DRIVING EXPERIMENTAL REPRODUCIBILITY AND LOT-TO-LOT BIOLOGICAL CONSISTENCY IN HUMAN IPSC-DERIVED CELLS ENABLED BY OPTI-OX TECHNOLOGY

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Abstract

Transcription factor (TF)-mediated cellular programming has introduced a novel paradigm in developmental biology, challenging traditional methods and facilitating scientific innovation.

Due to a lack of standardised, easy-to-use and readily accessible human cell models, scientists often rely on animal models, primary cells, and/or cell lines that considerably differ from human biology and can be difficult to source at scale. Induced pluripotent stem cell (iPSC)-derived cells are an alternative to these, offering a scalable, human model for disease research.

Directed differentiation to generate the desired cell types from iPSCs through signalling with growth factors and small molecules involves lengthy, complex protocols that are challenging to reproduce, difficult to scale, and lead to heterogeneous populations. Moreover, despite the benefits of forward programming, several challenges remain associated with conventional vector-based methods of transgene expression impacting the efficiency, consistency and purity of the resulting cell populations, as the random integration of TFs can result in gene silencing.

The use of these models makes it difficult to generate consistent data from a scalable source of cells, with experimental variability often preventing scientists from being able to reproduce results over time or replicate other scientists' experiments.

Genomic safe harbour (GSH)-mediated optimised inducible overexpression (opti-ox™) of cell type-specific TFs enables highly controlled, consistent and scalable manufacturing of human iPSC-derived cells, addressing these challenges. This technology has been used to deterministically cell program iPSCs into different cell types, including ioGlutamatergic Neurons, ioGABAergic Neurons, ioMotor Neurons, ioSensory Neurons, ioMicroglia, ioOligodendrocyte-like cells, and ioAstrocytes. The resulting cell types are highly defined and consist of homogeneous populations, confirmed by ICC and RT-qPCR. Moreover, whole transcriptome analysis reveals consistent expression profiles across manufactured lots, demonstrating consistency of the cells.

The availability of consistent lots, manufactured at scale, of human iPSC-derived cells has the potential to address the lack of experimental reproducibility seen across research, allowing scientists to accelerate their studies and enhance the reliability of their findings.

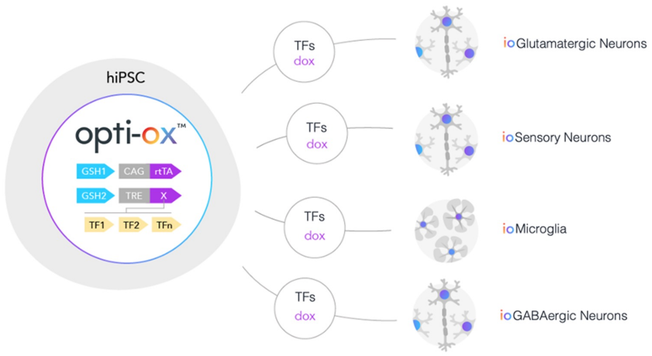
1. opti-ox deterministic cell programming of hiPSCs into defined human cell types

opti-ox is a dual cassette Tet-ON system that ensures tightly controlled and homogeneous expression of programming TFs by preventing silencing of the inducible expression cassette after genetic engineering of hiPSCs.

TF expression through opti-ox has been demonstrated to generate cell types from all three germ layers in a robust, consistent, and scalable manner.

We have developed a Discovery Platform that allows for the identification of core TF networks that drive cell fate acquisition from pluripotent stem cells.

