Optimised and scalable reprogramming of human iPSCs to generate nociceptor sensory neurons for the study of pain mechanisms and neuropathies

Authors

*D. Pacitti
M. Raman Srivastava
M. Bahri
M. Ortiz-Sanchez
K. Long
M. Byrne
M. Dermit-Salacar
A. Wilczynska
S. Milde
B. Newman
F. Patell-Socha
T. Moreau
W. Bernard
T. Oosterveen
M. Metzakopain
M. Kotter

*Poster presenter (dario.pacitti@bit.bio)

bit.bio

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

Abstract

Nociceptive sensory neurons, a subtype of somatosensory cells of the dorsal root ganglia, respond to diverse noxious and pruritic stimuli, and are critical for the study of pain mechanisms and neuropathies (1).

Chronic nociceptive and neuropathic pain is currently one of the leading causes of long-term disability affecting approximately 1 in 5 adults globally, with 30% of these patients reporting refractory pain to available analgesics (2).

Many promising drugs with efficacy in animal models of pain have failed in the clinic due to interspecies differences in nociception (3).

Conventional differentiation methods to generate nociceptors from human induced pluripotent cells (hiPSC) offer a more physiologically relevant alternative to animal models, but are limited by complex, inconsistent, and long protocols.

Thus, there is a need for a consistent, scalable human *in vitro* model to study the pathophysiology of nociceptive sensory neurons and develop new, efficacious, and safe pain therapeutics.

We used transcription factor mediated precision cellular reprogramming technology, opti-ox™ (4), to rapidly and consistently generate mature, functional and physiologically relevant sensory neurons that display critical features of nociceptors, named ioSensory Neurons, at scale from hiPSCs.

Transcriptomic and phenotypic characterisation demonstrated that reprogrammed hiPSCs acquired a sensory nociceptor identity.

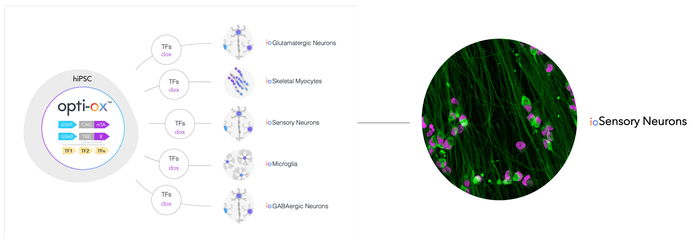
Within 7 days post-revival, the cells expressed the pan-sensory neuron markers ISL1, POU4F1 and PRPH, and key nociceptor markers NTRK1, TRPV1, TRPM8, and SCN9A.

Neurotrophic factors influence subtype specification of sensory neurons, and thus by optimising culture conditions we enriched for cells expressing key sensory genes, including peptidergic nociceptor markers TAC1 and ADCYAP1.

Multi-Electrode Array and calcium assays demonstrated asynchronous spontaneous activity and responsiveness to diverse noxious stimuli, revealing the sensory neurons' functionality.

In conclusion, with opti-ox™ precision reprogramming, iPSCs are rapidly converted into functional sensory neurons offering a robust and scalable source of human nociceptors which can be used as a relevant in vitro model to study the biology of pain and to develop novel therapies for neuropathies.

1. Precise opti-ox™ cellular reprogramming of hiPSCs into ioSensory Neurons

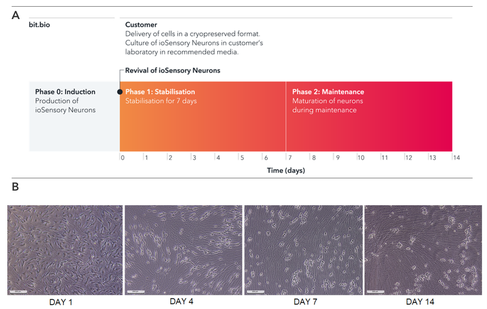


opti-ox™ technology for the optimal cellular reprogramming of human iPSCs into defined human cell types, including ioSensory Neurons. opti-ox™ dual cassette Tet-ON system ensures tightly controlled and homogeneous expression of reprogramming transcription factors (TFs) by preventing silencing of the inducible expression cassette after genetic engineering of hiPSCs .

2. Human ioSensory Neurons are ready to use by day 7

A) ioSensory Neurons are delivered in a cryopreserved format and are programmed to rapidly mature upon revival. Cells are revived and cultured in a single medium, with fully disclosed composition allowing modifications to fit customers' bespoke experiments. The protocol for the generation of these cells is a three-phase process: Induction, which is carried out at bit.bio (Phase 0), Stabilisation for 7 days (Phase 1), and Maintenance (Phase 2) during which the ioSensory Neurons mature. Phases 1 and 2 after revival of cells are carried out at the customer site.

