**CASE STUDY** Benefits of Controlled Rate freezing

A laboratory was looking at optimum freezing of fibroblasts, 1 million cells per vial, in mechanical freezers. The cells are typically used in regenerative medicine for growing dermal substitutes in vitro. Their measurement of post thaw viability at different storage times produced unexpected results and the researchers investigated the use of controlled rate freezing with a view to ultimate storage in liquid nitrogen.

A range of simple studies using a Planer 10.16 controlled rate freezing machine was initiated. To simply demonstrate the effect that a pre programmed ramp or protocol might have, three basic freezing runs were made. The demonstration highlighted the behaviour of cells during freezing and the need for using specific protocols.

Figure (A) shows the temperature/time profile of water as it is frozen at a steady rate of -1$^{o}$C per minute.

A. Freezing profile of water – no CRF

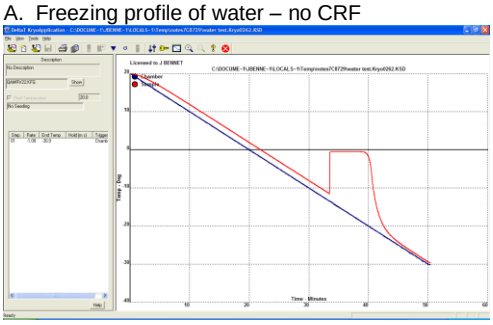

Figure (B) shows the temperature/time profile of cells in a cryoprotectant during an uncontrolled freezing rate of 1 $^{o}$C per minute

B. Freezing profile of cells in cryoprotectant – no CRF

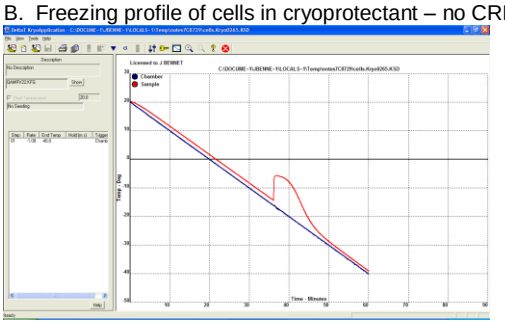

Figure (C) shows the temperature/time profile of cells in a cryoprotectant during a controlled freezing run using a basic protocol of

| Step | Rate | End Temp |
|---|---|---|
| | | |
| 1 | -1.00 | -7.5 |
| 2 | -35.00 | -40.0 |
| 3 | +6.50 | -13.0 |
| 4 | -1.00 | -40.0 |

C. Controlled program: cells in cryoprotectant