opti-ox™ deterministic cell programming to enable the industrialisation of New Approach Methodologies

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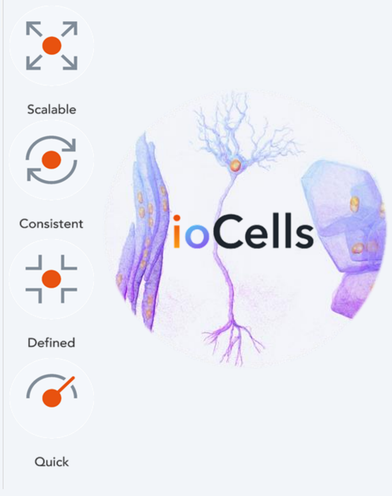
Abstract

Human induced pluripotent stem cell (hiPSC)-derived cells are a critical cell source for New Approach Methodologies (NAM), which industrialisation and acceptance by the pharmaceutical industry and regulatory agencies will require rigorous standardisation and scaling, especially of their core cellular components.

Directed differentiation to generate the desired cell types from hiPSCs through signalling with cytokines and small molecules involves lengthy, complex protocols that are often challenging to reproduce, difficult to scale, and lead to heterogeneous populations.

Conversely, transcription factor (TF)-driven cell programming using optimised inducible overexpression (opti-ox) of cell type-specific TFs enables highly controlled, consistent and scalable manufacturing of hiPSC-derived cells. This technology has been used by bit.bio to deterministically programme a variety of cell types: ioCells are defined functional human cells with highly consistent biological characteristics across large manufactured cell batches.

Powered by unique cell programming technologies overcoming the prevailing difficulty in manufacturing iPSC-derived cells at scale, bit.bio's growing catalogue of human cells and engineered derivatives provide the defined and consistent cellular components for application in multiple NAM.

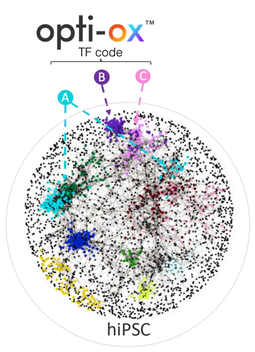


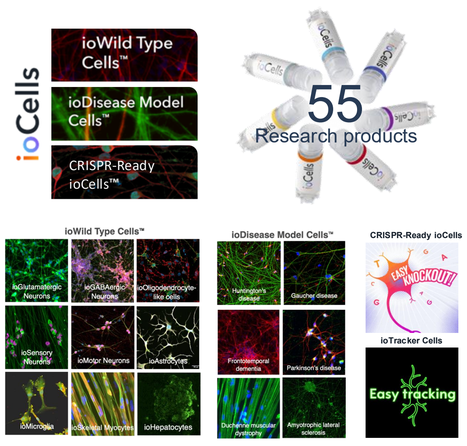
1. Technology & cell portfolio

opti-ox is a dual cassette Tet-ON system precisely integrated into genetic safe harbours 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 further developed a Discovery Platform that allows for the identification of core TF networks that drive cell fate acquisition from pluripotent stem cells.



