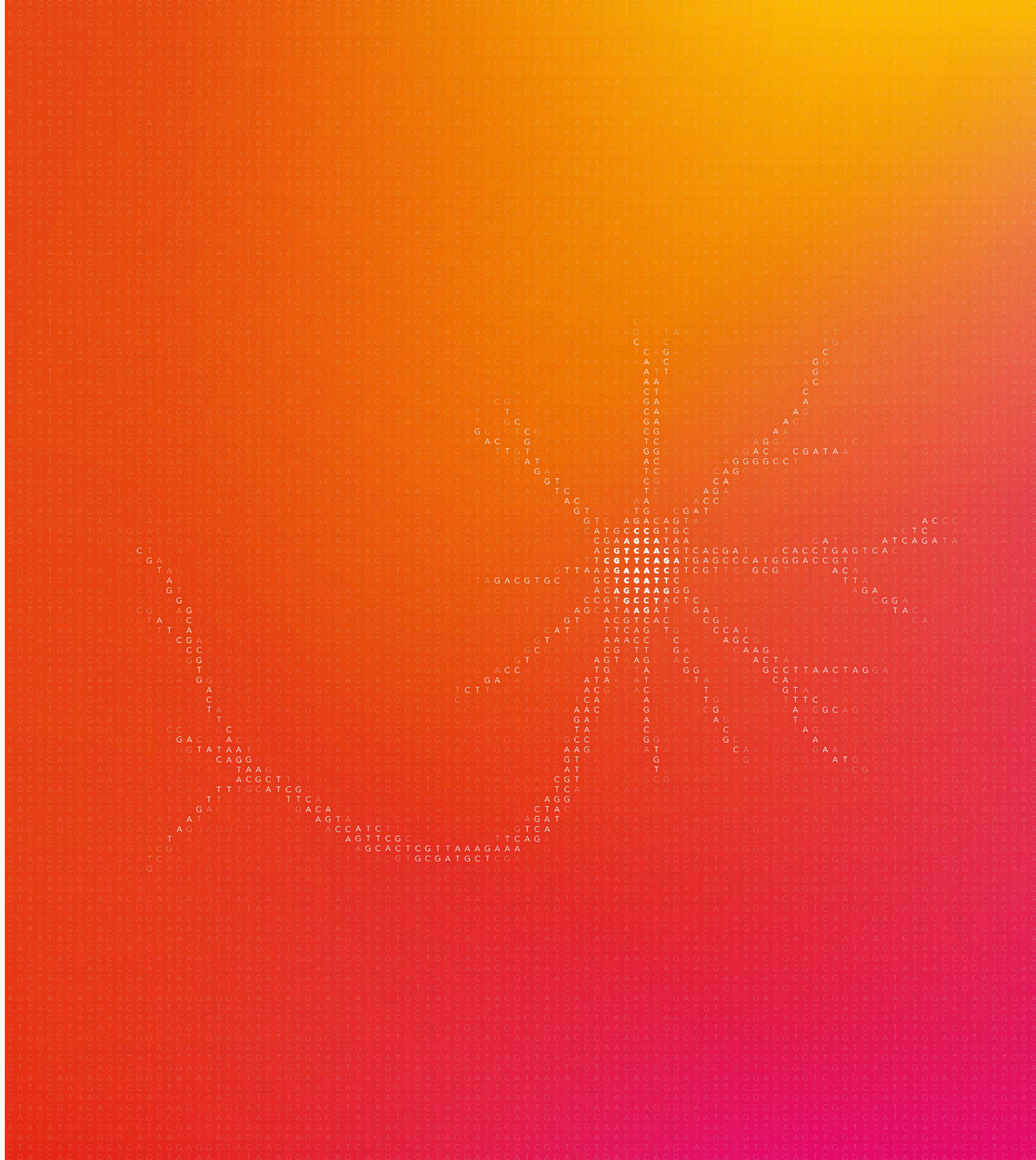
ioGlutamatergic Neurons

HTT·50CAG/WT

Cat. No. ioEA1004

bit.bio

THE CELL CODING COMPANY



Discover ioCells™

Programmed identity in every cell

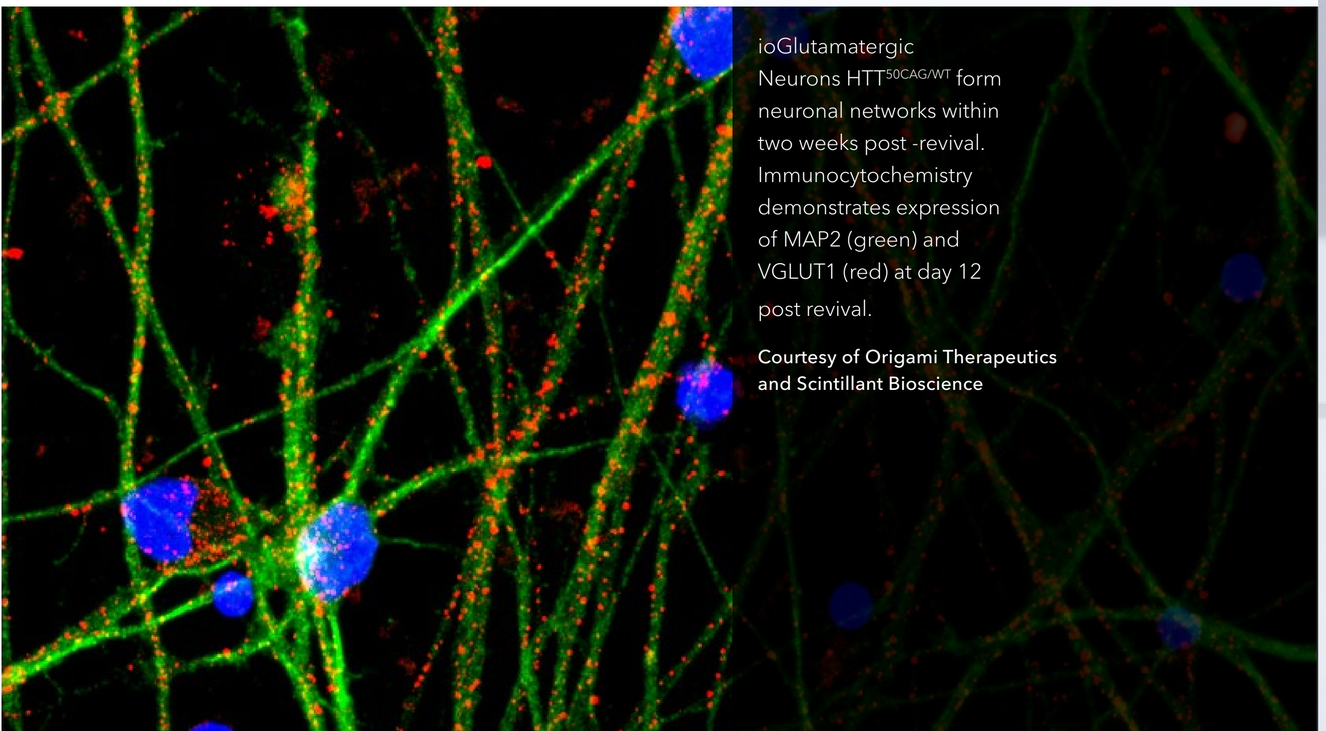
Next-generation iPSC-derived human cells driven by opti-ox™ precision reprogramming technology, offering speed, reliability and consistency at scale for research and drug discovery.

ioWild Type

ioWild Type cells are human iPSC-derived cells, powered by opti-ox™. On delivery, the cells mature rapidly and are ready-to-use within days of culture. The portfolio of cells are highly characterised and consistent at scale with batch-to-batch reproducibility, making them ideally suited to high throughput screening applications.

ioDisease Model

