Molecular and functional characterization of stem cells-derived glutamatergic neurons ioNEURONS/glut in support of drug discovery applications

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1 ABSTRACT

Central Nervous System (CNS) disorders are widely recognized as major economic, emotional and physical burden to patients, their families and society. Although progress has been made in the basic neuroscience research, there are still several challenges to overcome in order to find novel therapies and treatments for CNS diseases. One major limitation in current neurological research and drug discovery is the lack of human neuronal disease models which are biological relevant, scalable and reproducible.

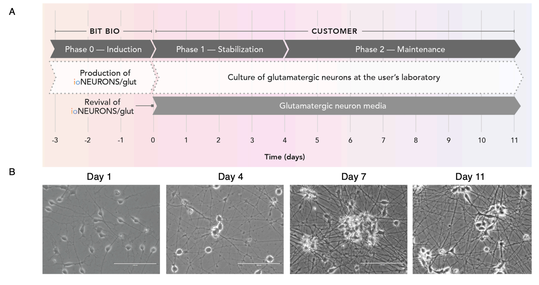
The advent of induced Pluripotent Stem Cell (iPSC) technology has offered new opportunities for disease modelling and drug discovery, as patient derived iPSCs and their derivatives represent more relevant in vitro models than those currently available involving other animal cells. Although protocols for iPSC differentiation into different lineages have been established, they are normally time consuming and often need further optimization to reproducibly generate highly consistent and scalable specific cell types for high throughput drug screening (HTS).

Charles River has performed molecular and functional characterization of human glutamatergic neurons (ioNEURONS/glut) differentiated from iPSCs using the forward reprogramming technology developed by Bit Bio[1].

The advantage of forward reprogramming technology, consisting of forced expression of transcription factors, provides an alternative to conventional differentiation protocols and accelerates lineage conversion and stem cell specification. Additionally, Bit Bio technology has overcome the limitation of deficient or heterogeneous inducible gene expression in hPSCs observed using alternative direct reprogramming methods, such as lentiviral transduction, and has established inducible gene expression in hPSCs using a dual genomic safe harbour gene-targeting strategy described in M Pawlowski et al. Stem Cell Rep, 2017[1].

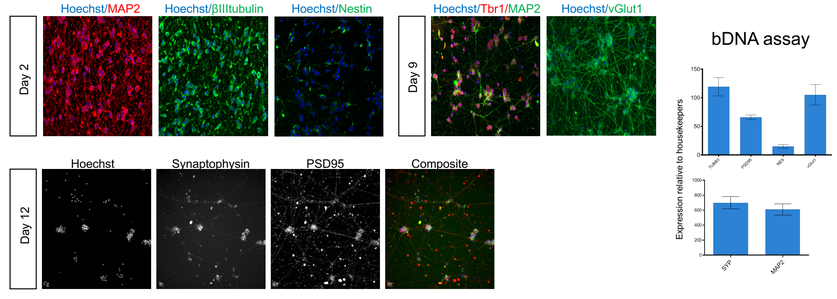
Here we have tested Bit Bio ioNEURONS/glut for the expression of markers of the neuronal lineage, functional readouts, and their ability to be used for HTS. Preliminary immunocytochemistry and branched DNA data showed expression of pan neuronal markers in glutamatergic (ioNEURONS/glut) neurons after only 2 days in culture. Moreover, functional MaxOne High-Resolution multi electrode array (MEA) data showed the presence of spontaneous activity after 3 weeks of differentiation when neurons were cultured in Brain Phys media. Finally, when applying to HTS applications (TR-FRET assay) including a cytotoxicity assay (Cell Titer Glo), ioNEURONS/glut neurons plated in 384 microplates and treated with tool compounds showed good assay statistics and higher suitability for HTS.

2 METHODS



Glutamatergic neurons (ioNEURONS/glut) were differentiated from human NGN2 opti-ox™ iPSCs generated by Bit Bio according to their published protocol[1]. This figure shows a schematic representation of the neuronal differentiation protocol and example images of neurons during differentiation.

