PLANER

Controlled rate freezing of stem cells: the importance of profiles.

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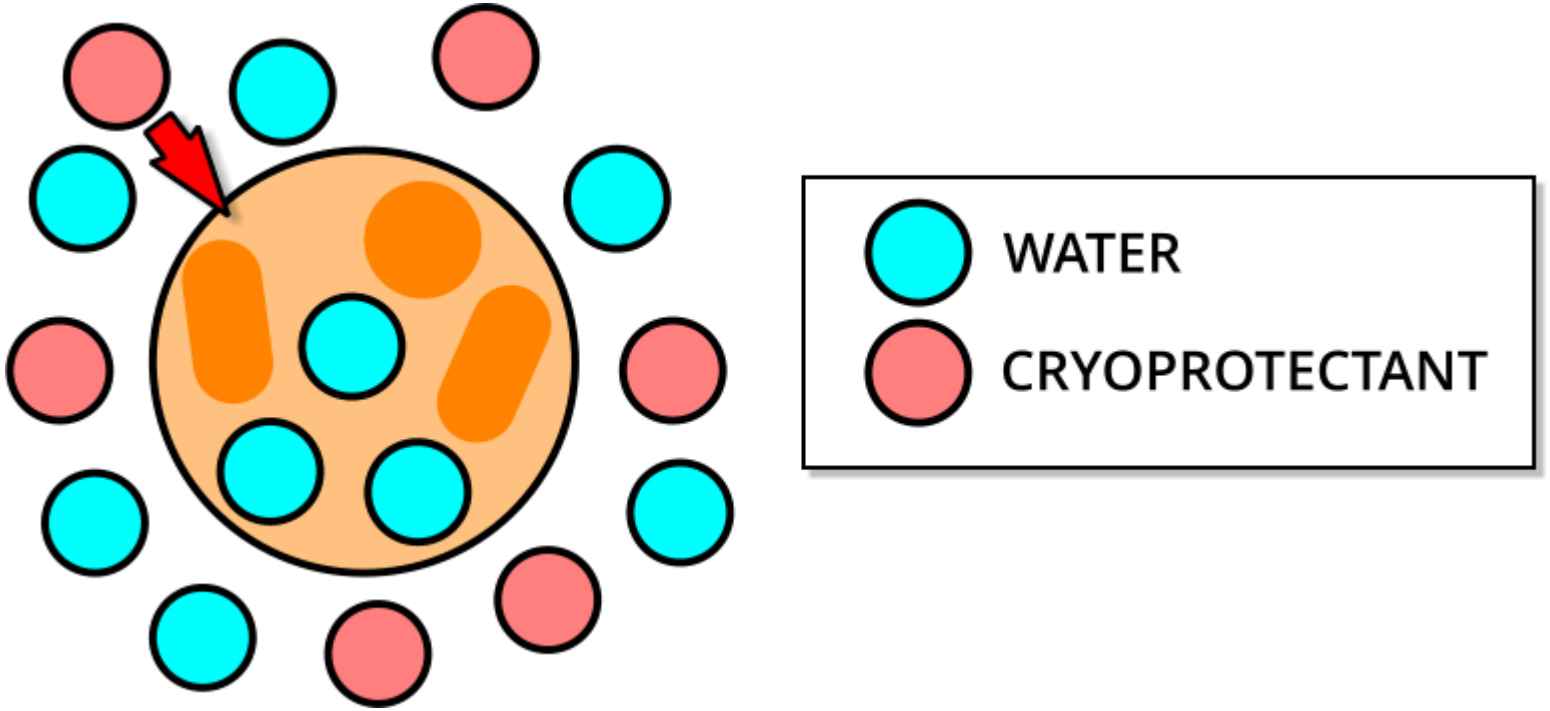
The process

- Cell are preserved at cryogenic temperatures; typically -80 °C or -196 °C.
- Biological activity is slowed or stopped at these ultra-low temperatures.
- The controlled-rate freezer is used to get the cells down to the cryopreservation temperature without damage.