ioDisease Model cells are a range of precision reprogrammed human iPSC-derived cells with disease-relevant mutations for studying disease-driving mechanisms in CNS and muscle disorders. Disease-relevant mutations have been engineered into opti-ox™ reprogrammed wild type ioCells.



ioGlutamatergic Neurons HTT$^{50CAG/WT}$ form neuronal networks within two weeks post -revival. Immunocytochemistry demonstrates expression of MAP2 (green) and VGLUT1 (red) at day 12 post revival.

Courtesy of Origami Therapeutics and Scintillant Bioscience

ioDisease Model

A next-generation approach to study Huntington's disease

A rapidly maturing, consistent and scalable isogenic system to study Huntington's disease

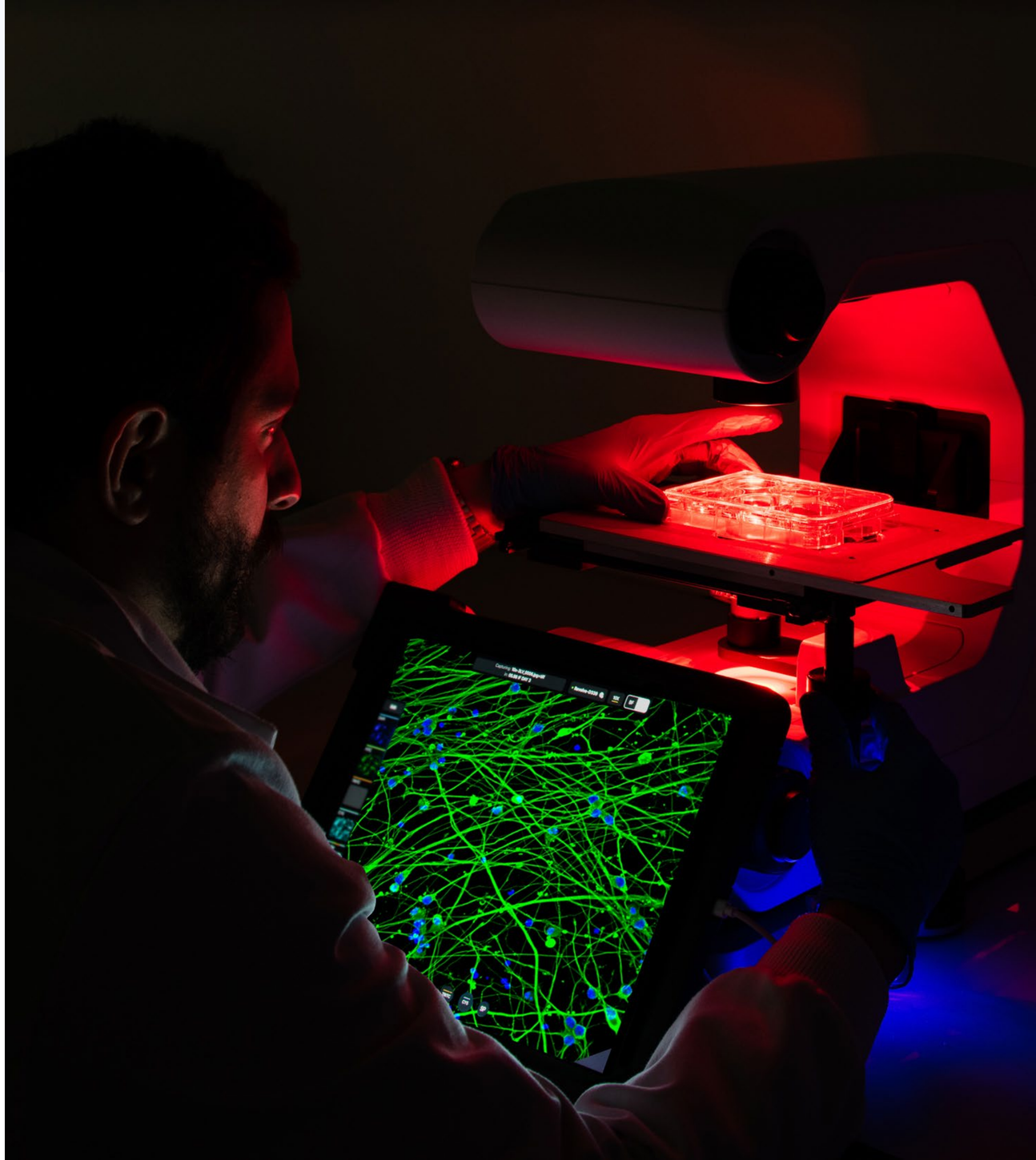
Patients with Huntington's disease present an abnormal expansion of CAG repeats in their Huntingtin (*HTT*) gene. Alleles with greater than 40 repeats are pathogenic, and there is an inverse correlation between the extent of the CAG trinucleotide repeat expansion and the age of disease onset[1].

