A novel human skeletal muscle in vitro model using opti-ox mediated cellular reprogramming of induced pluripotent stem cells

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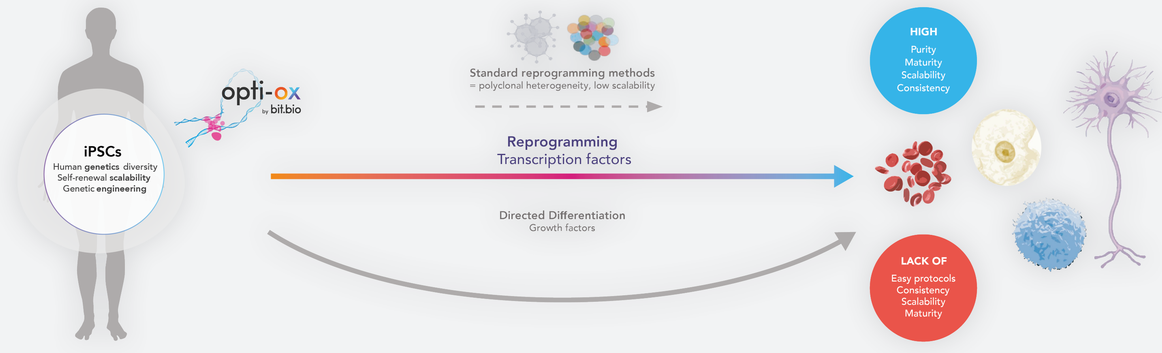
Abstract

Skeletal myocytes play roles in many biological processes ranging from limb movement to the regulation of nutritional homeostasis, and are implicated in diseases involving muscle dysfunction and wasting. There is a pressing need for reliable models of human skeletal muscle to permit investigations into physiological and disease mechanisms, and to facilitate the development of therapeutics. While human induced pluripotent stem cells (hiPSCs) offer a promising starting material, their broad use has been hampered by complex differentiation protocols. We have developed an optimised inducible system (opti-ox™) that enables tightly controlled expression of transcription factors (TFs) improving reprogramming approaches for the differentiation of hiPSCs. By targeting genomic safe harbour loci, we used opti-ox to achieve homogenous, inducible expression of the myogenic regulator MYOD1. Induction of MYOD1 leads to rapid shutdown of the core pluripotency network, and activation of core myogenic factors. We demonstrate robust expression of myosin heavy chain isoforms, coupled with the transition of immature myosin heavy chain isoform (MYH3 and MYH8) to mature isoforms (MYH1) expression, in a time dependent manner. Skeletal myocytes express Desmin, Dystrophin and Titin, and form striated multinucleated myotubes that contract in response to acetylcholine. Critically for metabolic studies, preliminary data suggests robust expression of the insulin-regulated glucose transporter GLUT4 is detected. opti-ox reprogramming provides a scalable model of human skeletal myocytes, opening avenues for high throughput screening and research.

bit.bio's approach to cellular reprogramming

Precise control of transcription factor expression through iPSC engineering



The use of human iPSC derived cell models has been hindered by the lack of consistency and scalability of differentiation methods. Novel reprogramming technology, opti-ox, is opening new avenues by allowing controlled expression of transcription factor combinations for optimal cellular reprogramming of human cell types from hiPSCs.

1. Precise reprogramming of iPSCs into defined human cell types

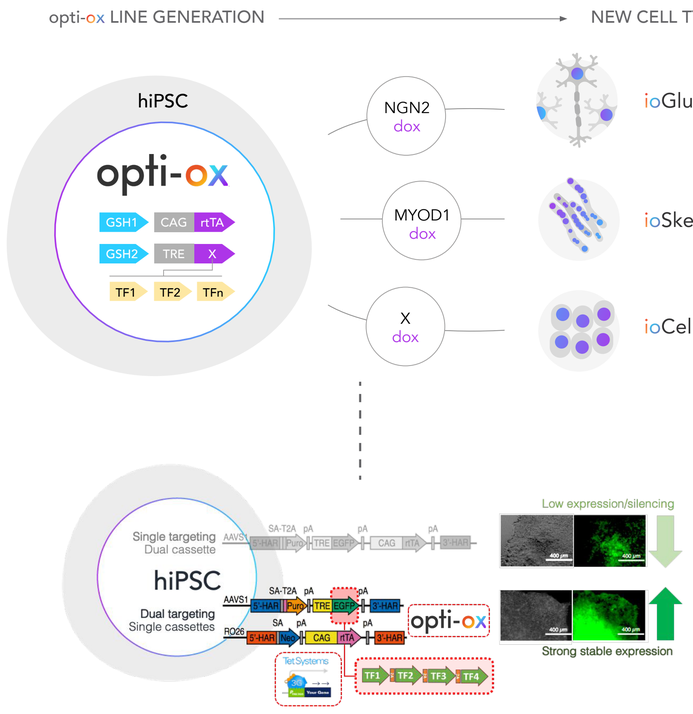
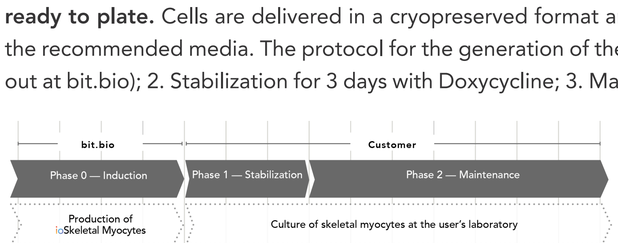


