Shipment of Human Transduced Live iPSC-derived Motor Neurons

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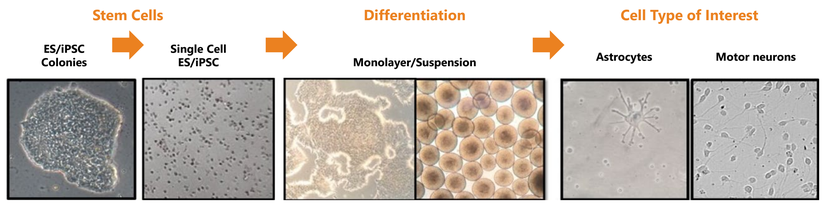
iPSC Background

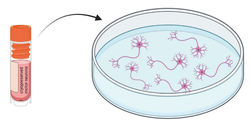
- In vitro neuroscience research often requires the use of human cells that are the correct cell type (neurons) to model disease phenotype and cell response. As neurons are post-mitotic, the use of immortal cell lines to understand disease biology remains limited.
- To solve this issue, induced pluripotent stem cells (iPSC) derived typically from fibroblasts have been key to in vitro discovery in neuroscience as they provide many key advantages; they can replicate and be expanded prior to differentiation[1] (important to cells that do not divide such as neurons), any cell type can be formed, and patient disease genotype can be retained and modeled.
- iPSC differentiation into motor neurons takes up to 6 weeks and can be variable in purity site to site. Therefore, a method to transfer pre-differentiated and matured motor neurons to a second site can enable reproducibility and collaboration into workflows.
- Currently, there are well established procedures for shipping genetic material, cellular isolates, small molecules, and cryopreserved cells with little published on the shipment on live iPSC-derived motor neurons[2]



Methods

Thaw Post-mitotic iPSC-derived Motor Neurons

- Thaw vial of cryopreserved cells and add 1mL NMM with ROCK inhibitor.
- Add more NMM, spin, then resuspend.
- Plate cells in NMM with ROCK inhibitor.



Pre-Shipment Culture and Shipment Preparation

- Wash cells 1X with PBS-/- before lifting them with accutase.
- Spin and resuspend cells in NMM with ROCK inhibitor.
- Assess cell viability and store/ship at 4 ^{0}C for 24 or 48 hours

Post-Shipment Restoration and Culture

- Resuspend cells after 24-48 hours at 4 ^{0}C. Spin and resuspend cells in NMM without ROCK inhibitor.
- Count cells and seed in poly-ornithine and laminin coated plates, change media after 72h.

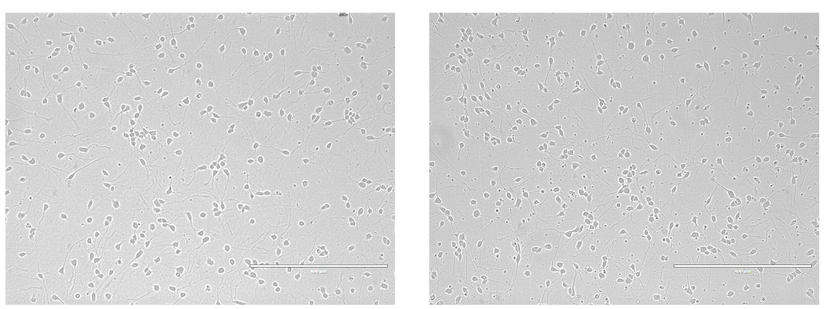


Figure 1: Neurons 24 hours after refrigeration and replating at 2.5x10^{4} cells per well, left in neuronal maturation media, right in Hibernate E. Cells show little debris indicating minimal cell death and within the range of normal (qualitatively minimal) cell clumping. Neurons in neuronal maturation media appear to be slightly more dense.

Results

RLU Normalized to Seeded Cell Count

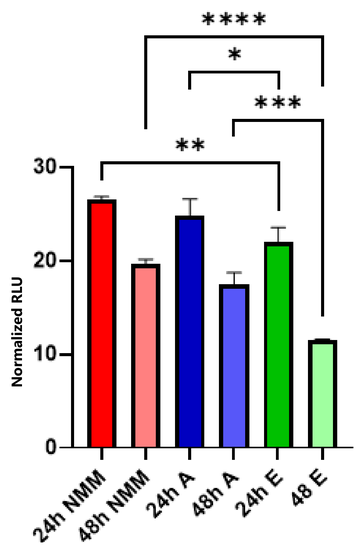


Figure 2: CellTiter-Glo of cells in neuronal maturation media (NMM), Hibernate A, and Hibernate E show no significant difference in viable cells at both 24 hours and 48 hours between NMM and Hibernate A but at both 24 and 48 hours, Hibernate E media led to significantly lower quantity of viable cells. In subsequent runs, only NMM and Hibernate A media were used.