bit.bio recognises that to advance Huntington's disease research and to improve efficiencies in drug discovery, a disease model that is isogenic, easy to culture, and quick to mature with batch-to-batch consistency is essential. To meet this need, we have developed ioGlutamatergic Neurons HTT$^{50CAG/WT}$ – opti-ox™ precision reprogrammed[2] glutamatergic neurons containing a heterozygous 50 CAG trinucleotide repeat expansion in exon 1 of the *HTT* gene.

Our wild type ioGlutamatergic Neurons form the genetically matched control for the ioGlutamatergic Neurons HTT$^{50CAG/WT}$ disease model. This physiologically-relevant isogenic pairing offers a powerful next-generation model to study Huntington's disease in research and drug discovery.

1. Capiluppi *et al.* Neurol Sci 2020 https://doi.org/10.1007/s10072-019-04177-8

2. Pawlowski *et al.* Stem Cell Reports 2017 www.ncbi.nlm.nih.gov/pmc/articles/PMC5390118



Benchtop benefits

EASY-TO-USE
Cells are programmed to mature rapidly upon revival with a simple two-phase protocol using an open-sourced medium required.

QUICK
Cells are ready for experimentation within days post-revival, expressing key neuronal markers and forming structural networks within 11 days post-revival.

DEFINED
The cell has been characterised by ICC, RT-qPCR and bulk RNA-seq.

CONSISTENT
opti-ox™ precision reprogramming results in batch-to-batch homogeneity, overcoming the challenge of reproducibility when using iPSCs for research and drug discovery.

SCALABLE
Industrial scale quantities are available with industry-leading seeding densities, and at a price point that allows the cells to be used from research to high throughput screening.

COST-EFFECTIVE
Available in two vial sizes, tailored to suit your experimental needs with minimal waste.

MAKE TRUE COMPARISONS
Be confident in your data. ioDisease Model cells can be paired with ioWild Type cells to provide a genetically matched, highly characterised background for the precise analysis of gene function.

ioWild Type and ioDisease Model: A true comparison

Be confident in your data by pairing ioDisease Model cells with the genetically matched ioWild Type control.

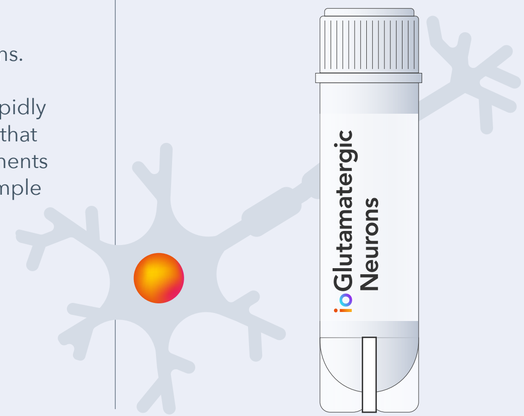
Wild type glutamatergic neuron parental iPSC line

opti-ox™

ioWild Type

Cryopreserved 'primed' wild type glutamatergic neurons.

Upon revival, cells rapidly mature into neurons that are ready for experiments in 11 days using a simple protocol.



Disease model development

An abnormal expansion of 50 CAG repeats is engineered into the first exon of the Huntingtin gene in the glutamatergic neuron parental iPSC line.

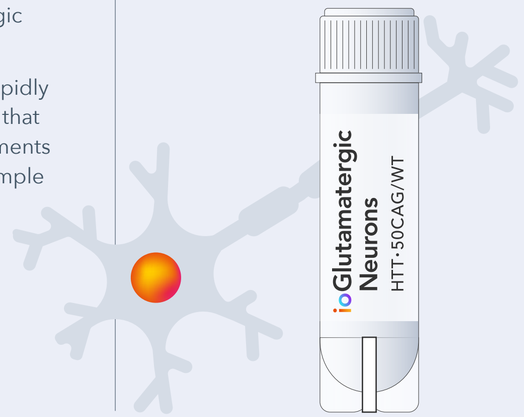
CRISPR/Cas9
50 CAG

opti-ox™

ioDisease Model

Cryopreserved 'primed' glutamatergic neurons $HTT^{50CAG/WT}$.

Upon revival, cells rapidly mature into neurons that are ready for experiments in 11 days using a simple protocol.



