

# 細胞冷凍學--細胞之保存

黃效民



食品工業發展研究所

Food Industry Research and Development Institute



# Reasons for cryobanking cells (I)

---

Obvious reasons (**SAVE**):

- ▶ **S**ave work, including time and money
- ▶ **A**ssurance of self-cell supply
- ▶ **V**alidated cells, tested and qualified
- ▶ **E**mergency, a back up supply

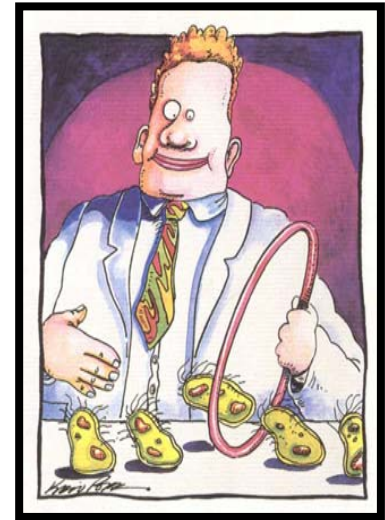


# Reasons for cryobanking cells (II)

---

Less obvious reasons (another **SAVE**):

- ▶ **S**hipping,
- ▶ **A**ging,
- ▶ **V**ariation,
- ▶ **E**volution,



Keeps your cells young  
and performing



# 細胞庫系統 (Banking System)

---

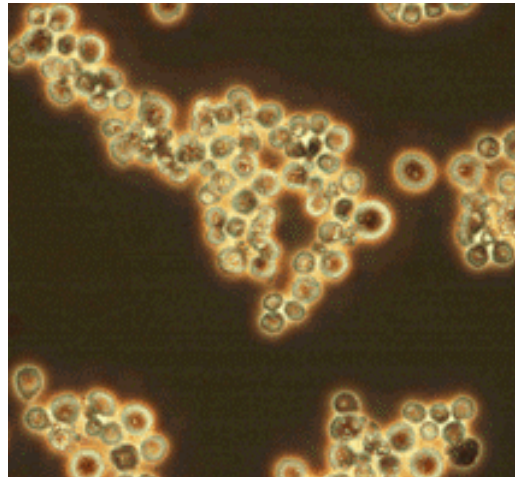
- ▶ 實驗/研究期間足夠量的細胞
- ▶ 降低污染機會
- ▶ 細胞之貯存
  - ▶ token, MCB, WCB in liq./vapor phase of liq. Nitrogen
  - ▶ Documentation: location, identity and inventory of individual ampoule of cells
  - ▶ at least 2 separate storage areas



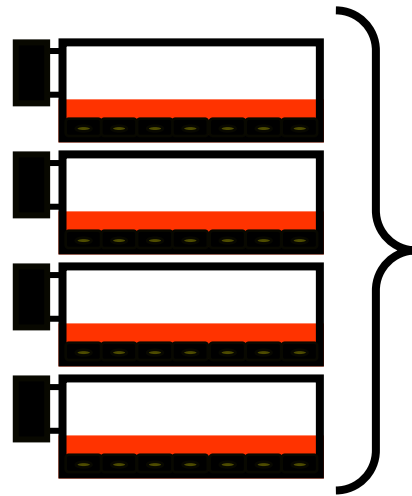
# Preparation of Cell Banking

---

- ▶ This works **if** you remember:
  - ▶ Two stocks: **M** & **W** (master & working cell bank)
  - ▶ Store cultures appropriately (cells & document)