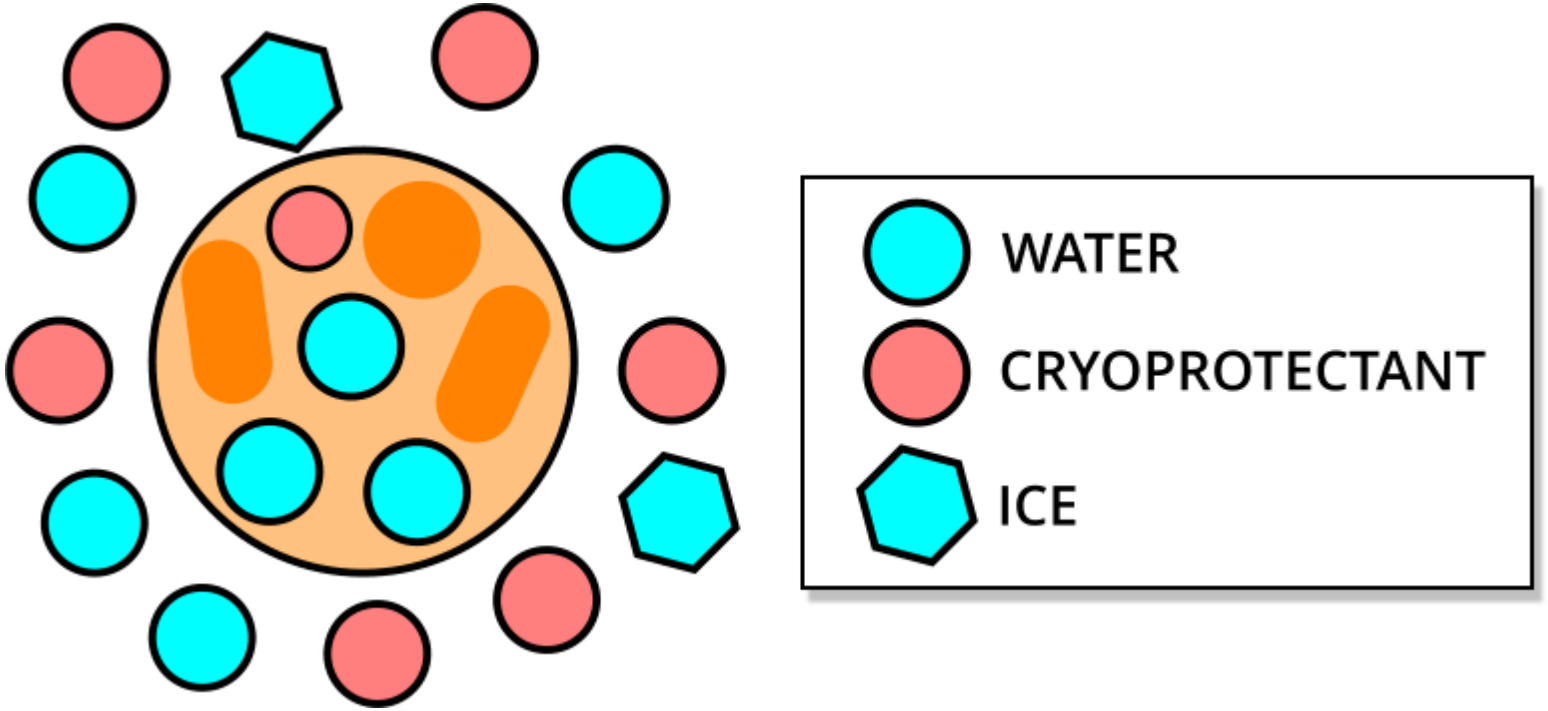
What occurs during preservation?

- At the start, the cell is surrounded by cryoprotectant and water.
- Penetrating cryoprotectants diffuse into the cell.



