A Controlled Framework for Modeling Temperature Excursions in Cryopreserved Cells

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Background and Introduction

Cryopreserved cell products rely on tightly controlled and consistent temperature storage and transport to maintain viability and functional integrity. However, during shipping, handling, or transfer between storage systems, samples can experience transient warming events or temperature excursions that may compromise quality. Despite their importance, these excursions are typically stochastic, poorly characterized, and difficult to reproduce, limiting our ability to systematically compare biological sensitivity or evaluate mitigation strategies.

To address this gap, we developed a controlled, programmable framework to model temperature excursions using a defined temperature–duration matrix, enabling reproducible assessment of excursion severity across multiple cell types.

Materials and Methods

Cell lines: A549 (ATCC® CCL-185™), THP-1 (ATCC® TIB-202™), U937 (ATCC® CRL-1593.2™), HepG2 (ATCC® HB-8065™), and SH-SY5Y (ATCC® CRL-2266™) cells were cultured according to ATCC-recommended protocols, expanded, and harvested at ≥2×10⁶ cells per vial.

Explore ATCC Cell Lines

Freezing & excursion protocol: Samples were cryopreserved using a CryoMed controlled-rate freezer (Thermo Fisher Scientific). Temperature excursion profiles were applied using a CytoSAVER FZ-2100 (STREX) with defined temperature holds.

Viability assessment: Cell count and viability were measured using a Vi-CELL BLU analyzer (Beckman Coulter) during growth, immediately pre-freeze, and post-thaw.

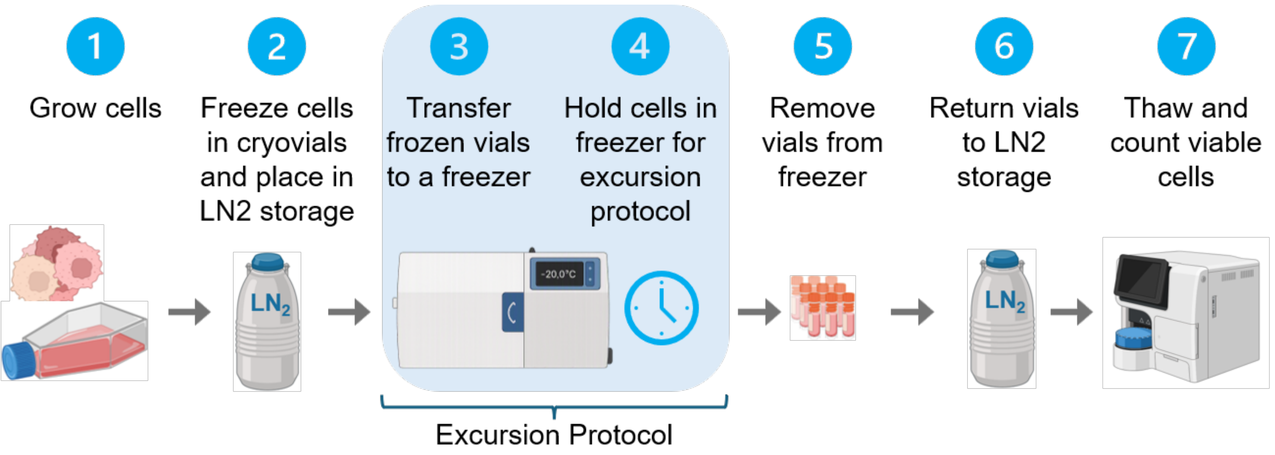


Figure1: Workflow for cryopreservation and temperature excursion testing. Created with BioRender.com.

