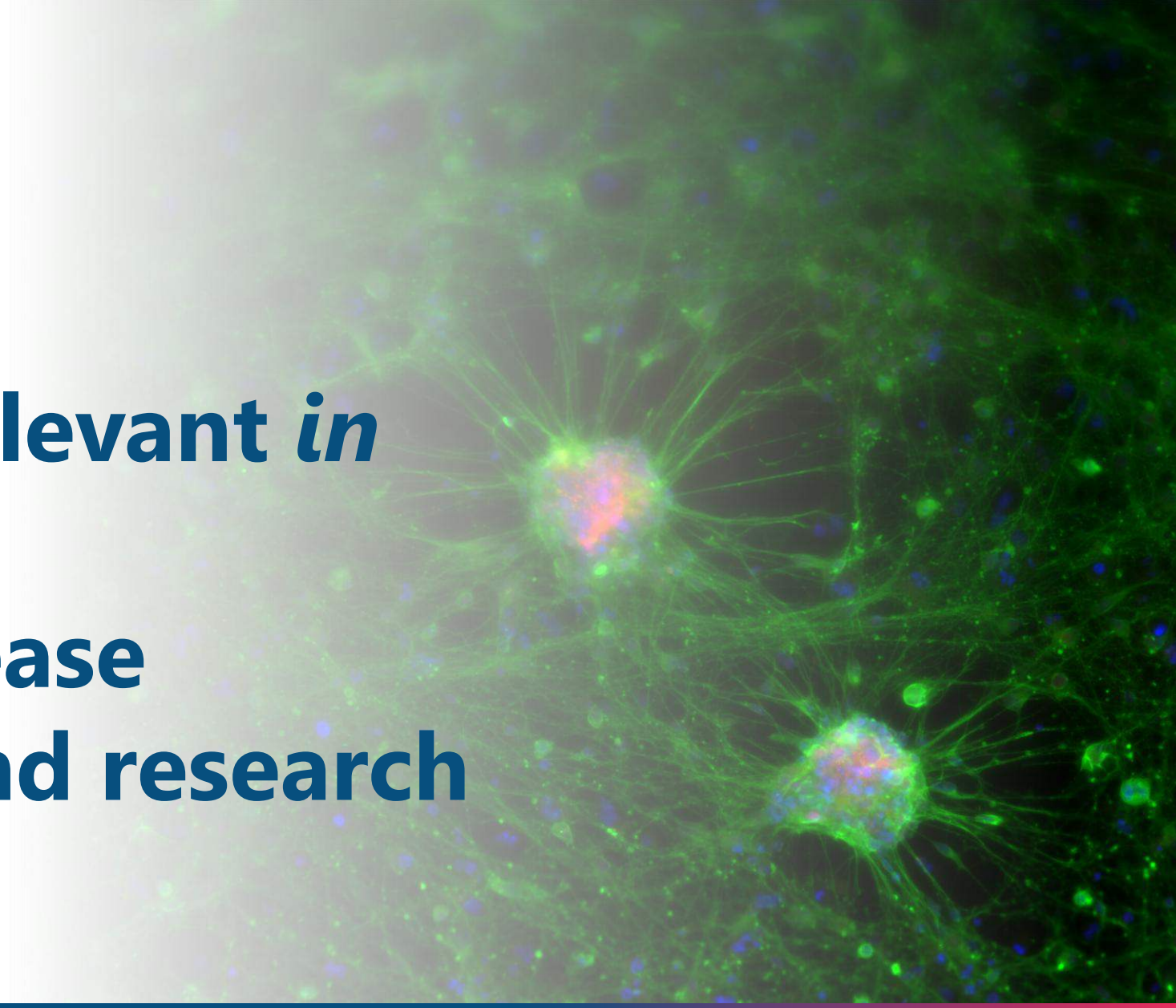
AXOL

Physiologically relevant *in vitro* models for Huntington's disease drug discovery and research



Human iPSC-derived products and specialist services

eBook

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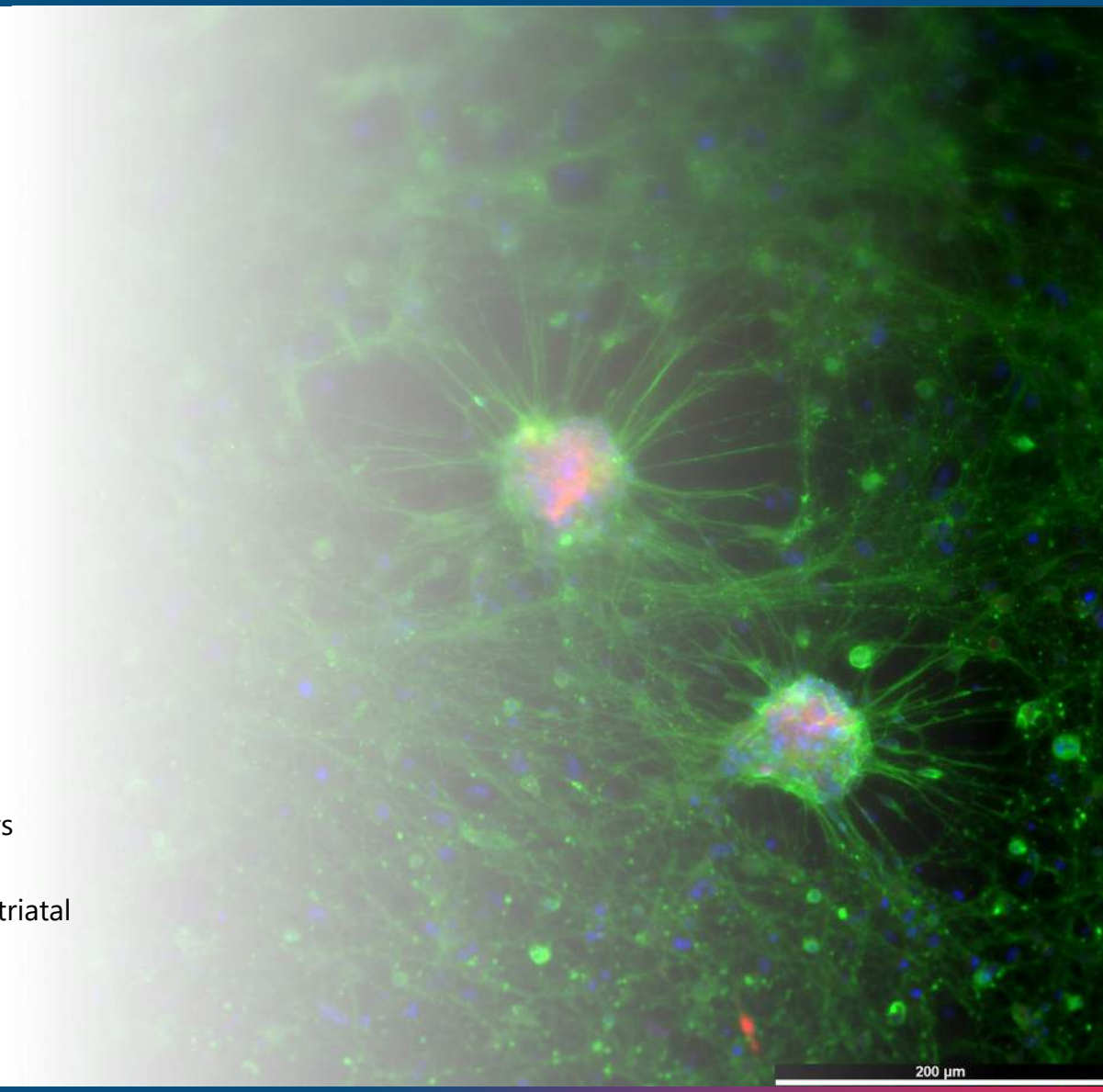