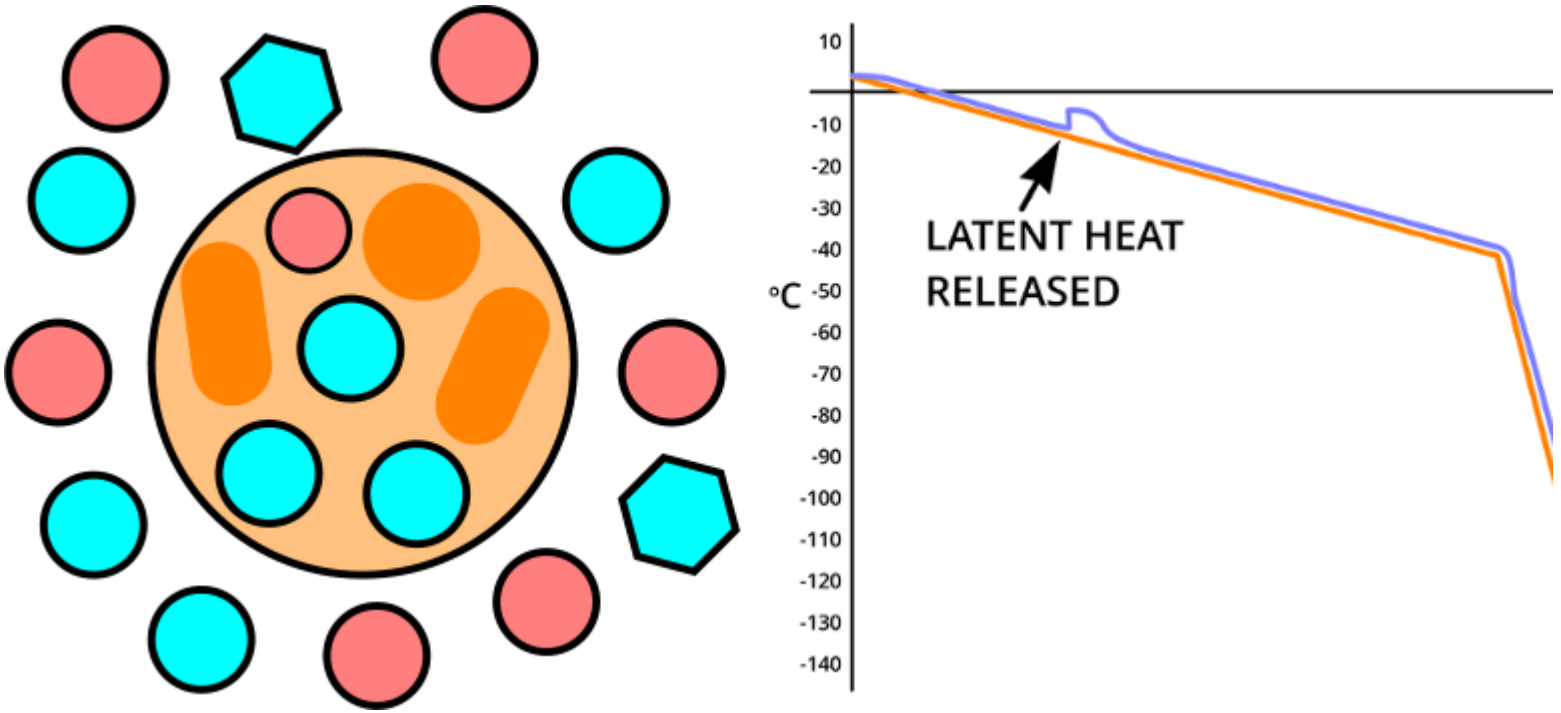
Ice starts to form

- Below the freezing point, extracellular ice forms if nucleated.



