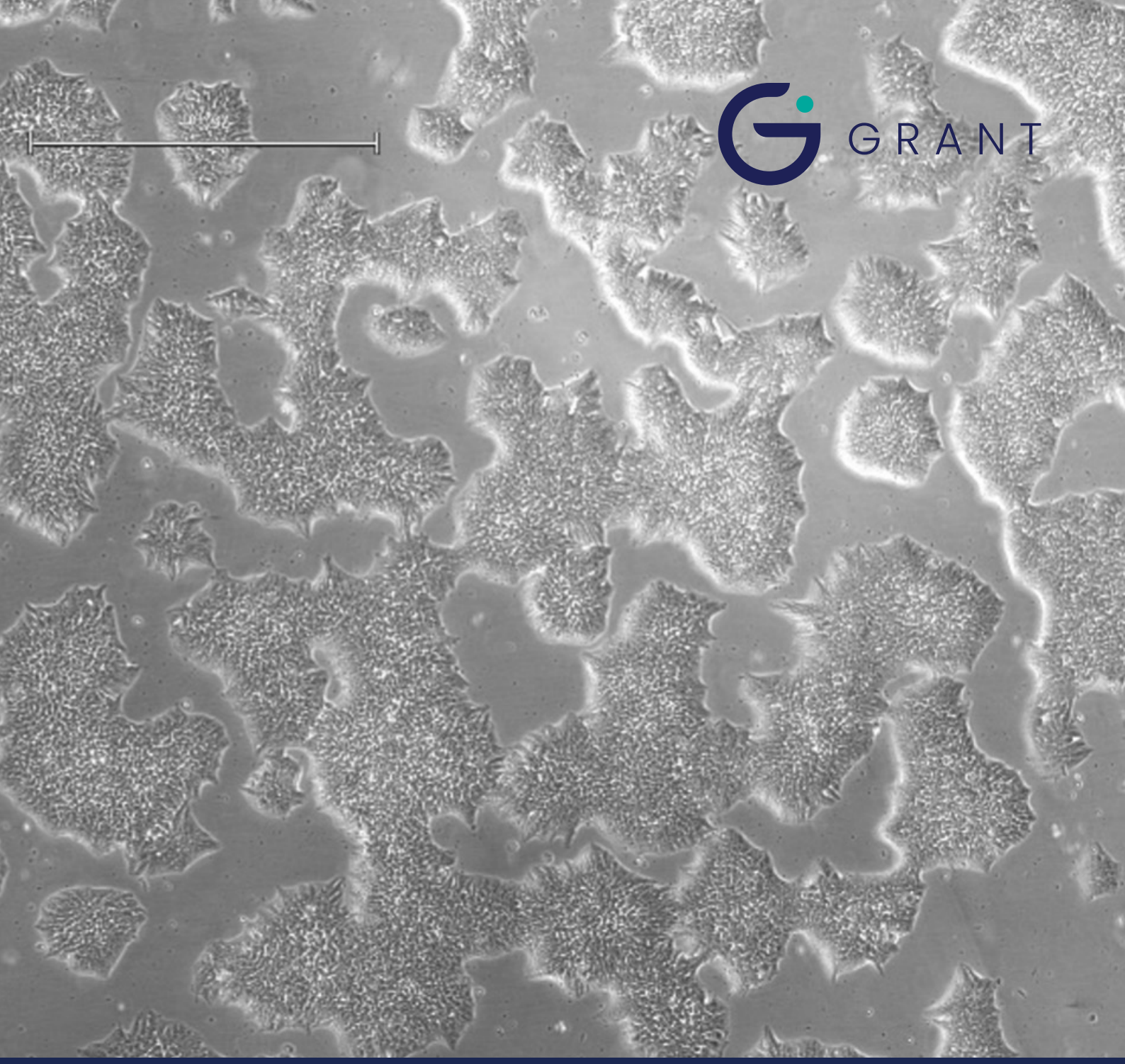

GRANT

CATAPULT Cell Therapy

CRFT evaluation report

The Evaluation of Grant Instruments Controlled Rate Freeze Thaw Device for the Cryopreservation and Thawing of hiPSC

EDN-REP-018

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Grant ultrasonic baths are manufactured in the United Kingdom and conform to exacting international standards.

CONTENTS

1. Abbreviations

ATMP	Advanced therapy medicinal products
CGTC	Cell and Gene Therapy Catapult
CPA	Cryoprotective Agent
CRF	Controlled rate freezer
CRFT	Controlled rate freeze-thaw
CS10	Cryostor CS10
E8	Essential 8
GMP	Good manufacturing practice
hiPSC	Human induced pluripotent stem cell
SOP	Standard operating procedure
LN2	Liquid nitrogen
ROCKi	Rho Kinase inhibitor

2. Introduction

Preserving living cells at ultra-low (< -100°C) temperatures slows cell processes, including degradation, thus allowing cells to be revived, even after long-term storage (Bojic *et al*., 2021). For cryopreserved cells to be successfully revived, the freezing and thawing processes must be well controlled and optimised to minimise cellular injury such as altered cell metabolism, morphology and characteristics or cell death (Bojic *et al*., 2021; Meneghel *et al*., 2020). There are numerous devices designed to facilitate slowed and controlled cooling of cells for cryopreservation.

The Corning CoolCell is an alcohol-free freezing container. The CoolCell XL has a capacity of 12 × 1.8 - 2 mL cryovials, and the CoolCell FST30 has a capacity of 30 × 1.8 - 2 mL cryovials. When cryovials containing cells are placed in the CoolCell container and stored in a -80°C freezer, cryovial contents are cooled at a rate of -1°C/minute. The CoolCell XL containers utilise insulation foam, radial symmetry, and a solid-state core to passively regulate sample heat loss. To compensate for the increased capacity, the CoolCell FST30 has 2 vents and a diffuser plate allowing additional airflow, cooling samples via convection (Corning Incorporated, 2019). While CoolCell containers have been demonstrated to successfully freeze cells, the small capacity, minimal control, lack of data traceability, and vulnerability to extraneous factors severely limit wider applications (Meneghel *et al*., 2020).

Controlled rate freezers (CRF) actively cool samples based on a user defined profile and real time temperature data. Traditional controlled rate freezers, utilise liquid nitrogen (LN2) vapours to cool samples through convection. These devices are well established in the advanced therapy medicinal products (ATMP) field, but LN2 poses health and safety risks to operators and is not suitable for use in graded good manufacturing practice (GMP) environments due to the contamination risk (Grout & Morris, 2009). LN2-free CRFs are a relatively new development in cryobiology, and actively cool samples to ultra-low temperatures without the use of LN2; the Grant Instruments Controlled Rate Freeze-Thaw device (CRFT) utilises an electrically powered, integrated Stirling engine to freeze samples through conduction (Grant Instruments, n.d.). The increased complexity of CRF devices allows for greater control and traceability of freezing processes, but requires more capital, maintenance, and expertise.

In a typical thaw procedure, cryopreserved cells are rapidly rewarmed using a water bath or dry thawing device until a small ice pellet remains, usually between 1-5 minutes, then cells are processed for further use (culture, analysis, manufacturing, administration, etc). This process is highly operator dependent and poorly controlled. The Grant Instruments CRFT has controlled rate thaw function, allowing operators to warm vessels from ultra-low temperatures (> -80°C) to 30°C based on a user defined profile (Grant Instruments, n.d.). With little systematic study of relationships between thawing rate, cell recovery and cell type, controlled-rate thawing is relatively novel (Baboo *et al*., 2019; Gurina *et al*., 2016). However, with further investigation and optimisation, controlled-rate thawing could be an opportunity to reduce variability and increase robustness.

This study assessed the use of the Grant Instruments CRFT for the cryopreservation and thawing of human induced pluripotent stem cells (hiPSCs), originally expanded in 2D tissue culture flasks using single cell passaging facilitated by Rho-kinase inhibitor (ROCKi) treatment, resuspended in cryopreservation medium. The CRFT was compared to passive freezing and thawing devices, a Corning CoolCell XL and 37°C water bath respectively (Table 2). This study marked the first experimental work examining the controlled-thaw function of the Grant Instruments CRFT for the recovery of cryopreserved mammalian cells.

Table 2. Summary of Experimental Groups

Treatment Group	Freezing Protocol	Thawing Protocol	Name
1	Grant Instruments CRFT	Water Bath	CRFT/Water Bath
2	Grant Instruments CRFT	Grant Instruments CRFT	CRFT/CRFT
3	BioCision CoolCell	Water Bath	CoolCell/Water Bath
4	BioCision CoolCell	Grant Instruments CRFT	CoolCell/CRFT

2.1 OBJECTIVES

1. Determine if the Grant Instruments CRFT is capable of slow freezing hiPSC samples for cryopreservation by assessing sample temperature during the slow freezing process and post-thaw recovery as compared to Corning CoolCell.
2. Determine if Grant Instrument CRFT is capable of thawing hiPSC samples for recovery by assessing post-thaw recovery as compared to a 37°C water bath.