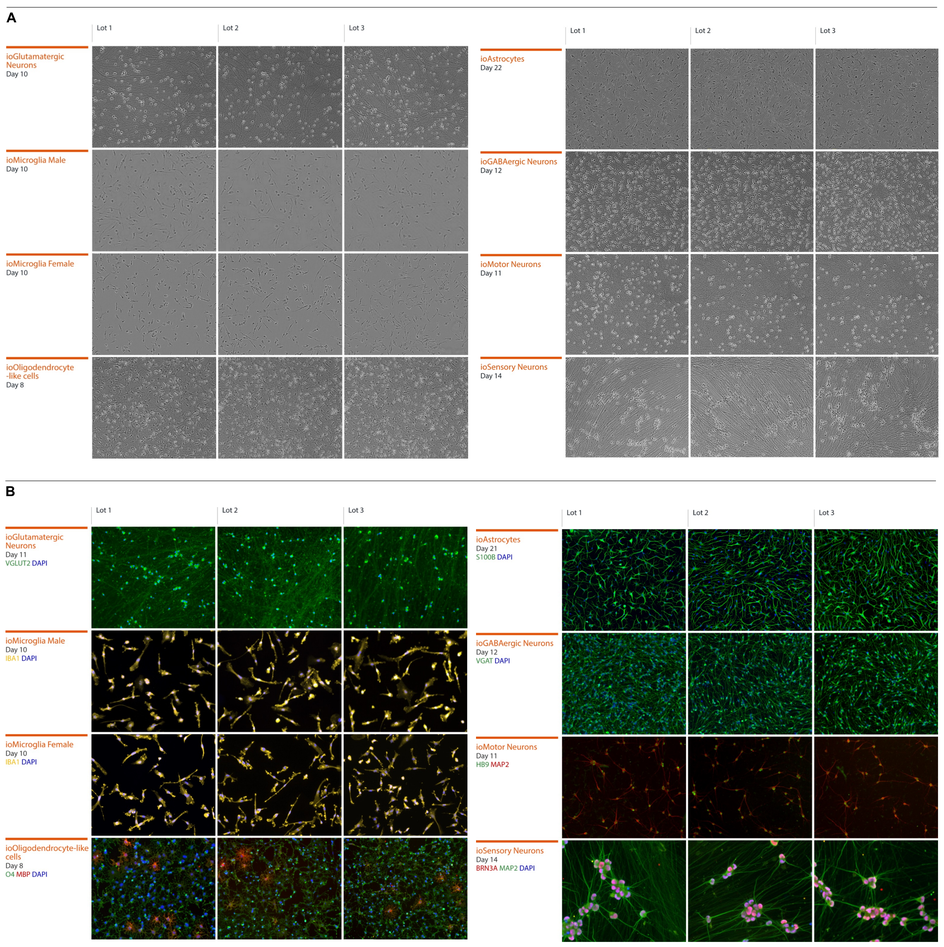
opti-ox powered cells show a level of consistency across differently manufactured lots which has not before been reported for hiPSC-derived cells.



2. hiPSC-derived cells display consistent morphology and marker expression across different lots

We have generated multiple cell types, including ioGlutamatergic Neurons, ioMicroglia, ioAstrocytes, ioOligodendrocyte-like cells, ioGABAergic Neurons, ioMotor Neurons, and ioSensory Neurons.

Brightfield imaging (A) and immunostaining (B) demonstrate consistent morphology and marker expression across manufactured lots. Data is shown for the endpoint specified in the cell type user manual.

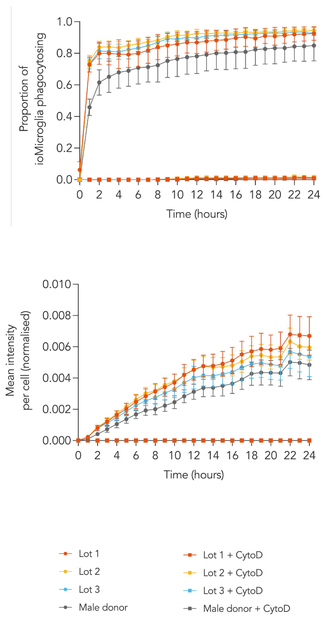


3. Consistent functional activity is shown across lots

ioMicroglia Male show key phagocytic function at a consistent degree across three manufactured lots.

The cells were incubated with pHrodo™ RED labelled Zymosan particles for 24 hours with or without cytochalasin D (CytoD) control. The proportion of cells phagocytosing Zymosan particles (left) and the degree of cells phagocytosing (right) is consistent across three independent lots. Images were acquired every 30 mins on the Incucyte® looking at red fluorescence and phase contrast.

Three technical replicates were performed per lot. Equivalent data is available for ioMicroglia Female.



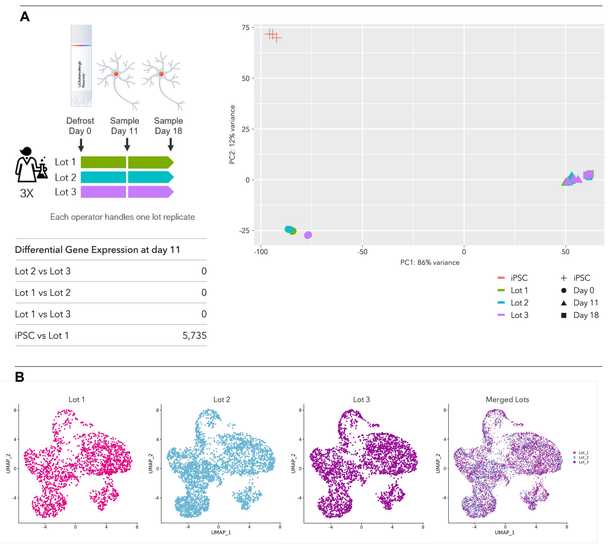
4. Genome-wide consistency in cell programming

Using comprehensive transcriptome analysis, bulk RNA-sequencing and single cell RNA-sequencing, the following data reveal how different users, manufacturing lots, and time periods do not affect the consistency of hiPSC-derived cells deterministically programmed with opti-ox technology.