GeneX Relative Gene Expression

*Ordinary One-way ANOVA

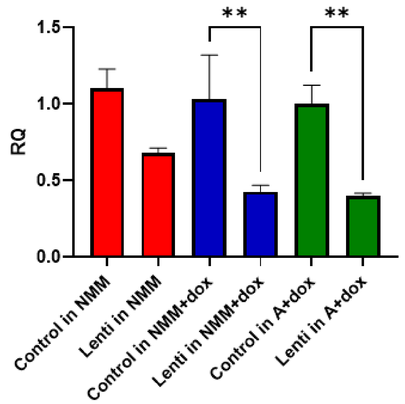


Figure 3: On cells treated with shRNA expressing lentivirus designed to knock down GeneX and induced with doxycycline, qRT-PCR probing for GeneX shows that cells without active shRNA, without doxycycline, have a non-significant difference in the amount of GeneX present whereas in both NMM and Hibernate A media with doxycycline, cells with virus and doxycycline had significantly less GeneX.

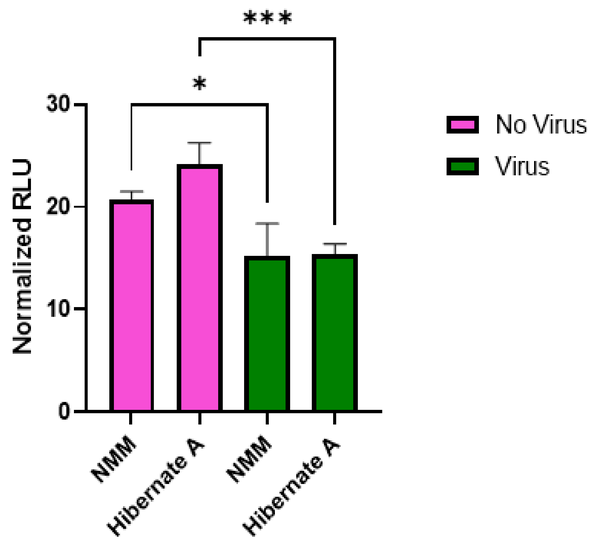
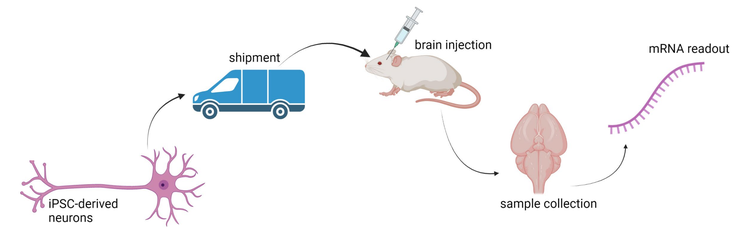


Figure 4: When virus was added to cells and shRNA was activated with doxycycline, cells transduced with virus had significantly less viability than cells without virus but no difference was observed in viability between media.

Xenograft Pilot Study

Goal : To determine whether human RNA can be detected in a mouse xenograft model

QurAlis iPSC-derived neurons were shipped live via our developed protocol. The cells were spun and resuspended in vehicle for unilateral injection to the mouse brain.



		Count 1	Count 2	*Average*
Tube 1	Viability	54.8%	57.6%	*56.2%*
	Viable cells/mL	1.53x10^{6}	2.18x10^{6}	*1.86x10^{6}*
Tube 2	Viability	66.7%	65.2%	*66%*
	Viable cells/mL	1.5x10^{6}	2.02x10^{6}	*1.76x10^{6}*

Shipped Neurons Survive Post-grafting to the Right Mouse Brain

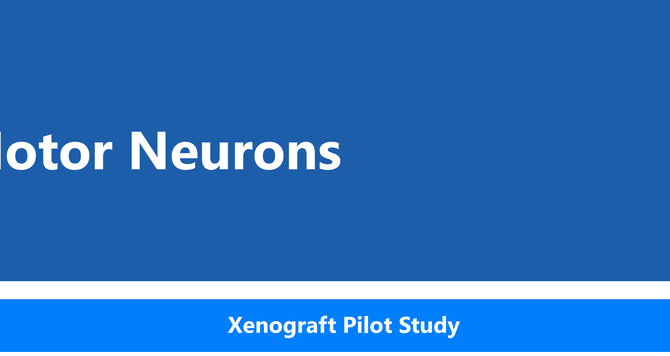


Figure 4 : At collection, RNA levels of human *GAPDH* were detectable only on the grafted side of the brain, indicating specificity of the assay. Shipping live iPSC-derived neurons is a viable option for studies requiring minimal processing at a second site.

Conclusions

- This method for preparing and shipping iPSC-derived motor neurons shows high levels of cell survival when refrigerated for 24 to 48h.
- Gibco Hibernate A media with ROCK inhibitor is the recommended shipment media due to higher levels of survival and no observed adverse effects in terms of morphology.
- This shipment method has also been proven to be effective in terms of functionality. Using the protocol above, human *GAPDH* RNA was detected in all mice injected, establishing a successful xenograft pilot study using shipped iPSC-derived neurons.

References

1. Nat Biotechnol, Watanabe et al., 2007
2. NIH, Laboratory Shipping Protocols, 2021