Figure 1. opti-ox technology for the optimal cellular reprogramming of human iPSCs into defined human cell types. opti-ox dual cassette Tet-ON system ensures tightly controlled and homogeneous expression of reprogramming transcription factors by preventing silencing of the inducible expression cassette after genetic engineering of hiPSCs[1].

2. ioSkeletal Myocytes, a novel human muscle model

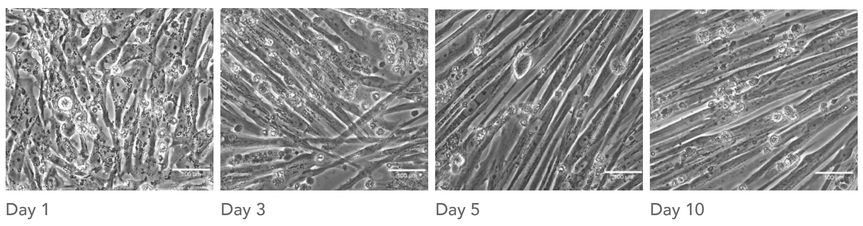
Figure 2. ioSkeletal Myocytes are derived from hiPSCs by MYOD1[1,2] driven opti-ox reprogramming and arrive ready to plate. Cells are delivered in a cryopreserved format and are programmed to rapidly mature upon revival in the recommended media. The protocol for the generation of these cells is a three-phase process: 1. Induction (carried out at bit.bio); 2. Stabilization for 3 days with Doxycycline; 3. Maintenance during which the skeletal myocytes mature.



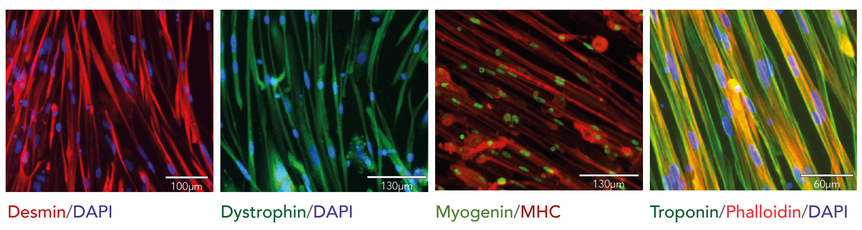
3. ioSkeletal Myocytes form contractile, elongated fibres over 10 days and express mature myogenic markers

Figure 3: Characterization of ioSkeletal Myocytes. (A) ioSkeletal Myocytes after revival over the course of the first 10 days of culture. Day 1 to 10 post-thawing; 400X magnification; scale bar: 100µm. **(B)** Immunofluorescence staining at day 10 post revival demonstrates robust expression of components of the contractile apparatus such as Desmin, Dystrophin, and Myosin Heavy Chain (MHC), along with the muscle transcription factor Myogenin. Cells also demonstrate expression of Troponin with visible striated fibres, and multinucleation.