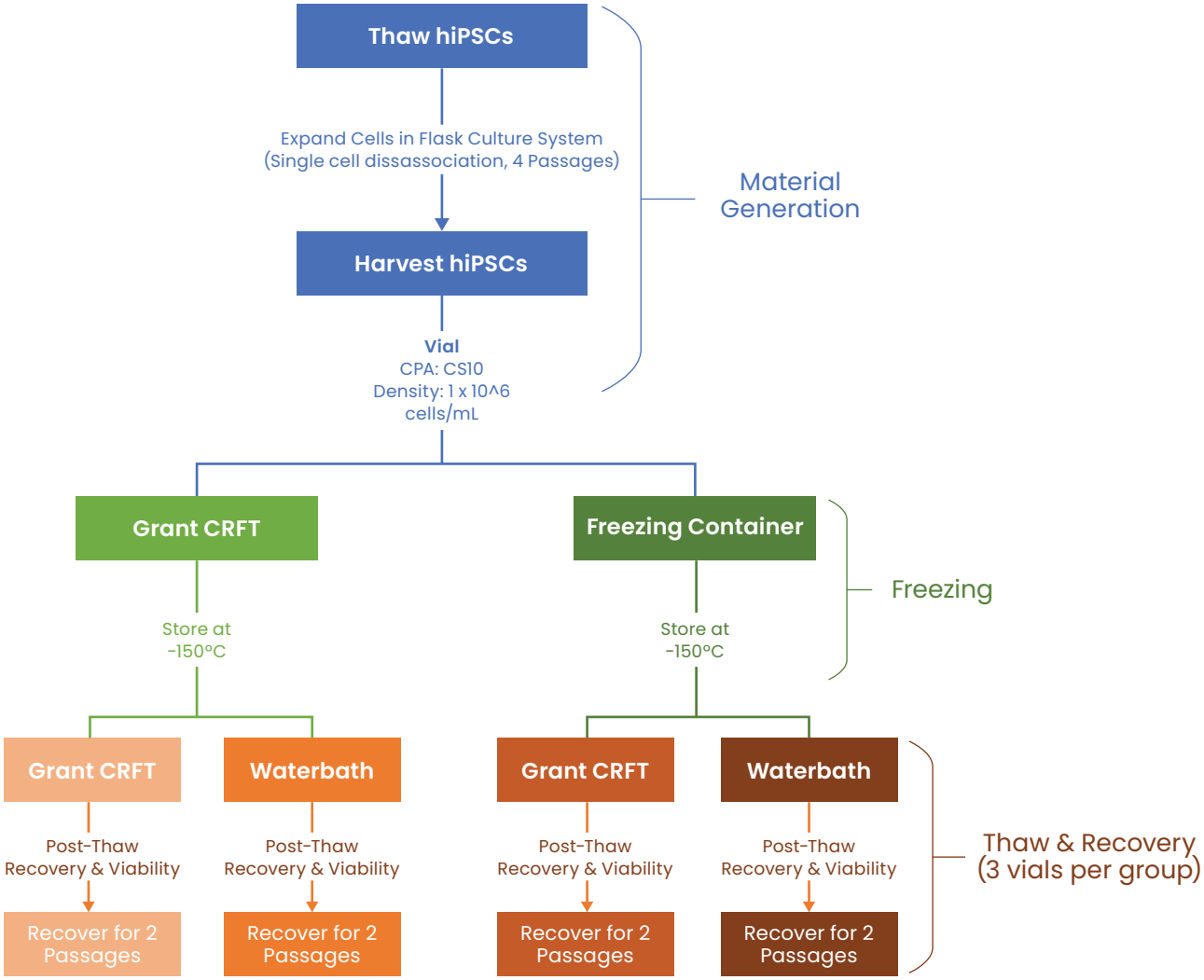


Figure 1. Experimental workflow for the evaluation of the Grant CRFT for the freezing and thawing of hiPSC.

3. Methods

3.1 MATERIAL GENERATION & FREEZING

Starting material was expanded from a single vial (1×106 cells) of hiPSC (CGT Catapult's proprietary RCiB10 line at passage 35 (P35)) in T-flasks. Cells were maintained in Complete Essential 8 (E8) medium on a vitronectin (VTN) matrix in accordance with CGTC standard operating procedure (SOP) (**EDN-SOP-012**). Complete E8 medium was changed daily until vessels reached their required confluency for passage or harvest at which point cells were dissociated with Accutase and treated with 10 mM Rocki.

Cells were harvested (P38) and formulated in Cryostor CS10 (containing 10% v/v dimethyl sulfoxide as the cryopreservation agent (CPA) at 1×106 viable cells/mL. Subsequently, 1.8mL cryovials were filled with 1 mL of cell formulation per vial. Vials were transferred to their designated freezing device (CoolCell or Grant CRFT) in a Cool Rack (Corning) on a cold pack (approximately 4°C). The freezing profiles were executed without incident or deviation, and the vials were transported on dry ice to the -150°C freezer for storage. The formulation, freezing and storage process flow is summarised in **Figure 2**.

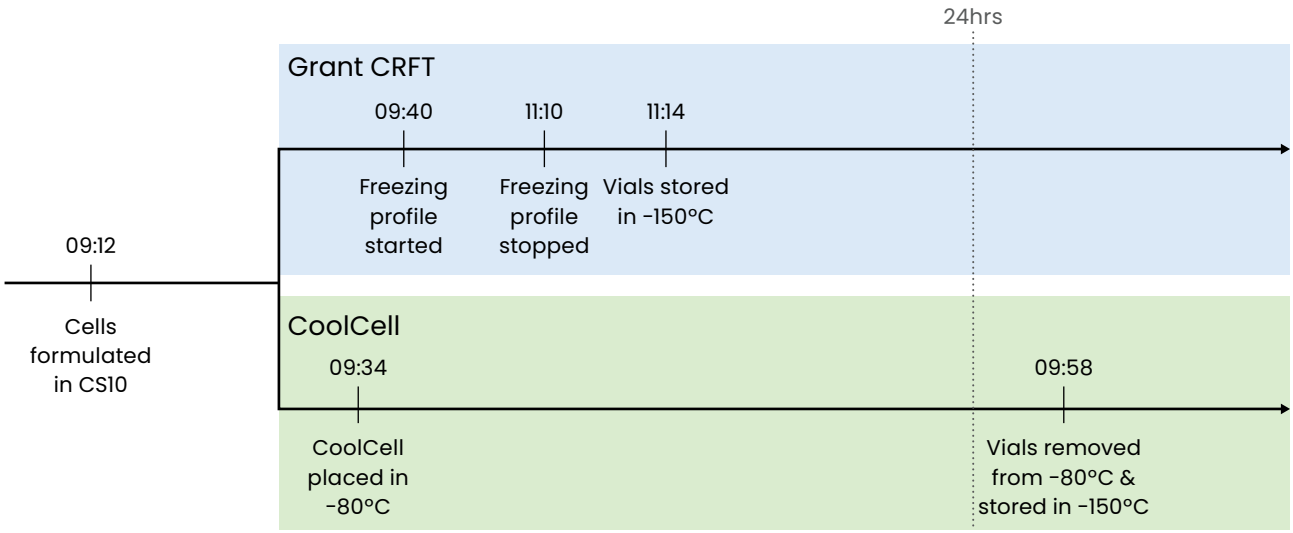


Figure 2. Cell formulation, freezing and storage process flow. Note, the CoolCell vials were transferred to -150°C approximately 24hrs after being placed in -80°C in accordance with manufacturer's instructions.

Table 3. Grant CRFT Freezing Profile

Step	Parameter
Starting Temperature	4°C
Step 01	-2°C/minute to -12.0°C
Step 02	Hold 10 minutes 0 seconds
Step 03	-1°C/minute to -80.0°C

In addition to the cell containing vials, 6 dummy vials containing 1mL Cryostor CS10 only and a thermocouple were placed in the Grant CRFT (**Figure 3**). The temperature of the dummy vials was sampled and logged every second for the duration of the run.

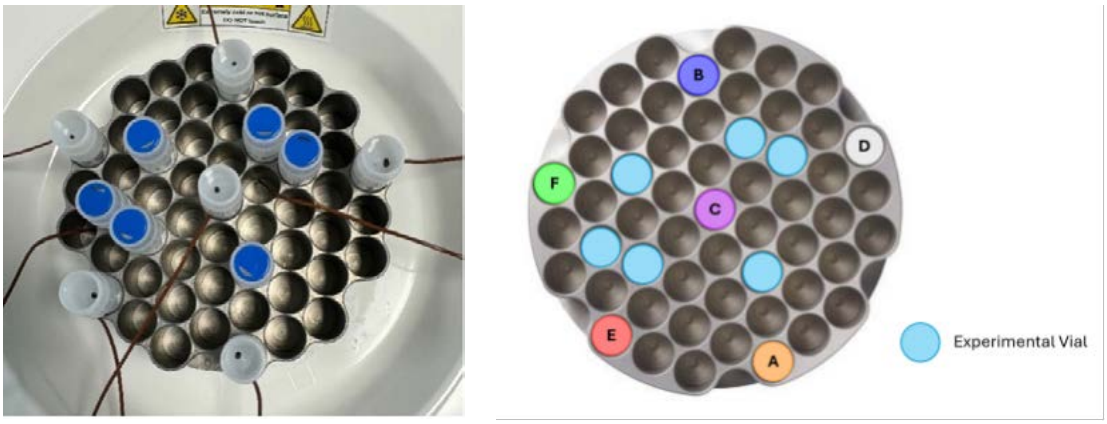


Figure 3. Schematic and images of sample location within CRFT head. Vials containing cells are indicated in blue, and dummy vials are indicated with the associated data logging channel colour code and channel name noted in bold.

