Harnessing CRISPR-Ready ioGlutamatergic Neurons* for drug discovery in neurodegenerative diseases

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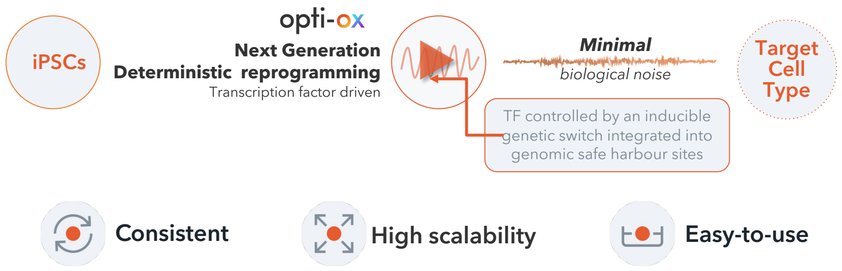
About bit.bio's opti-ox* technology



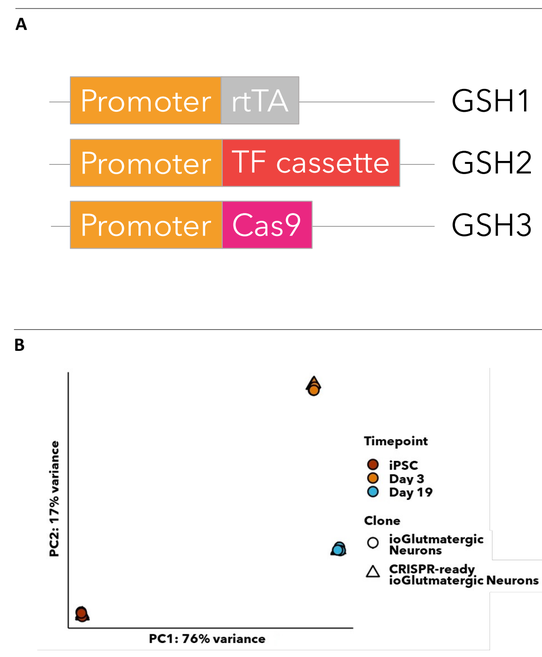
CRISPR-Ready ioGlutmatergic Neurons*

CRISPR-Ready ioGlutamatergic Neurons are precision reprogrammed human iPSC-derived neurons containing a constitutively expressed Cas9 nuclease. This product has been designed for scientists looking to generate gene knockouts and perform CRISPR screens in a physiologically relevant human cell model, without the need to spend months engineering and characterising their own Cas9 stable iPSC lines.

These cells share the same user benefits as bit.bio's well-established ioGlutamatergic Neurons, offering users a well-defined and characterised human neuron that shows a rapid gain of functional activity. Their high lot-to-lot consistency, and their simplicity in handling and culturing support experimental scalability.

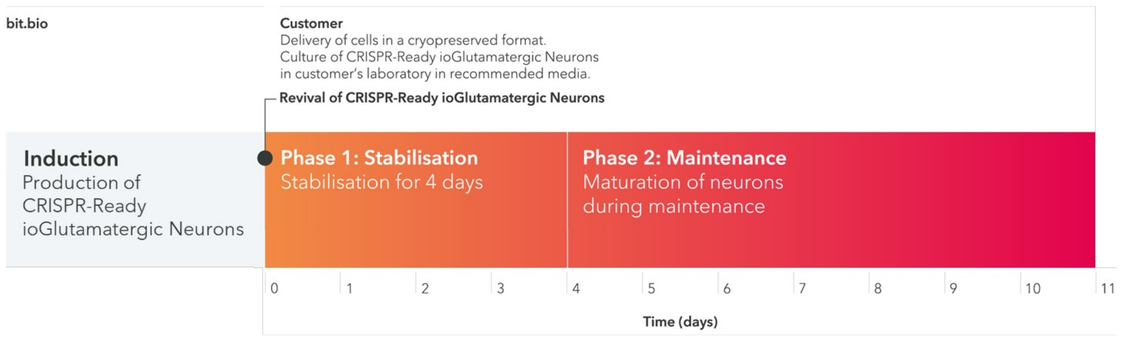
CRISPR-Ready ioGlutamatergic Neurons offer easy disease-specific model generation for drug development. Using bit.bio's simple cell culturing protocol with optimised gRNA delivery, researchers can achieve high gene knockout efficiencies and obtain functional experimental readouts within a few days post-thaw. The cells arrive ready to use and offer the opportunity to perform single gene knockouts, and scale to high throughput pooled or arrayed CRISPR screens.