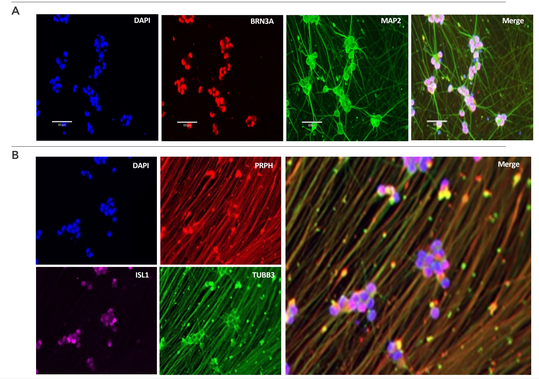
B) Upon reprogramming, ioSensory Neurons show rapid morphological changes with neurons being identified by day 4 and forming visible neuronal networks by day 7. Day 1 to 14 post revival; 10X magnification; scale bar: 200 µM.



3. ioSensory Neurons homogeneously express key pan-sensory markers

A) Immunofluorescent staining on day 14 post-thaw, demonstrates that reprogrammed ioSensory Neurons are all positive for BRN3A (red), MAP2 (green), and DAPI counterstain (blue). MAP2 positive neurons colocalize with the sensory marker BRN3A suggesting a high purity of neurons with a sensory identity. 10X magnification, scale bar: 60µm.

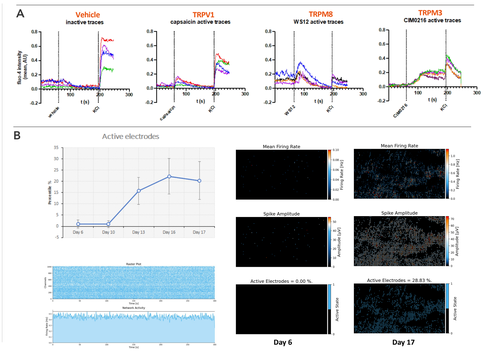
B) Immunofluorescent staining on day 14 post-thaw, demonstrates that reprogrammed ioSensory Neurons are all positive for ISL1 (magenta), PRPH (red), TUBB3 (green), and DAPI counterstain (blue). TUBB3 positive neurons co-localize with the sensory markers ISL1 and PRPH indicating that neurons have a sensory identity. 10X magnification.



4. Rapid gain of functional nociceptor phenotype

A) Calcium mobilisation imaging showing that ioSensory Neurons respond to pharmacological agonists targeting key thermosensitive TRP channels such as TRPV1 (Capsaicin), TRPM3 (CIM0216) and TRPM8 (WS-12). Active traces represent the increase in intracellular calcium mobilisation of individual cells upon exposure to noxious agonists but not to vehicle, indicating that cells display features of functional nociceptors. Note, this data is from cells in continuous culture and not cryopreserved cells.

B) Multi-Electrode Array (MEA) analysis of ioSensory Neurons over a period of 17 days. ioSensory neurons display increased spontaneous activity over time, with neurons firing as early as day 13 of reprogramming. Note, this data is from cells in continuous culture and not cryopreserved cells



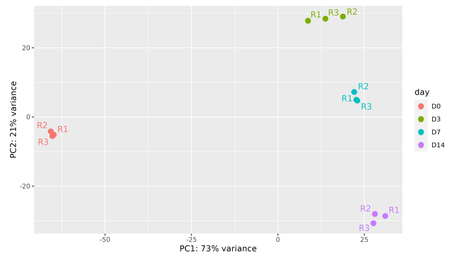
5. Whole transcriptome analysis demonstrates high batch-to-batch consistency of ioSensory Neurons

Bulk RNA sequencing analysis was performed on three different batches (R1, R2 and R3) of ioSensory Neurons at different time points (days 0, 3, 7 and 14) throughout the reprogramming protocol.

Principal component analysis represents the variance in gene expression between batches of ioSensory Neurons. This analysis shows high consistency between each batch of ioSensory Neurons at each given timepoint.

Pure populations of ioSensory Neurons with equivalent expression profiles can be generated consistently from every vial, allowing confidence in experimental reproducibility.

Note, this data is from cells in continuous culture and not cryopreserved cells.