3.2 POST-THAW ASSESSMENT

After 2 days in -150°C storage, 2 control vials and 2 experimental vials were retrieved from -150°C storage and transported on dry ice to the lab to be thawed and recovered. One vial from each freezing treatment group (control and CRFT) were thawed using the Grant CRFT and one using a water bath resulting in 4 treatment groups (**Table 2**). Immediately after thawing, the CPA-containing cell suspension was diluted with complete E8 medium supplemented with 10 µM ROCKi, washed, sampled for post-thaw recovery cell counts, and seeded on VTN. Thawing and recovery was performed on 3 consecutive days, with one replicate from each group thawed each day totalling 12 samples across 4 groups.

Table 4. Grant CRFT Thawing Profile

Step	Parameter
Starting Temperature	-80°C
Step 01	+5°C/minute to 30.0°C

SAMPLE TEMPERATURE

To assess sample temperature, data logging was performed during thawing. Dummy vials containing 1mL CS10 only and a thermocouple were frozen on dry ice. A single dummy vial was thawed in parallel with cell containing vials (**Figure 4**) in accordance with the protocol in **Table 4**. To assess sample temperature during a water bath thaw, a dummy vial was swirled in the water bath, alongside an experimental vial and removed when ice pellets in the experimental vial were the appropriate size. The temperature of the dummy vials was sampled and logged every second for the duration of thawing. Data logging was performed separate 3 times for each thaw protocol.

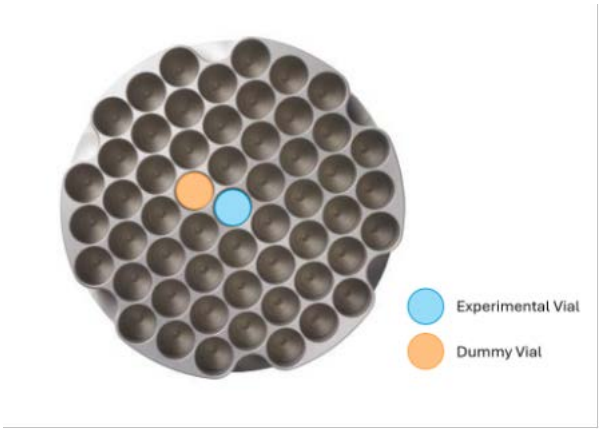


Figure 4. Location of sample vials and dummy vials for thawing protocol.

PERCENT VIABILITY AT THAW

Percent viability is the percentage of viable cells (cells with a competent membrane) in a sample (ChemoMetec A/S, 2022; Kepp *et al*., 2011). This value is determined by the automated cell counter (NC-202) and is calculated as per **Equation 1** where Ct equals the total concentration (cells/mL) of acridine orange (AO) positive cells and Cnv equals the concentration (cells/mL) of 4′,6-diamidino-2-phenylindole (DAPI) positive (non-viable) cells (ChemoMetec A/S, 2022).

Equation 1. Percent Viability

$$\%\ Viability = \frac{C_t - C_{nv}}{C_t} * 100$$

TOTAL RECOVERY

Total recovery is the proportion of the total number of viable cells post-thaw compared to total number of viable cells initially frozen (Murray & Gibson, 2020). The total recovery is calculated as per **Equation 2** where Cv equals the total number of viable cells and Cpf is the total number of viable cells in the vial prior to freezing.

Equation 2. Total Recovery

$$\%\ Recovery = \frac{C_v}{C_{pf}} * 100$$

MORPHOLOGY & PROLIFERATION

Cells were seeded post-thaw (P38) and cultured as per internal SOPs for 2 passages to assess for any gross morphological changes or deviation from previously established hiPSC RCiB10 growth patterns before harvesting, counting with the NC-202 to determine yield, and discarding (P40).

3.3 EQUIPMENT

Table 5. Equipment

Equipment	Manufacturer	Model
Aspirator	Integra	VacuSafe
Automated Cell Counter	Chemometec	Nucleocounter NC-202
Centrifuge	Sigma	4-16KS
Passive Cooling Container	Corning	CoolCell LX
CoolRack	Corning	CoolRack XT CFT24
Data Logger	Grant Instruments	Squirrel SQ16PLUS
Freezer (-20±5°C)	LabCold	RLVF0417
Freezer (-80±5°C)	PHCbi	TwinGuard MDF-DU302VX-PE
Incubator	NuAire	NU-5820E
Microbiological Safety Cabinet	Contained Air Solutions	BioMAT 2 -S2
Microscope	ThermoFisher Scientific	EVOS M500
P10 Micropipette	Gilson	PipetMan P10
P1000 Micropipette	Gilson	PipetMan P1000
P2 Micropipette	Gilson	PipetMan P2
P20 Micropipette	Gilson	PipetMan P20
P200 Micropipette	Gilson	PipetMan P200
Pipette Aid	Integra	Pipetboy 2
Refrigerator (5±3°C)	LabCold	RLPR0517
Thermocouple	Tempcon	Type T Exposed Junction T/C 2 m Flat Pair PFA
1/0.2 mm + Tail		
Water Bath	Nickel Electro	Clifton NE2-4D
Controlled Rate Freeze-Thaw Device	Grant Instruments	CRFT
Controlled Rate Freeze-Thaw Device Head	Grant Instruments	H06

3.4 MATERIALS

Table 6. Materials

Material	Brand	Supplier	Catalogue Number
Essential 8 medium	Gibco	Fisher Scientific	15190617
Vitronectin	Gibco	Fisher Scientific	15134499
Accutase	STEMCELL Technologies	STEMCELL Technologies	07920
Cryostor CS10	STEMCELL Technologies	STEMCELL Technologies	07930
Y-27632 dihydrochloride	Tocris Bioscience	Tocris Bioscience	1254
DPBS (-Ca/-Mg)	Biowest	VWR	L0615-100
6-Well plate	Corning	Fisher Scientific	10578911
T25 Flask	Thermo Scientific	Fisher Scientific	12034917
T75 Flask	Thermo Scientific	Fisher Scientific	10364131
2 mL Aspiration stripette	Falcon	Fisher Scientific	10248470
5 mL Stripette	Corning	Fisher Scientific	10127400
10 mL Stripette	Corning	Fisher Scientific	10677341
25 mL Stripette	Corning	Fisher Scientific	10732742
50 mL Stripette	Corning	Fisher Scientific	10636391
P10 Tips	Gilson	Gilson	F171203
P30 Tips	Gilson	Gilson	F171303
P200 Tips	Gilson	Gilson	F171503
P1000 Tips	Gilson	Gilson	F171703
0.5 mL Tube	VWR	VWR	525-0642
25 mL Centrifuge tube	Eppendorf	Fisher Scientific	16356485
50 mL Centrifuge tube	Corning	Fisher Scientific	10604551
250 mL Sterile bottle	Corning	Fisher Scientific	10738212
5 mL Syringe	BD Plastipak	Camlab	309649
Via2 Cassettes	Chemometec	Chemometec	941-0024
0.2 µm Syringe filter	Cytiva	VWR	514-4131
1.8mL cryovials	Nunc	Thermo Scientific	363401PK

3.5 DEVIATIONS

DEVIATION 1: POOR LIFTING OF CELLS AT REPLICATE 1, P39 PASSAGE

Nature of Deviation: Replicate 1 for each of the 4 experimental groups were passaged on P39, Day 3. Prior to passage, cells were observed under phase contrast microscopy and their confluency assessed. All groups exhibited normal morphology and cell confluency was appropriate for passage (90.2%-95.0%).

Passage was performed as per standard operating procedure, however operator noted poor cellular detachment after Accutase (Lot: 3B1718A) treatment across all experimental groups. All culture vessels had approximately 50% of cells remaining adhered to culture surface. Cell counts were performed as per SOP, and percent aggregates were higher than acceptable (>30%), total cells and viability were within acceptable ranges, but lower than expected. Cell suspension was pipetted up and down 3 times to break up aggregates, and new cell count samples taken. Percent aggregates remained greater than 25%, in repeated cell counts despite intervention. To preserve viability of harvested cells, process proceeded, and cells were seeded based on first 3 cell counts.

A new batch of Accutase (Lot: RNBM6303) was used for passage of replicates 2 and 3, and no passaging issues were observed.

Root Cause: The subsequent investigation of reagents and processing was not able to determine the specific root cause with certainty. It is presumed that the Accutase enzymatic activity was reduced, impacting disassociation. The Accutase was within the open expiry date and no issues with the lot to date have been reported to the supplier.

Notably, the supplier shipping procedures have recently changed and the Accutase arrived from the supplier thawed and was then stored at -20°C. According to the supplier this should not impact product performance.

Impact Assessment: In summary, the poor lifting was observed in all experimental groups and only impacted Replicate 1, P39. High aggregation can impact the accuracy of cell counts and as a result the cell count results are likely not reflective of the culture. After seeding the cells recovered and confluency, morphology, and proliferation appeared normal. The cells lifted without issue at harvest and the yield and viability were in line with Replicates 2 and 3. This deviation was therefore considered low impact.