# How to do it - Step 1 (*Master stock*)

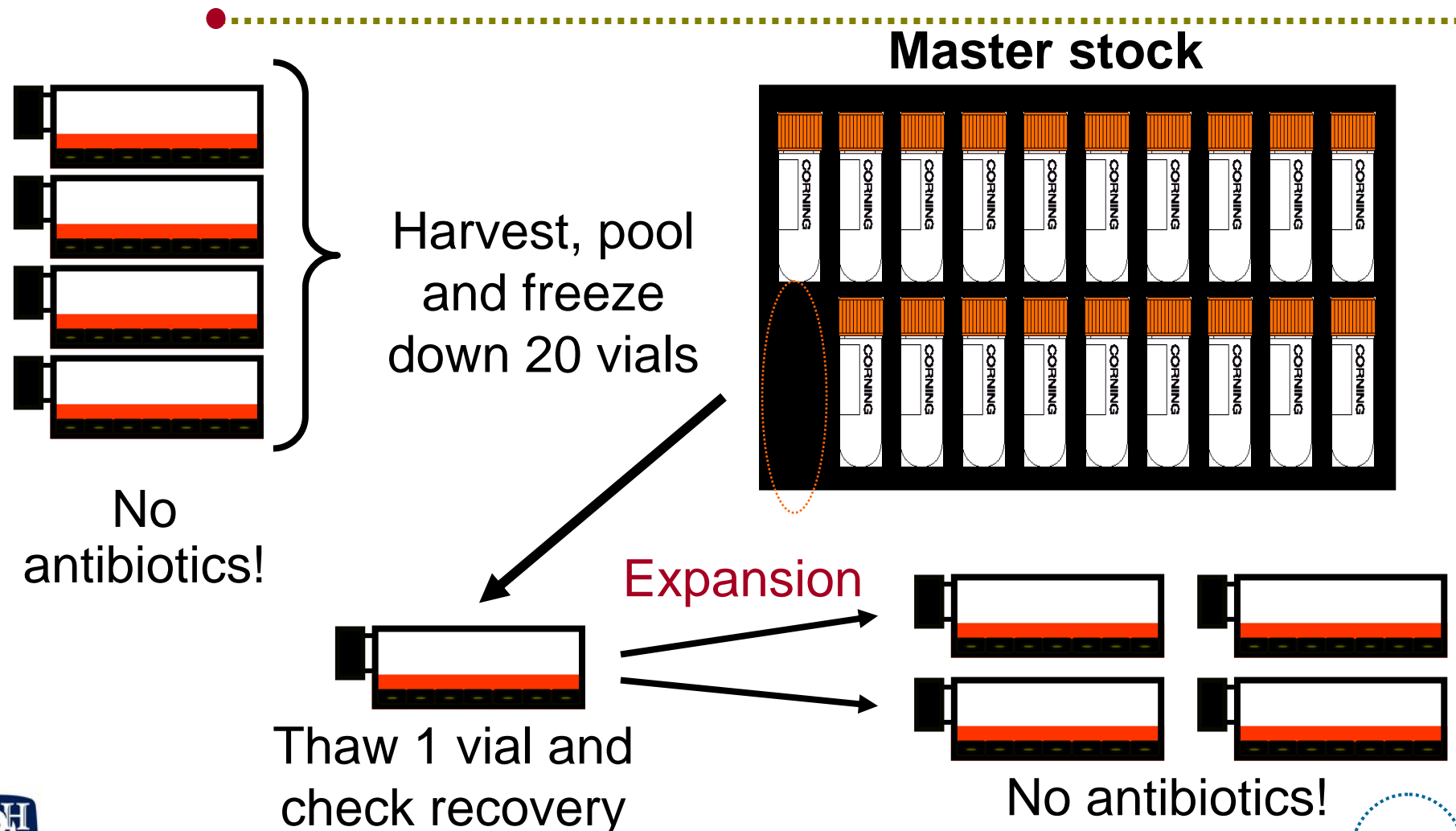


No  
antibiotics!

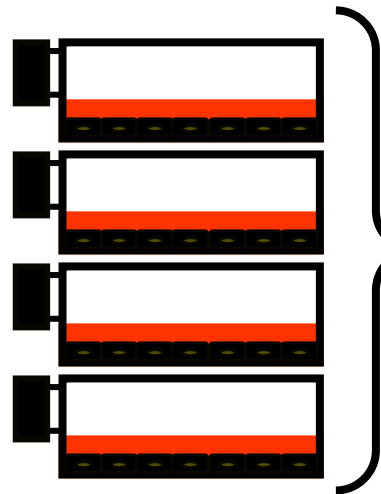
Harvest, pool  
and freeze  
down 20 vials  
**(master stock,  
passage:N)**