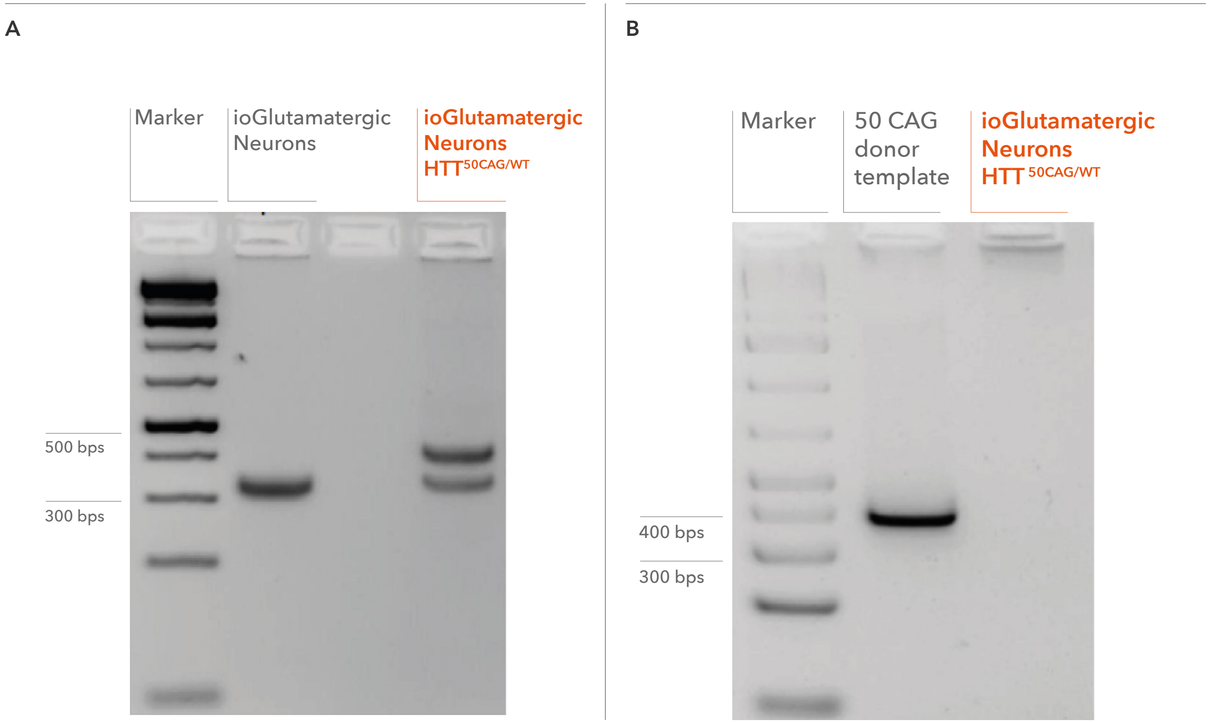
Genotype validation

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ contain the pathogenic mutation associated with Huntington's disease

The CAG repeat expansion was successfully integrated into one *HTT* allele, as confirmed by gel electrophoresis.

A. Confirmation of the on-target integration of a 50 CAG repeat expansion into one *HTT* allele. Genotyping primers flanking the endogenous *HTT* CAG repeat region produce a band at approximately 320 base pairs by PCR. Bands at 395 base pairs detect on-target gene editing and the introduction of a 50 CAG repeat.

B. Amplicon PCR of the donor plasmid backbone reveals no random integration of the 50 CAG repeat expansion into the genome of targeted colonies via gel electrophoresis.



DEFINED
ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are genetically identical to wild type ioGlutamatergic Neurons, apart from a heterozygous 50 CAG repeat expansion in one *HTT* allele.

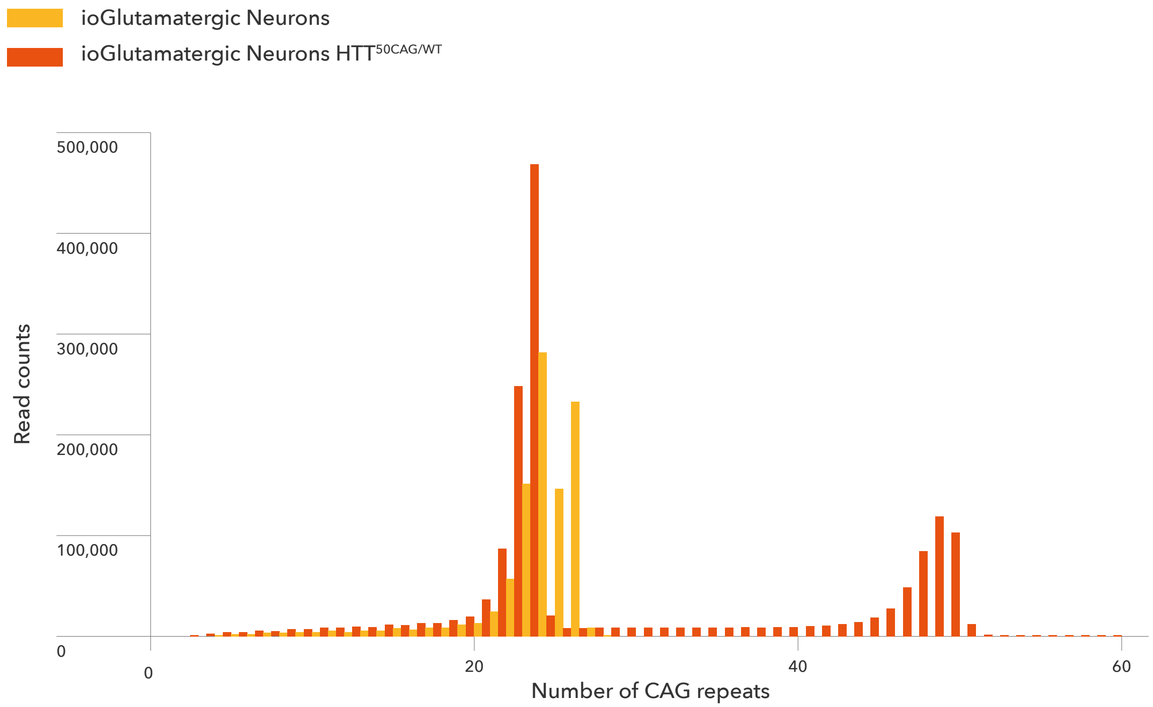
Genotype validation

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ contain an abnormal expansion of CAG repeats consistent with Huntington's disease

A 50 CAG repeat expansion has been successfully introduced into the *HTT* gene, confirmed by NGS-amplicon sequencing.

