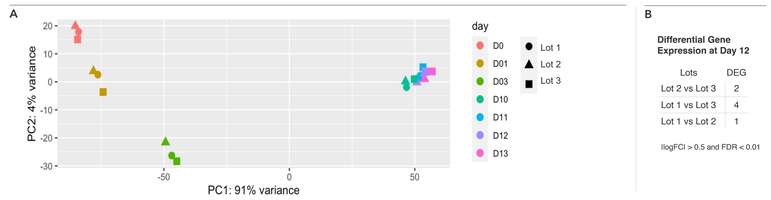
3. ioGABAergic Neurons show visible neuronal networks and express GABAergic neuron-specific markers

A) Upon reprogramming, ioGABAergic Neurons show rapid morphological changes, with neurons identified by day 7 post-revival. Visible neuronal networks are observed by day 12. Images show day 1 to 12 post-thawing; 10X magnification; scale bar: 200 µm.

B) Immunofluorescent staining on day 10 post-revival demonstrates that ioGABAergic Neurons are positive for GABA (yellow), the pan-neuronal marker MAP2 (red), and the DAPI counterstain (blue).



4. Whole transcriptome analysis demonstrates high lot-to-lot consistency of ioGABAergic Neurons



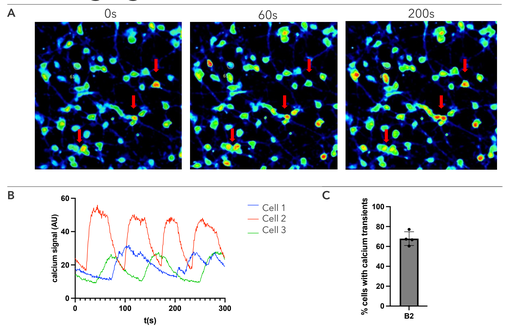
Bulk RNA sequencing analysis was performed on three different lots of ioGABAergic Neurons at different time points throughout the reprogramming protocol. **A)** Principal component analysis represents the variance in gene expression between the lots of ioGABAergic Neurons. This analysis shows high consistency between each lot of ioGABAergic Neurons at each given timepoint. **B)** Differential gene expression analysis between three independent lots of cells show less then 5 differentially expressed genes (DEG) between lots. Pure populations of ioGABAergic Neurons with equivalent expression profiles can be generated consistently from every vial, allowing confidence in experimental reproducibility.

5. ioGABAergic Neurons rapidly gain spontaneous functional activity shown by calcium imaging

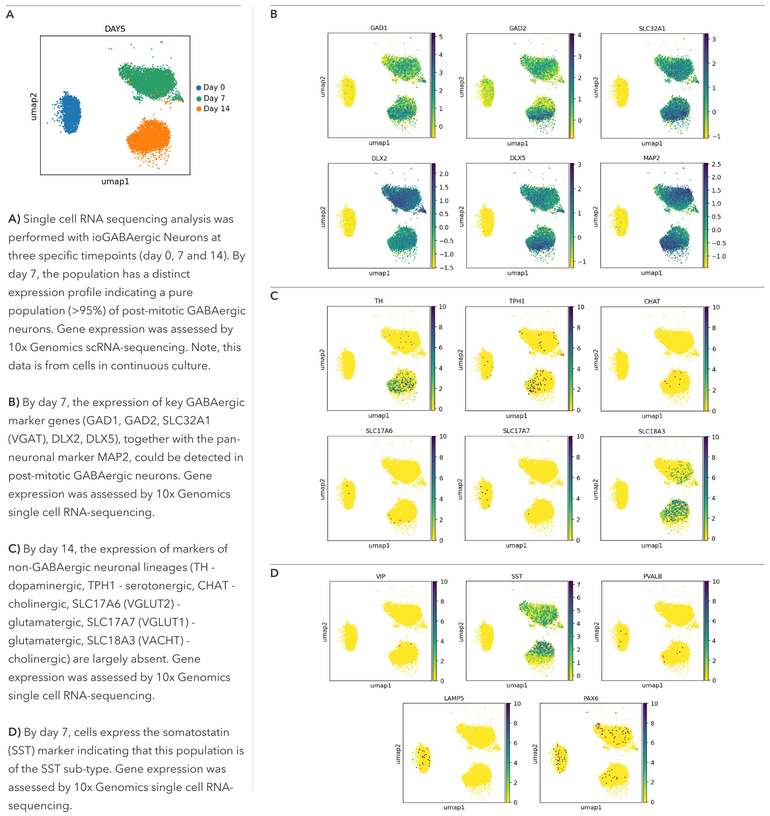
A) Images at different time points showing spontaneous activity of ioGABAergic Neurons, subjected to calcium imaging at day 16 post-revival. Red arrows points at cells showing calcium transients.

B) Example of individual cell traces for calcium imaging over time.

C) Quantification demonstrating that >60% of cells displayed calcium transients.



6. Single cell RNA-sequencing shows ioGABAergic Neurons form a pure population (>95%)



A) Single cell RNA sequencing analysis was performed with ioGABAergic Neurons at three specific timepoints (day 0, 7 and 14). By day 7, the population has a distinct expression profile indicating a pure population (>95%) of post-mitotic GABAergic neurons. Gene expression was assessed by 10x Genomics scRNA-sequencing. Note, this data is from cells in continuous culture.

B) By day 7, the expression of key GABAergic marker genes (GAD1, GAD2, SLC32A1 (VGAT), DLX2, DLX5), together with the pan-neuronal marker MAP2, could be detected in post-mitotic GABAergic neurons. Gene expression was assessed by 10x Genomics single cell RNA-sequencing.

C) By day 14, the expression of markers of non-GABAergic neuronal lineages (TH - dopaminergic, TPH1 - serotonergic, CHAT - cholinergic, SLC17A6 (VGLUT2) - glutamatergic, SLC17A7 (VGLUT1) - glutamatergic, SLC18A3 (VACHT) - cholinergic) are largely absent. Gene expression was assessed by 10x Genomics single cell RNA-sequencing.

D) By day 7, cells express the somatostatin (SST) marker indicating that this population is of the SST sub-type. Gene expression was assessed by 10x Genomics single cell RNA-sequencing.

Summary & conclusions

Human iPSC derived ioGABAergic Neurons are highly pure (>95%), consistent, and functional GABAergic neurons that provide a high-quality human model for neurological disease research and drug development.

Highly pure and defined cell identity – GABAergic neurons of the SST subtype, characterized by single cell RNA-sequencing and ICC.
Lot-to-lot consistency shown by whole transcriptome analysis – ensures high experimental reproducibility.
Rapid functionality – calcium imaging shows that cells display >60% spontaneous activity at 16 days post-revival.

Rapid maturity – ready for experimentation within 12 days post-revival, compared to classical differentiation that takes several weeks to achieve similar results.
Ready-to-culture cells are easy to use – simple two-step protocol using open-source media.

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