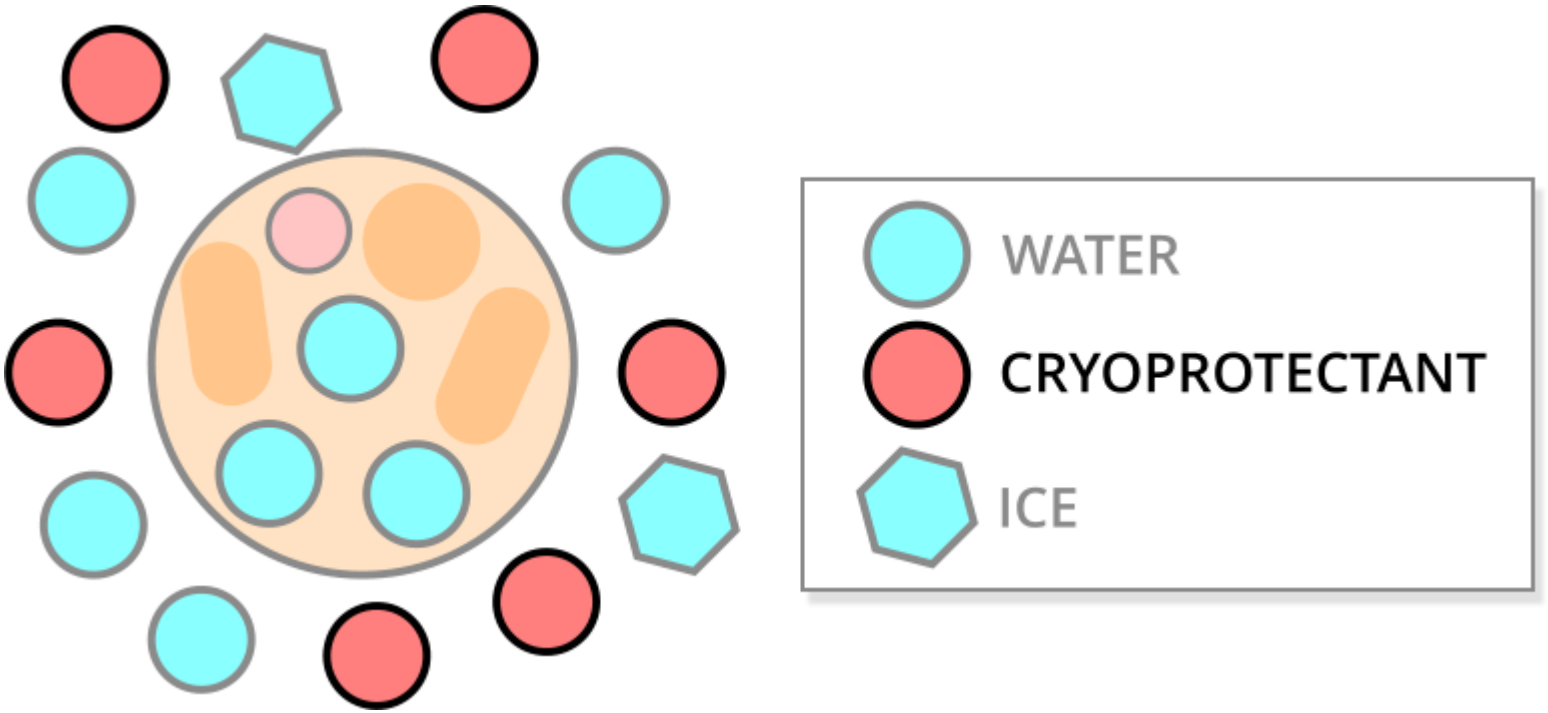
Latent heat is released

- When extracellular ice starts to form, there is a release of latent heat.