1. CRISPR-Ready ioGlutamatergic Neurons express neuron-specific markers and form structural neuronal networks by day 11



A. Schematic representation of CRISPR-Ready cells engineered to express Cas9 constitutively. Cas9 is inserted into a genomic safe harbour locus (GSH), ensuring stable expression.
B. Principal component analysis (PCA) of bulk RNA sequencing data comparing CRISPR-Ready ioGlutamatergic Neurons and ioGlutamatergic Neurons at different time points during forward programming. Analysis shows comparable gene expression profiles.
C + D. Immunofluorescent staining on post-revival day 11 demonstrates similar homogenous expression of **(C)** pan-neuronal proteins MAP2 and TUBB3 (100x magnification) and **(D)** glutamatergic neuron-specific transporter VGLUT2 (200x magnification) in CRISPR-Ready ioGlutamatergic Neurons compared to ioGlutamatergic Neurons (io1001)

2. CRISPR-Ready ioGlutamatergic Neurons are ready for gRNA delivery from day 1 and allow to perform a readout within days



CRISPR-Ready ioGlutamatergic Neurons are delivered in a cryopreserved format and are programmed to rapidly mature upon revival in the recommended media.

The protocol for culturing these cells has two phases:
1. Stabilisation for 4 days
2. Maintenance phase

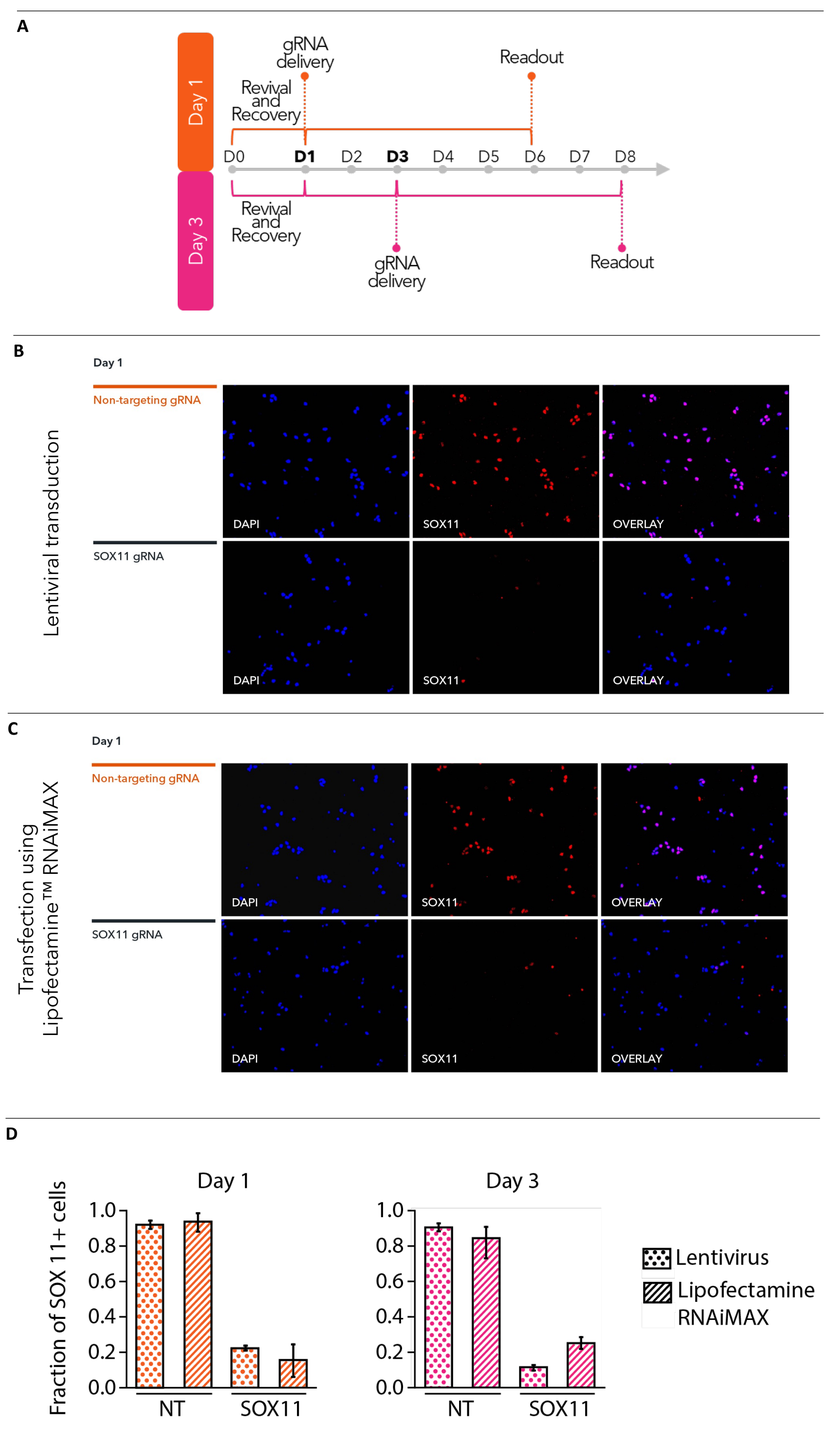
gRNAs may be delivered between day 1 and day 11. Protocols have been optimised for lipid- or lentivirus-based guide RNA delivery to ensure maximal knockout efficiency. Readouts may be performed within 5 days after the guide delivery.