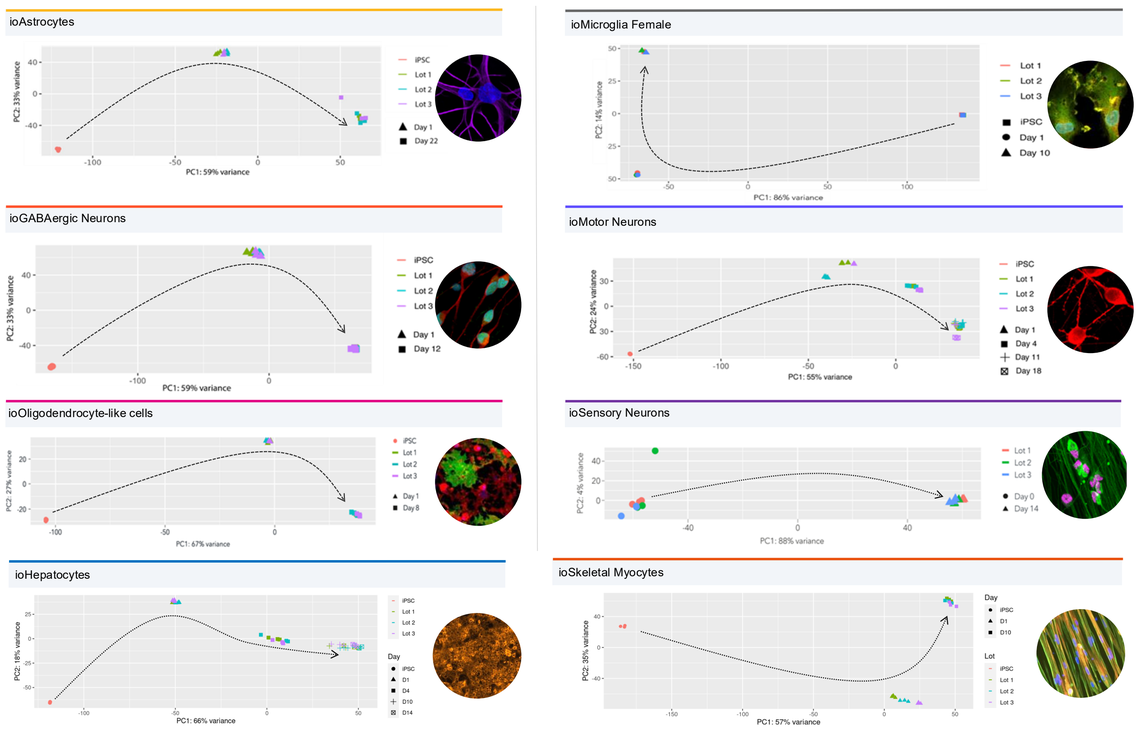


2. Consistency at scale across human cell types

Manufacturing consistency study design

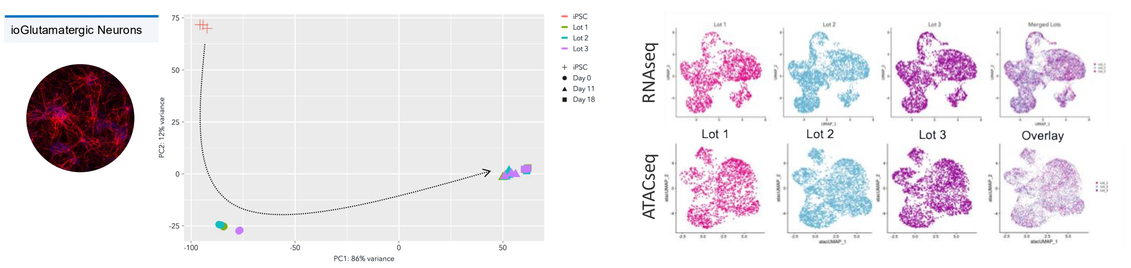
ioMicroglia male example

A. Bulk RNA-sequencing analyses were performed on 3 manufacturing lots of each cell type at different timepoints post-revival. The timepoints were selected based on the recommended culturing conditions provided in the user manuals.



Bulk RNA-seq PCA shows tight clustering of the samples at each timepoint, demonstrating high consistency between the 3 manufactured lots, across multiple cell types. The lot-to-lot consistency of ioCells helps reduce experimental variation and increases the reproducibility of experiments.