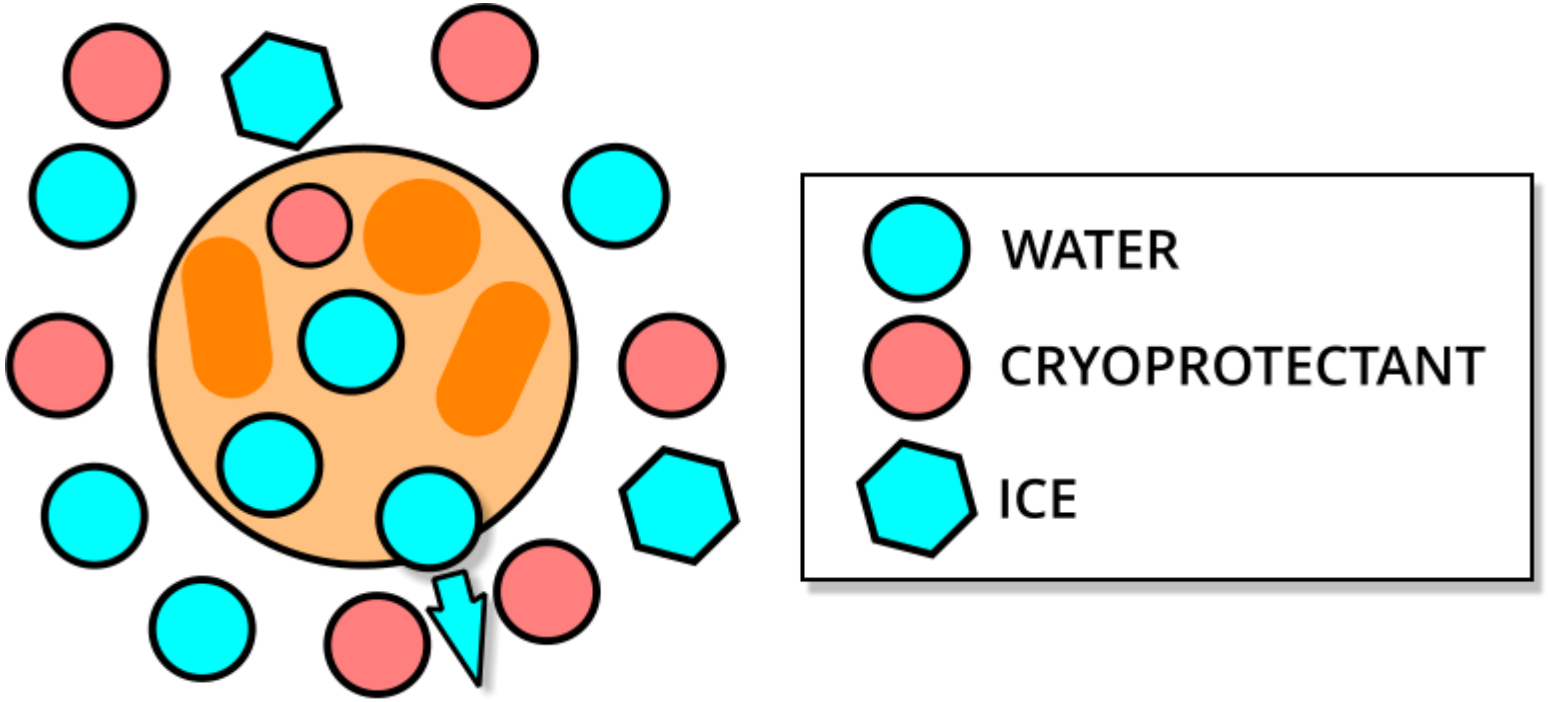
Concentration increases

- The formation of ice causes the solute concentration to increase in the remaining liquid.
- This depresses the freezing point further.