3. Immunofluorescence staining demonstrate high knockout efficiency of SOX11 by both lentiviral transduction and lipid-based transfection

A. gRNAs were introduced into the cells either 1 or 3 days after thawing using two methods: lentiviral transduction and synthetic gRNA delivery with Lipofectamine™ RNAiMAX transfection reagent. A non-targeting gRNA was used as a control. Immunofluorescence staining of SOX11 was conducted five days post sgRNA delivery.

B + C. Immunofluorescence staining of CRISPR-Ready ioGlutamatergic Neurons, subjected to a SOX11-targeting gRNA, demonstrates a highly efficient knockout of SOX11. The gRNAs were delivered by **(B)** lentiviral transduction or **(C)** transfection of synthetic gRNA using Lipofectamine™ RNAiMAX on day 1 post-revival. Immunofluorescence staining of SOX11 was conducted five days post gRNA delivery. Similar knockout efficiencies were achieved. A non-targeting gRNA was used as a control.

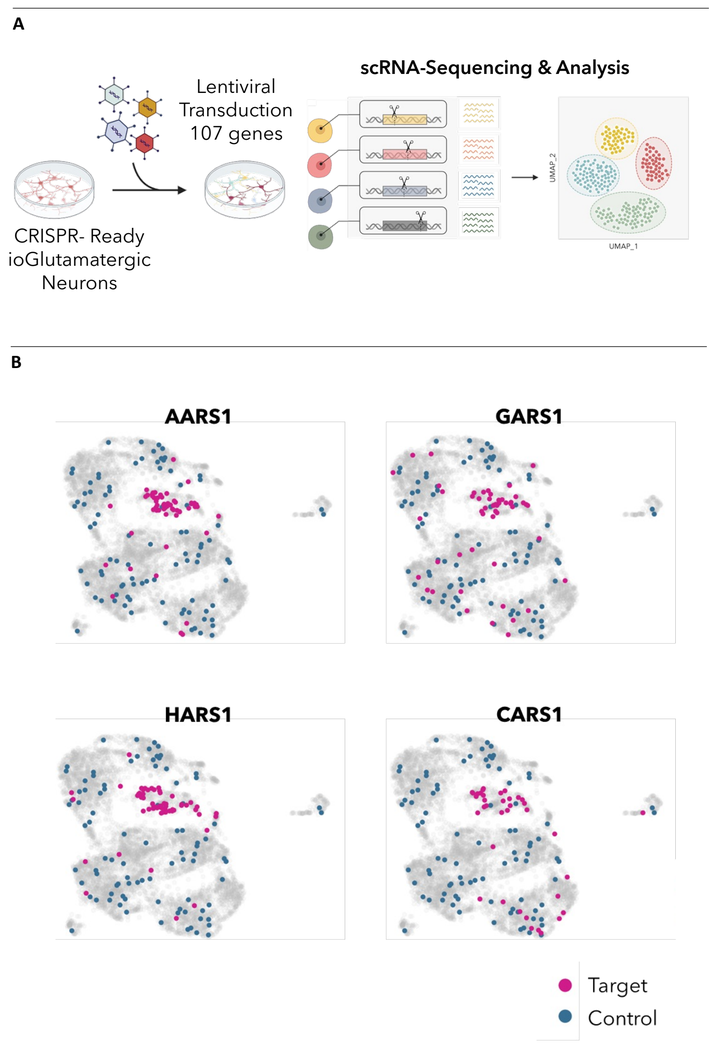
D. Quantification of images of CRISPR-Ready ioGlutamatergic Neurons after gRNA delivery. Graph is showing the ratio of SOX11 positive cells five days post virus or lipid-based guide delivery. Timepoint of gRNA delivery was at day 1 (left panel) or at day 3 (right panel). Non-targeting gRNAs (NT) were used as controls for both delivery methods.



4. A pooled knockout screen of neurodegenerative disease-relevant genes in CRISPR-Ready ioGlutamatergic Neurons shows clustering of aaRS genes in UMAPs

A. For a pooled scCRISPR screen, 107 genes known to be involved in neurodegenerative diseases were selected. Lentiviral transduction of the sgRNAs was carried out on day 3, and single-cell gene expression analysis was performed on day 15.

B. Single cells were clustered on uniform manifold approximations and projections (UMAPs) based on their shared nearest neighbour's gene expression. Clustering of aminoacyl-tRNA synthetase (aaRSs) knockouts including AARS1, HARS1, CARS1, and GARS1 was observed. In contrast, cells transduced with non-targeting control sgRNAs were evenly distributed among clusters. Pathway analysis showed gRNAs targeting aaRSs activated the unfolded protein response (UPR), the mechanism by which cells control endoplasmic reticulum protein homeostasis. In many neurodegenerative diseases, signs of UPR activation have been reported. The most common aaRS-associated monogenic disorder is the incurable neurodegenerative disease Charcot–Marie–Tooth neuropathy (CMT).



Summary & conclusions

Ready to use

Defined and characterised human neurons constitutively expressing Cas9, ready for knockout experiments from day 1.

High knockout efficiency

Optimised protocols for lipid- or lentivirus-based guide RNA delivery ensure maximal knockout efficiency.

Quick and easy

Generate readouts within days using a simple protocol for cell maturation and guide RNA delivery.