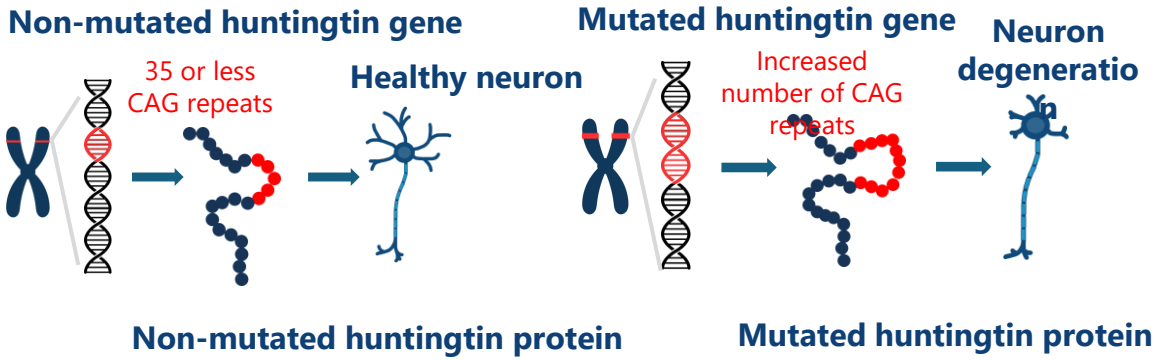
Understanding Huntington's disease

Huntington's disease (HD) is a progressive neurodegenerative disorder that leads to neuron loss in the striatal part of the basal ganglia, resulting in cognitive, behavioral, and motor decline. HD is an autosomal dominant disorder caused by the expansion of a $(CAG)_n$ trinucleotide repeat in exon 1 of the huntingtin protein (*HTT)* gene. The *HTT* gene encodes the huntingtin protein, which is essential for neuronal function and development.

The neuronal degeneration pathways caused by mutant HTT protein aggregates are not yet fully understood. There are currently no FDA-approved therapies that halt or reverse HD progression. Therapeutic development has focused on symptomatic management, however recent advances in genetic targeting and small-molecule discovery offer potential for disease-modifying interventions.

Drug discovery researchers utilize both animal (*in vivo*) and cell culture (*in vitro*) models to study HD. *In vivo* models primarily help evaluate therapeutic efficacy and systemic effects, while *in vitro* models enable human-specific investigations such as customized disease models and identification of potential therapeutics that may alter disease progression.

Human induced pluripotent stem cell (iPSC)-derived models are showing promise as a scalable and physiologically relevant platform for HD research helping to reveal disease mechanisms and contribute to the development of new therapies. Importantly, these models retain the donor's genetic background, CAG repeat mutations and can be differentiated into disease-relevant cell types including striatal neurons.



Expansion of CAG repeats in exon 1 of the Huntingtin gene (*HTT*) produces a mutant version of the huntingtin protein. Accumulation of the mutant form of the protein leads to cell death and underpins the progressive pathology of Huntington's Disease.

Most people have <36 consecutive CAG repeats in the *HTT gene*, which is within the normal range and non-pathogenic.

- The more CAG repeats present, the earlier the onset of symptoms and the more severe the disease
- The expanded CAG repeat region shows somatic instability – increasing in length throughout patients' life-span.

Understanding CAG expansion is crucial for diagnosing and managing HD. Many potential treatments and therapies aim to halt or slow the expansion of CAG repeats.

Hope for new Huntington’s disease therapies

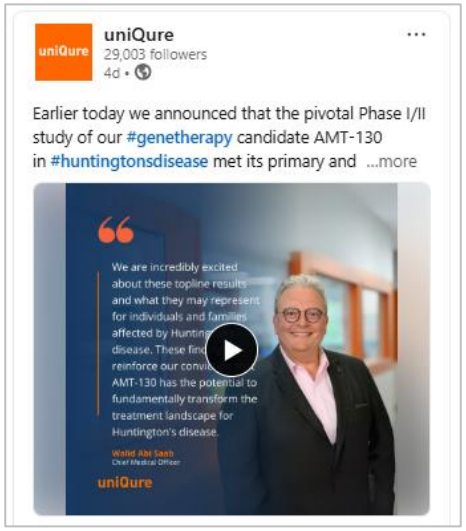
Non-profit foundations and patient advocacy groups are working tirelessly to support and champion new therapy development for HD. One of these, Cure Huntington's Disease Initiative (CHDI), is an influential funder and advocate for Huntington’s disease research. At Axol, we are proud to have collaborated with the dedicated team at CHDI for over a decade.

The HD research community is focused on two main approaches to halt disease progression.

- Reducing the expression of the mutant HTT protein
- Reducing the somatic instability of the CAG repeat region

Recently there was a glimmer of hope in the race to find a widely available cure when in a recent clinical trial from uniQure of AMT-130, a gene therapy delivered via brain surgery which lowers both the regular and toxic forms of huntingtin, they showed slowing of disease progression at 36 months.

Positive progress for several other ongoing trials of potential HD therapy were reported at the annual Huntington's Disease Therapeutics conference.



Supporting HD research & drug discovery with iPSC technology

1. iPSC lines from patients and unaffected donors

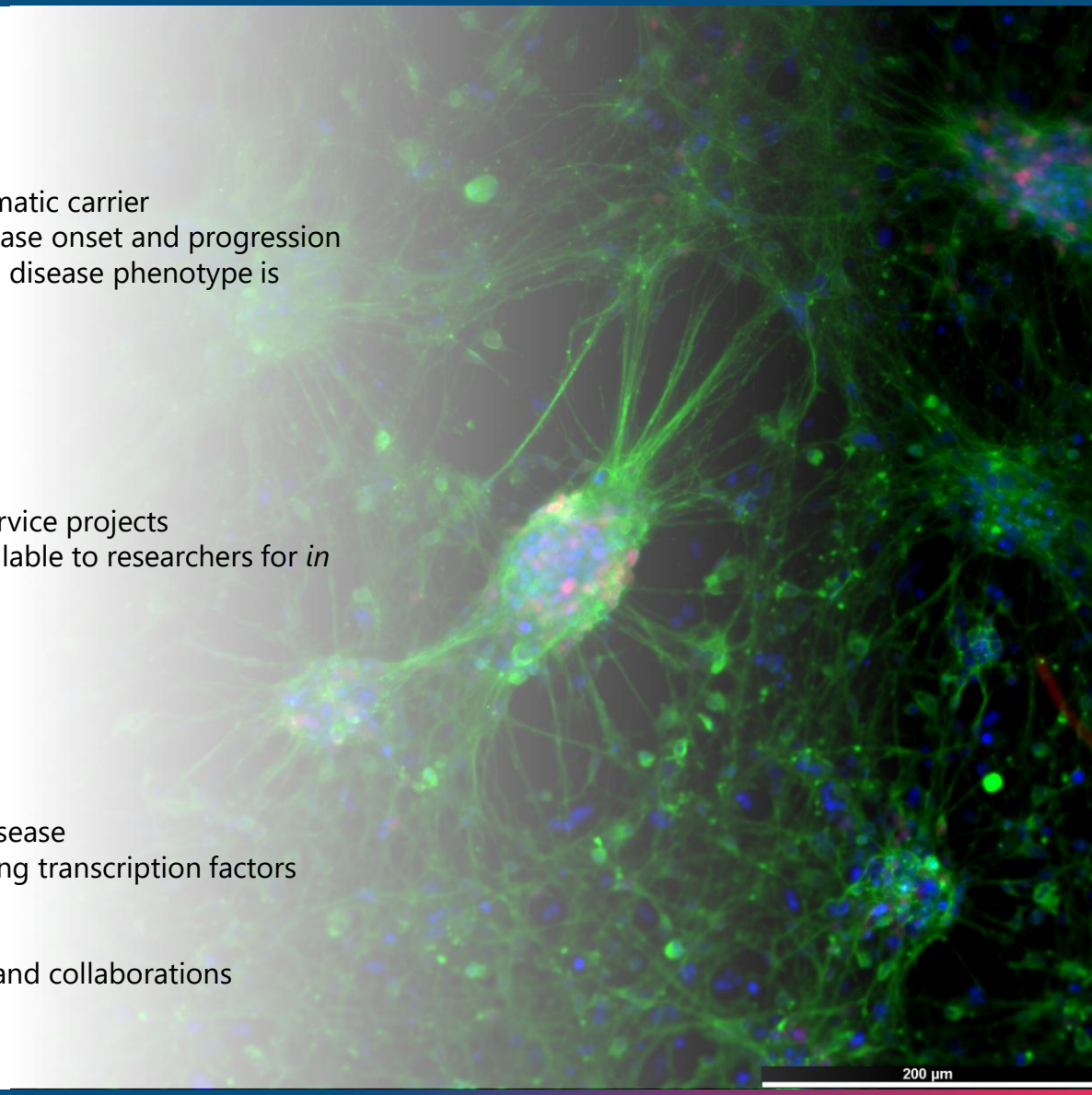
- Five iPSC lines derived from Huntington's disease patients and one from an asymptomatic carrier
- CENSOi019-B (HTT CAG 14/125) line reveals somatic instability, a key modifier of disease onset and progression
- Generation of patient / isogenic control line: a powerful tool for understanding how a disease phenotype is expressed in an *in vitro* model