NGS-amplicon sequencing confirms the number of CAG repeats in wild type ioGlutamatergic Neurons (yellow) and ioGlutamatergic Neurons HTT$^{50CAG/WT}$ (orange).

The number of CAG repeats shows a peak at the normal physiological range of 24 for both the wild type and mutant cells. The 50 CAG repeat was detected only in the mutant cells (orange) confirming the successful introduction of a heterozygous 50 CAG repeat expansion in ioGlutamatergic Neurons HTT$^{50CAG/WT}$.



DEFINED
ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are genetically identical to wild type ioGlutamatergic Neurons, apart from a heterozygous 50 CAG repeat expansion in one *HTT* allele.

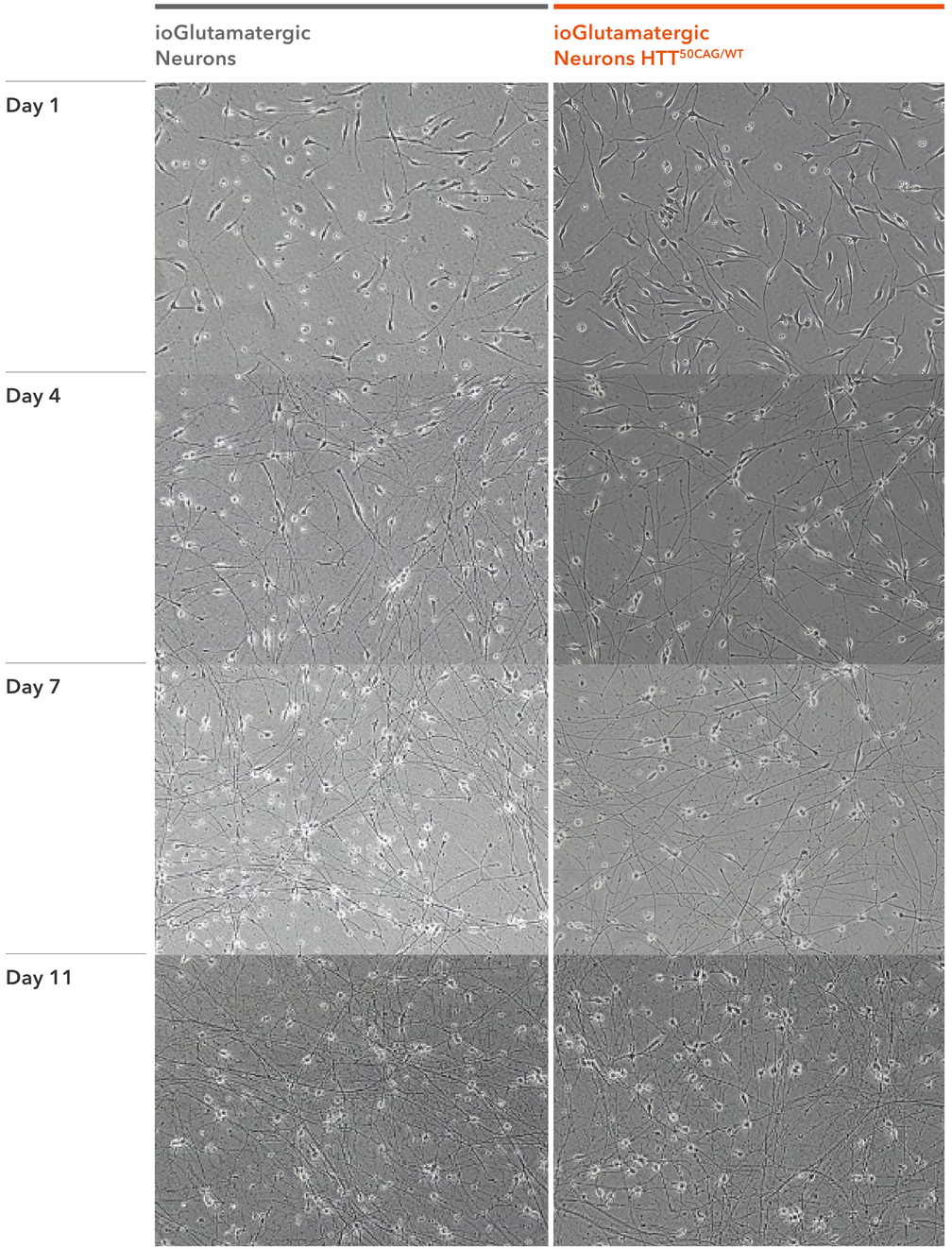
Characterisation

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are ready for experimentation within days

Brightfield images show neuronal networks form within days.

Brightfield images compare the morphological changes in cultures of ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and the wild type isogenic control at day 1, 4, 7 and 11 post-revival (100X magnification).

Neuronal cultures of both the ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and wild type ioGlutamatergic Neurons mature at identical rates forming more complex neuronal network structures over time.



