Modelling neurodegeneration using a human isogenic system: A next generation approach to study frontotemporal dementia and amyotrophic lateral sclerosis

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Abstract

Patient-derived induced pluripotent stem cells (iPSCs) enable the generation of in vitro models that can recapitulate human disease phenotypes. However, conventional human iPSC differentiation protocols are often lengthy, inconsistent, and difficult to scale. The lack of genetically matched controls for patient-derived models further complicates the investigation of disease phenotypes.

bit.bio has developed opti-ox™, a precise and highly controlled iPSC reprogramming technology that overcomes these limitations and enables the generation of mature cell types and isogenic disease models. Our objective was to generate disease models for frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) for use with isogenic, wild type ioGlutamatergic Neurons to improve screening specificity and accelerate drug discovery for these neurodegenerative disorders.

We used CRISPR/Cas9 gene editing to introduce the disease-relevant mutation M337V in the *TDP-43 (TARDBP)* gene, and the mutations P301S or N279K in the *MAPT* gene, encoding Tau.

During the pathogenesis of FTD and ALS, mutant TDP-43 and Tau proteins are prone to misfolding, aggregation, phosphorylation and mislocalisation, and have been reported to affect a range of neuronal subtypes, including cortical glutamatergic neurons.

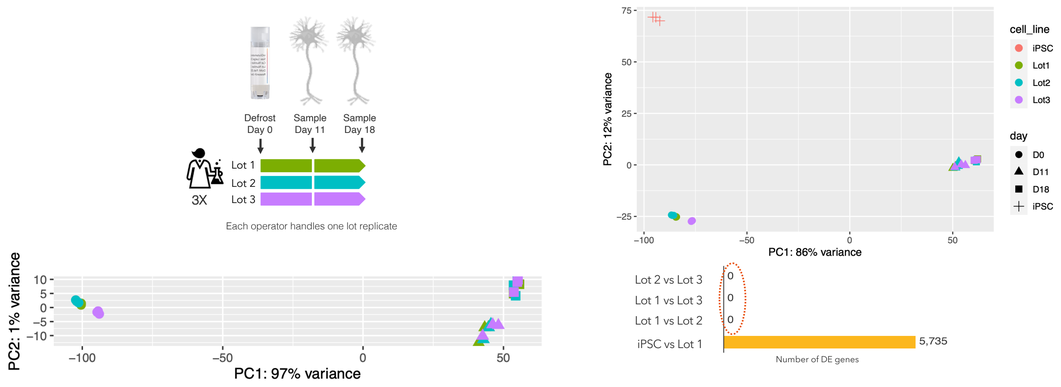
We have characterized these models and their isogenic controls to show the differences in their neuronal activity and proteinopathy.

opti-ox technology permits the generation of human iPSC-derived isogenic disease models and provides physiologically-relevant, robust, tools for neurodegenerative research and drug discovery.

1. Wild-type ioGlutamatergic Neurons demonstrate high lot-to-lot consistency and rapid maturation, ideal for the development of reliable disease models

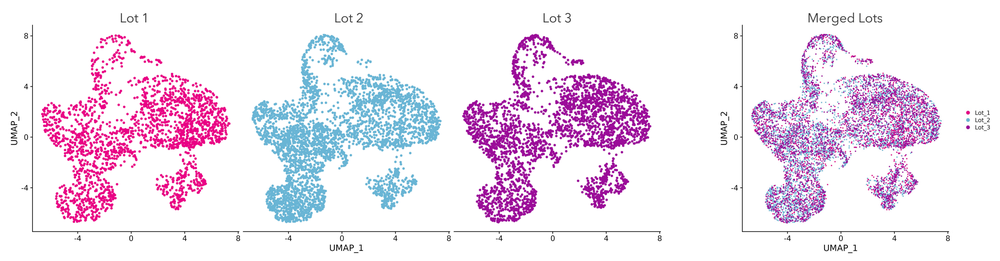
A

High lot-to-lot consistency of the opti-ox reprogrammed ioGlutamatergic Neurons is revealed by bulk RNA-sequencing analysis.
The experimental design includes three operators, each handling one lot replicate, of the different manufactured lots. A principle component analysis clusters the samples tightly at each timepoint, showing that precision reprogramming by opti-ox enables the manufacturing of ioGlutamatergic Neurons in a consistent manner. No statistically significant differentially expressed (DE) genes could be detected across the three lots at day 11 (|logFC| > 0.5 and FDR < 0.01). Day 11 - end of user manual; day 18 – extended culture condition.