A

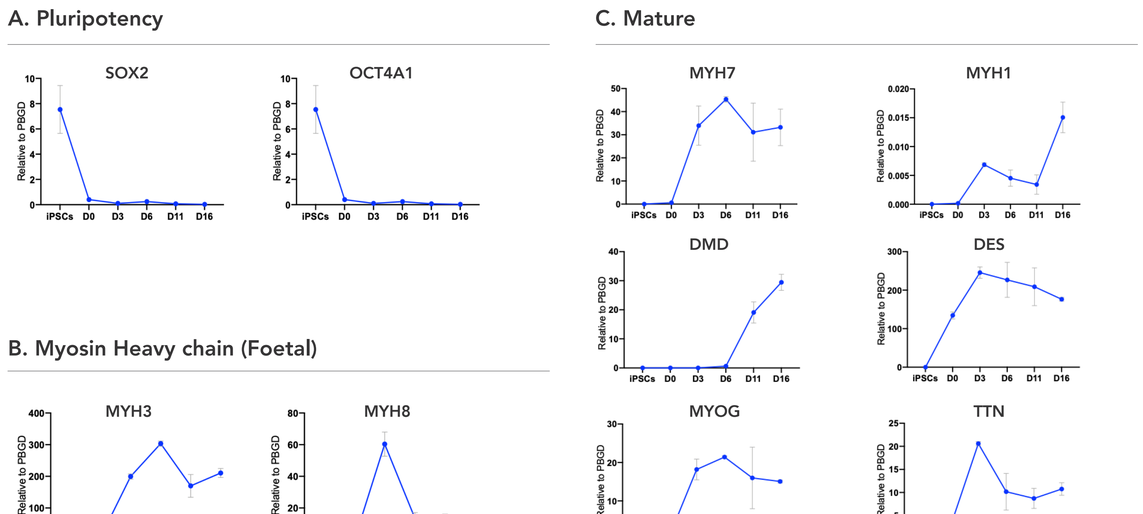


B



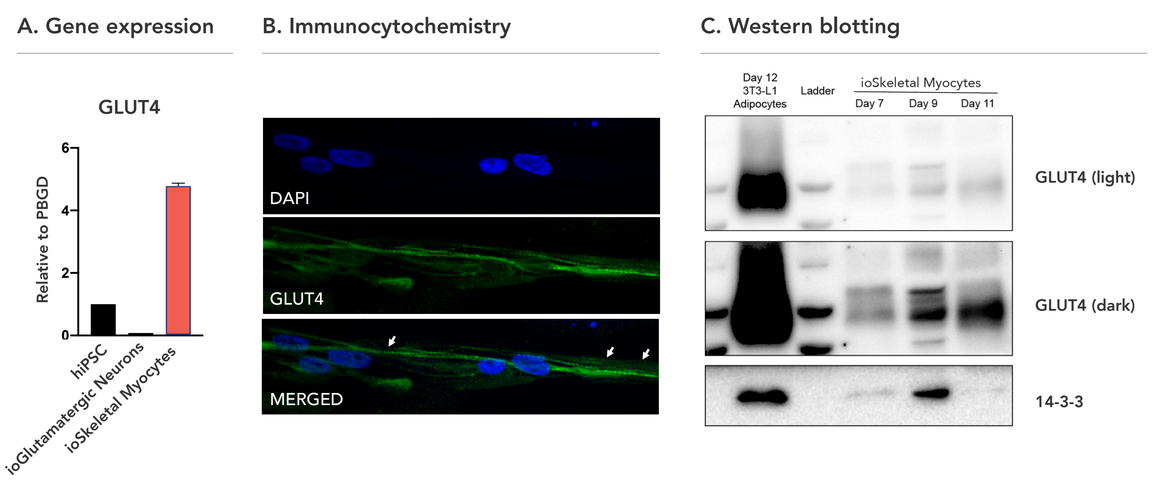
4. Cells demonstrate timewise gene expression of key myogenic markers

Figure 4: ioSkeletal Myocytes gene expression. Following reprogramming, ioSkeletal Myocytes robustly downregulate expression of pluripotency markers **(A)**, and begin to express myosin heavy chain isoforms MYH3 and MYH8 **(B)**. Through continued culture, ioSkeletal Myocytes demonstrate expression of mature myosin isoforms MYH7 and MYH1, along with DESMIN, DYSTROPHIN, MYOGENIN, and TITIN **(C)**. Gene expression levels assessed by RT-qPCR (data expressed relative to parental iPSC, normalised to PBGD). Data represents day (Dx) post-thaw.



5. ioSkeletal Myocytes - human iPSC derived model for the study of metabolic disease?

Figure 5: Preliminary data demonstrates GLUT4 expression in ioSkeletal Myocytes. (A) RT-qPCR demonstrating expression of GLUT4 compared to hiPSCs and ioNEURONS/glut. **(B)** Immunocytochemistry of ioSkeletal Myocytes revealing expression of GLUT4 in peri-nuclear regions, and striations[3]. **(C)** Western blotting of differentiated 3T3-L1 adipocytes and maturing ioSkeletal Myocytes demonstrates GLUT4 expression in a time-dependent manner[3].



Summary

- Human iPSC-derived skeletal myocytes, generated using opti-ox, form contractile, elongated fibres over 10 days.
- ioSkeletal Myocytes are highly characterized and demonstrate timewise expression of mature myocyte markers.
- GLUT4 expression in ioSkeletal Myocytes suggests they could provide a unique human cell model for metabolic research.
- ioSkeletal Myocytes provide a reliable human cell model for disease modelling (e.g. Muscular dystrophy) and drug discovery applications.

1. Pawlowski M, et al., Stem Cell Reports, 2017. **2.** Yang N, et al., Nature Methods, 2017. **3.** Dougall Norris & Daniel Fazakerley, Wellcome-MRC Institute of Metabolic Science

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