Modelling neurodegeneration using a human isogenic system:
A next generation approach to study Huntington's disease

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Abstract

The development of therapies to treat patients with neuronal indications is currently hampered by the use of animal models as less than 10% of findings derived from these preclinical models can be translated to humans. Patient-derived induced pluripotent stem cells (iPSCs) offer the possibility to generate *in vitro* systems to model neurological diseases that can recapitulate relevant human disease phenotypes. However, conventional human iPSC (hiPSC) differentiation protocols are often lengthy, inconsistent, and difficult to scale.

More importantly, the lack of genetically matched controls for patient-derived models further complicates the investigation of disease-relevant phenotype and study of molecular mechanisms underlying neurodegeneration.

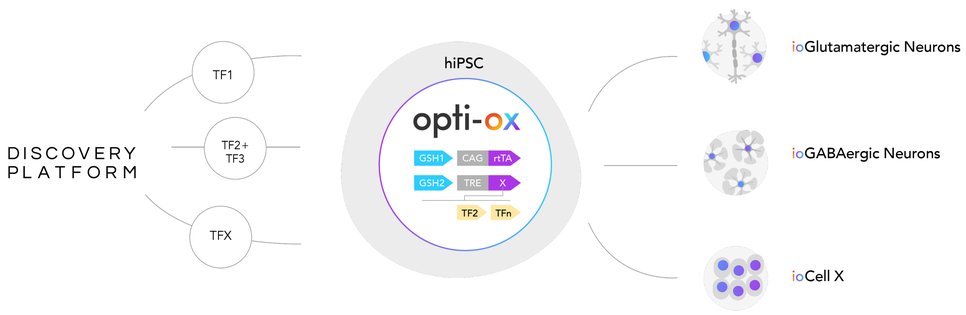
To overcome these problems, we developed a proprietary gene-targeting strategy (opti-ox™) that enables highly controlled expression of transcription factors to rapidly reprogram hiPSCs into pure somatic cell types in a scalable manner. Combined with CRISPR/Cas9-mediated genetic engineering enabled us to introduce specific mutations in these hiPSC-lines and create isogenic disease models that will improve screen specificity and accelerate drug development.

opti-ox™ based isogenic ioGlutamatergic Neurons Huntington's disease (HD) model carrying a 50CAG expansion in the huntingtin (*HTT*) gene have been developed using this approach. Mutant huntingtin proteins containing elongated polyglutamine (PolyQ) stretches are aggregation-prone and have been reported to affect a range of neuronal subtypes, including the cortical glutamatergic neurons.

Characterisation of these neurons by immunochemistry and RT-qPCR showed that the expression profile of pan-neuronal (MAP2 and TUBB3) and glutamatergic (VGLUT1 and VGLUT2) marker genes as well as of the HTT transcript itself are highly similar between ioGlutamatergic Neurons HTT 50CAG and the isogenic control, wild type (WT) ioGlutamatergic Neurons. We are currently performing an in-depth phenotypic characterisation of this disease model and the genetically matched control to determine the differences in their transcriptome, neuronal activity and mitochondrial functions.

Beside the 50CAG mutation in HTT, we have generated mutations in the MAPT, TARDBP, GBA and PRKN to provide isogenic disease models for FTD, FTD/ALS and Parkinson's disease. Our novel strategy to use the opti-ox™ technology for the scalable and consistent production of hiPSC-derived isogenic disease models, offers new avenues into drug discovery and can accelerate research and the development of new therapeutics.

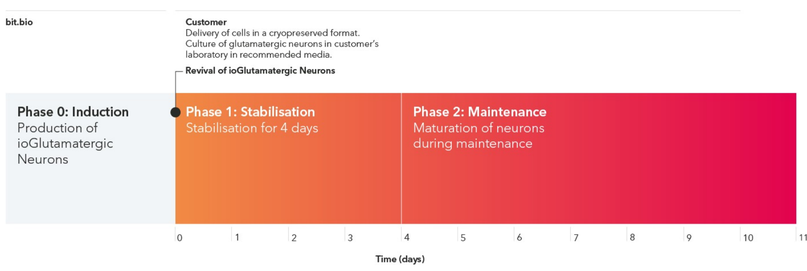
1. Precise opti-ox™ reprogramming into ioGlutamatergic Neurons



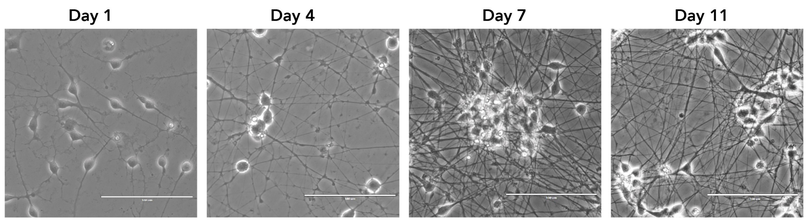
opti-ox™ technology for the optimal cellular reprogramming of human iPSCs into defined human cell types. opti-ox™ dual cassette Tet-ON system ensures tightly controlled and homogeneous expression of reprogramming transcription factors (TFs) by preventing silencing of the inducible expression cassette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