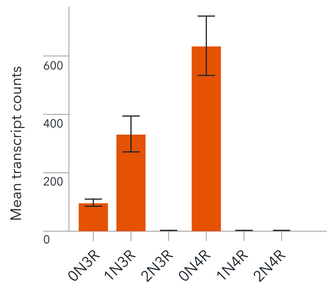
B

Transcriptomic similarity between cells from three lots of ioGlutamatergic Neurons.
Single cell RNA-sequencing demonstrates overlapping UMAPs across three independently generated lots of ioGlutamatergic Neurons at day 11, with a significant overlap when merged.



C

3R/4R Tau ratio at day 11 indicates mature ioGlutamatergic Neurons.
Human Tau, encoded by the *MAPT* gene, undergoes alternative splicing of exons 2, 3, and 10, which leads to the expression of six Tau isoforms in the adult human brain. Tau isoforms differ in their tubulin-binding domains, varying between three-repeat (3R) or four-repeat (4R) Tau. The inclusion of exon 10 leads to the expression of 4R Tau, while its exclusion generates the 3R isoforms. In the human adult brain, 3R and 4R isoforms of Tau are found in an equimolar ratio.
Bulk RNA-sequencing data demonstrates a similar ratio, circa 1:1 at day 11, indicating rapid maturation of ioGlutamatergic Neurons.

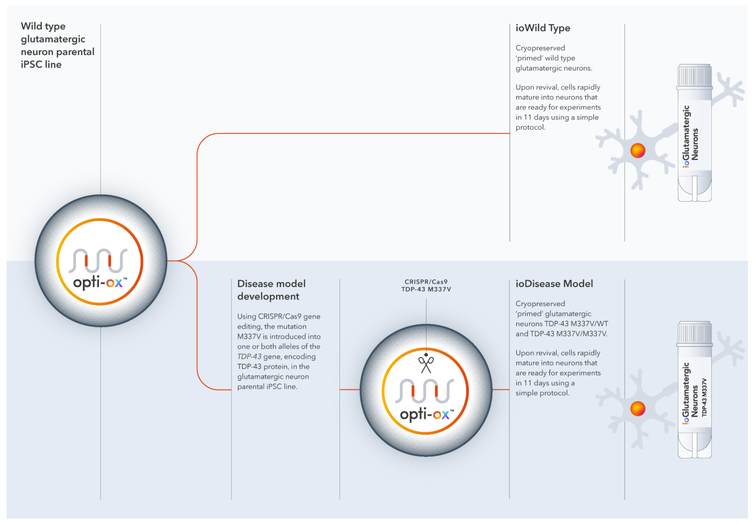


2. Generation of disease-relevant point mutations in the *MAPT* or *TDP-43* genes and their characterisation

A

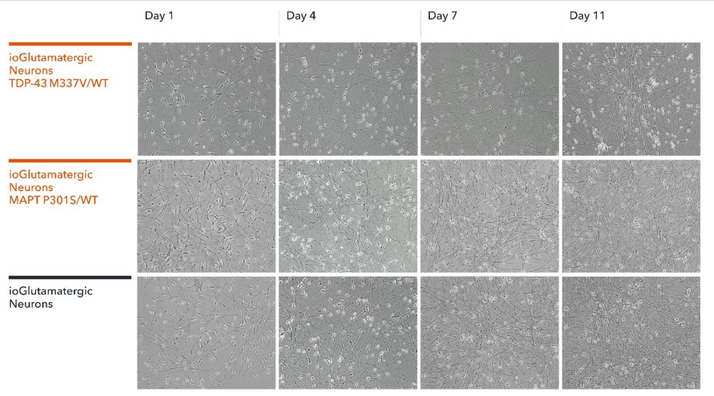
The schematic diagram shows an example of the development of ioDisease Model Cells™, in this instance, the ioGlutamatergic Neurons TDP-43 M337V disease model.
A homozygous or heterozygous M337V missense mutation in the *TDP-43* gene was engineered into a parental (wild type) iPSC line using CRISPR/Cas9 gene editing to generate an isogenic disease model. The same method was used to generate homozygous and heterozygous P301S or N279K mutations in the *MAPT* gene.