# How to do it - Step 2 (expansion)



# How to do it - Step 3 (Working stock)



Cells from seed stock

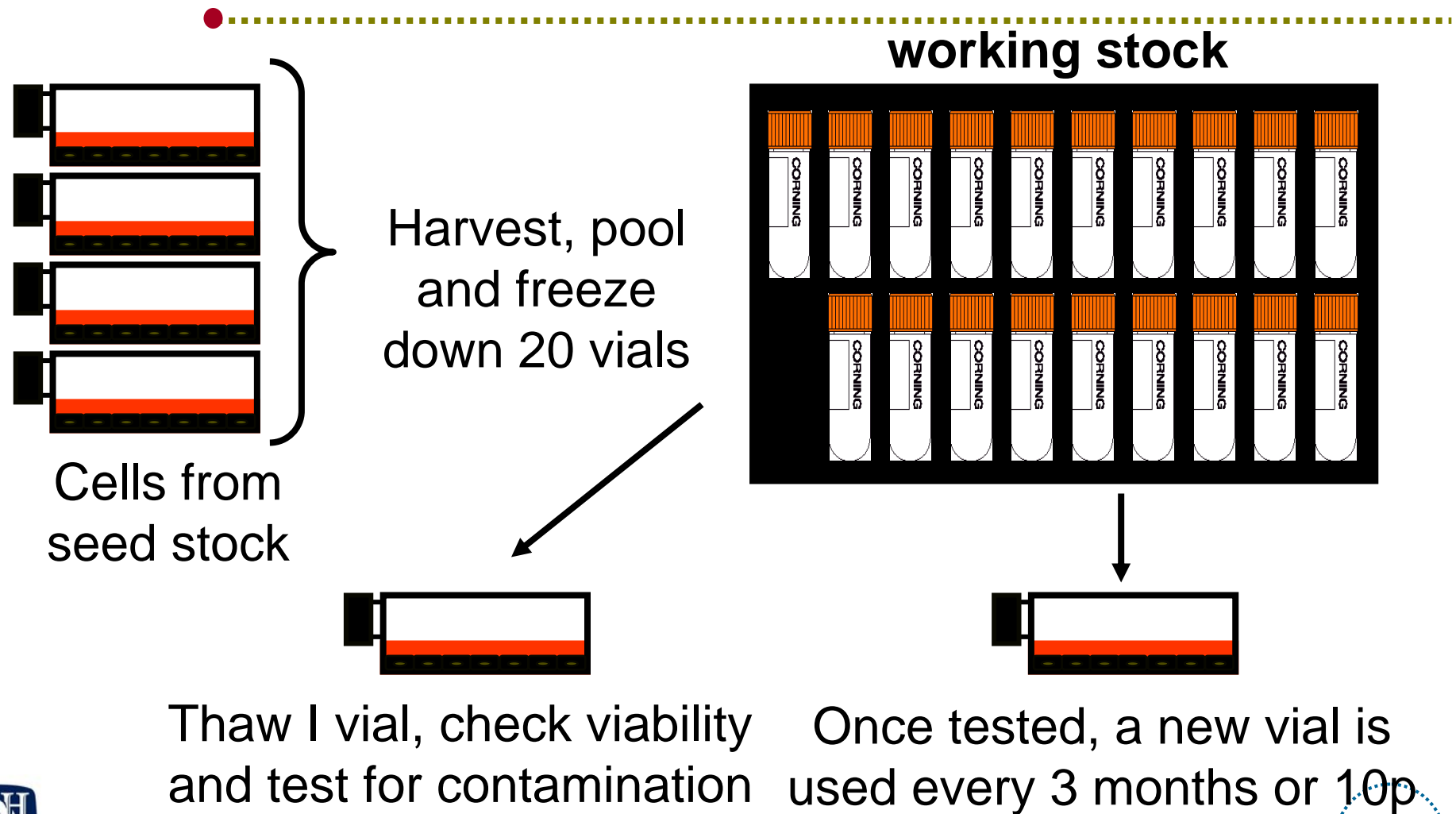
Harvest, pool and freeze down 20 vials

**(Working stock, passage:N+2)**





# How to do it - Step 4 (check)



# Simple calculations

---



- ▶ So how many vials you have now:
  - ▶ Master stock (20-1) plus Working stock (20-1)
- ▶ Total vials you will have at the same passage number (p:N+2):
  - ▶ 20 (Master) x 20 (working) = 400 (vials)
- ▶ How long can you handle this cell line? (if: 1 vial for 3 months)
  - ▶ 3 month x 400 = 1200 months (100 years!!!)



