Harnessing CRISPR-Ready ioCells as Functional Genomics Tools for Drug Target Identification and Validation

Authors

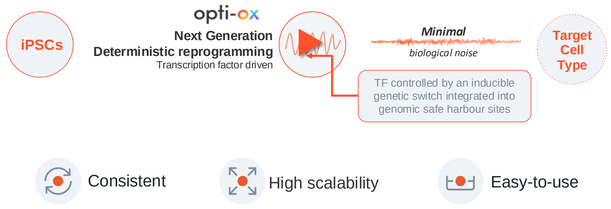
L. Grabner, C. Schmidt, H. Ceylan, K. Arat, M. Gamperl, B. Könye, T. Perlova, N. Papai, S. Sandner, A. Grandcolas, G. Belli Valletta, G. Shipley, K. Firth, M. Metzakopian, J. Conde-Vancells, C. Talbot-Cooper, B. Klapholz, L. Spencer, A. Byrne *, F. Patell-Socha, T. Buerckstuemmer, S. Salic- Hainzl, M. Kotter

*Poster presenter (ann.byrne@bit.bio)

bit.bio

The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
info@bit.bio | www.bit.bio

About bit.bio's opti-ox™ technology



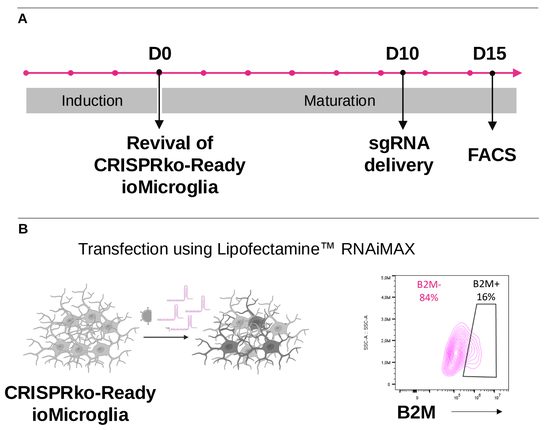
CRISPRko-Ready ioMicroglia and ioGlutamatergic Neurons

CRISPRko-Ready ioCells have been built for scientists looking to generate high-efficiency gene knockouts in physiologically relevant human cells. Based on our opti-ox powered ioWild Type Cells, these cells offer users a well-defined and characterised human model with high lot-to-lot consistency and simple protocols for handling and culturing. Each model can be used with an optimised protocol for guide RNA (sgRNA) delivery that helps maximise knockout efficiency. Scale from single gene knockouts to pooled or arrayed CRISPR screens for applications including functional genomics, disease model generation, drug target identification and fundamental human biology research.

1. Lipid-based sgRNA delivery results in high gene knockout efficiency in CRISPRko-Ready ioMicroglia

A. CRISPRko-Ready ioMicroglia are delivered as a cryopreserved product. The protocol for culturing these cells requires a 10-day maturation phase. sgRNA targeting B2M was delivered by lipid-based transfection on day 10 post thawing, followed by FACS analysis on day 15.

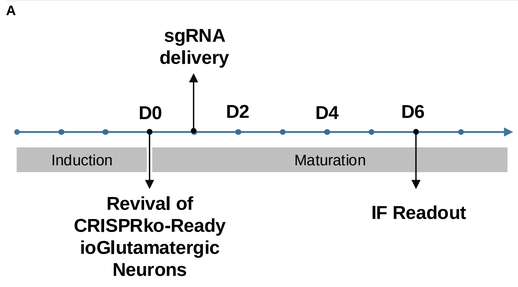
B. Flow cytometry analysis of CRISPRko-Ready ioMicroglia following sgRNA transfection revealed 84% knockout efficiency of B2M.

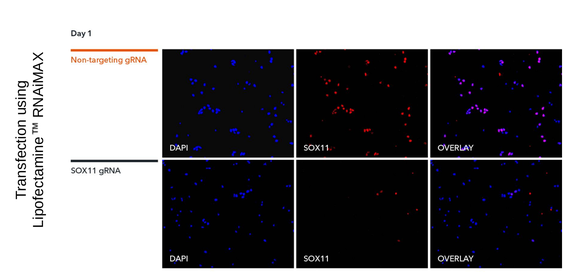


2. Immunofluorescence staining demonstrates high knockout efficiency of SOX11 by lipid-based transfection of sgRNA in CRISPRko-Ready ioGlutamatergic Neurons

A. Synthetic sgRNAs were introduced into the cells either 1 or 3 days (data not shown) post-revival by transfection using Lipofectamine™ RNAiMAX. A non-targeting sgRNA was used as a control. Immunofluorescence staining of SOX11 was conducted five days post sgRNA delivery.

B. Immunofluorescence staining of CRISPRko-Ready ioGlutamatergic Neurons, subjected to a non-targeting (top panels) or a SOX11-targeting gRNA (bottom panel) demonstrates a highly efficient knockout of SOX11.