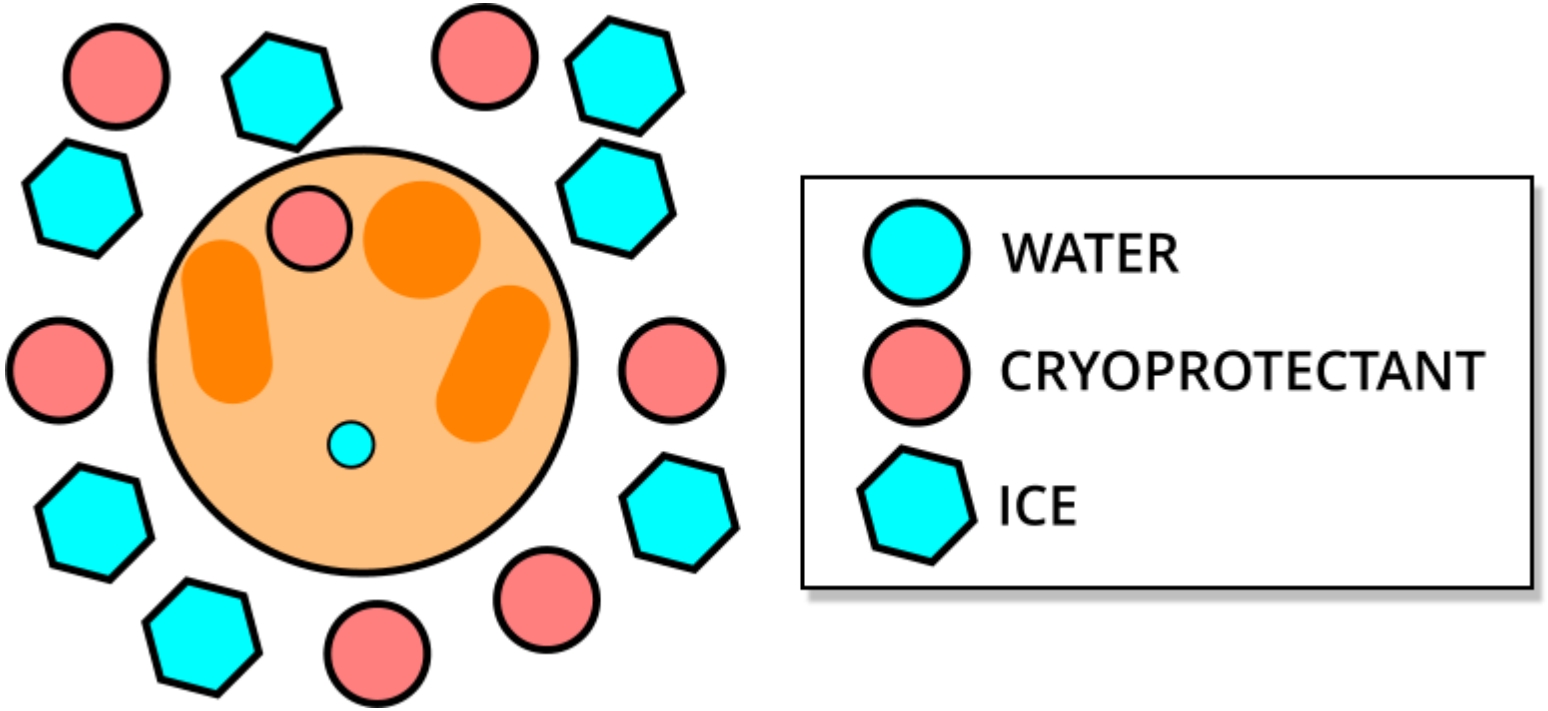
The cell begins to dehydrate

- The increased concentration of solute draws water out of the cell by the increased osmotic pressure.



The cell can be safely cooled

- Finally the cell is sufficiently dehydrated to prevent the formation of lethal intracellular ice.

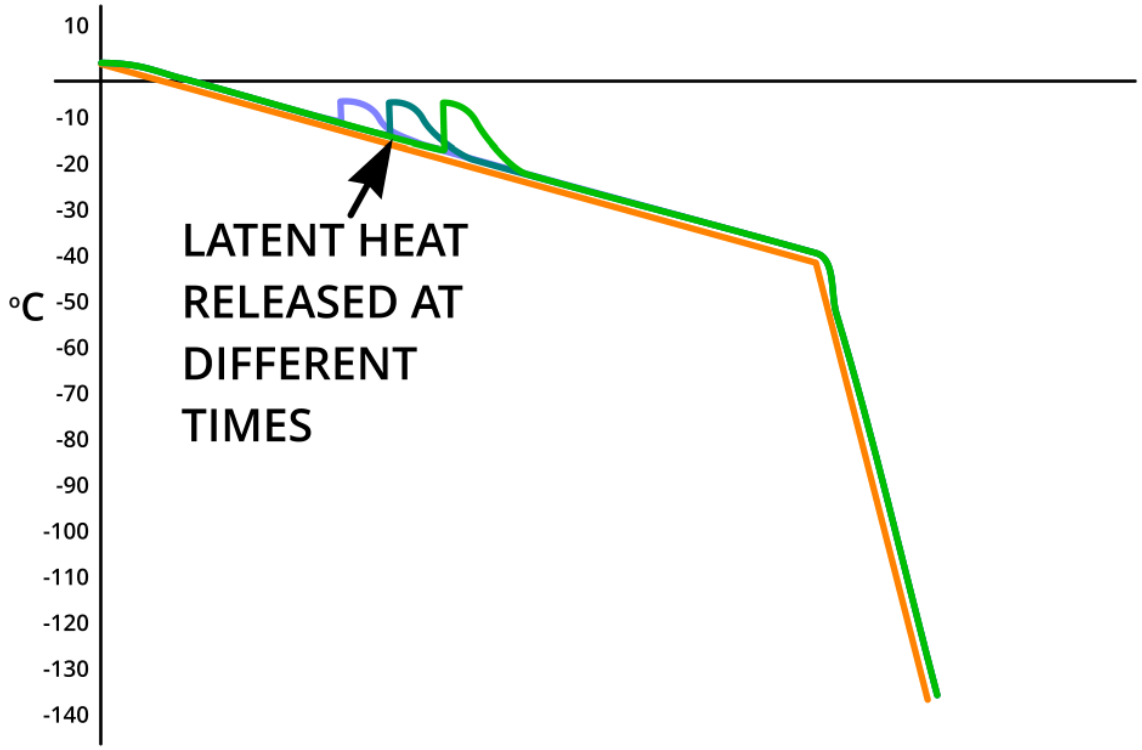


But how fast?

- Cool too slowly:
  - Cells are damaged by long exposure to damaging concentrations of solutes.
- Cool too quickly:
  - Lethal intracellular ice is formed.
- Once sufficiently dehydrated, the cells can be rapidly cooled to the final storage temperature.