QUICK

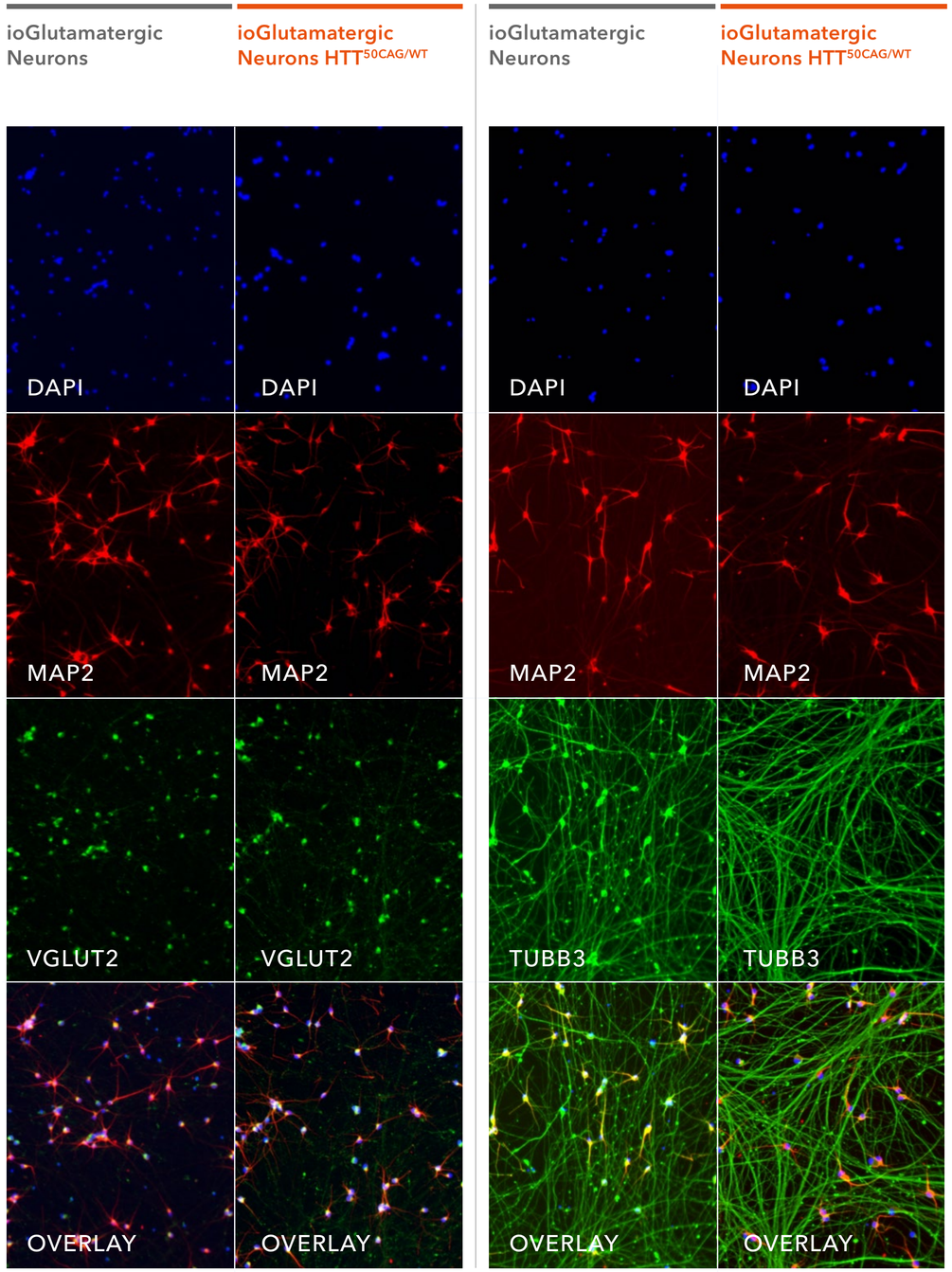
ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and wild type ioGlutamatergic Neurons mature quickly, at identical rates, so you can start collecting meaningful data sooner.

Characterisation

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ express key neuronal markers within 11 days post-revival

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and the wild type isogenic control have highly similar protein expression patterns.

Immunocytochemistry staining on day 11 post-revival demonstrates similar homogenous expression of pan-neuronal proteins (MAP2 and TUBB3) and glutamatergic neuron-specific transporter (VGLUT2) in ioGlutamatergic Neurons HTT$^{50CAG/WT}$ compared to wild type ioGlutamatergic Neurons.



MAKE TRUE COMPARISONS

The expression of key neuronal markers is highly similar between the isogenic pairs, ensuring biological comparability of the models.

Characterisation

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ express pan-neuronal and glutamatergic-specific markers by day 11 post-revival

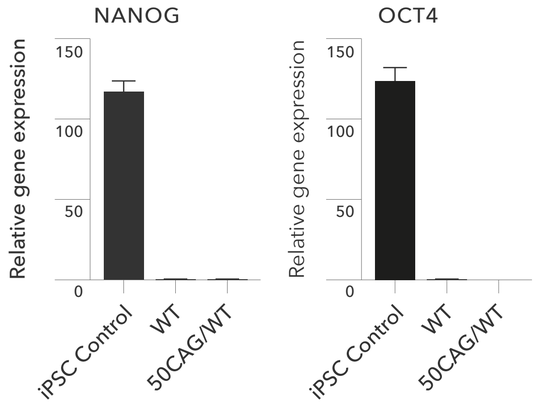
DEFINED
The expression of key neuronal markers is highly similar between the isogenic pairs, ensuring biological comparability of the models.

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and the wild type isogenic control have highly similar gene expression patterns.