Lot-to-lot consistency will help reduce experimental variation and increase the reproducibility of experiments.

(A) Bulk RNA-sequencing analysis was performed on three different lots of ioGlutamatergic Neurons on day 0, day 11 and day 18 post-revival. A principal component analysis (PCA) plot to assess gene expression variance between three different manufactured lots showed a tight clustering of the samples at each timepoint, demonstrating high consistency between these lots. Differential gene expression analysis reveals no statistically significant differentially expressed (DE) genes across the three lots at day 11 (|logFC| > 0.5 and FDR < 0.01).

(B) Single cell RNA-sequencing analysis was performed on three different lots of ioGlutamatergic Neurons on day 11. UMAP plots represent the cell-to-cell variation in gene expression profiles of cells, each dot representing an individual cell. Cells from each of the three lots a re equally distributed across the body of the plot. Merging the UMAP plots creates a tight overlay, showing a strong transcriptional relationship between cells from three independently manufactured lots. Gene expression was assessed by 10x Genomics scRNA-sequencing.

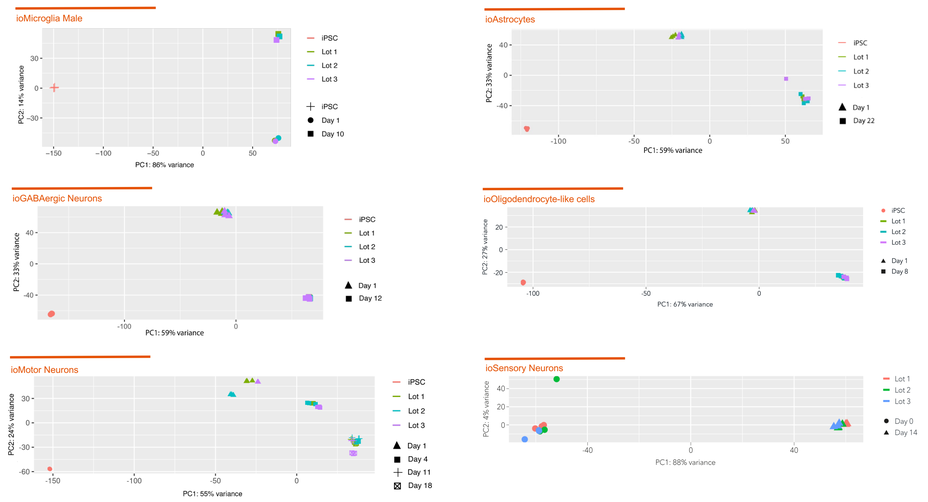


Differential Gene Expression at day 11	
Lot 2 vs Lot 3	0
Lot 1 vs Lot 2	0
Lot 1 vs Lot 3	0
iPSC vs Lot 1	5,735

5. Whole transcriptome analysis demonstrates high lot-to-lot consistency across three manufactured lots

Bulk RNA-sequencing shows tight clustering of the samples at each timepoint, demonstrating high consistency between three different manufactured lots, across multiple cell types. This allows for high signal to noise ratio in experiments and reducing the need for lot-to-lot validation by the user.

Bulk RNA-sequencing analysis was performed on three different lots of at different timepoints post-revival. The timepoints were selected for each cell type based on the recommended culturing conditions provided in the user manuals.



Summary | opti-ox technology drives lot-to-lot consistency in cell programming

- opti-ox technology has been successfully applied to deterministically cell program a range of human cell types from hiPSC-derived cells. The resulting highly defined, homogeneous cell populations can be used as physiologically relevant cell models for research and drug discovery
- Furthermore, the generated **cell populations show high levels of consistency across different manufactured lots**, as evidenced by morphology, key marker expression and functional activity data.
- Whole transcriptome analysis reveals **how different users, manufacturing lots, and time periods do not affect the consistency** of the cell populations
- The availability of a consistent source of hiPSC-derived cells has the **potential to address the lack of experimental variability and reproducibility** seen across research, allowing scientists to have higher confidence in their experimental findings.