Characterization of a human iPSC derived Huntington's disease cell line suitable for disease modelling and drug screening

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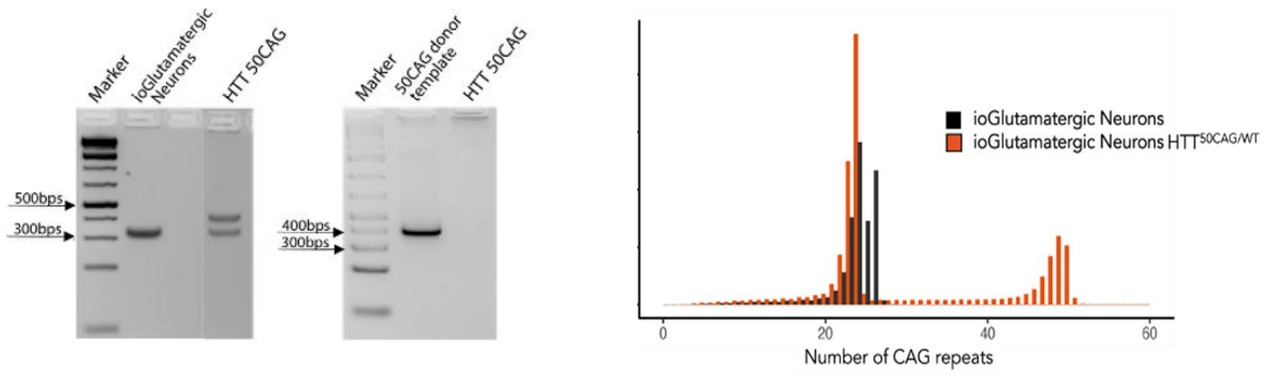
1 Background

- Huntington's disease (HD) is a genetically inherited autosomal dominant neurodegenerative disorder caused by a trinucleotide CAG (glutamine) repeat in the Huntingtin (HTT) gene.
- Age of onset is strongly correlated to CAG repeat length.
- Symptoms are characterised by motor, cognitive and psychiatric deficits and no effective treatment exists for preventing onset or delaying progression.
- Precision cell reprogramming technology, opti-ox™, in combination with CRISPR-Cas9 gene editing has been used to develop iPSC-derived ioGlutamatergic Neurons carrying a 50 CAG repeat expansion in the HTT gene.

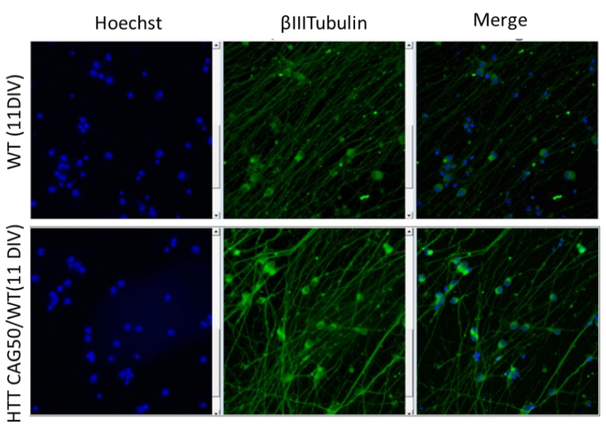
2 Cell line generation and characterization

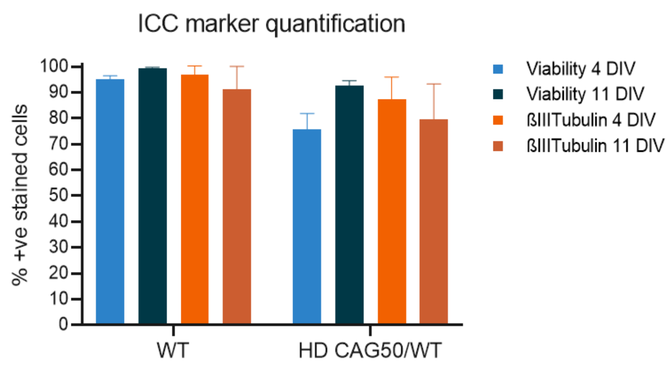


ioGlutamatergic Neurons carrying a50 CAG repeat expansion in the HTT gene were generated by bit bio using CRISPR-Cas9 technology. Gel electrophoresis and NGS amplicon sequencing were then used to confirm the heterozygous 50CAG mutation in the HTT gene.