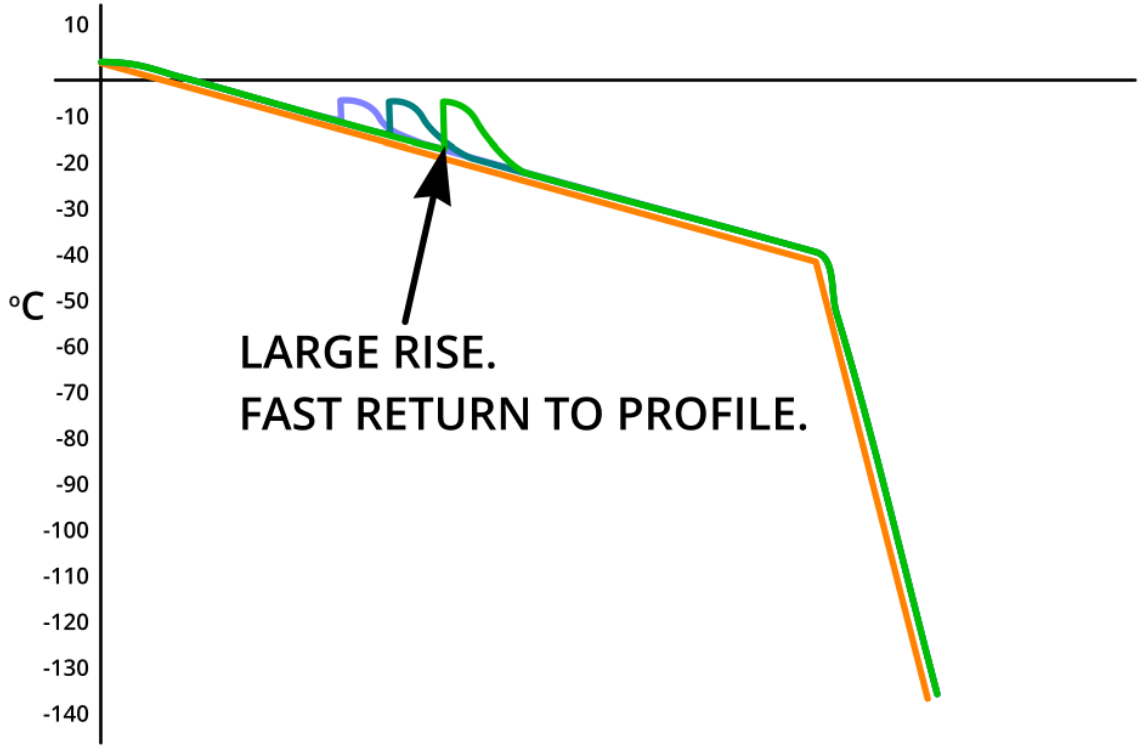
Latent heat release

- The point at which freezing actually starts varies between samples.
- It depends on the number of nucleation sites.



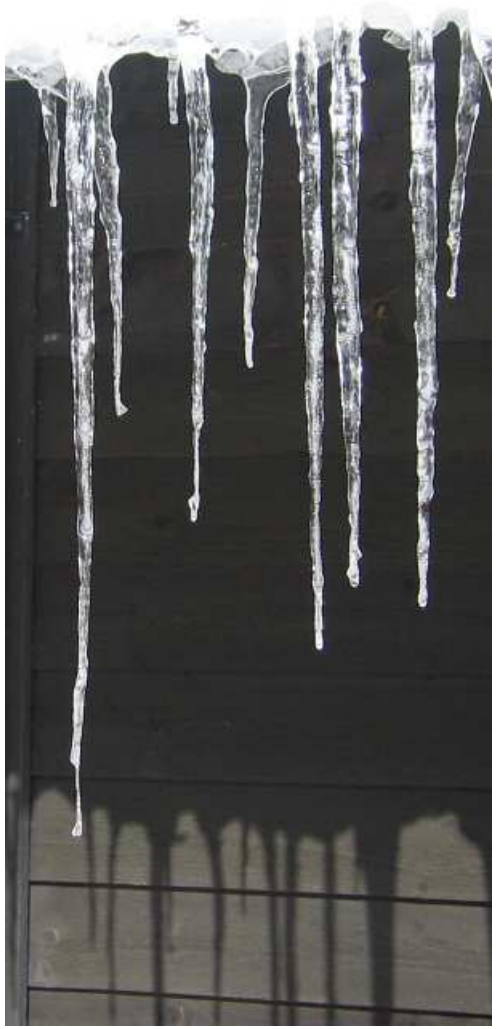
Risk of intracellular ice

- The chance of intracellular ice forming is increased if freezing starts later.