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

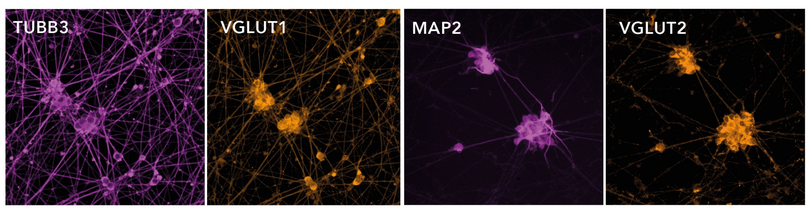
ioGlutamatergic Neurons show rapid maturation (A) and form visible structural networks by day 11 post-revival (B). Cultures consist mainly of glutamatergic neurons (>80%) characterised by the expression of the glutamate transporter genes VGLUT1 and VGLUT2 (C, D, E). Four days after initiation of reprogramming, ioGlutamatergic Neurons show no expression of pluripotency markers and express pan-neural genes (data not shown).



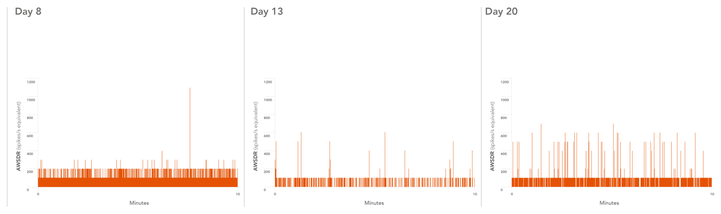
(A) Arrive ready to plate.
The 3 phase protocol for generating ioGlutamatergic Neurons: 1. Induction (carried out at bit.bio), 2. Stabilisation (4 days), 3. Maintenance during which the neurons mature.



(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.



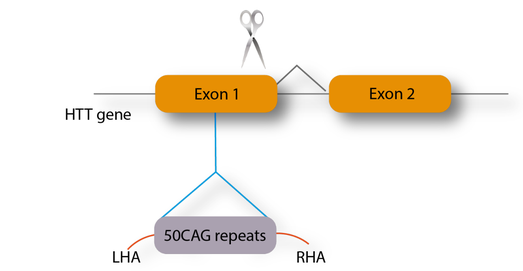
(C) Express glutamatergic neuron-specific markers & exhibit neurite outgrowth.
Immunofluorescent staining 11 days post-revival demonstrates homogenous expression of pan-neuronal proteins (MAP2 and TUBB3) and glutamatergic neuron-specific transporters (VGLUT1 and VGLUT2).



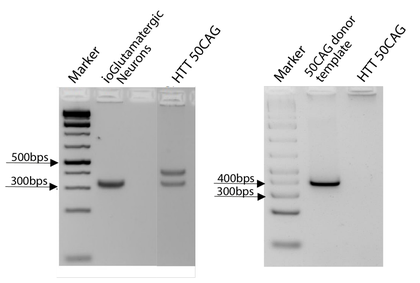
(D) Time-dependent increase in synchronous activity upon co-culture with rat astrocytes.
Results show prominent synchronicity on Day 13, exemplified by the 'spikier' nature of the associated Array Wide Spike Detection Rate (AWSDR) histograms, which increases at Day 20.

3. Generation of 50CAG repeat expansion in huntingtin gene

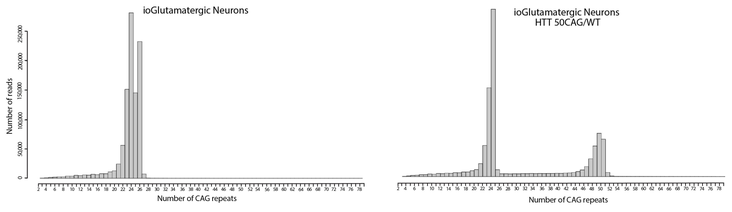
A heterozygous 50CAG mutation in the *HTT* gene was introduced into a WT ioGlutamatergic Neuron cell line using CRISPR/Cas9 gene editing to generate an isogenic disease model.



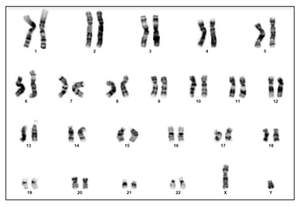
(A) Targeting strategy.
A plasmid donor containing left and right arms of homology, and the 50 CAG repeat template was targeted to exon 1 of the *HTT* gene using CRISPR/Cas9 delivered by RNP.



(B) On-target integration.
Confirmation of the on-target integration of 50CAG expansion into one *HTT* allele (left panel). Amplicon PCR of the plasmid donor reveals no random integration in genomic DNA from targeted colonies (right panel) via gel electrophoresis.