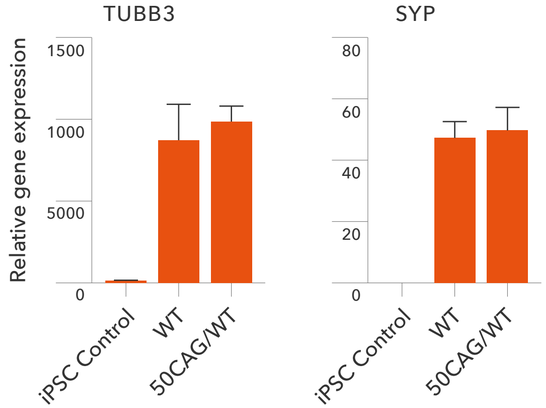
RT-qPCR analysis was performed on cultures of the wild type ioGlutamatergic Neurons (WT) and ioGlutamatergic Neurons HTT$^{50CAG/WT}$ (50CAG/WT) at day 11. cDNA samples of the parental iPSC line (iPSC) were included as reference.

ioGlutamatergic Neuron HTT$^{50CAG/WT}$ cultures, like the wild type isogenic control, loose the expression of pluripotency makers (NANOG and OCT4) whilst robustly expressing pan-neuronal (TUBB3 and SYP) and glutamatergic specific (VGLUT1 and VGLUT2) genes, as well as the glutamate receptor GRIA4.

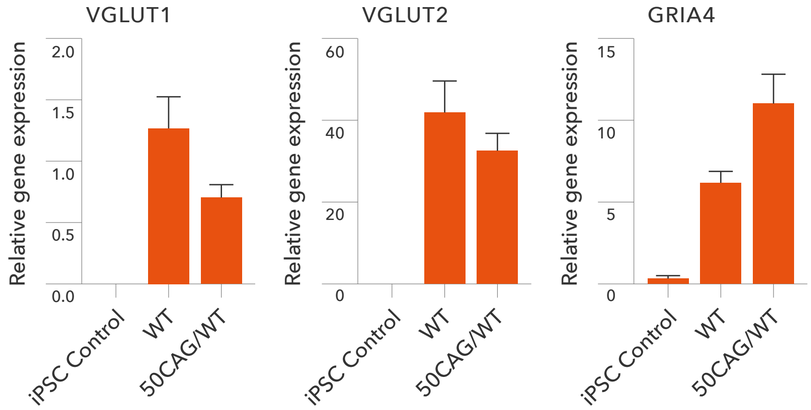
Pluripotency markers*



Pan-neuronal markers*



Glutamatergic markers*



* Average gene expression calculated relative to the house keeping gene HMBS.

Characterisation

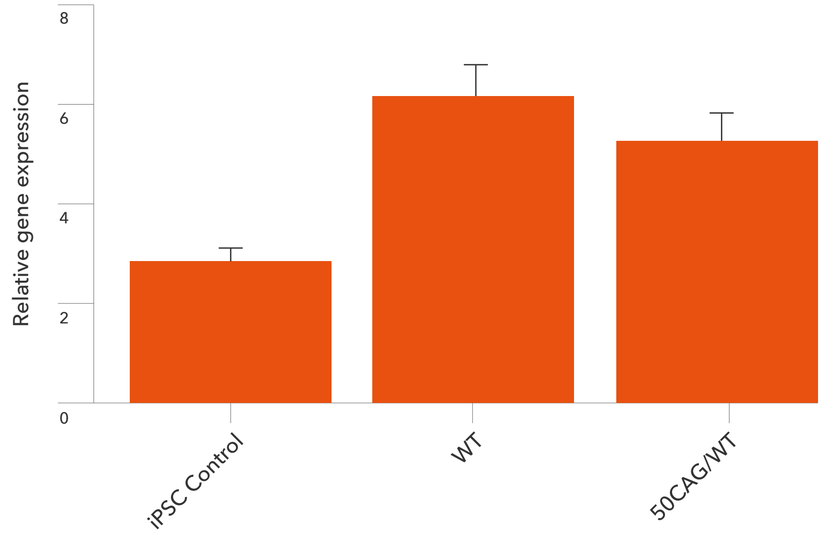
ioGlutamatergic Neurons HTT$^{50CAG/WT}$ robustly express of the disease-relevant protein Huntingtin

Huntingtin is expressed in both the wild type isogenic control and the disease model.

A. RT-qPCR analysis shows similar expression levels of the Huntingtin gene in both wild type ioGlutamatergic Neurons (WT) and ioGlutamatergic Neurons HTT$^{50CAG/WT}$ (50CAG/WT) at day 11 post-revival (n=2 replicates). cDNA samples of the parental iPSC line (iPSC Control) were included as reference.

B. Immunocytochemistry showing similar protein expression of Huntingtin in both wild type ioGlutamatergic Neurons (WT) and ioGlutamatergic Neurons HTT$^{50CAG/WT}$ at day 20 post-revival. Data provided by Origami Therapeutics and Scintillant Bioscience.

A



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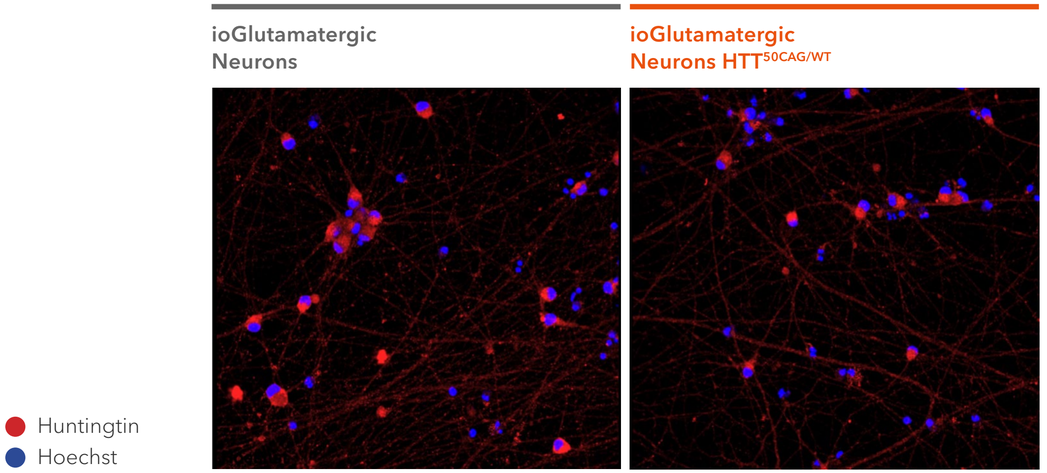

DEFINED
ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and the wild type similarly express disease-relevant markers. Genetic engineering has not damaged the *HTT* locus, providing an accurate model for Huntington's disease.