# 冷凍速率細胞之影響

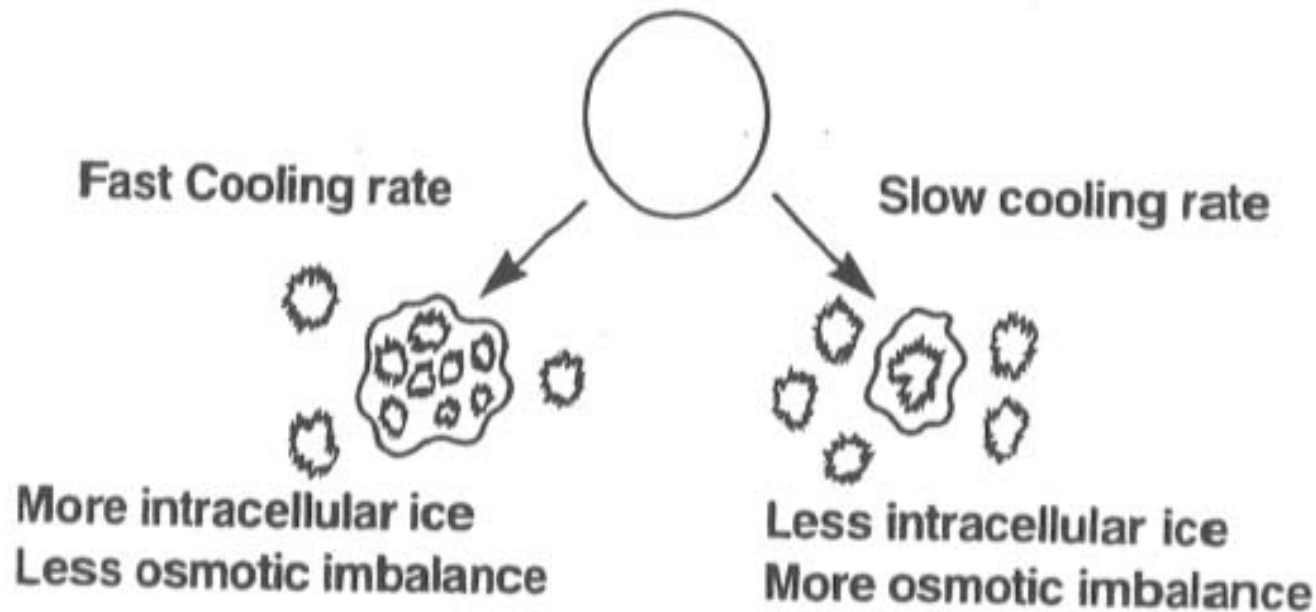


Fig. 1 Use a cooling rate of  $1^{\circ}\text{C}/\text{minute}$  and a cryoprotective agent to minimize damage due to osmotic imbalance and ice crystal formation.



# 冷凍保護劑

---

- ▶ 改變溶液的物理性質
  - ▶ Solution effect
- ▶ 穩定細胞膜
  - ▶ Cell permeability
- ▶ 穩定細胞內的巨分子
  - ▶ Enzyme systems

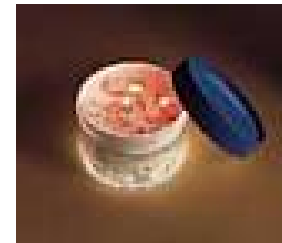
目前使用最多者為：DMSO (dimethyl sulfoxide); glycerol

---



# 動物細胞的冷凍操作

- ▶ 配製冷凍培養基
  - ▶ DMSO (5-10%)
- ▶ 細胞濃度 ( $5 \times 10^5 \sim 1 \times 10^7 / \text{ml}$ )
- ▶ 等速降溫機或階段手動降溫
  - ▶  $-20^\circ\text{C}$ , 30min// $-80^\circ\text{C}$ , 隔夜//液態氮筒
  - ▶ 使用 Mr. Frosty (Nalgene 產品或相當替代品)
  - ▶ 善用細胞寄送之寶麗龍盒,  $-80^\circ\text{C}$  隔夜
  - ▶ 利用程式降溫儀
- ▶ 仔細記錄細胞存放位置 (tank/rack/box/no.)



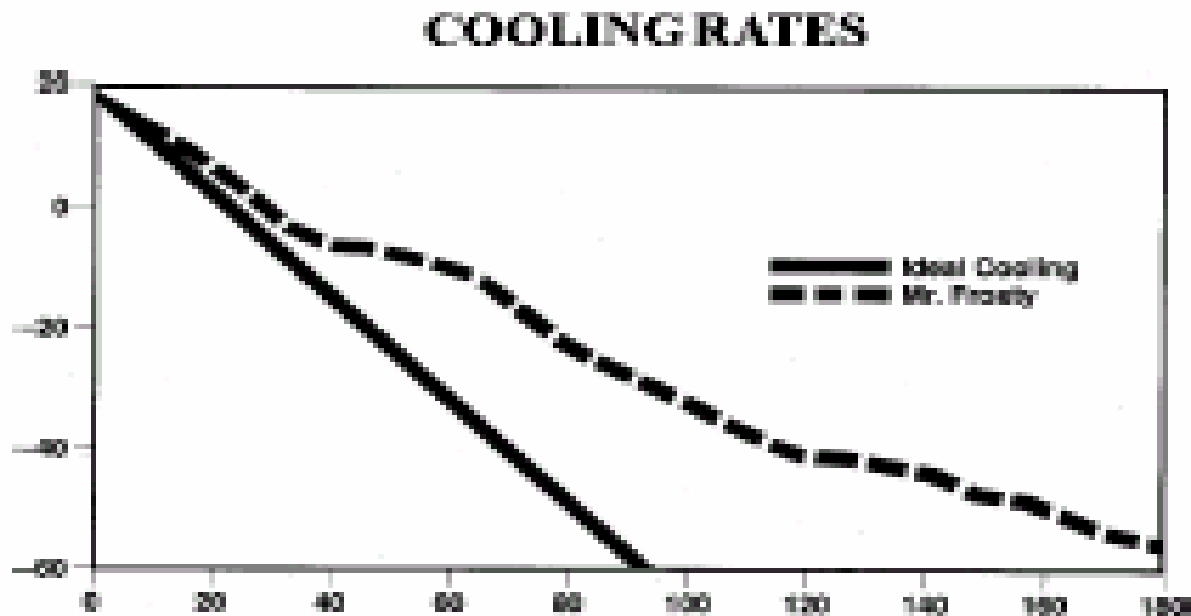
## Mr. Frosty (from Nalgene)