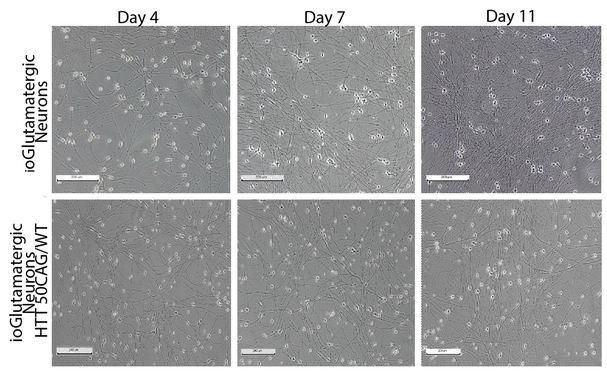


(C) NGS-Amplicon sequencing.
NGS-Amplicon Sequencing confirms both WT and 50CAG repeat alleles. The number of CAG repeat reads peak at 24 (WT, left panel) and 50 (50CAG, right panel).

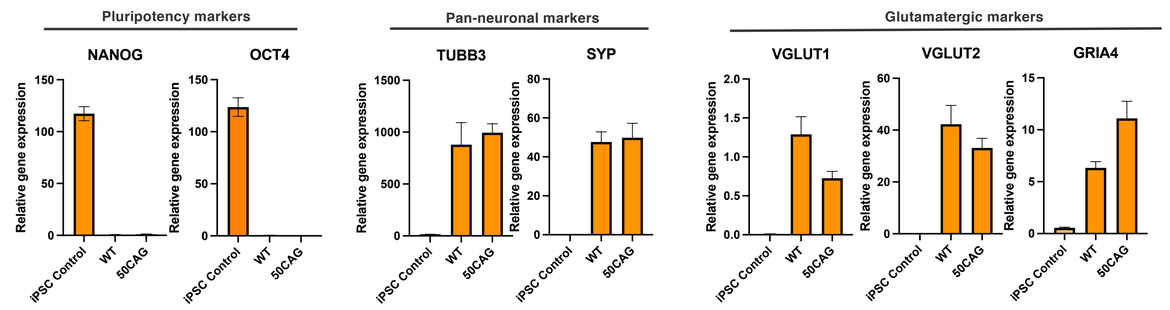


(D) No chromosomal abnormalities were detected following CRISPR/Cas9 editing of *HTT* gene.
G-banding analysis confirmed no chromosomal abnormalities and expected male karyotype.

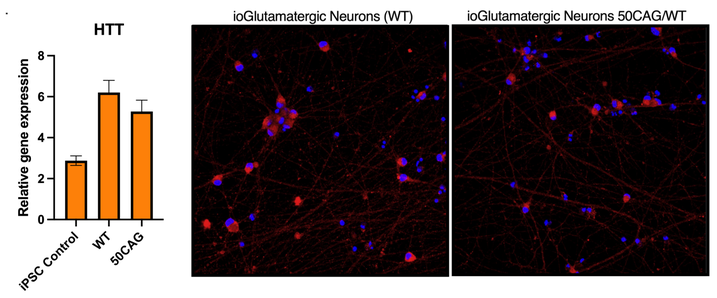
4. Characterisation of the isogenic Huntington's disease model



(A) Neuron-like morphology and formation of structural networks in HTT 50CAG glutamatergic neurons.
No morphological changes between the ioGlutamatergic Neurons HTT 50CAG/WT cells and their genetically matched parental cells were observed for up to 11 days in culture. Mutant cells matured over time by showing complex neuronal network structures (10x).



(B) HTT 50CAG/WT cells express glutamatergic markers by day 11.
The expression of the TUBB3, MAP2 and VGLUT2 markers in the ioGlutamatergic Neurons HTT 50CAG/WT cell were analysed at day 11 post-thawing by ICC and compared to WT cells to assess the ability of these clones to give rise to mature glutamatergic neurons.



(C) Huntingtin expression in the HTT 50CAG cells and their isogenic control.
Huntingtin was expressed in both WT and 50CAG glutamatergic neurons at day 11 as assessed by qPCR (left panel) and ICC analysis as day 20 post-revival (right panel; red= *HTT*; blue= Hoechst). ICC images provided by Origami Therapeutics.

Conclusions

- opti-ox™ reprogramming converts hiPSC to ioGlutamatergic Neurons in a highly synchronised and consistent manner
- Reprogrammed cells show rapid maturation and give rise to functional glutamatergic neurons in 2-3 weeks
- Using CRISPR/Cas9 editing we successfully introduced a 50CAG expansion in exon 1 of the *HTT* gene in an opti-ox™ enabled ioGlutamatergic Neuron cell line creating a novel isogenic human Huntington's disease model
- This isogenic disease model, ioGlutamatergic Neurons HTT 50CAG/WT, will enable detection of even the most subtle phenotypic differences associated with Huntington's disease

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