2. iPSC-derived cells for advanced *in vitro* modeling

- Striatal neurons differentiated from Huntington's disease patient iPSCs are used in service projects
- Neural stem cells, along with full protocols for maturation to striatal neurons, are available to researchers for *in vitro* disease modelling and drug discovery research
- Extensive characterization data

3. Assays and disease modeling services

- Service projects for clients to support research and drug discovery in Huntington's disease
- Includes long range (100 day+) instability studies and accelerate studies by modulating transcription factors such as FAN1
- Novel, human-relevant *in vitro* model development for and compound testing
- Provision of specialist banking, reprogramming, sourcing, and gene-editing services and collaborations

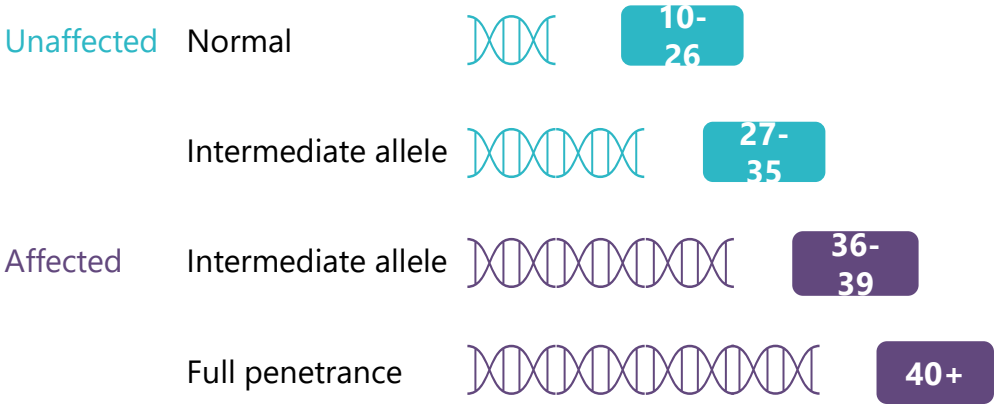


1. Disease and control iPSC lines: Axol Bioscience HD iPSC collection

Axol Bioscience provides five iPSC lines derived from Huntington's disease patients and one from an asymptomatic carrier. These are used to generate relevant iPSC-derived cells at scale and for delivery of specialist drug discovery services. Each line undergoes rigorous quality control such as assessments of morphology, karyotype, pluripotency, sterility and CAG repeat sizing. From these iPSCs we can provide striatal neurons, supplied as neural stem cells. Characterization and longitudinal studies of these cell lines allow the development of physiologically relevant cellular models with clear disease phenotypes for target identification and drug screening.

Cell line name	Disease status	Sex	Age	Confirmed genetic mutation
CENSOi019-B	Huntington's Disease	F	7	CAG14/CAG125
CENSOi019-A	Huntington's Disease	F	7	CAG14/CAG125
CENSOi052-A	Huntington's Disease	M	16	CAG28/CAG66
CENSOi017-A	Huntington's Disease	F	51	CAG17/CAG42
CENSOi011-D	Huntington's Disease	F	40-50	CAG18/CAG39
CENSOi053-A	Asymptomatic Carrier	M	64	CAG17/CAG38

CAG repeat length and Huntington's disease status



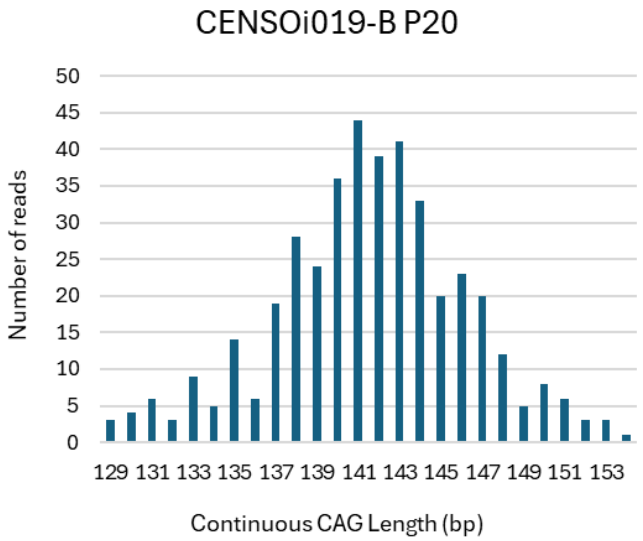
iPSC model to study repeat expansion over time: CENSOi019-B iPSC line

The CENSOi019-B iPSC line was derived from a seven-year-old juvenile HD patient with 125 CAG repeats in the *HTT* gene. Striatal neurons derived from this iPSC line exhibit CAG repeat instability, a hallmark of HD. This instability is critical for studying disease progression, as CAG repeats can expand over time, contributing to neuronal dysfunction and degeneration—particularly in the striatum.

After reprogramming and generation of the master bank, the number of CAG repeats was 143. CAG expansion analysis during 15 passages showed an increase of 1 x CAG repeat every 5 passages. Further study suggested that this line has an atypical allele associated with hastening the onset and progression of the disease. Striatal neurons derived from the CENSOi019-B iPSC line provide a powerful and highly relevant model for studying HD. They replicate key aspects of the disease, including CAG repeat instability, striatal degeneration, and neuronal dysfunction, offering essential insights into disease mechanisms. This model is invaluable for therapeutic development, drug screening, and the discovery of biomarkers to aid in the diagnosis and treatment of HD.

The CENSOi019-B line (HTT: 14/125 CAG) has enabled the study of repeat expansion dynamics over time and provides a model for early-onset HD, which is often more severe and less understood than the adult-onset form of the disease. When differentiated into striatal neurons, these cells closely replicate the key features of HD pathology, making them an ideal system for studying the selective degeneration of striatal neurons in this condition.

CENSOi0190B HD iPSC line contains 143 CAG repeats after banking and exhibits repeat expansion



CAG repeat size of the bank was assessed by nanopore sequencing. Consensus sequence contains 143 CAG repeats.