4. Results & Discussion

4.1 DATA LOGGING

FREEZING

The report generated by the Grant CRFT is appended in **Section 7**. The Grant CRFT performed the protocol inline with the freezing profile (**Table 3**), without deviation. The temperature of the 6 dummy samples as measured by an external data logger is presented in **Figure 5**, with the mean sample temperature presented in **Figure 6**. There were no notable hot spots or cold spots among the sampling locations.

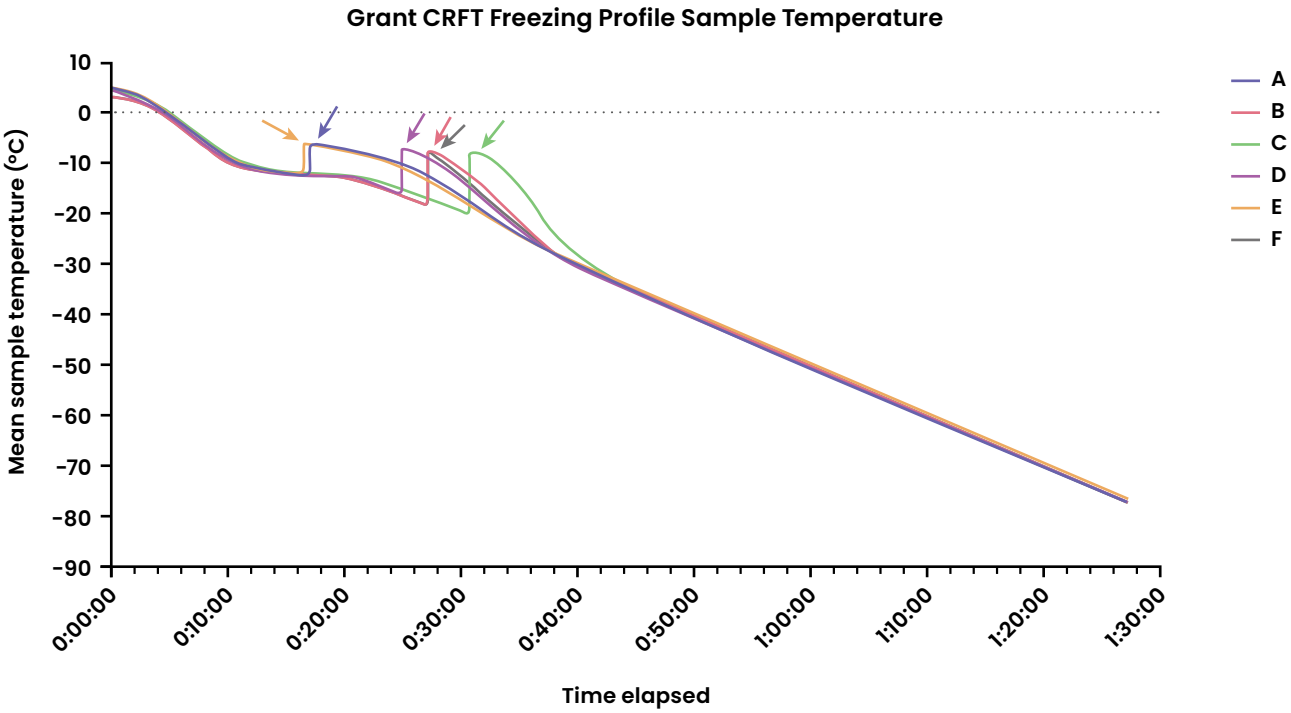


Figure 5. Dummy vials sample temperatures across 6 sampling points (Figure 3). Sample nucleation is indicated by a sudden jump in sample temperature due to latent heat release, sample nucleation points are annotated with an arrow.

Nucleation, represented by a transient spike in sample temperature, was observed in all 6 dummy vials. Nucleation occurred between -11 and -20°C, with approximately 14 minutes between the first and last nucleation.

Nucleation is a stochastic event, and therefore it is normal to see some variation across samples (Tan *et al*., 2021). Excessive supercooling can negatively impact cell viability (Murray & Gibson, 2022), however it is impossible to determine if cell containing vials supercooled and the subsequent impact. Later nucleation events are subsequently associated with a more rapid cooling profile, this is seen most clearly in Vial C. The freezing profile (**Table 3**), has a 10-minute hold step at -12°C to encourage nucleation and sample consistency.

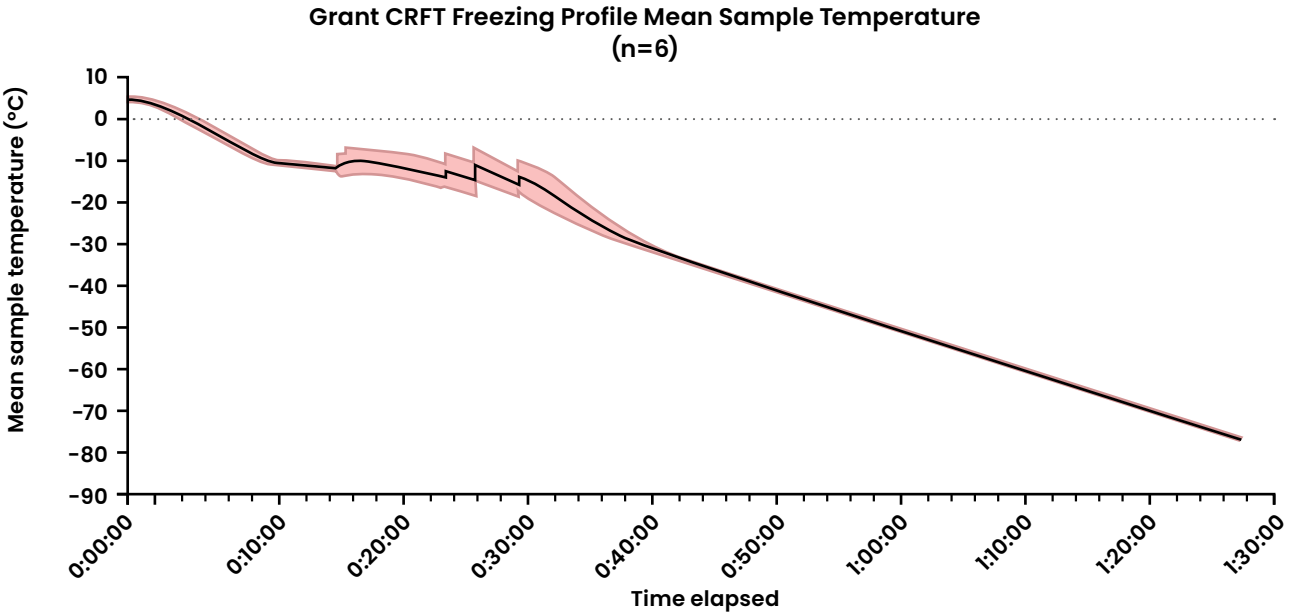


Figure 6. Grant CRFT mean dummy samples temperatures as measured by an independent data logger. Standard deviation is represented in red. The increased variability between -10 and -20°C is a result of latent heat release during nucleation.

THAWING

As demonstrated by Grant CRFT reports (**Section 10**), the Grant CRFT performed the thawing in accordance with the defined protocol (**Table 4**) and without deviation. The mean sample temperature is presented in **Figure 7**. The sample temperature was the temperature consistent between runs (Pearson's r = 0.99, $p < 0.001$). While the defined thawing protocol was linear, +5°C per minute, the measured sample warming rate was non-linear (**Figure 8**). After an initial cooling period, the warming rate appears relatively linear until the sample temperature reached approximately -10°C, at which point the warming rate rapidly increases. The rate of temperature change ranged from -7.8 to 10.0°C/min ($\bar{x}$ = 3.0 ± 2.4°C).