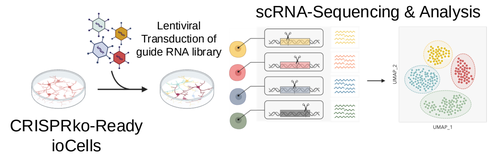


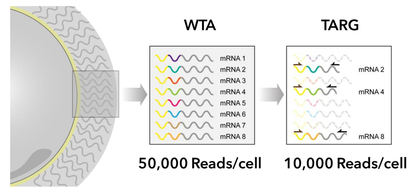


3. CRISPRko-Ready ioCells can be used in pooled CRISPR/Cas9 based knockout screens

A. CRISPRko-Ready ioCells cells are used in CRISPR/Cas9-based knockout screening workflows. A library of guide RNAs can be delivered via lentiviral transduction. Single-cell RNA sequencing (scRNA-seq) serves as a powerful readout to study gene function across a range of biological questions.

B. scRNA-seq can be conducted as Whole Transcriptome Analysis (WTA) or Targeted (TARG) scRNA sequencing. The latter offers a lower-cost alternative by focusing only on genes of interest.

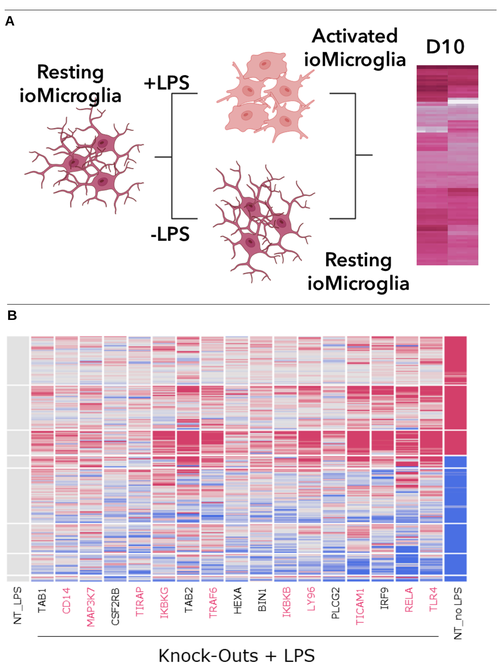




4. Targeted single-cell RNA sequencing uncovers genes involved in microglia activation following a pooled CRISPR/Cas9 based knockout screen

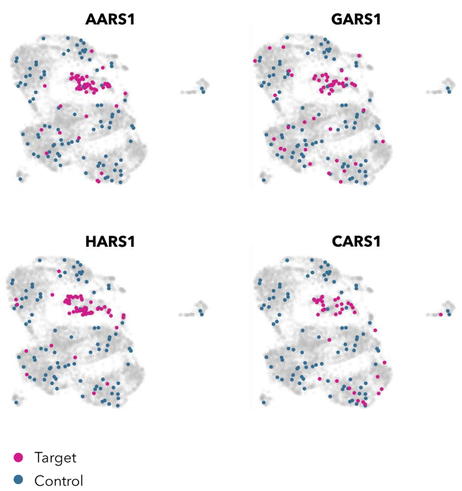
A. An activation signature of LPS-treated CRISPRko-Ready ioMicroglia was identified using bulk RNA sequencing. A total of 1,610 genes were differentially expressed between the LPS-treated and untreated conditions. Out of these, 258 genes were selected for the targeted sequencing readout. This signature served as a benchmark in the pooled scCRISPR knockout screen to identify modulators of LPS-induced activation.

B. 110 candidate genes were selected for the pooled scCRISPR screen based on their known roles in neurodegeneration and neuroinflammation. Guide RNAs were delivered via lentiviral transduction on day 10, aiming for a single integration per cell. The cells were treated with +/- LPS for 24 hours before single cell processing on day 15. Cosine similarity analysis compared knockouts in LPS-treated CRISPRko-Ready ioMicroglia to both resting and activated states. The analysis identified 17 gene knockouts that altered responses to LPS stimulation. The heatmap shows Log2FC profiles for gene knockouts that had a cosine similarity above 0.3 (arbitrarily chosen threshold) compared to cells with non-targeting guides in the unstimulated condition. Knockouts are sorted based on their cosine similarity to the non-LPS condition. CD14, MAP3K7, TIRAP, IKBKG, TRAF6, IKBKB, LY96, TICAM1, RELA, and TLR4 are genes known to be involved in LPS activation mediated via the TLR4 signalling pathway.



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

For a pooled scCRISPR screen, 100 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 12. To visualise the knockout phenotypes, single cells were projected onto the UMAP embedding. Clustering of aminoacyl-tRNA synthetase (aaRSs) knockouts including AARS1, HARS1, CARS1, and GARS1 was observed. Pathway analysis showed sgRNAs 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 neurodegenerative disease is the Charcot–Marie–Tooth neuropathy (CMT).



Summary & conclusions

Functional CRISPRko-Ready ioCells

Well characterised and functional human microglia and glutamatergic neurons with constitutive Cas9 expression.

High knockout efficiency

Deliver sgRNA by lipid-based transfection or lentiviral-based transduction.

Compatible with knockout screening workflows

Perform large-scale experiments for target identification and validation.