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are ready-to-culture and easy-to-use

One medium required in a simple protocol

EASY-TO-USE
ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and the wild type both mature rapidly and at the same pace, giving you access to an accurate isogenic disease model that requires minimal effort to culture.

Easy-to-use

Both ioGlutamatergic Neurons HTT$^{50CAG/WT}$ and the wild type isogenic control are delivered in a cryopreserved format and are programmed to rapidly mature upon revival with a simple two-phase protocol.

One medium required

The cells require a single culture medium formulation with a fully disclosed composition allowing for modifications to fit your experimental needs.

bit.bio
Precision reprogrammed cells are primed before shipping.

Customer
Cells are delivered in a cryopreserved format. ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are cultured in your laboratory in recommended media.

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Phase 0: Induction

Production of ioGlutamatergic Neurons HTT$^{50CAG/WT}$

Phase 1: Stabilisation

Stabilisation for 4 days

Phase 2: Maintenance

Maturation of neurons

ioGlutamatergic Neurons HTT$^{50CAG/WT}$ are cost-effective and flexible

Two vial sizes are available to suit your experimental needs

COST-EFFECTIVE
ioCells offer market leading seeding densities, so the cost-per well is significantly lower when choosing bit.bio.

Industrial scale quantities to support high throughput applications

Recommended seeding density is 30,000 cells/cm^2.

One Small vial can plate a minimum of:

- 0.7 × 24-well plate
- 1 × 96-well plate
- 1.5 × 384-well plate

One Large vial can plate a minimum of:

- 3.6 × 24-well plate
- 5.4 × 96-well plate
- 7.75 × 384-well plates

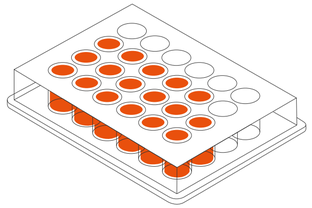
Recommended seeding density

30,000 cells/cm^2

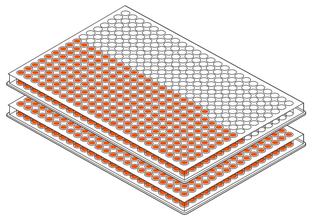
Small vial size

1 × 10^6 cells

24-well plate



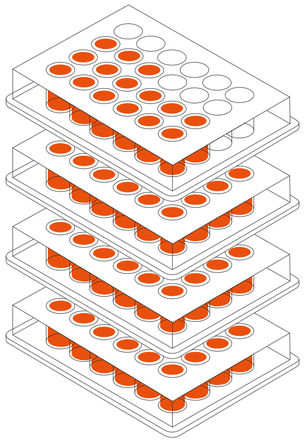
384-well plate



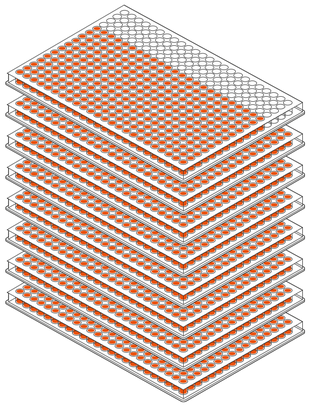
Large vial size

5 × 10^6 cells

24-well plate



384-well plate



Upcoming products

Our ioDisease Model portfolio is constantly expanding. Coming soon are iPSC-derived ioGlutamatergic Neurons with disease-relevant mutations for modelling Parkinson's disease, frontotemporal dementia, amytrophic lateral sclerosis and Gaucher's disease.

- ioGlutamatergic Neurons MAPT P301S
- ioGlutamatergic Neurons MAPT N279K
- ioGlutamatergic Neurons TARDBP M337V
- ioGlutamatergic Neurons PRKN R275W
- ioGlutamatergic Neurons GBA Null
- ioGlutamatergic Neurons GBA N409S

Request an invite to be the first to know about our latest products by emailing info@bit.bio.

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To order or speak with a member of our team, email orders@bit.bio.

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THE CELL CODING COMPANY

Product specificiations

Starting material
human iPSC line

Donor
Caucasian adult male
(skin fibroblast)

Differentiation method
opti-ox™ precision reprogramming

Karyotype
Normal (46, XY)

Vial size
Small: > 1 × 10^6 viable cells
Large: > 5 × 10^6 viable cells

Recommended seeding density
30,000 cells/cm^2

Seeding compatibility
6, 12, 24, 96, 384 well plates

Quality control
Sterility, protein expression (ICC) and gene expression (RT-qPCR)

User storage
LN2 or -150°C

Shipping info
Dry ice

Product use
These cells are for research use only

Genetic modification
Heterozygous – *HTT* 50 CAG repeat expansion

Product applications

ioGlutamatergic Neurons $HTT^{50CAG/WT}$ enable

- Disease modelling
- Academic research
- Drug discovery
- High throughput screening
- Co-culture studies with astrocytes

Validated techniques include

- ICC/IF
- Bulk RNAseq & single cell RNAseq
- qPCR & bDNA
- Electrophysiological assays (e.g. MEA)