- ▶ Provides the critical, repeatable 1 °C/minute cooling rate required for successful cell cryopreservation and recovery.
- ▶ Requires only 100% isopropyl alcohol and mechanical freezer. Labeled with graphic, step-by-step instructions. Holds up to 18 vials.



# Mr. Frosty in -80 degree freezer



\* 實際細胞冷凍保存管內溫度與此曲線差異極大

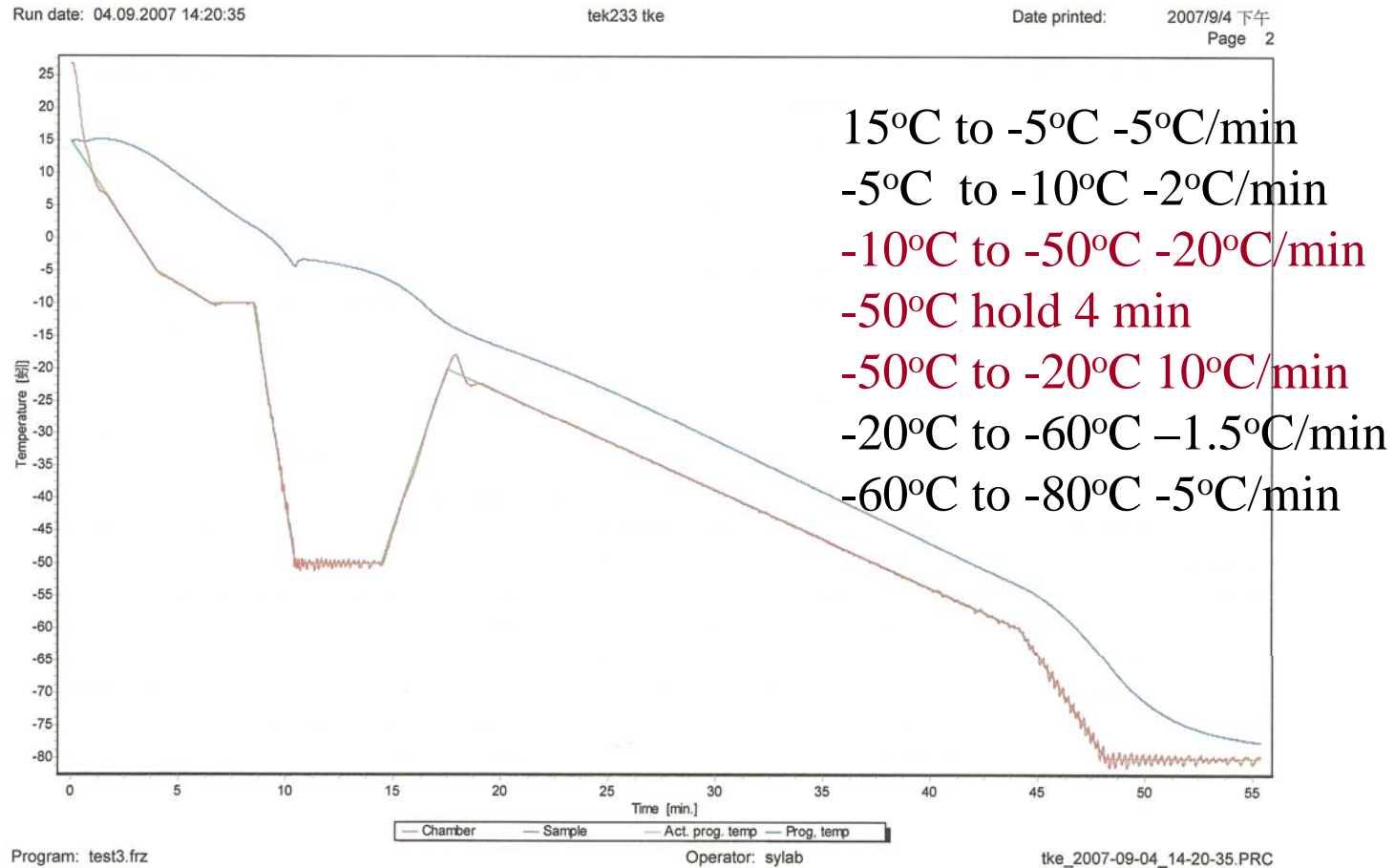


# 程式降溫機：細胞之冷凍溫控

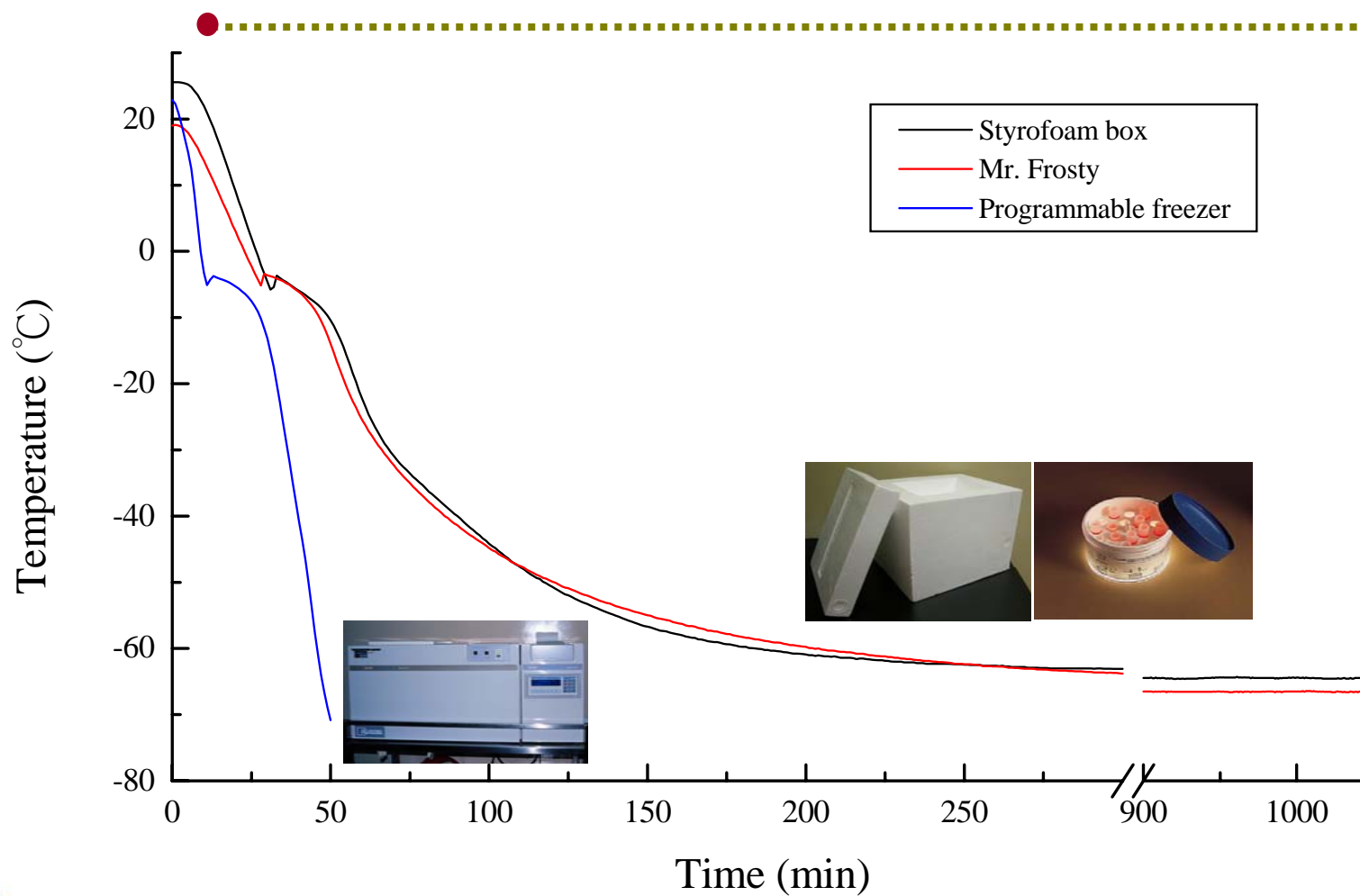




# Programmed cell-freezing

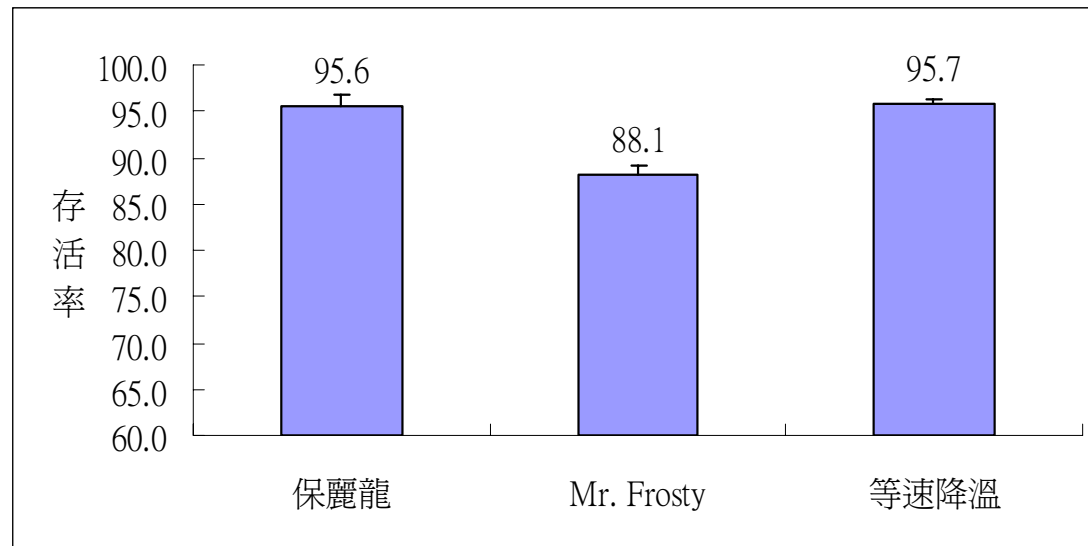


# 等速降溫機取代物之測試



# 3T3-L1 cells之測試~凍後存活率(五個月)

	保麗龍	Mr. Frosty	等速降溫
1	94.3	87.2	95.3
2	95.9	89.3	95.4
3	96.6	87.7	96.4
平均	95.6	88.1	95.7
SD	1.2	1.1	0.6



# Liquid Nitrogen Storage

- ▶ Liquid phase

- ▶ Constant temperature (-196°C)
- ▶ Less watching labor
- ▶ Good for sealed vials (ampules)



- ▶ Vapor phase

- ▶ Plastic vials
- ▶ Convenient
- ▶ Less contamination





# Cryovials

---

- ▶ **WARNING!** Do not use cryogenic vials for storage in the liquid phase of liquid nitrogen. Such use may cause entrapment of liquefied nitrogen inside the vial and lead to pressure build-up resulting in possible explosion or biohazard release.