CAG repeat size was assessed at each passage using fragment analysis. iPSCs expand 1x CAG repeat every 5 passages ~0.4 CAG/week

Genomic characterization of the CENSOi019-B line

Gaining an understanding of disease modifiers, drivers of somatic instability, factors that reduce age of onset, or alter HD progression is essential to inform the development of new therapeutic interventions.

iPSCs reprogrammed from HD patients, such as the CENSOi019-B line described here, enable more personalized disease models and capture clinical heterogeneity related to the donor's genetic background. These lines contain the CAG repeat disease mutations and the genetic background that contributes to disease onset and severity.

Capturing the impact of genetic makeup in these *in vitro* models provides a platform to screen potential genetic modifiers that may influence onset, progression, and symptoms, and advance targeted therapeutic development. Genetic modifiers themselves are also molecular targets for drug discovery.

The CENSOi019-B line is a crucial tool to study the repeat instability and expansion dynamics of the CAG repeat and can be used to provide an *in vitro* model for early-onset HD, offering essential insights into disease mechanisms for therapeutic development, drug screening, and the discovery of HD biomarkers.

Whole Genome Sequencing (WGS) data of the CENSOi019-B suggests loss of the CAA interruption and presence of certain SNPs/INDELS in the mutant *HTT* allele providing insights into potential contribution to disease severity and progression.

Allele	Sequence
Allele 1	$(CAG)_{18}$ CAACAGCCGCCA(CCG)n
Allele 2	$(CAG)_{138}$ CAGCAGCCGCCG(CCG)n

Mutant allele has variant associated with increased instability

INDELS

- 44 identified
- Most in introns (39/44)
- One identified as pathogenic

SNPs

- 199 identified
- Most in introns (188/199)
- Some identified as pathogenic

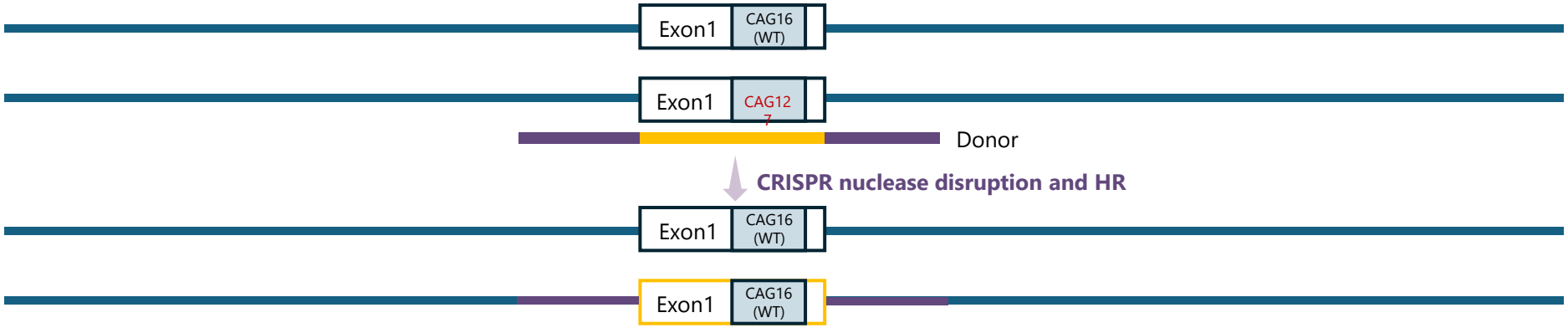
Isogenic control cell line for CENSOi019-B

Licensed CRISPR-Cas9 technology is used to edit the CENSOiO19-B iPSC line by reducing the number of CAG repeats to fewer than 36. This modification generates a cell line carrying a non-pathogenic CAG repeat allele while maintaining the genetic background of the original pathogenic line. These isogenic cell line pairs, which differ only in CAG repeat length, can then be differentiated into multiple disease-relevant cell types, including striatal neurons, astrocytes, and microglia.

Patient line
CENSOiO19-B iPSC cell line with CAG14/ CAG127 genotype

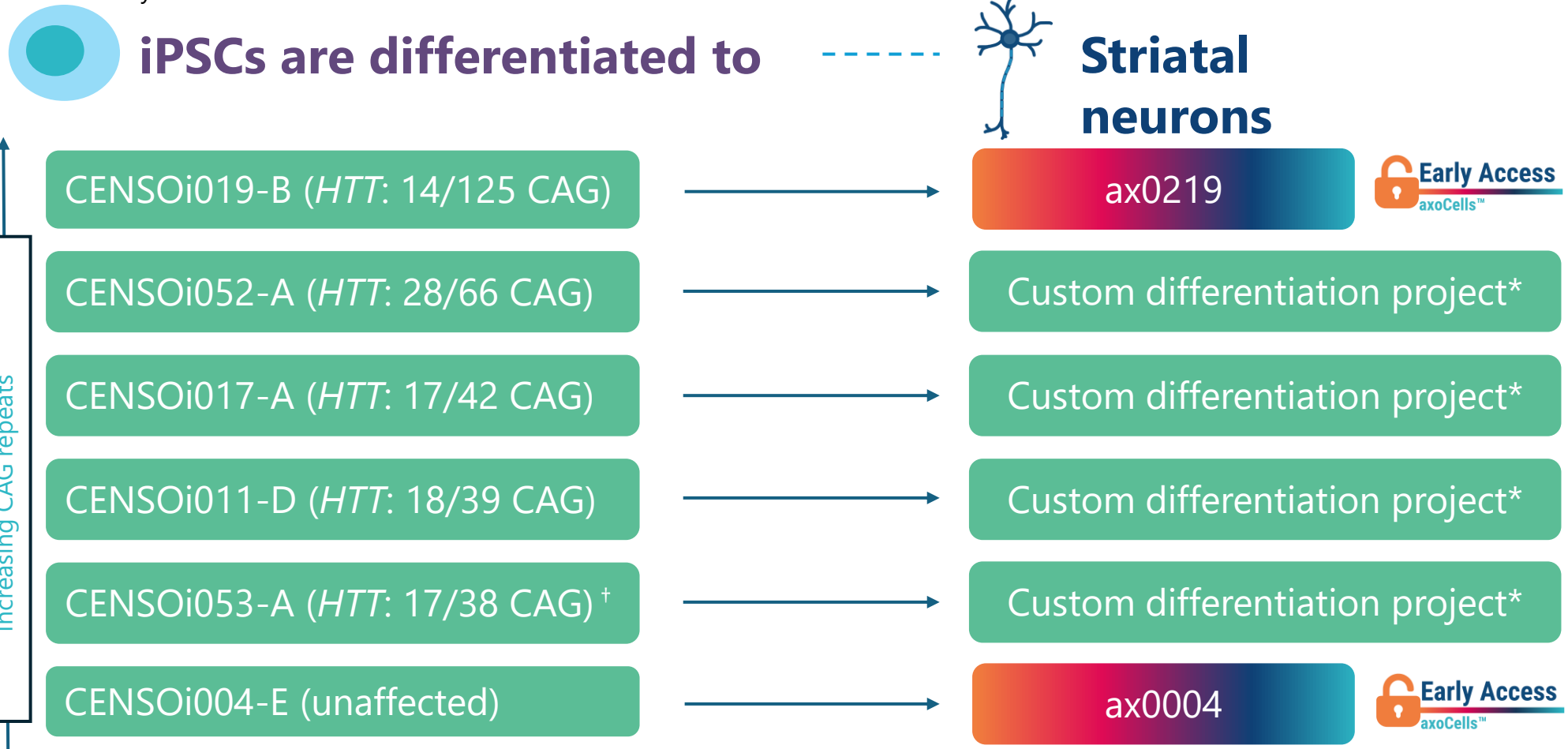
Isogenic control
Edited to contain the wild type allele of the variant of interest

Ideal *in vitro* model disease phenotype control



2. axoCells™ human iPSC-derived striatal neurons

Striatal neurons are supplied to you as frozen neuronal stem cells. These are thawed and matured using Axol's supplements and media to final assay-ready state in around 21 days.