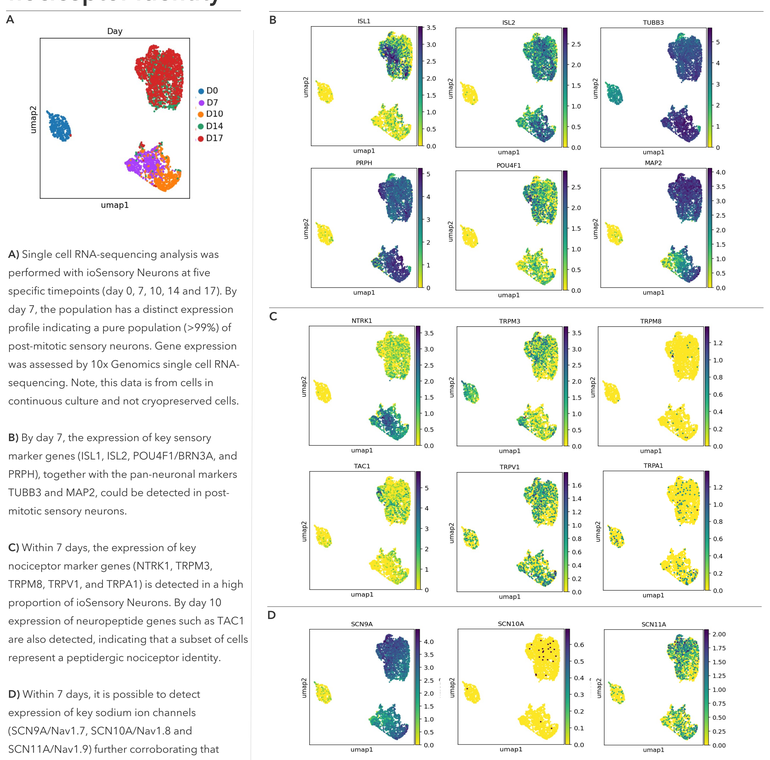
6. Single cell RNA-sequencing shows ioSensory Neurons form a pure population (>99%) of sensory neurons with a defined nociceptor identity

A) Single cell RNA-sequencing analysis was performed with ioSensory Neurons at five specific timepoints (day 0, 7, 10, 14 and 17). By day 7, the population has a distinct expression profile indicating a pure population (>99%) of post-mitotic sensory neurons. Gene expression was assessed by 10x Genomics single cell RNA-sequencing. Note, this data is from cells in continuous culture and not cryopreserved cells.

B) By day 7, the expression of key sensory marker genes (ISL1, ISL2, POU4F1/BRN3A, and PRPH), together with the pan-neuronal markers TUBB3 and MAP2, could be detected in post-mitotic sensory neurons.

C) Within 7 days, the expression of key nociceptor marker genes (NTRK1, TRPM3, TRPM8, TRPV1, and TRPA1) is detected in a high proportion of ioSensory Neurons. By day 10 expression of neuropeptide genes such as TAC1 are also detected, indicating that a subset of cells represent a peptidergic nociceptor identity.

D) Within 7 days, it is possible to detect expression of key sodium ion channels (SCN9A/Nav1.7, SCN10A/Nav1.8 and SCN11A/Nav1.9) further corroborating that ioSensory Neurons display a nociceptor identity.



Summary & conclusions

ioSensory Neurons show >99% purity for the expression of key sensory neuron markers including PRPH, POU4F1 (BRN3A), ISL1, and TUBB3 as characterized by single cell RNA sequencing and ICC.

ioSensory Neurons have a defined nociceptor identity as shown by the expression of key nociceptor marker genes, NTRK1 and TRP ion channels, including TRPV1.

Rapid functionality – spontaneous activity demonstrated by MEA.

Functional nociceptors – calcium mobilisation in response to specific TRP agonists.

Cells are easy to culture using a simple 1-medium, 2-step mitomycin C-free protocol – ideal for researchers without iPSC expertise.

ioSensory neurons show batch-to-batch reproducibility and homogeneity, as shown by bulk RNA-sequencing.

Rapidly maturing sensory neurons that are ready to use by 7 days, post-revival.

References:
1. Haberberger, *et al.* Front Cell Neurosci. 2019;13:271.
2. Goldberg, *et al.* BMC Public Health. 2011 Oct 6;11(1):770.
3. Wilson, *et al.* PloS One. 2018 Jun 28;13(6):e0199996–e0199996.
4. Pawlowski, *et al.* Stem Cell Reports. 2017 Apr 11;8(4):803-812.

May 2023