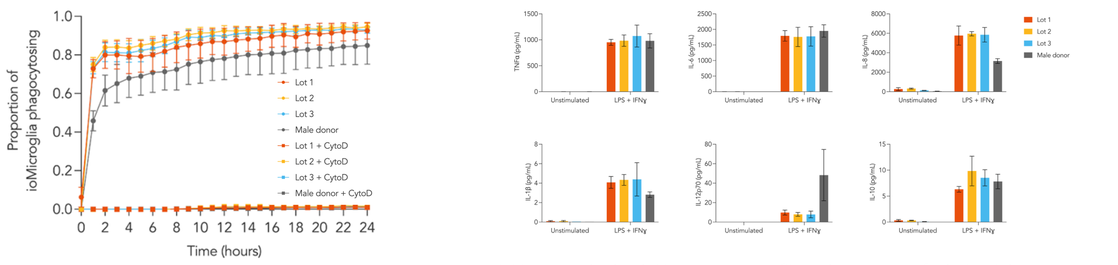
B. Bulk and Single cell RNA-sequencing performed on 3 manufacturing lots of ioGlutamatergic Neurons



Bulk RNA-seq PCA shows a tight clustering of the samples at each timepoint post-revival, demonstrating high consistency between lots (no statistically significant differentially expressed genes at d11 (|logFC|>0.5; FDR<0.01)).

Single cell RNA- & ATAC-seq analyses show strong overlay of both transcriptional and open chromatin profiles between cells from 3 independently manufactured lots.

C. Functional characterisation of 3 manufacturing lots of female ioMicroglia



Highly consistent phagocytosis activity between ioMicroglia lots (day10 cells were incubated with pHrodo RED labelled Zymosan particles for 24 hours +/- cytochalasin D control; images analysed by Incucyte).

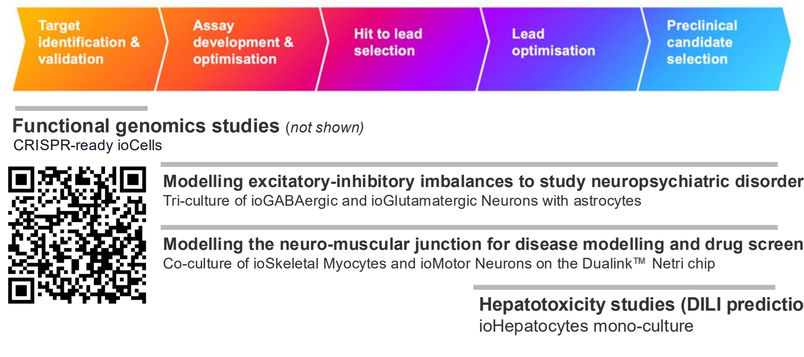
Highly consistent cytokine release profile between manufacturing lots (day 10 cells from 3 lots and a male ioMicroglia control were stimulated with LPS (100 ng/ml) and IFNγ (20 ng/ml) for 24 hours. Supernatants were analysed using MSD V-PLEX Proinflammatory Kit).

3. Cellular models to support drug discovery

ioWild Type Cells and their derivatives (CRISPR-Ready ioCells, ioDisease Model Cells, ioTracker Cells) can comprehensively support drug discovery pipelines.

They are being assessed internally and collaboratively in multiple biological models as cellular components of NAM development.

***Scan this QR code** to access further in vitro model studies with ioCells !*



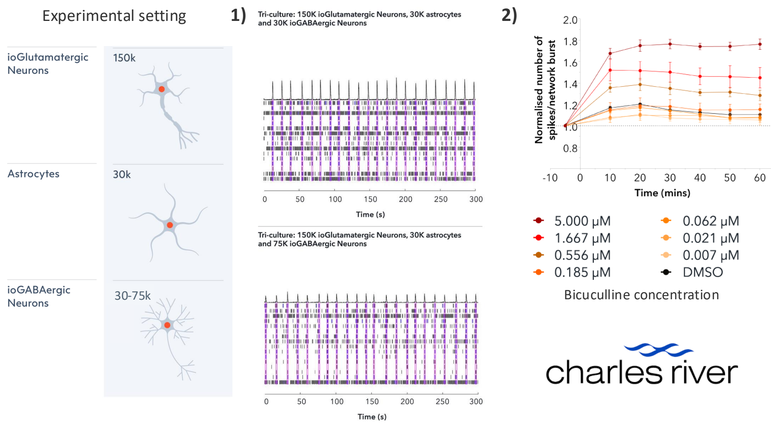
Functional genomics studies *(not shown)*
CRISPR-ready ioCells