† Asymptomatic carrier

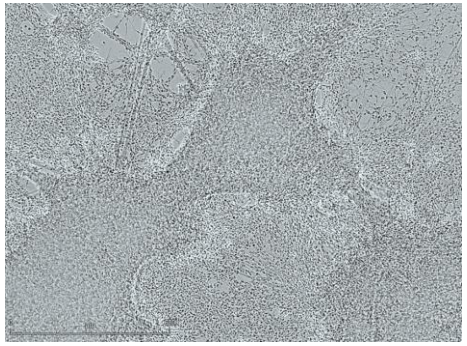
**Custom differentiation projects provide cells to be used at client site, by a CRO partner or in an Axol service project.

Characterization of HD and control striatal neurons

Phenotypic characterization: morphology

iPSC-derived striatal neurons derived from an unaffected donor (ax0004) cluster after day 25, showing large cell clusters by day 30, while HD striatal neurons (ax0219) derived from CENSOi019-B remain very neuronal throughout the maturation period. These phenotypic differences have been observed repeatedly in these lines.

Control striatal neurons (ax0004)

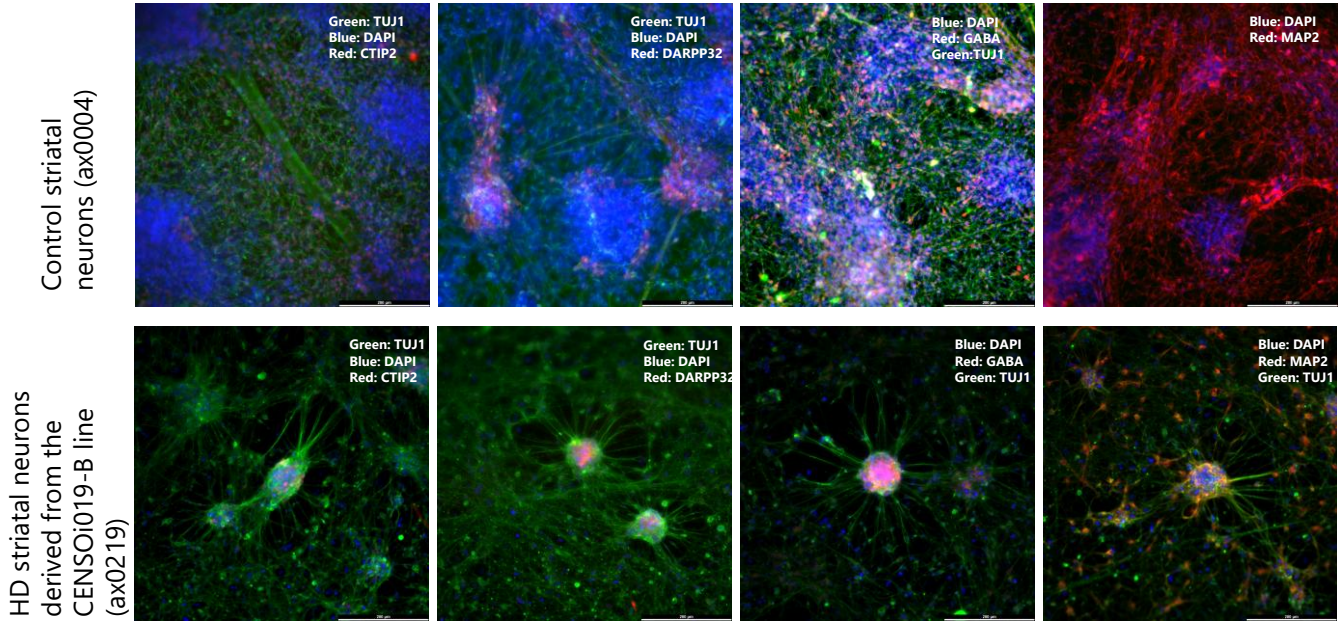


HD striatal neurons derived from the CENSOi019-B line (ax0219)

Control iPSC-derived striatal neurons and HD striatal neurons were matured for 30 days. Morphology was monitored on an IncuCyte® and showed phenotypic differences between the two lines. Images shown represent cells matured over 30 days. Scale bar = 600µm

Phenotypic characterization: immunocytochemistry

HD striatal neurons (ax0219) express CTIP2, DARPP32 and GABA, markers indicative of mature striatal neurons in addition to neuronal markers like TUJ1 and MAP2.



Immunocytochemistry of control iPSC-derived striatal neurons and HD striatal neurons at Day 31.

axoCells™ human iPSC-derived striatal neurons

axoCells™ human iPSC-derived striatal neurons are designed for use in advanced *in vitro* models

- Assay-ready from day 21 onwards
- Express key markers, including DARPP32, CTIP2, and GABA
- Disease and unaffected donor iPSC-derived neural stem cells

Cells and kits

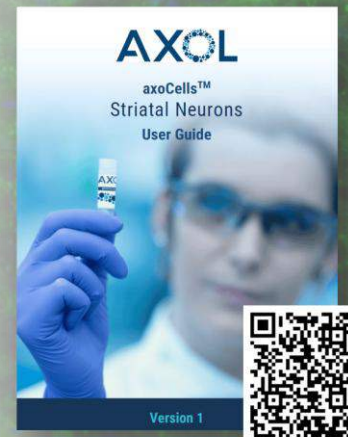
Product Name	Cells only code / 1 vial	Quantity* / per vial	Kit** code	Catalog / Early Access Product
axoCells™ human iPSC-derived neural stem cells, newborn male donor, ≥1.5 million cells	ax0015	≥1.5 million cells	**ax3115**	Catalog product
axoCells™ human iPSC-derived neural stem cells (HD, CAG125), female donor, aged 7, ≥1.5 million cells	ax0219	≥1.5 million cells	-	Early Access product
axoCells™ human iPSC-derived neural stem cells, unaffected male donor, aged 40-50, ≥1.5 million cells	ax0004	≥1.5 million cells	-	Early Access product

*Number of viable cells post thaw
**Kit contains cells and one of each item listed in the media and supplements table

Media and supplements

Product Name	Product code	Quantity
axoCells™ striatal neuron medium kit, 500ml	ax0333	250 ml + 250 ml + 7.5 ml + 7.5 ml + 2 ml
axoCells™ human BDNF supplement, 10 µg	ax139800	10 µg
axoCells™ human GDNF supplement, 10 µg	ax139855	10 µg
axoCells™ SureBond-XF coating, 1 ml	ax0053	1 ml

Additional third-party components may be required. Please refer to the user guide for full list



3. End-point assays and services

Specialized services for Huntington's disease (HD) drug discovery and research

We conduct specialized service projects utilizing our proprietary iPSC differentiation and manufacture processes and extensive scientific expertise in drug discovery, compound testing, neurodegeneration and iPSC biology.

For HD, these include longitudinal studies to look at somatic instability and modulation of mismatch repair genes such as FAN1 to accelerate instability.