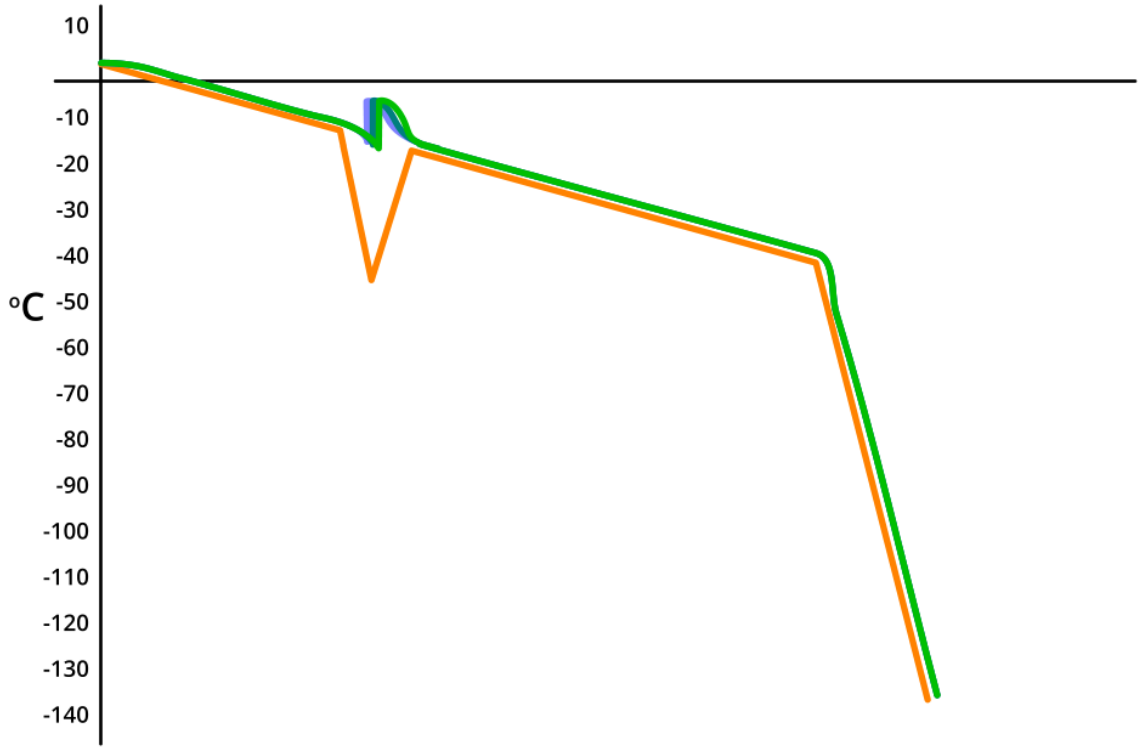
Repeatability

- Inconsistency in the release of latent heat leads to a loss of repeatability.
- Without intervention, the start of ice formation depends on the presence of nucleation sites.
- The further below the freezing point, the greater the probability of ice forming.
- How can we ensure repeatable results?



Seeding dip

- By introducing a dip, ice nucleation can be initiated in a consistent and repeatable manner.



Example run with a seeding dip

- In this example the user has programmed the freezer to move to next step when the sample has reached the end temperature. This is indicated in the trigger column.

Rate °C/min	End temperature °C	Trigger
-2.0	-4	sample
-35.0	-60	chamber
-8.0	-20	chamber
-2.5	-45	chamber
-10.0	-80	sample

Recap

- Cryopreservation in a controlled-rate freezer removes water from the cells.
- A seeding dip can improve repeatability by initiating ice nucleation.
- Further sources of information:
  - Berz D, McCormack E, Winer E, Colvin G, Quesenberry P. Cryopreservation of hematopoietic stem cells. American Journal of Hematology [Internet]. 2007 [31 May 2014];82(6):463-472. Available from: http://dx.doi.org/10.1002/ajh.20707
  - Gao D, Critser J. Mechanisms of Cryoinjury in Living Cells. ILAR Journal [Internet]. 2000 [31 May 2014];41(4):187-196. Available from: http://dx.doi.org/10.1093/ilar.41.4.187
  - Hubel A. Cryopreservation of hematopoietic stem cells: how did we get here and where are we going? [Internet]. 1st ed. 2007 [30 May 2014]. Available from: https://secure.emmes.com/pactweb/system/files/07workshop_12_hubel.pdf

The end.
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