Gene	Mutation	Disease relevance
TDP-43	M337V	ALS, FTD
MAPT	P301S	FTD
MAPT	N279K	FTD



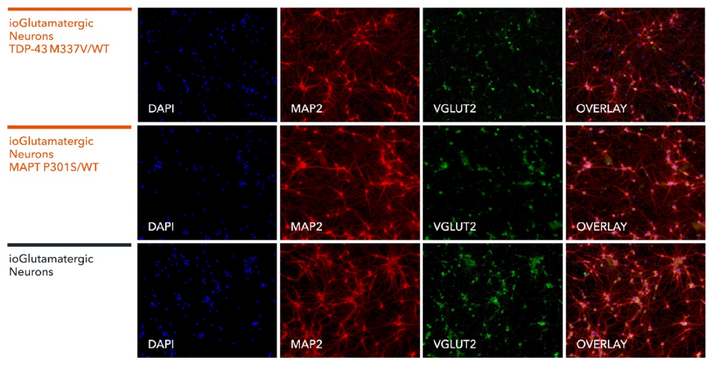
B

Formation of structural neuronal networks over 11 days.
Neuronal cultures of both isogenic control and disease models mature at identical rates forming more complex neuronal network structures over time.



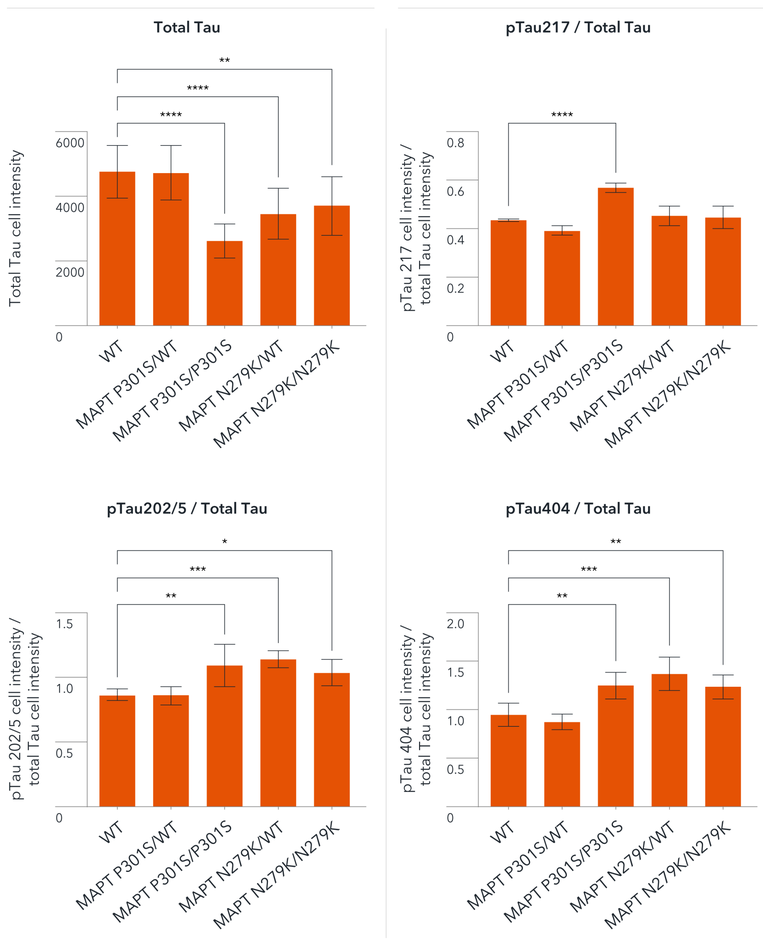
C

Disease model cells express neuron-specific markers reminiscent to the isogenic control.
Immunofluorescent staining on post-revival day 11 demonstrates similar homogenous expression of pan-neuronal proteins MAP2 and TUBB3 (not shown) and glutamatergic neuron-specific transporter VGLUT2 in disease model cells compared to the isogenic control; representative examples shown for ioGlutamatergic Neurons TDP-43 M337V/WT and ioGlutamatergic Neurons MAPT P301S/WT.