# LN<sub>2</sub> invasion

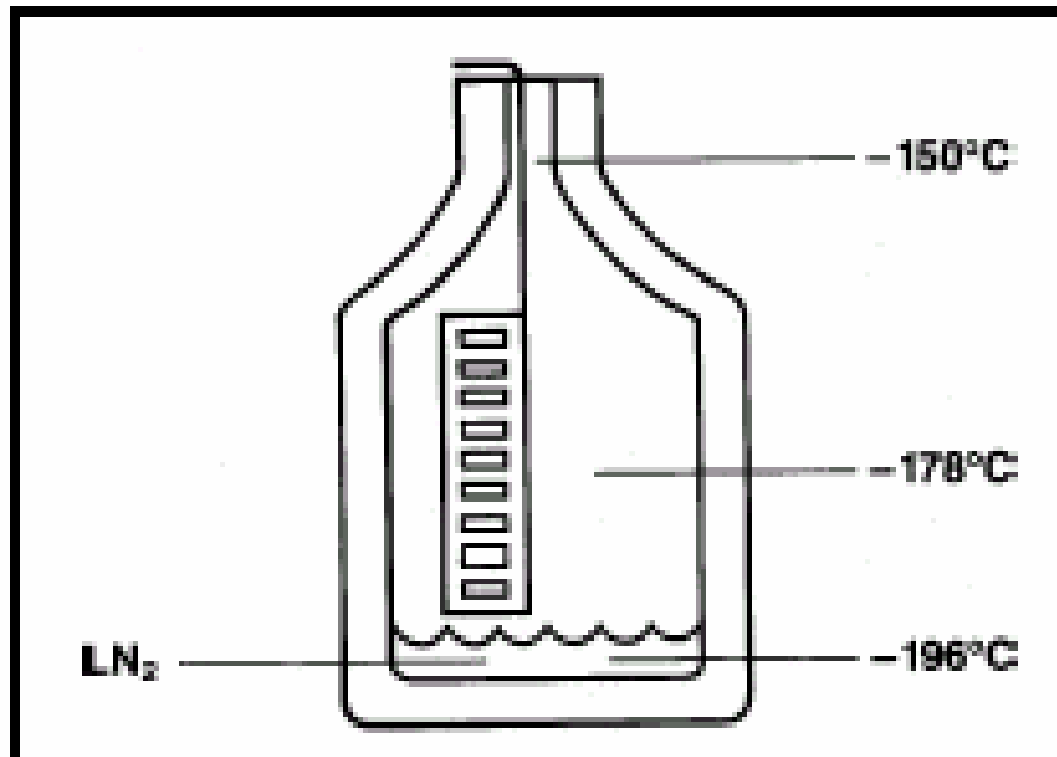
---

- ▶ **Explosion** of cryovials due to the rapid evaporation of penetrated liquid nitrogen during retrieving them from liquid nitrogen vessels.
- ▶ **Microbial contamination** due to the penetration of non-sterile liquid nitrogen.
- ▶ **Cross-contamination** between cryovials due to the in and out of liquid nitrogen.



# Temperature gradient in LN tank

---







**Taylor-Wharton**

## LABS Series

The LABS Series is designed to provide near LN<sub>2</sub> Temperatures at the top of the Inventory System and all racks are in vapor.



- **LABS Series** - All Stainless Steel Freezers designed for -190°C vapor storage in box type racks.





液氮筒



大型液氮槽內部



# 冷凍細胞之 儲存管理

1. 單核細胞(MNC) 2. LN2 3. 種子庫 保存位置圖

Tank: L17 Box: BA42 Pos: 99 Detail

	1	2	3	4	5	6	7	8	9
1	CM02515	CM02516	CM02517	CM02518	CM02519	CM02520	CM02521	CM02522	CM02523
2	CM02524	CM02525	CM02526	CM02527	CM02528	CM02529	CM02530	CM02531	CM02532
3	CM02533	CM02534	CM02535	CM02536	CM02537	CM02538	CM02539	CM02540	CM02541
4	CM02542	CM02543	CM02544	CM02545	CM02546	CM02547	CM02548	CM02549	CM02550
