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

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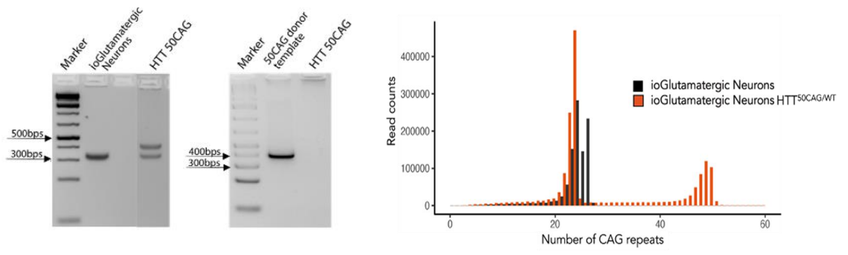
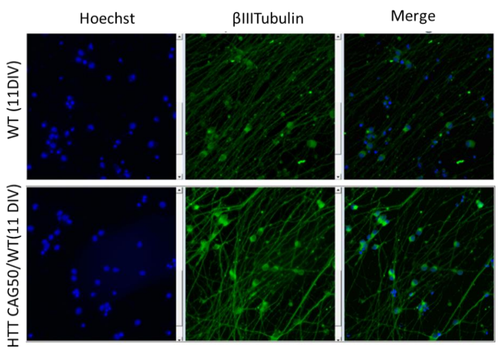
1 Background

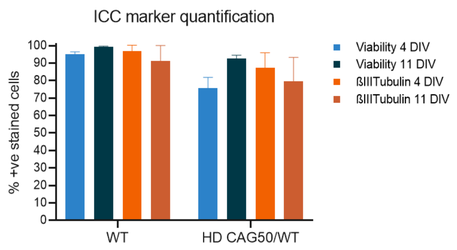
- Huntington's disease (HD) is a genetically inherited autosomal dominant neurodegenerative disorder caused by a trinucleotide CAG (glutamine) repeat in the Huntingtin (HTT) gene.
- Age of onset is strongly correlated to CAG repeat length.
- Symptoms are characterised by motor, cognitive and psychiatric 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

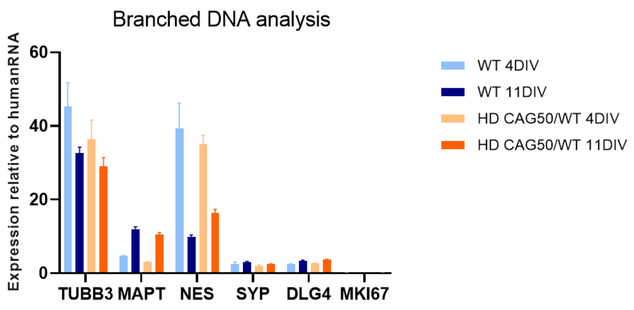


ioGlutamatergic Neurons carrying a 50 CAG repeat expansion in the HTT gene were generated by bit bio using CRISPR-Cas9 technology. Gel electrophoresis and NGS amplicon sequencing were then used to confirm the heterozygous 50CAG mutation in the HTT gene.



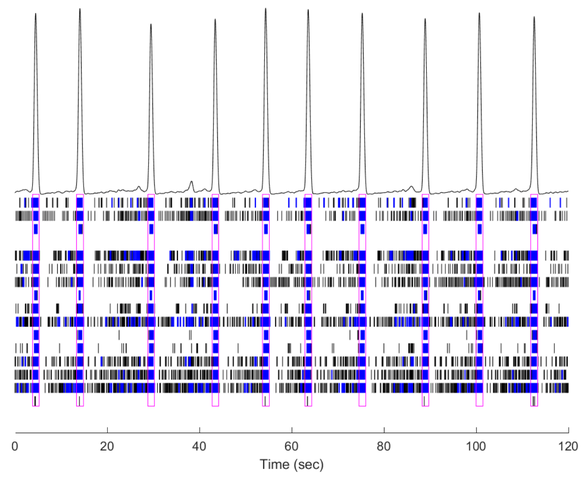


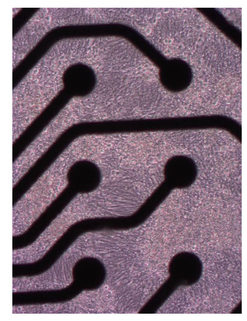
ioGlutamatergic Neurons from WT and HTT 50 CAG/WT showed a complex neurite network already at 11DIV when stained with βIIITubulin. High content image analysis showed a high level of expression of βIIITubulin neuronal marker already at 4DIV. The graph also shows that viability measured by Hoechst staining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