Results

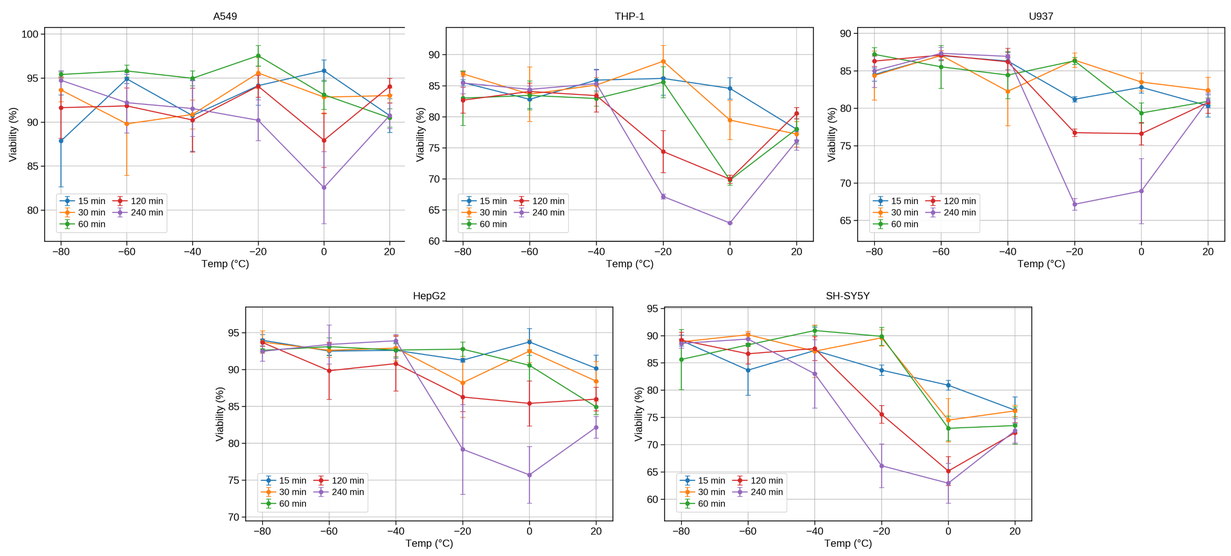


Figure 2: Excursion temperature defines a vulnerable warming range. Mean viability was measured across excursion temperatures from −80°C to 20°C at five exposure durations. Points represent condition means with ± SEM. Across cell lines, viability remained relatively stable from −80°C through approximately −40°C, consistent with the expectation that maintaining samples near dry-ice shipping temperatures limits warming-associated damage. Larger losses emerged at −20°C, 0°C, and 20°C, with THP-1 and SH-SY5Y showing the greatest sensitivity and A549 the greatest stability. In several cell lines, viability partially recovered at 20°C relative to 0°C. Because 20°C was the only condition expected to produce complete thawing, this pattern suggests that prolonged residence within a partially frozen or melting state may be more damaging than complete thawing itself.

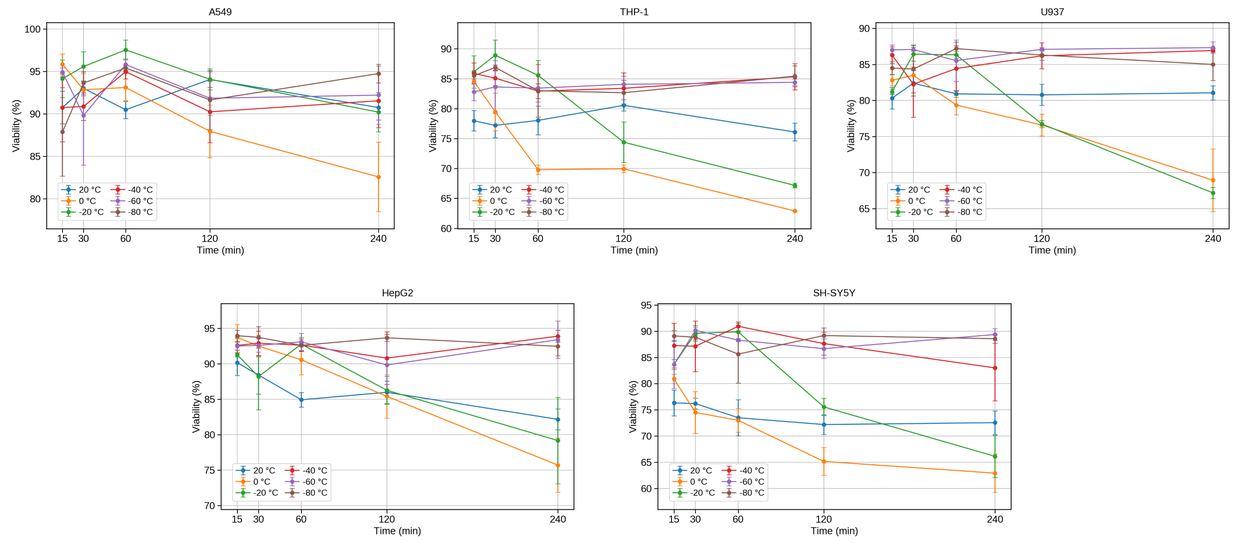


Figure 3. Excursion duration amplifies damage during partial thawing
Mean viability was measured after excursions lasting 15–240 min at each tested temperature. Points represent condition means with ± SEM. A549 remained comparatively stable, with its clearest time-dependent decline occurring at 0 °C. In the other cell lines, the strongest duration-dependent losses occurred at −20 and 0 °C, particularly at 120 and 240 min. By contrast, the reduction at 20 °C was comparatively consistent across time. This distinction is consistent with samples at 20 °C being fully thawed even at the shortest exposure, after which continued injury would more likely reflect cryoprotectant-associated cytotoxicity or osmotic stress. At −20 and 0 °C, progressive loss may additionally reflect partial melting, changing solute concentrations, osmotic volume shifts, and ice recrystallization.