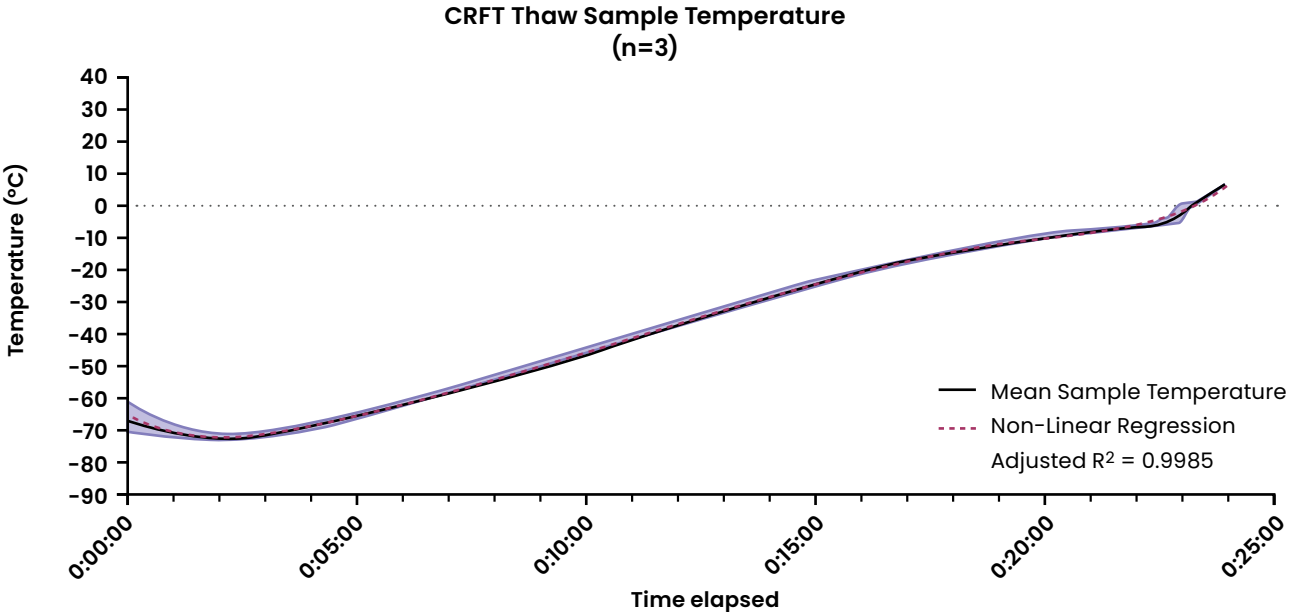


Figure 7. Mean sample temperature during sample thawing across 3 thawing procedures with the standard deviation is represented in blue. The sample temperature was modelled using non-linear regression and a sixth order polynomial equation was generated.

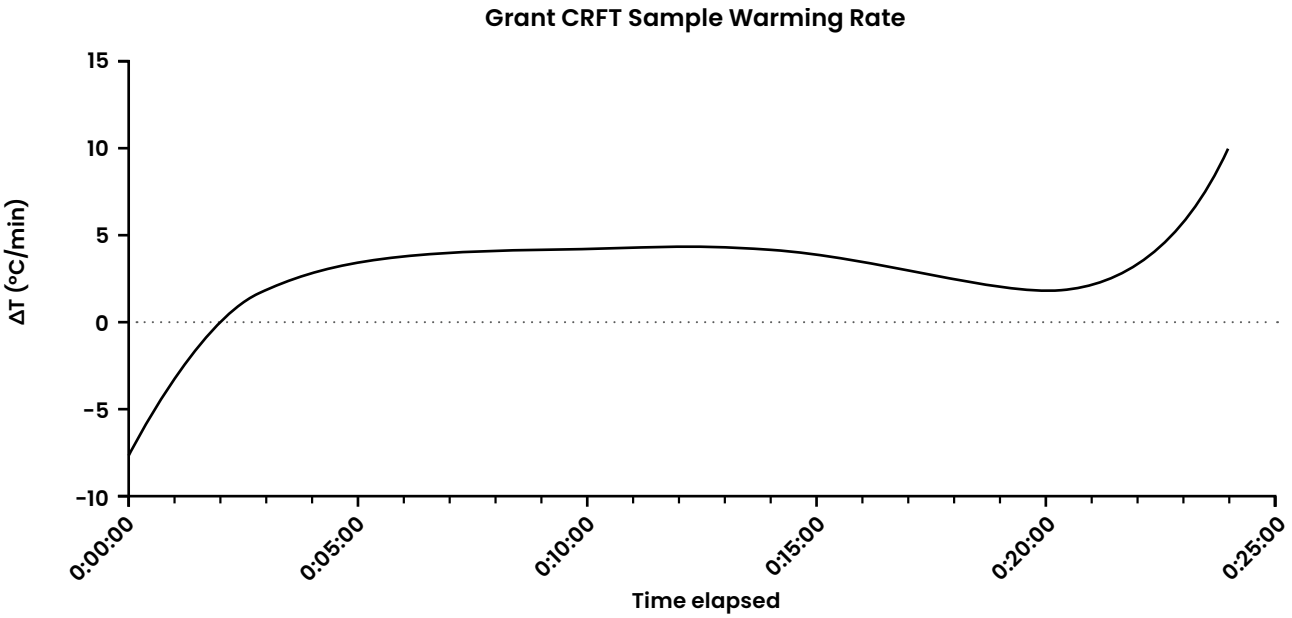


Figure 8. The CRFT sample warming rate, the first derivative of the non-linear regression.

The mean sample and mean water bath temperatures are presented in **Figure 9**. The water bath sample temperature was consistent between runs (Pearson's r = 0.91, p < 0.001), although not as consistent as the CRFT sample temperatures. Unsurprisingly, the rate of thaw was much faster in the water bath thaw (**Figure 10**), than the CRFT. The warming rate ranged from -46.6°C/min to 129.8°C/min ($\bar{x}$ = 31.8 ± 28.78°C/min). The most rapid warming was observed in the first minute of the thawing protocol.

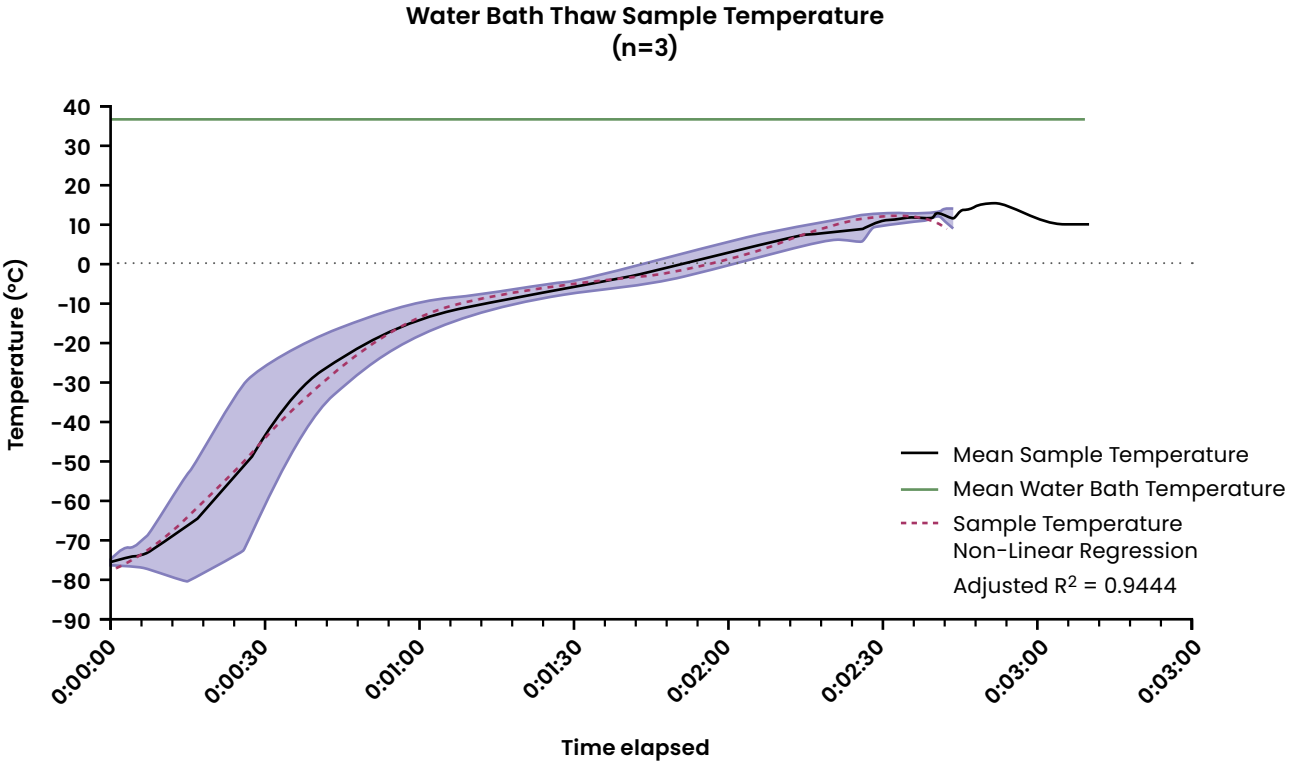


Figure 9. Mean water bath and sample temperature during sample thawing across 3 thawing procedures with the standard deviation is represented in blue. The sample temperature was modelled using non-linear regression and a fifth order polynomial equation was generated.