Modelling excitatory-inhibitory imbalances to study neuropsychiatric disorders
Tri-culture of ioGABAergic and ioGlutamatergic Neurons with astrocytes

Modelling the neuro-muscular junction for disease modelling and drug screening
Co-culture of ioSkeletal Myocytes and ioMotor Neurons on the Dualink™ Netri chip

Hepatotoxicity studies (DILI prediction)
ioHepatocytes mono-culture

A. Modelling excitatory-inhibitory imbalances: tri-culture of ioGlutamatergic and ioGABAergic Neurons



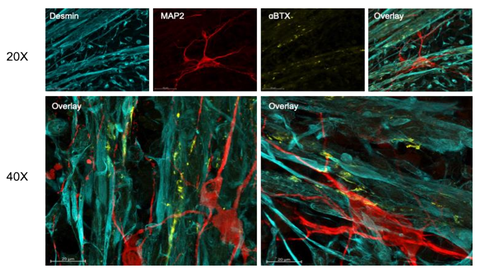
1) ioGABAergic Neurons exert an inhibitory effect on the excitatory ioGlutamatergic Neurons within the tri-cultures. Data show a dose response reduction of the number of spikes per network burst and increased neuron Network Burst Rate (MEA analysis at 53 DIV using 48-well CytoView MEA plate from Axion Biosystems).

2) The effect of adding bicuculline, a competitive antagonist of $GABA_A$ receptors, to the tri-cultures and control co-cultures was investigated by MEA analysis at 65 DIV. A dose dependent increase in the number of spikes per network burst is seen in the tri-culture.

These data were generated in partnership with Charles River Laboratories.

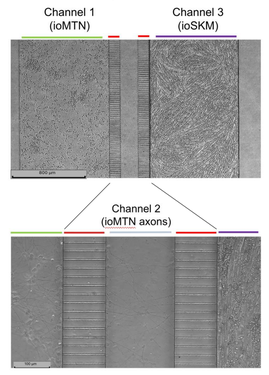
B. Neuromuscular junction model: co-culture ioMotor Neurons and ioSkeletal Myocytes (*in development*)

1) 2D co-culture method



High resolution confocal imaging of ioSkeletal Myocytes and ioMotor Neurons 2D co-culture at day 30 (Staining with alpha-bungarotoxin (aBTX) highlights acetylcholine receptor expression on ioSkeletal Myocytes. Desmin and MAP2 define ioSkeletal Myocytes and ioMotor Neurons respectively).

2) Culture on Dualink™ NETRI chip



The Dualink™ device enables separate culture of ioMotor Neurons and ioSkeletal Myocytes whilst allowing axonal projection through microchannels.

The availability of wild type matched ioDisease Model Cells and CRISPR-Ready ioCells has the potential to advance disease in vitro models for drug screening.

ioDisease Model Cells

Amyotrophic Lateral Sclerosis
ioMotor Neurons TDP-43 M337V
ioMotor Neurons TDP-43 A382T
ioMotor Neurons TDP-43 N352S
ioMotor Neurons FUS P525L
ioMotor Neurons SOD1 G93A
Duchenne muscular dystrophy
ioSkeletal Myocytes DMD Del Ex44/Y
ioSkeletal Myocytes DMD Del Ex52/Y
ioSkeletal Myocytes DMD Del Ex51/Y

C. Other examples of collaborative development of complex in vitro cell models

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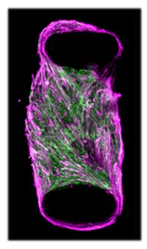
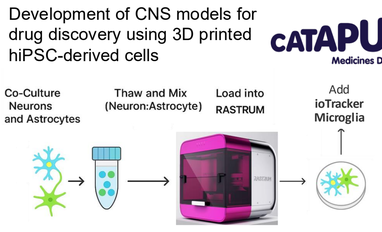
Collaboration for the generation of 3D muscle cell bundles using ioSkeletal Myocytes for modelling Duchenne Muscular Dystrophy.