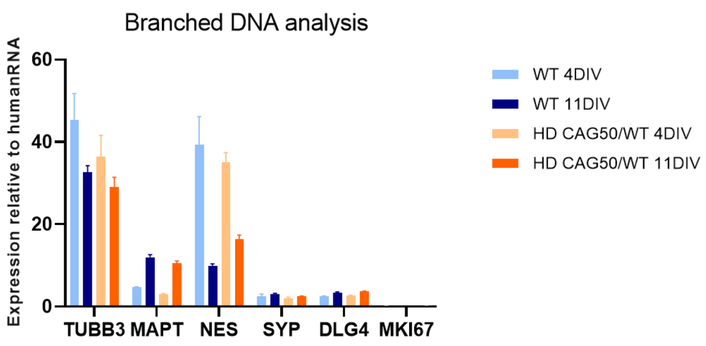
ioGlutamatergic Neurons (wild type (WT) and HTT CAG50/WT) → Assessment of cell viability and morphology during differentiation → Immunocytochemistry (ICC) and branched DNA (bDNA) analysis → Applications for drug discovery

Isogenic pairs of ioGlutamatergic Neurons (wild type (WT) and HTT 50 CAG/WT) were thawed and differentiated in parallel in 384 well plates. Neurons were monitored during differentiation and ICC and bDNA assays were performed after 4 and 11 days in vitro (DIV).
Results from those assays are indicated in the images below.



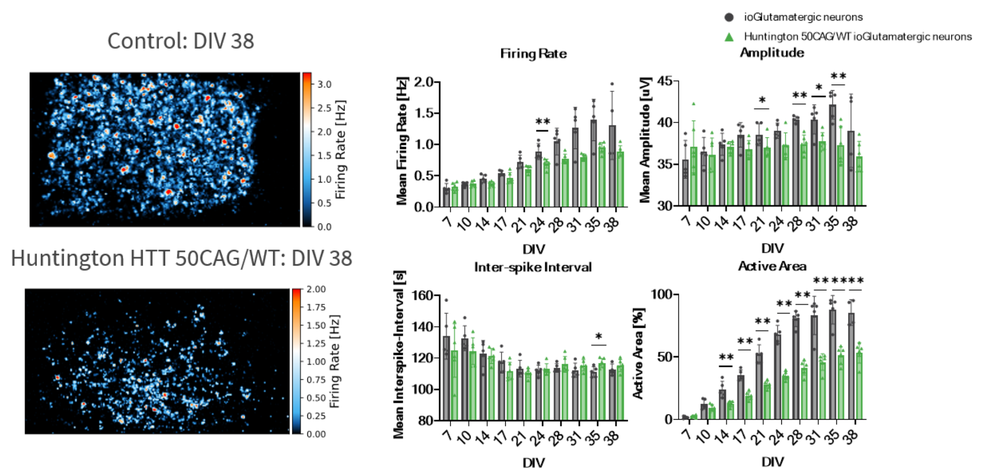


ioGlutamatergic Neurons from WT and HTT 50 CAG/WT showed a complex neurite network already at 11DIV when stained with βIIITubulin. High content image analysis showed a high level of expression of βIIITubulin neuronal marker already at 4DIV. The graph also shows that viability measured by Hoeschst staining remained above 70% during differentiation in both isogenic cells.