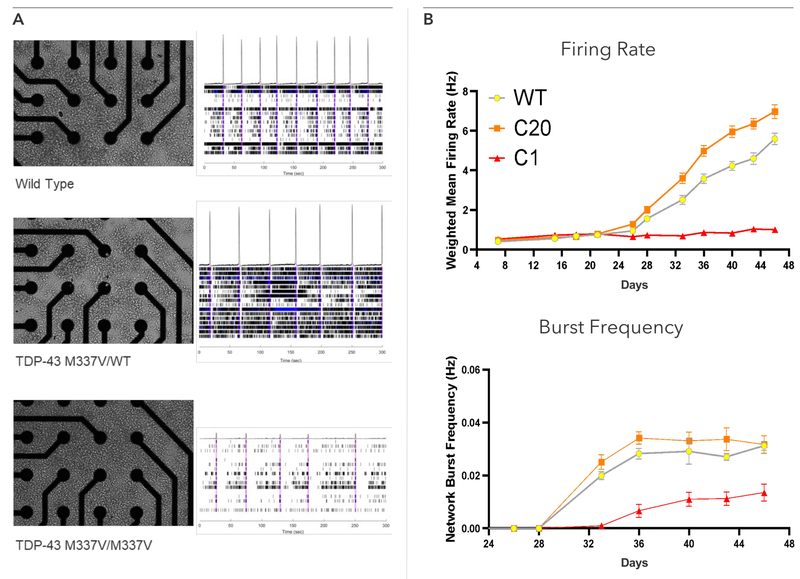
3. Hyperphosphorylation of Tau observed in the FTD disease model cells compared to the isogenic control

ioGlutamatergic Neurons MAPT P301S/P301S, MAPT N279K/WT and MAPT N279K/N279K show hyperphosphorylation when compared to isogenic control cells.
Bar graphs showing total Tau, pTau 217/total Tau, pTau 202/5/total Tau or pTau404/total Tau ICC analysis in cell bodies. Statistical analysis (one way ANOVA) are performed on 5 cellular replicates in the same plate. Bars, mean; error bars, SD. Statistics calculated by one way ANOVA and Tukey posthoc analysis. Data courtesy of Charles River Laboratories.



4. ALS disease model shows reduced neuronal activity

Reduced neuronal activity measured in TDP-43 M337V/M337V neurons compared to TDP-43 M337V/WT and isogenic control.
Microelectrode array (MEA) chips were spotted with 100K (~900K cells/cm^2) ioGlutamatergic Neurons (WT), TDP-43 M337V/WT (C20), or TDP-43 M337V/M337V (C1), along with 20K (~180K cells/cm^2) human iPSC-derived astrocytes. Brightfield at 26 DIV (**A**, left). Cells show good coverage of electrodes and produce clear burst and network burst activity as seen in the raster plot of activity (**A**, right). In the raster plot, each dash indicates a firing event, blue indicates a single electrode burst and the pink box indicates a network burst event. Quantification of raster plots over the course of culture shows that TDP-43 M337V/M337V neurons have a lower weighted mean firing rate, and network burst frequency than WT and TDP-43 M337V/WT neurons (**B**). No clear difference is noted between WT and TDP-43 M337V/WT neurons. Error bars indicate SEM, n=14 technical repeats. Data courtesy of Charles River Laboratories.



Summary & conclusions

ioGlutamatergic Neurons have been precision reprogrammed from human iPSCs into consistent, mature, functional neurons showing a high level of transcriptomic similarity between lots.

Morphological analysis demonstrated that ioGlutamatergic Neurons with disease-relevant mutations for FTD and ALS matured quickly, at identical rates to the wild type isogenic control.

The isogenic pairs also showed similar protein and gene expression of key neuronal markers ensuring biological comparability of the disease models.

Mutations in *MAPT* and *TDP-43* affect Tau phosphorylation and neuronal activity respectively and result in phenotypes relevant for FTD and ALS research.

Using opti-ox precision reprogramming technology to produce human iPSC-derived isogenic disease models provides physiologically and phenotypically-relevant in vitro tools for neurodegenerative research and drug discovery.