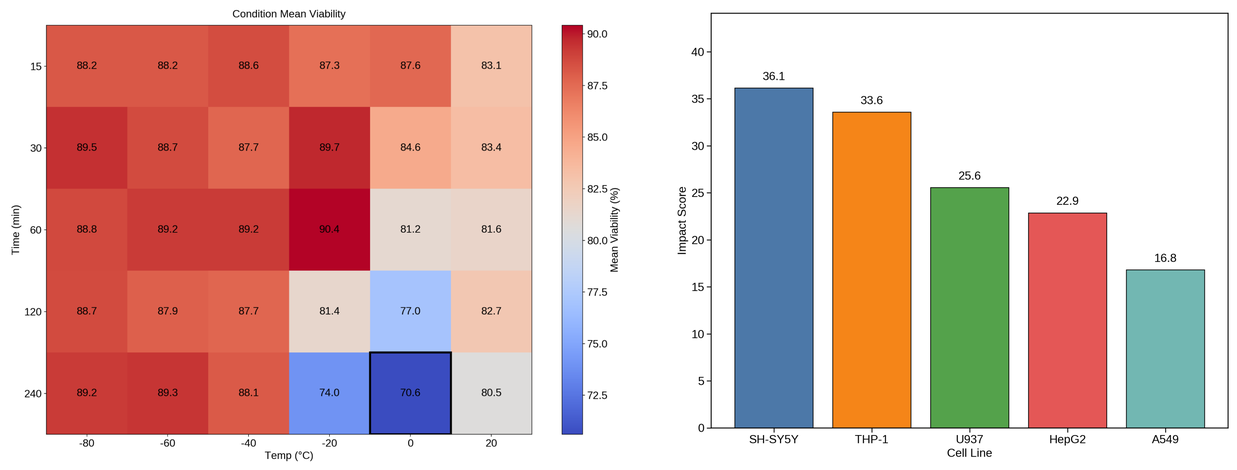
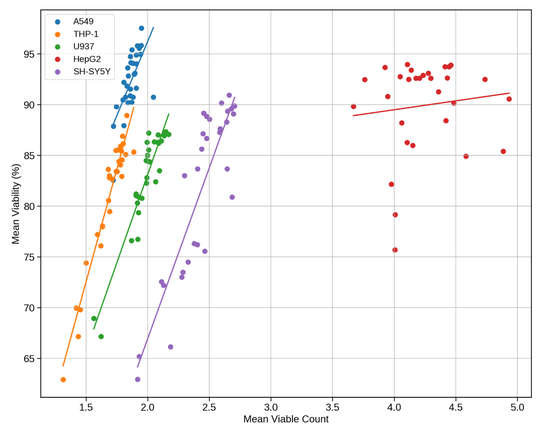


Figure 4: Excursion duration amplifies damage during partial thawing. Mean viability was measured after excursions lasting 15–240 min at each tested temperature. Points represent condition means with ± SEM. A549 remained comparatively stable, with its clearest time-dependent decline occurring at 0°C. In the other cell lines, the strongest duration-dependent losses occurred at −20°C and 0°C, particularly at 120 and 240 min. In contrast, the reduction at 20°C was comparatively consistent across time. This distinction is consistent with samples at 20°C being fully thawed even at the shortest exposure, after which continued injury would more likely reflect cryoprotectant-associated cytotoxicity or osmotic stress. At −20°C and 0°C, progressive loss may additionally reflect partial melting, changing solute concentrations, osmotic volume shifts, and ice recrystallization.



Cell Line	N	Pearson r
A549	30	0.667
THP-1	30	0.965
U937	30	0.932
HepG2	30	0.113
SH-SY5Y	30	0.872

Figure 5: Viability and viable count track differently by culture phenotype. Mean viability was compared with mean viable cell count across all 30 excursion conditions for each cell line. THP-1 and U937 showed very strong correlations, consistent with their dispersed suspension-culture phenotype, where loss of viability is expected to closely track loss of recoverable cells. SH-SY5Y showed a strong but slightly lower relationship, reflecting its mixed adherent and suspension-associated behavior. A549 showed a moderate correlation, likely influenced by detachment and recovery efficiency. HepG2 showed little correlation because it commonly forms dense aggregates; freeze–thaw and handling can disrupt these clumps and increase the number of individually detected particles without representing a true increase in cell number.

Conclusions and Future Directions

- Excursion sensitivity is strongly cell-line dependent, suggesting distinct response profiles that can be leveraged to group similar cell types (including primary and stem cells) and guide model shipping strategies to minimize damage across diverse systems.
- Excursion duration increases damage; however, complete thaw at 20°C did not produce the greatest loss in viability. Instead, the most damaging conditions occur in the −20°C to 0°C range, suggesting that partial thawing introduces additional cellular stress.
- A defined sublethal stress window, particularly one achievable using a standard −20°C freezer transfer, provides a practical and accessible challenge model.
- This framework can be used to test whether alternative cryoprotectants mitigate excursion-associated damage under controlled conditions and improve cold-chain robustness.

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