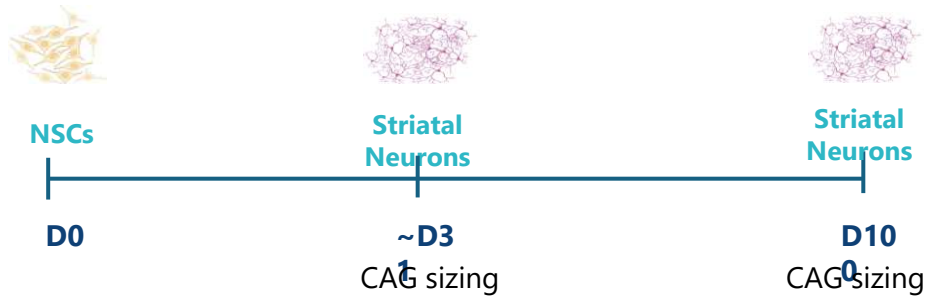
To functionally assess HD pathology *in vitro*, we use both morphological and functional end-points including trinucleotide instability, neurite outgrowth, and spontaneous firing. Additional endpoints, such as mitochondrial function and protein aggregation are available, and bespoke assays can be developed and tailored to clients' scientific needs.

Other services

We also provide technical services to support clients with banking, reprogramming, sourcing, gene-editing and custom differentiation of our iPSC lines, or client lines.

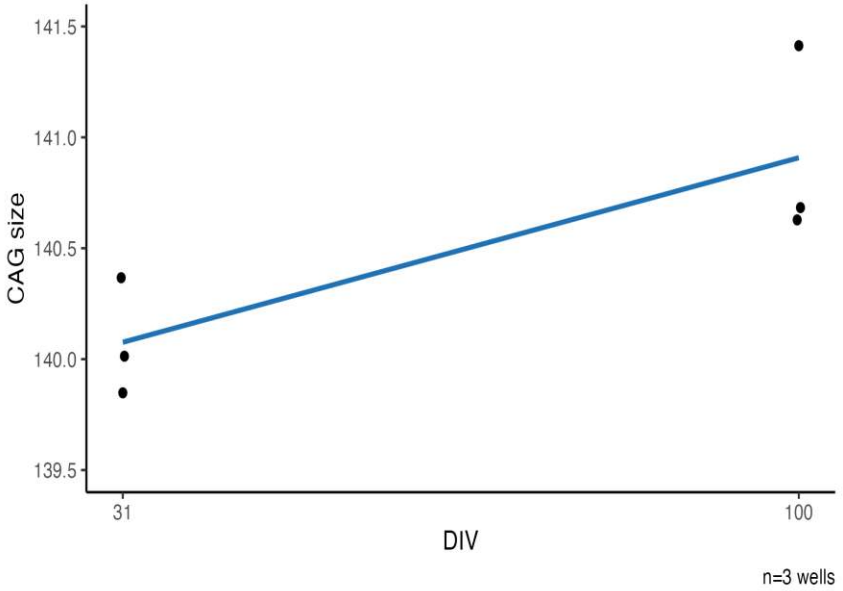
Application: using ax0219 striatal neurons to study HD phenotypes

We have completed a series of client service projects focused on maintaining long-term (100-day) iPSC-derived striatal neuron cultures to support genomic instability studies. We are currently generating transcriptomic data from these longitudinal cultures spanning over 100 days.

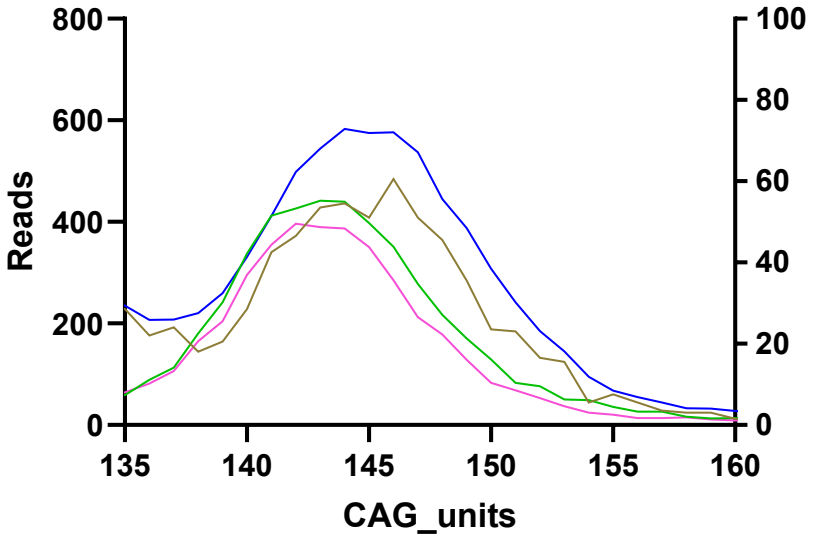


Striatal neurons derived from the CENSOi019-B line (ax0219) longitudinal studies

ax0219 (CENSOi019-B) and ax0004 (Control) were matured to striatal neurons and cultured for 100 days (the control line did not survive beyond 60 days). Samples were taken throughout the maturation process to assess CAG expansion.



HD striatal neurons expand at slower rate than iPSCs (0.13X CAG/week) when assessed during a 100 day-maturation. The number of CAG repeats was quantified using Nanopore sequencing data after quality control steps. The weighted arithmetic mean of the CAG distribution was then calculated to determine the CAG size for each sample. n=3 wells



The number of uninterrupted CAG repeats was quantified using Nanopore sequencing after PCR amplification.

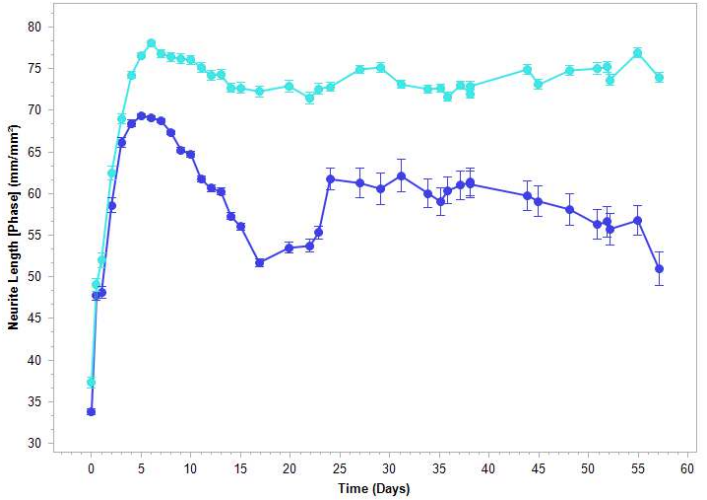
Application: morphology and neurite outgrowth: CENSOi019-B HD line vs unaffected donor

Morphology of HD striatal neurons and control striatal neurons at Day 20 recorded on IncuCyte®. The controls form many more large clusters and this becomes more prominent as they mature over time which is why it becomes more inaccurate as shown on the graphs.