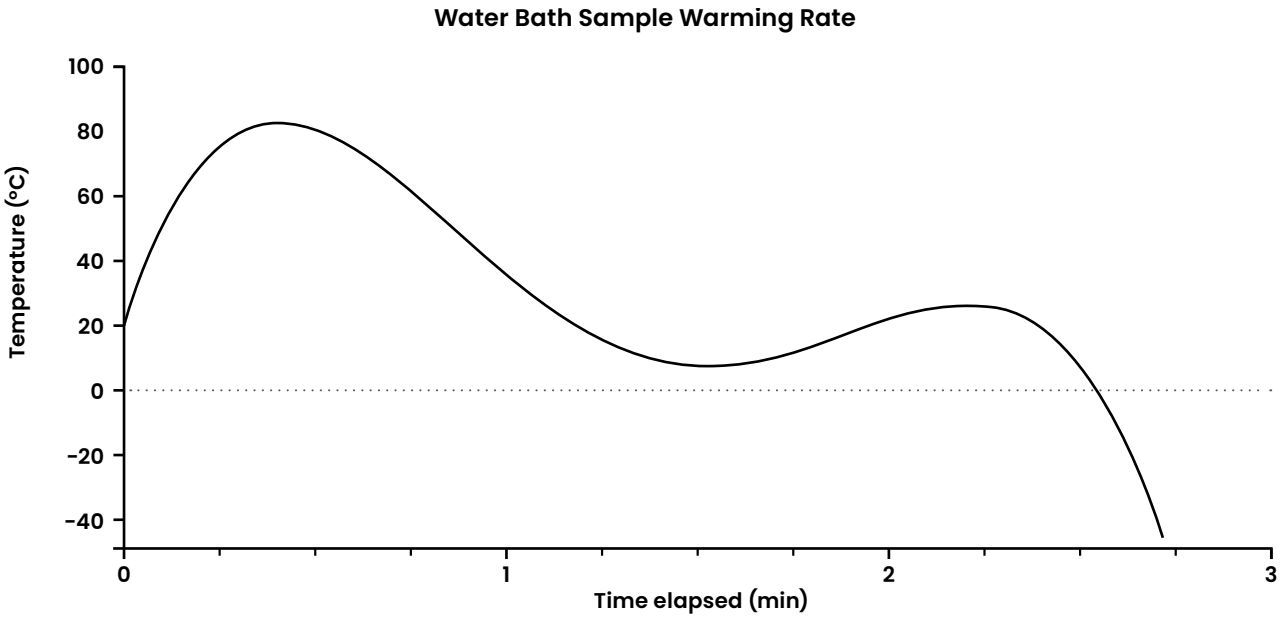


Figure 10. The water bath sample warming rate, the first derivative of the non-linear regression formula.

4.2 POST-THAW RECOVERY

VIABILITY & TOTAL CELL RECOVERY AT THAW

Viability and recovery results are presented in **Figure 11** and **Table 7**.

The percent viability at thaw for all 4 experimental groups met the ≥80% acceptance criteria (≥80%, **Table 7**). Furthermore, there was no statistically significant difference between experimental groups based on ANOVA analysis ($F(3, 7) = 0.1868$, $p = 0.90$).

To assess if cells were lost to necrotic processes, like ice crystal formation, and cell structure was too degraded to detect during cell counts, total cell recovery was calculated (Murray & Gibson, 2020). Like viability, ANOVA analysis demonstrated recovery was comparable across experimental conditions ($F(3, 8) = 1.354$, $p = 0.32$).

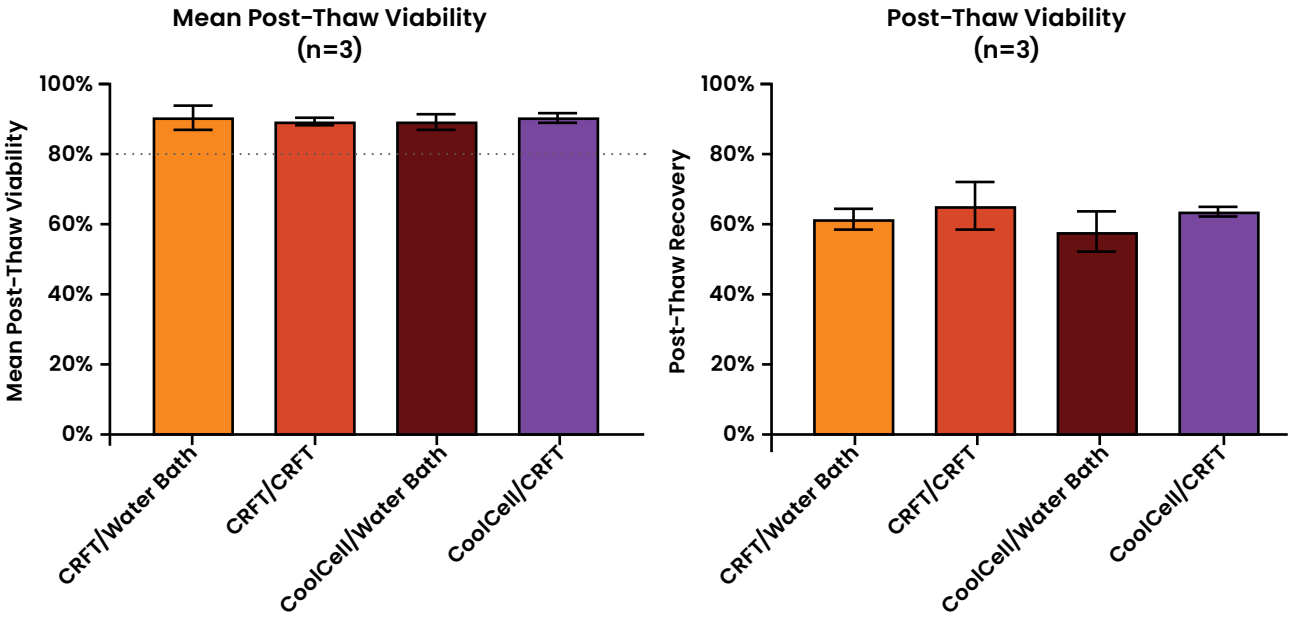
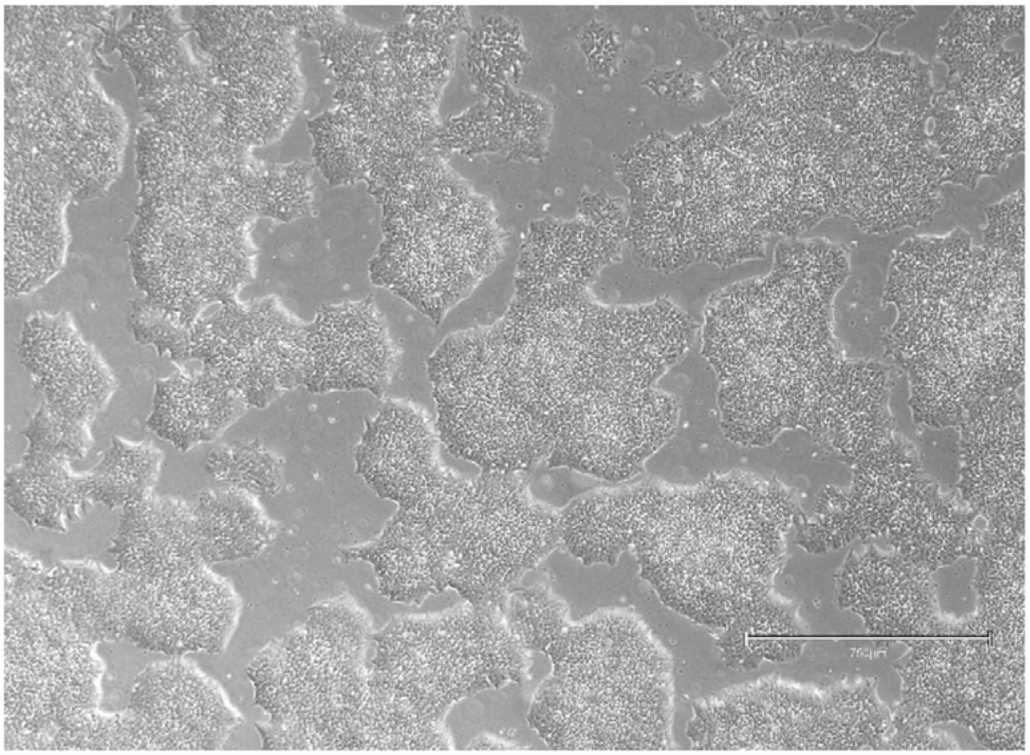


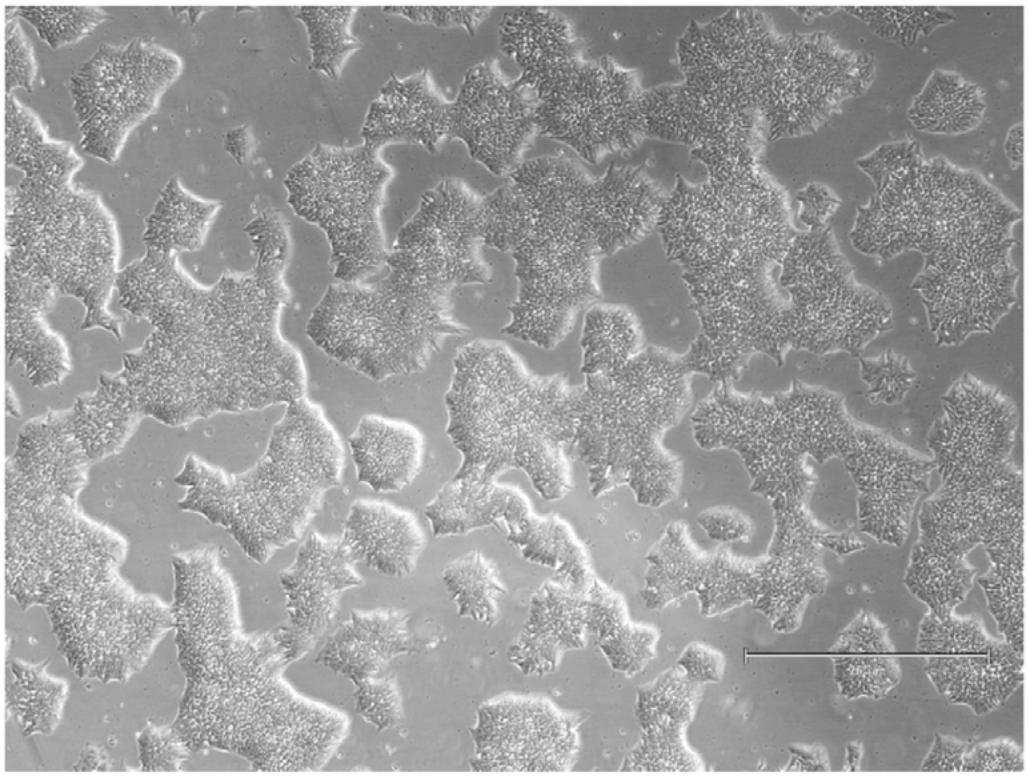
Figure 11. Mean viability and recovery at thaw for each experimental group.