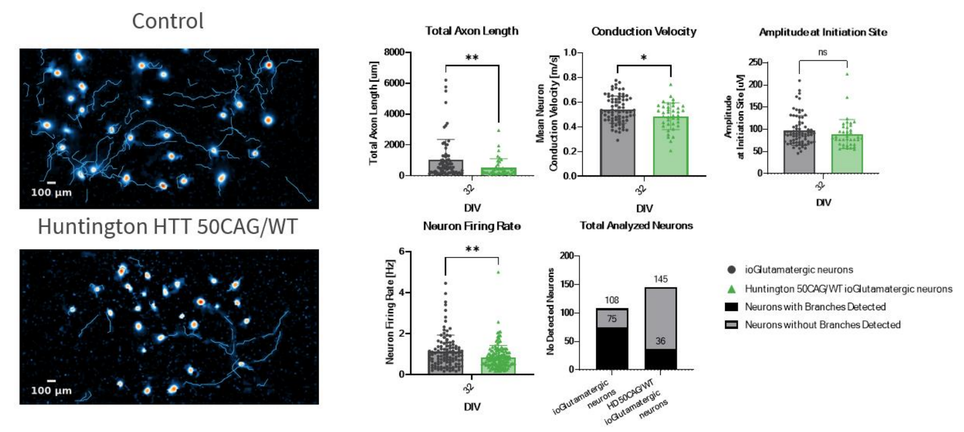


Branched DNA assay showed expression level changes of a small subset of selected genes in the WT and the HTT 50 CAG/WT cells at 4DIV and 11DIV suggesting comparable differentiation status. Gene expression was normalised against 3 different housekeeping genes and represented in the graph as expression level relative to total human RNA. Data was gathered using a Luminex MAGPIX.

3 Functional characterization

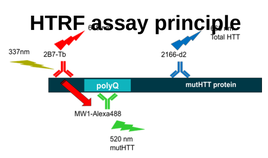


Neuronal activity was monitored in the ioGlutamatergic neurons WT and HTT 50 CAG co-cultured with astrocytes up to 38 DIV using the high-density microelectrode array MaxTwo (MaxWell Biosystems). ActivityScan Assay analysis showed that the HTT 50 CAG/WT disease model displays delayed network formation compared to the WT isogenic control. In particular, significant differences in amplitude and the active area were observed.



The AxonTracking Assay was also performed with the ioGlutamatergic Neurons WT and HTT 50 CAG/WT cells which showed that the total axon length, conduction velocity and firing rate were significantly reduced in the HTT 50 CAG/WT disease model when compared to WT control.

4 Neuronal HTRF assay development



Protocol workflow

Day 0
Thaw and plate neurons in 384 well plate

Day 2
Feed with neuronal media +Doxycycline

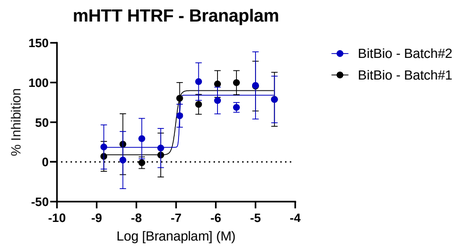
Day 4
Media change and compound addition for 7 days

Day 11
Cell lysed and HTRF and CTG assay performed

HTRF assays are routinely used at Charles River to test compounds that lower mutant HTT protein in patient derived cells. The assay is very robust and fully automated for HD derived pluripotent stem cells however it required further optimization when used with HD derived neurons. Therefore, a Design of Experiment (DoE) was performed, which included 36 variables, in order to establish the best conditions for a neuronal HTRF assay.

Potential Final Assay Conditions following DOE

Factors	Conditions
Seeding density	35k cells/well
Compound incubation time course	7 days
Number of washes before lysis	0
Lysis buffer	PBS + TX100
Lysis buffer volume	40 µl
Lysis buffer concentration	7%
Lysis incubation temperature	RT
Lysate	Fresh
Plate type	Black
Antibody detection buffer	CRL
Lysate volume	5 µl
Antibody volume	2 µl
2B7 antibody concentration	0.05 ng/µl
MW1 antibody concentration	2 ng/µl
Antibody/lysate incubation temperature	RT
Read time	4 hrs
Reader protocol	Optimised



ioGlutamatergic Neurons HTT 50 CAG/WT were treated for 7 days with a tool compound Branaplam, a splicing modulator, and showed a concentration response reduction of mutant HTT protein. Although the assay was reproducible in the two batches of cells tested further optimization is require for full automation of the assay for screening a larger number of compounds.