Control striatal neurons (ax0004)

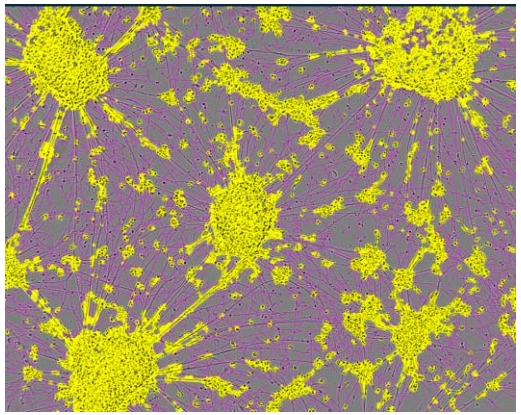
HD striatal neurons derived from the CENSOi019-B line (ax0219)

Neurite length

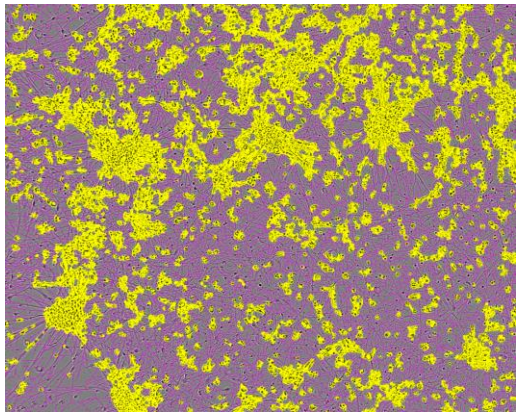


Day 20

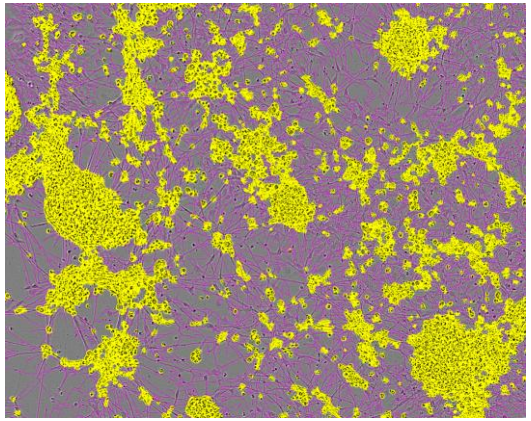
Control striatal neurons (ax0004)



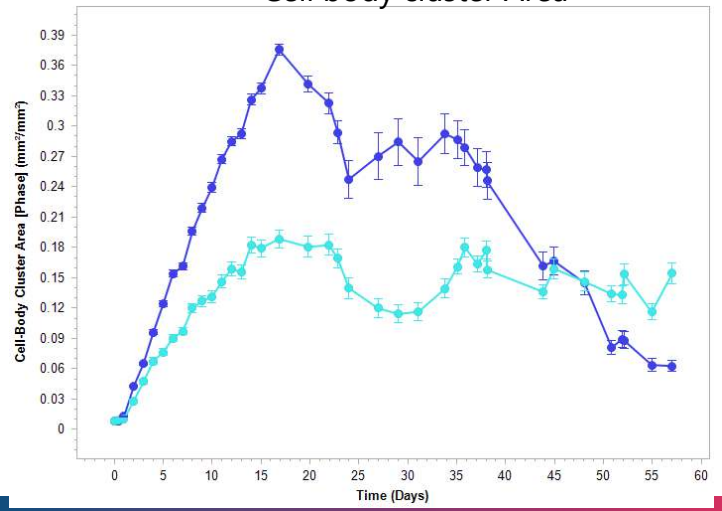
HD striatal neurons derived from the CENSOi019-B line (ax0219)



Control striatal neurons (ax0015)



Cell body cluster Area



Application: characterization of the hyperexcitability phenotype in iPSC striatal neurons

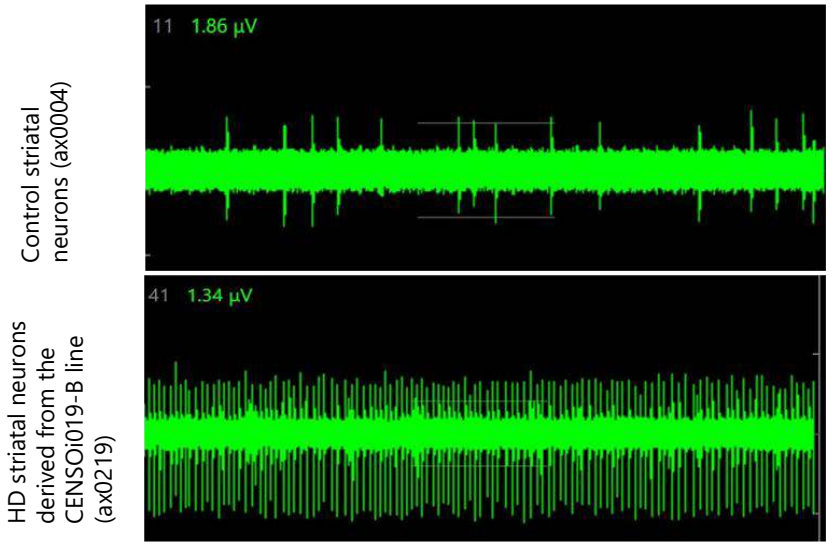
HD iPSC-derived striatal neurons line are more active than control iPSC-derived striatal neurons when assessed by Multi Electrode Array (MEA).

Representative sodium spike traces from one electrode of control striatal neurons and HD striatal neurons from the HD line at Day 25 of differentiation show a trend toward increased activity in the HD line.

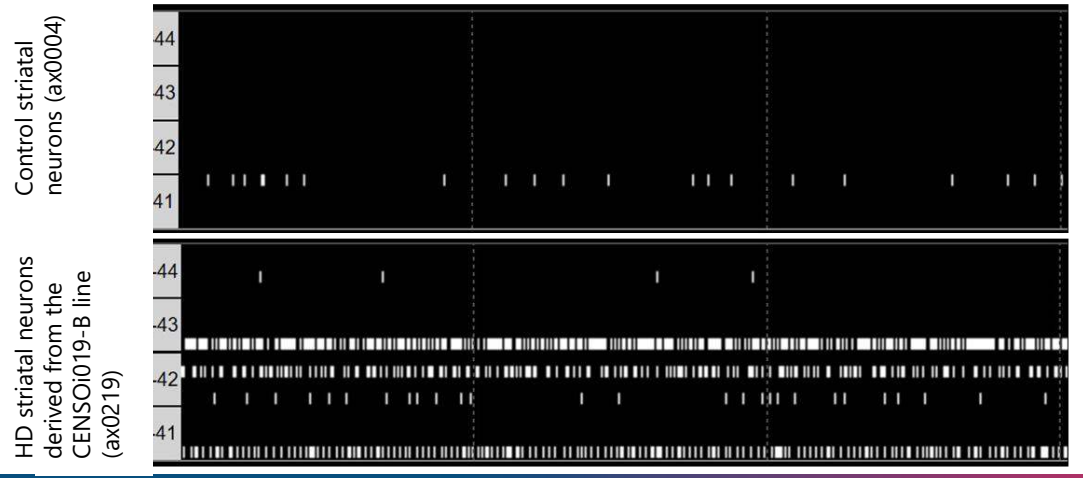
The HD line displays more frequent spikes, indicating greater neuronal activity. Since striatal neurons are inhibitory by nature, minimal activity is expected in neurons from unaffected donor backgrounds.

The HD line shows activity across most electrodes, whereas the control line exhibits activity in only one electrode, further supporting a trend toward hyperexcitability in the HD model.