This demonstrates that the tested Grant CRFT freezing and thawing protocols maintain hiPSC viability and recovery akin to established methods.

MORPHOLOGY & PROLIFERATION

All groups were recovered in culture and cells displayed normal hiPSC morphology. The cells were in tightly packed colonies with well-defined borders, a high nucleus to cytoplasm ratio, and prominent nucleoli. This morphology is consistent with hiPSC characteristics described in literature (Rivera *et al*., 2020) and the characteristics of the RCiB10 cell line.





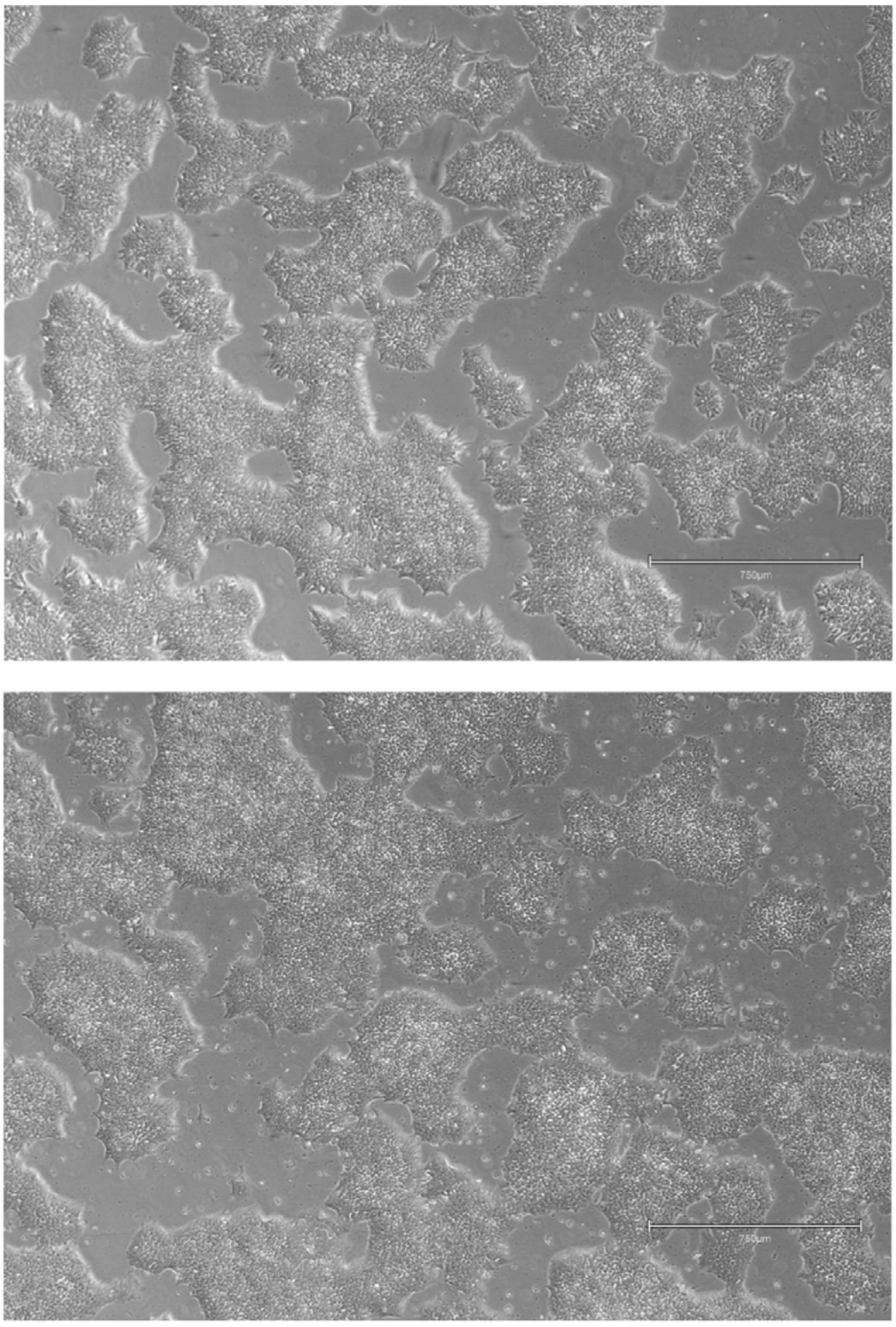


Figure 12. Representative image of cell morphology for each treatment group: (A) CRFT/Water Bath, (B) CRFT/CRFT, (C) CoolCell/Water Bath, (D) CoolCell/Water Bath. All images were taken on P40 D3 viewed under 4x with phase contrast.

After seeding, all groups established the expected proliferation rate, demonstrated by confluency. Additionally, all groups met the Day 1 post-seeding confluency acceptance criteria (> 10%). As seen in **Figure 13**, the confluency was consistent between treatment groups for the duration of culture.

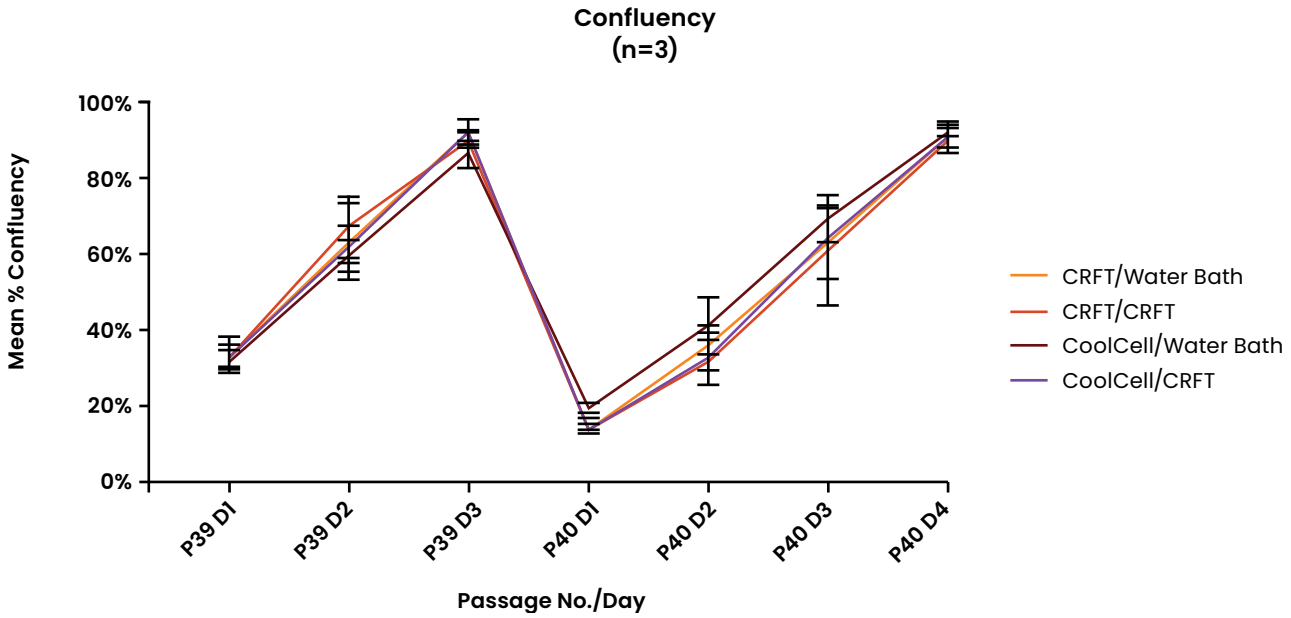


Figure 13. Mean confluency of vessels at media exchange, demonstrating consistent growth rates between the experimental groups.

Culture vessels were seeded at 3×104 cells/cm2 as per standard operating procedure and achieved appropriate confluency by Day 3 for passage, meeting the acceptance criteria (70-95%).

After recovering for 2 passages, cells were harvested and counted to determine the post-recovery yield. While there is no acceptance criteria for yield, based on historical data the yield was expected to be greater than 3.0×105 cells/cm2. All groups met the expected yield value (**Figure 14**) and ANOVA analysis demonstrated no statistically significant differences between groups ($F(3, 8) = 1.169$, $p = 0.38$).