5	CM02551	CM02552	CM02553	CM02554	CM02555	CM02556	CM02557	CM02559	CM02560
6	CM02561	CM02562	CM02563	CM02564	CM02565	CM02566	CM02567	CM02568	CM02569
7	CM02570	CM02571	CM02572	CM02573	CM02574	CM02575	CM02576	CM02577	CM02578
8	CM02579	CM02580	CM02581	CM02582	CM02583	CM02584	CM02585	CM02586	CM02587
9	CM02588	CM02589	CM02590	CM02591	CM02592	CM02593	CM02594	CM02595	CM02596

細胞庫研究中心  
Technologies Center

## 庫房管理系統

基本資料 統計

- 員工管理(E)
- 菌株基本資料(I)
- 出入庫型態(E)
- 出入庫原因(R)
- 入庫作業登錄(I)
- 出庫作業登錄(O)

- 菌株位置圖查詢
- 月-菌株入出庫
- 菌株數量
- 補庫菌株
- 系統功能
- 返回系統資料庫
- 結束庫房管理

NUM SCRL

Microsoft

Microsoft Access - [血液計畫藥品報表-bcr - 報表]

食品工業發展研究所  
Food Industry Research and Development Institute  
◎病毒轉染建立人類單核細胞株

檢體名稱編號: [Barcode]

檢體名稱	檢體資料	性質	資料用途
MNC	檢體編號	CM02515	2002/09/20 09:00
	檢體名稱	單核細胞	2002/09/20 09:00
	檢體來源	血液	2002/09/20 09:00
	檢體處理	凍存	2002/09/20 09:00
Immortalized MNC	檢體編號	CM02516	2002/09/20 09:00
	檢體名稱	永生化單核細胞	2002/09/20 09:00
	檢體來源	血液	2002/09/20 09:00
	檢體處理	凍存	2002/09/20 09:00

我的電腦 苗種庫房主... 上午 10:40

# 冷凍細胞之解凍

---

- ▶ 防護用具穿戴
- ▶ 37°C水浴槽中快速解凍
- ▶ 小心冷凍管中液氮之爆衝
- ▶ 水浴槽液面不可高過冷凍管蓋緣
- ▶ 完全解凍後注意無菌操作
- ▶ 不需立刻去除DMSO(建議第二天再更換培養基)



# 結語

---

- ▶ 好的開始是成功的一半
- ▶ 唯有正確的使用試驗材料，方有正確和符合品質的結果
- ▶ 唯有使用正確的試驗材料，方有可預期的目的結果