Day 25



Day 25



Huntington’s disease services delivery and scientific team

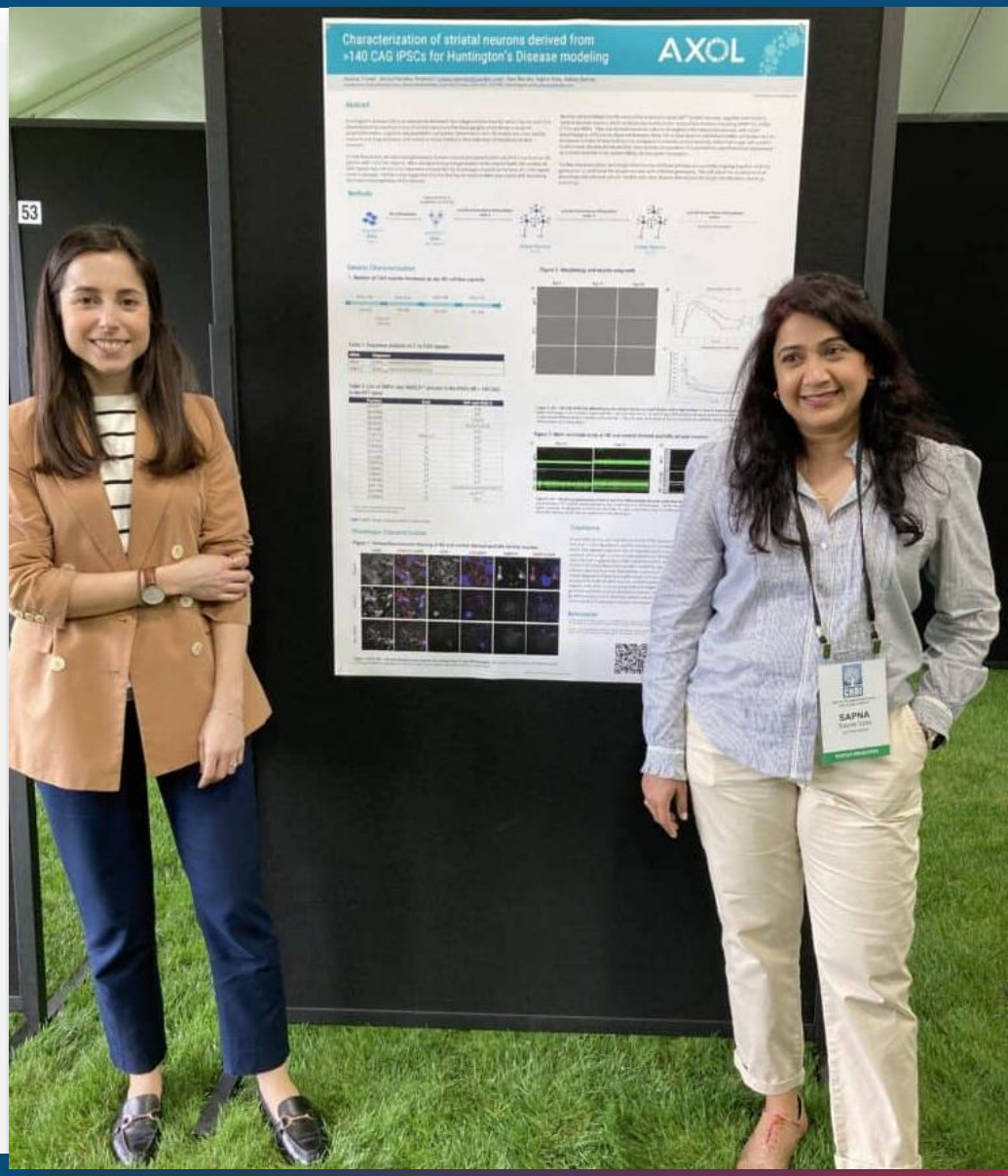
Collaboration is at the heart of everything we do, ensuring that we bring the best minds together to drive meaningful progress in supporting the industry with physiologically relevant models HD. Whether you're seeking advice, services, or support, our team is here ready to help you.

Our team is a dedicated group of experts that range across our sites, in Cambridge, Roslin, and Grasse. We bring an innovative approach to advancing research and drug development for HD, leveraging the diverse expertise and approaches across these sites. Many members of our team are recognised as scientific leaders in their fields. Our customers can leverage their experience to accelerate their programs of work.

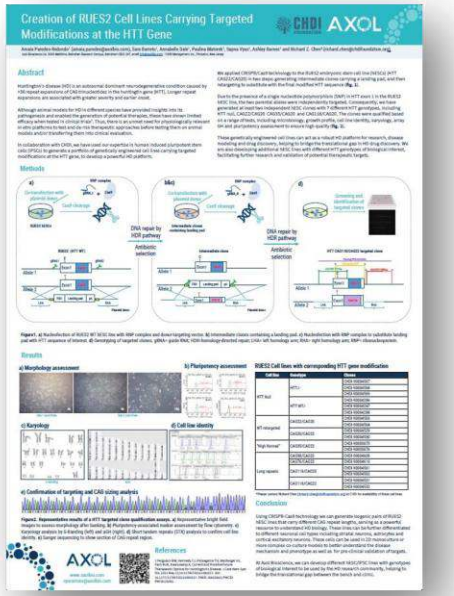
When you collaborate with Axol Bioscience, you gain access to a tailored approach that supports your research every step of the way. From the start, we facilitate open communication with our iPSC experts, ensuring that your project is guided by decades of experience.

We offer flexibility in experimental design, leveraging our in-house expertise to customize assays to meet your specific needs. Throughout the project, we maintain rigorous data management and ensure full transparency with detailed reporting and data-sharing meetings.

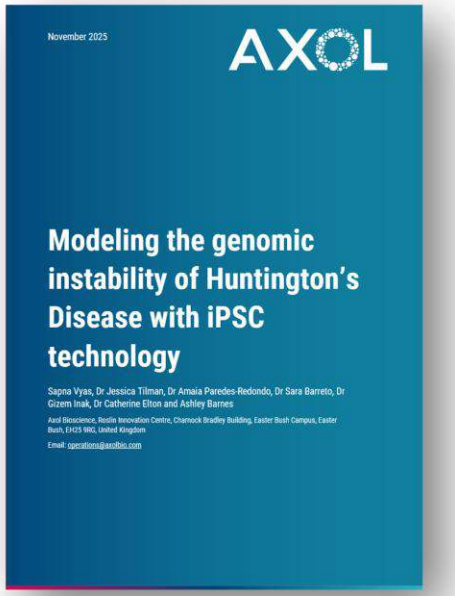
Our commitment doesn't end at project completion, we continue to offer ongoing support, helping you integrate cells into your systems and maintain an open channel for future collaborations.



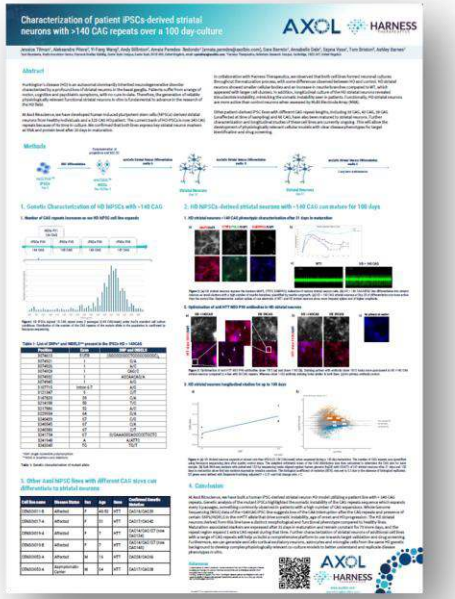
Additional resources



Poster
Creation of RUES2 Cell Lines Carrying Targeted Modifications at the *HTT* Gene



Whitepaper
Modeling the genomic instability of Huntington's Disease with iPSC technology



Poster
Characterization of patient iPSCs-derived striatal neurons with 140 CAG repeats over a 100 day-culture

Webinar
Human iPSC-derived models for Huntington's Disease with large CAG repeat expansion

AXOL

If you would like to discuss the adoption of iPSC-derived cells into your workflow, or our specialist services provision, please contact us.

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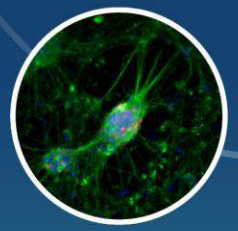
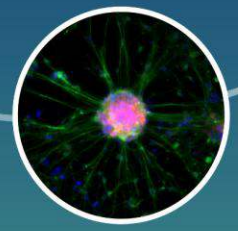
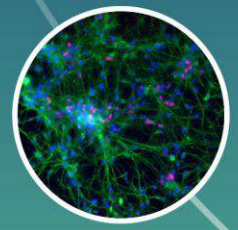
Axol Bioscience Ltd
www.axolbio.com







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