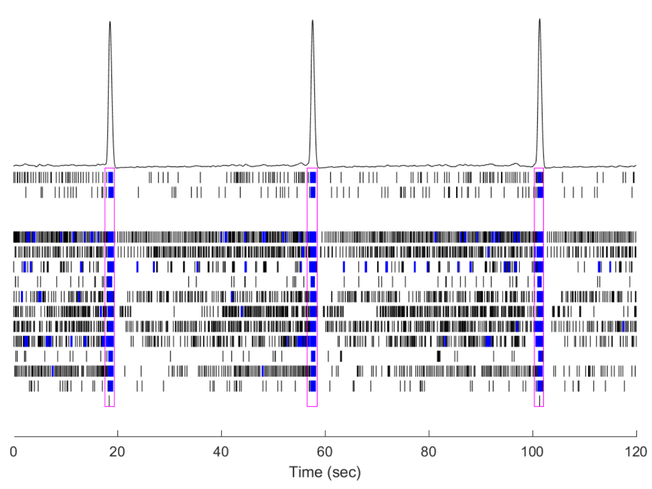


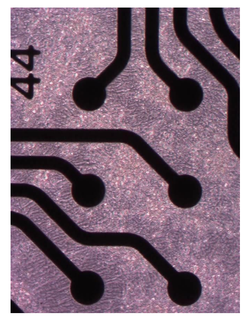
Branched DNA assay showed expression level changes of a small subset of selected genes in the WT and the HTT 50 CAG/WT cells at 4DIV and 11DIV suggesting comparable differentiation status. Gene expression was normalised against 3 different housekeeping genes and represented in the graph as expression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

3 FUNCTIONAL CHARACTERIZATION – MULTI ELECTRODE ARRAY (MEA)

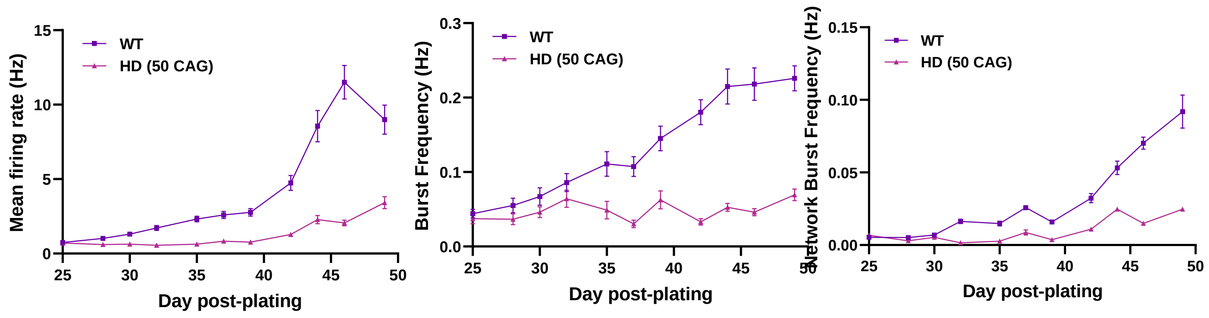








50K ioGlutamatergic Neurons WT and HTT 50CAG/WT co-cultured with 10K iCell Astrocytes (Fujifilm CDi) spotted to MEA plates. At 44 DIV in culture cells show good coverage of electrodes and produce clear burst and network burst activity as seen in the raster plot of activity (left). In the raster plot, 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 HTT 50CAG/WT neurons have a lower mean firing rate, burst and network burst frequency than WT neurons.
Error bars indicate SEM, n=24 technical repeats.

4 CONCLUSIONS

- ioGlutamatergic Neurons carrying a 50 CAG repeat expansion in the HTT gene were successfully generated by bit bio using CRISPR-Cas9 technology.
- Neurons from both WT and HTT 50 CAG/WT repeat showed neuronal morphology and expression of neuronal markers after few days in culture.
- Functional characterization using MEA platform showed electrophysiological differences between the WT and HTT CAG50/WT cell line.
- ioGlutamatergic Neurons HTT 50 CAG/WT are suitable for compounds screening however further optimization is required for high-throughput screening applications.