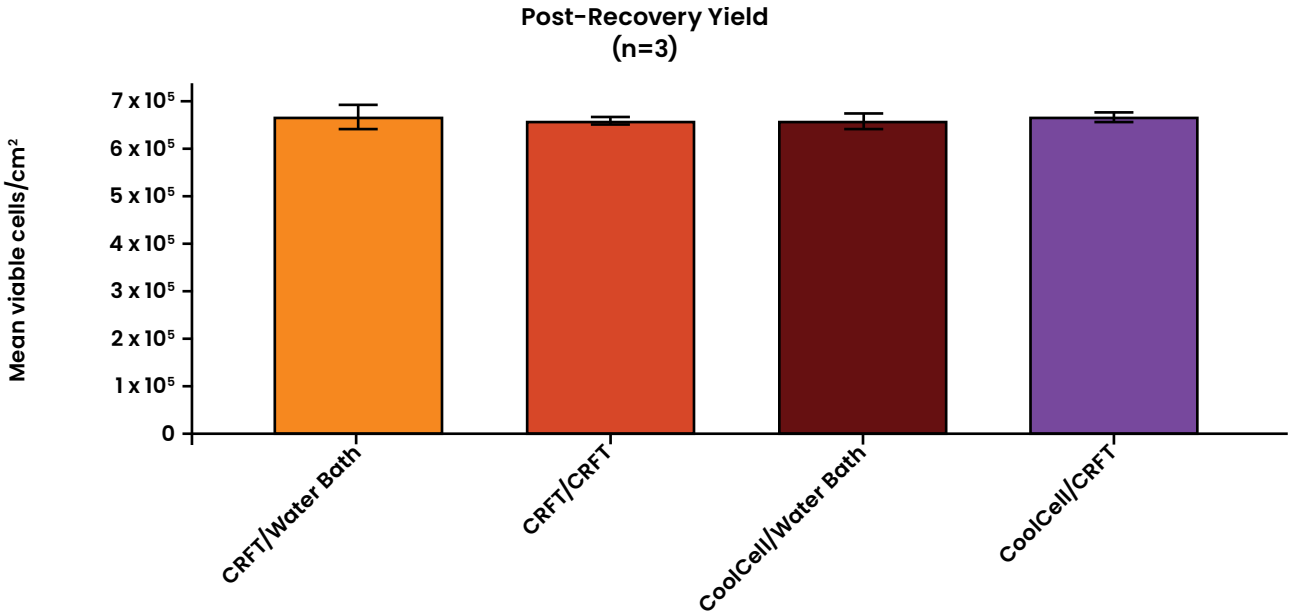


Figure 14. Mean post-recovery yield, cultures were harvested at P40 D4, 2 passages post-thaw, to perform cell counts.

Table 7. Post-Thaw Recovery Acceptance Results & Summary

Process Step	Assay	Acceptance Criteria	CRFT/Water Bath		CRFT/CRFT		CoolCell/Water Bath		CoolCell/CRFT	
			Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD
Vial Thaw	Cell viability at thaw assessed with NC-202	≥80%	90.01%	2.43%	90.51%	2.20%	90.41%	0.26%	89.20%	1.90%
Vial Thaw	Cell recovery at thaw assessed with NC-202	Reported Value	61.51%	2.48%	65.18%	5.51%	57.89%	4.65%	63.71%	1.16%
Cell Attachment Post-Seeding	Cell confluency on Day 1 assessed with CGTC confluency tool	>10%	33.70%	3.76%	33.30%	2.43%	32.43%	3.34%	33.57%	2.39%
2D iPSC expansion	Mean cell confluence at passage across the vessel assessed with the CGTC confluency tool	70%-95%	92.27%	2.75%	90.43%	1.82%	87.40%	3.82%	92.60%	2.27%
2D iPSC expansion	Yield at harvest assessed with NC-202 (Viable cells/ cm^2).	Reported Value	4.96×10^5	4.40×10^4	4.42×10^5	3.49×10^4	4.82×10^5	7.04×10^4	5.42×10^5	5.88×10^4

*n=3

5. Conclusion

The Grant Instruments CRFT successfully performed the programmed freezing protocol, for slow freezing of hiPSCs for cryopreservation. Post-thaw, cell viability and recovery were comparable to the CoolCell control. While this investigation demonstrated that the Grant Instruments CRFT is as effective as the CoolCell XL, it also highlighted opportunities for further optimisation. Notably, only 2 of the vials, A and E, nucleated during the hold step at -12°C, suggesting that there may be an opportunity to further optimise the freezing profile, specifically the hold step, to better control ice nucleation. Given the impact of intracellular and extracellular ice crystal formation on membrane integrity and post-thaw viability (Murray & Gibson, 2022) and the importance of consistency when preserving cellular products (Yu & Hubel, 2019), reducing nucleation point variability may be an area for future study.

Controlled rate thawing is relatively novel, and the prolonged protocol tested in this experiment is a departure from the standard rapid thawing procedures (Baboo *et al*., 2019). Water bath thawing is highly operator dependent and poses a contamination risk to both the GMP environment and the cell product; an automated, dry thawing system could be an opportunity to reduce variability and increase robustness. The Grant Instruments CRFT controlled thaw profile successfully thawed cryopreserved hiPSCs with results that are comparable to the water bath control. The process required no input from the operator to determine thaw end point, and the sample temperature was more consistent across runs than the water bath. Further investigation of the impact of controlled rate thawing on hiPSC identity and metabolism would enable optimisation of the thawing protocol and, potentially, the development of a standard thawing profile.

The Grant Instruments CRFT was used to successfully freeze and thaw vials containing hiPSCs. The post-thaw recovery was comparable to the controls, CoolCell and water bath, and required less operator intervention. One of the key advantages of the CRFT is increased control over the freezing and thawing protocol, which allows for more in-depth investigation and optimisation of the freezing process than passive devices.

6. References

Baboo, J., Kilbride, P., Delahaye, M., Milne, S., Fonseca, F., Blanco, M., Meneghel, J., Nancekievill, A., Gaddum, N., & Morris, G. J. (2019). The Impact of Varying Cooling and Thawing Rates on the Quality of Cryopreserved Human Peripheral Blood T Cells. *Scientific Reports*, 9(1). https://doi.org/10.1038/s41598-019-39957-x

Bojic, S., Murray, A., Bentley, B. L., Spindler, R., Pawlik, P., Cordeiro, J. L., Bauer, R., & de Magalhães, J. P. (2021). Winter is coming: the future of cryopreservation. In *BMC Biology* (Vol. 19, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12915-021-00976-8

ChemoMetec A/S. (2022). *Application Note No.2026 v. 1.7*. www.chemometec.com

Corning Incorporated. (2019). *Corning ® CoolCell ® Containers*.

Grant Instruments. (n.d.). *Controlled Rate Freeze Thaw unit CRFT Operating Manual*.

Grout, B. W. W., & Morris, G. J. (2009). Contaminated liquid nitrogen vapour as a risk factor in pathogen transfer. *Theriogenology*, 71(7), 1079–1082. https://doi.org/10.1016/j.theriogenology.2008.12.011

Gurina, T. M., Pakhomov, A. V., Polyakova, A. L., Legach, E. I., & Bozhok, G. A. (2016). The development of the cell cryopreservation protocol with controlled rate thawing. *Cell and Tissue Banking, 17*(2), 303–316. https://doi.org/10.1007/s10561-015-9533-6

Kepp, O., Galluzzi, L., Lipinski, M., Yuan, J., & Kroemer, G. (2011). Cell death assays for drug discovery. In *Nature Reviews Drug Discovery* (Vol. 10, Issue 3, pp. 221–237). https://doi.org/10.1038/nrd3373

Meneghel, J., Kilbride, P., & Morris, G. J. (2020). Cryopreservation as a Key Element in the Successful Delivery of Cell-Based Therapies—A Review. *Frontiers in Medicine, 7*. https://doi.org/10.3389/fmed.2020.592242

Murray, K. A., & Gibson, M. I. (2020). Post-Thaw Culture and Measurement of Total Cell Recovery Is Crucial in the Evaluation of New Macromolecular Cryoprotectants. *Biomacromolecules*, 21(7), 2864–2873. https://doi.org/10.1021/acs.biomac.0c00591

Murray, K. A., & Gibson, M. I. (2022). Chemical approaches to cryopreservation. In *Nature Reviews Chemistry* (Vol. 6, Issue 8, pp. 579–593). Nature Research. https://doi.org/10.1038/s41570-022-00407-4

Rivera, T., Zhao, Y., Ni, Y., & Wang, J. (2020). Human-Induced Pluripotent Stem Cell Culture Methods Under cGMP Conditions. *Current Protocols in Stem Cell Biology*, 54(1). https://doi.org/10.1002/cpsc.117

Tan, M., Mei, J., & Xie, J. (2021). The formation and control of ice crystal and its impact on the quality of frozen aquatic products: A review. In *Crystals* (Vol. 11, Issue 1, pp. 1–17). MDPI AG. https://doi.org/10.3390/cryst11010068

Yu, G., & Hubel, A. (2019). The Role of Preservation in the Variability of Regenerative Medicine Products. *Regenerative Engineering and Translational Medicine*, 5(4), 323–331. https://doi.org/10.1007/s40883-019-00110-9

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