Development of CNS models for drug discovery using 3D printed hiPSC-derived cells

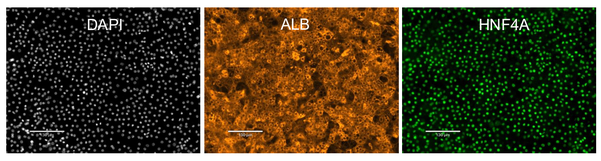
CATAPULT Medicines Discovery



*Live cells imaging of **ioTracker GFP Microglia***

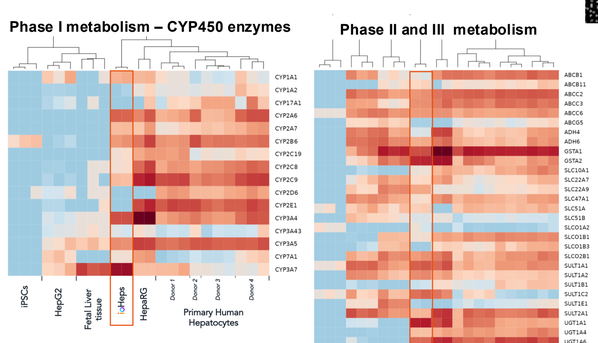
4. ioHepatocytes to model drug-induced liver toxicity

1) Immunofluorescent staining showing homogenous expression of the key pan-hepatocyte markers Albumin (ALB, orange) and HNF4A (green), and the DAPI counterstain (white). Day10 post-revival; scale bar: 130µm.

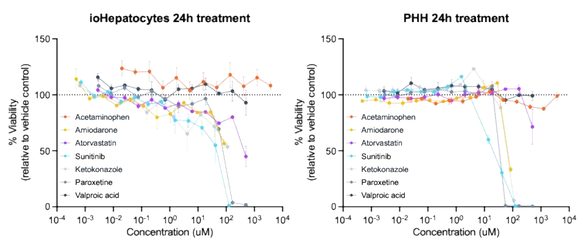


Phase I metabolism – CYP450 enzymes

Phase II and III metabolism



2) Bulk RNA-seq performed on ioHepatocytes and HepaRG after 8 days in culture. Data from fetal liver, plated primary human hepatocytes (PHH) were used for comparison.



3) Viability of ioHepatocytes after 24h exposure to drugs known to cause DILI. Primary human hepatocytes (PHH) were used as reference. Data shows similar cytotoxic response in both cell types. *No response was observed in either ioHepatocytes or PHH after treatment with Valproic Acid (used as control, known to not cause cytotoxicity in vitro) and Acetaminophen (which usually requires a higher dose to cause cytotoxicity).*

**ioHepatocytes product is currently in development. Its performance characteristics has not been fully established; therefore, the product format, user manual and presentation may be subject to change in the final catalogue product.*

Summary | ioCells provide scalable consistent cellular components for NAM development

- bit.bio has a unique cell programming technology that enables the deterministic manufacturing of human cells with unprecedented consistency at scale.
- ioCells provide defined and consistent cellular components to support the standardisation and scalability required to realise NAM's potential.
- ioCells derivatives and bit.bio's extensive cellular engineering capabilities further enable the development of advanced NAM cell components anchored into our consistent cell type manufacturing workflow.
- This includes CRISPR-Ready ioCells to enable genomic screens in complex models, isogenic ioDisease Model Cells and further engineering avenues.
- ioCells and derivatives are being assessed in multiple *in vitro* models, internally and in collaboration with industry or academic institutes, supporting ongoing NAM development.

Do reach out if you are interested in testing ioCells and bit.bio's technologies for your human model development!