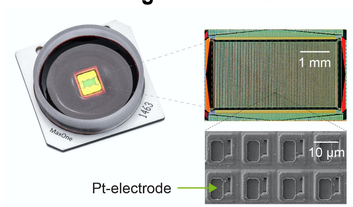
3 MORPHOLOGICAL AND MOLECULAR PROFILE



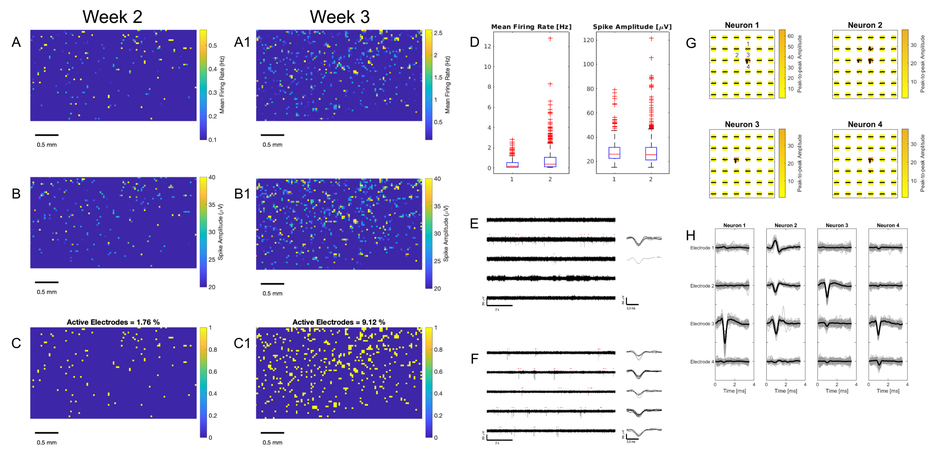
Representative images of ioNEURONS/glut neurons at day 2, 9 and 12 of differentiation. Cells show presence of pan-neuronal markers already after 2 days of differentiation and Tbr1 and vGlut1 at day 9. Synaptic markers PSD95 and Synaptophysin were detected at day 12 of differentiation. Branched DNA assay confirmed gene expression profile of key neuronal and synaptic markers.

4 FUNCTIONAL CHARACTERIZATION

MaxOne High-Resolution MEA

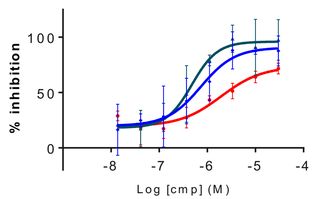


- **26400** electrodes
- **2x4 mm²** recording area
- **17.5 µm** electrode pitch
- Low-noise readouts, **2.4 µV$_{rms}$**
- Electrical stimulation



Examples of activity maps based on firing rate (A and A1), spike amplitude (B and B1) and % of active electrodes (C and C1) show a time-dependent increase of activity during neuronal maturation from 2 to 3 weeks post plating. (D) Distributions of all electrode mean firing rates and electrode spike amplitudes at these two time points. Representative voltage traces from five electrodes at week 2 (E) and week 3 (F), with relative spike waveforms. (G) Single-cell footprints plot traces and (H) average spike traces for 4 selected electrodes (Neurons 1 & 2 from week 2 and Neurons 3 & 4 from week 3). All recordings were made in BrainPhys media.

5 HTS COMPATIBILITY



	Signal/Background
CTG	327
HTT HTRF	1.65
AKT HTRF	1.95

HTRF and CTG assays in ioNEURONS/glut neurons treated with tool compound. Compound titration shows concentration response curve in all three assays (mean±sd of 2 replicates). Average signal/background of 2 experiments is excellent for CTG, but lower for both HTRF assays due to lower assay sensitivity.

6 CONCLUSIONS

Charles River has characterised glutamatergic neurons (ioNEURONS/glut) derived by Bit Bio using the forward reprogramming technology. Preliminary molecular data using immunocytochemistry and bDNA assays showed expression of pan neuronal markers MAP2 and βIIItubulin already 2 days post plating and synaptic markers after 12 days of differentiation. Moreover MEA analysis using the MaxOne high resolution MEA system showed presence of spontaneous activity at 3 weeks post plating when neurons were cultured in Brain Phys media. Finally TR-FRET (HTRF) assays for Huntington (HTT) and protein kinase B (AKT) proteins and CTG assay showed lower signal but low variability indicating a good suitability for HTS platforms. Further work will be required to fully characterise ioNEURONS/glut function (e.g. co-cultures with astrocytes) and compound response. However, ioNEURONS/glut provide a promising platform to advance drug discovery programs in physiologically relevant cell type of the CNS.

ACKNOWLEDGEMENTS

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REFERENCES

1. M Pawlowski et al. Inducible and Deterministic Forward Programming of Human Pluripotent Stem Cells into Neurons, Skeletal Myocytes, and Oligodendrocytes. Stem Cell Reports j Vol. 8 j 803–812 j April 11, 2017.