

# Koruma / stoklama teknikleri

# Devamlı üretim

# Dehidrasyon

# Dondurarak saklama

Biyobankacılık

En uygun yöntemin seçimi organizmaya göre yapılmalı  
— Mümkünse denemeler gerçekleştirilmeli

# # Devamlı üreme gerçekleşen yöntemler #

Madeni yağ / parafin tabakası altında yaşam 

— Steril parafin tabakası altında sıvı kültür

Oksijen yokluğunda yavaşlamış metabolizma —

15-20°C sıcaklıkta saklama

Çoğu bakteri kültürü 2-4 yıl dayanır.

Mantarlar genellikle daha uzun süre dayanır 

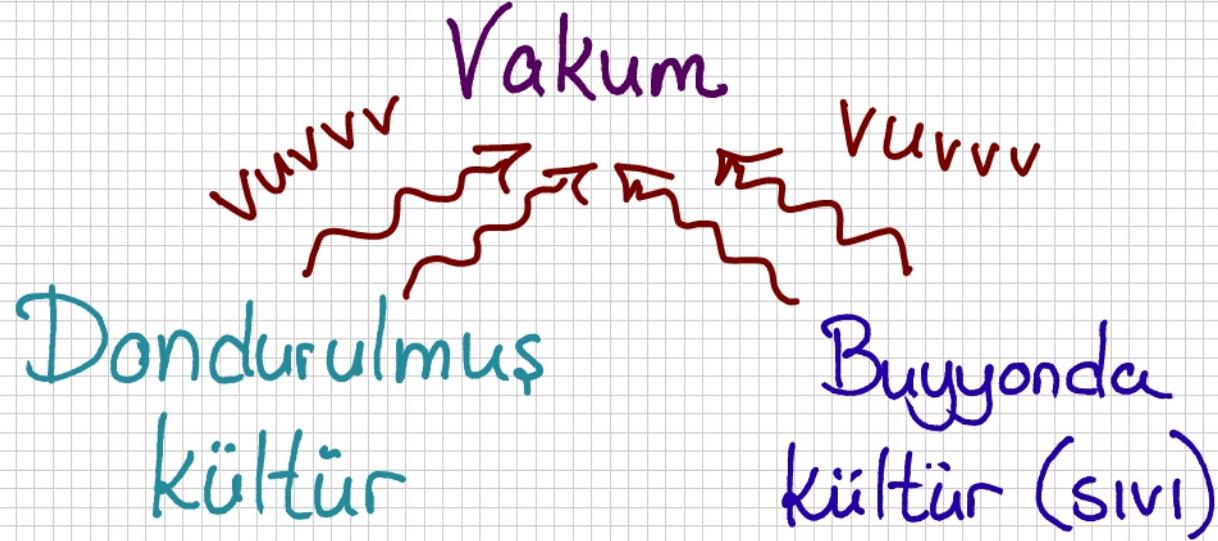
 Bu yöntem ile ilgili sorunlar nelerdir?

Su içinde saklama

Bir başka çokook yavaş üremeye dayalı teknik (hatta, durma noktasında ---)

Küf, maya, fitopatogen bakteriler, aktinomyetler için uygun

# # Dehidrasyona dayalı stok teknikleri #



Freeze-Dryer  
Nasıl Çalışır?

Skimmed milk + inositol içinde  
süspanse edilerek dondurulan  
hücreler

Destekleyici ortam:  
toprak, kum, silika-jel,  
porselen, skimmed milk,  
inositol, at serumu,  
nutrient broth, glukoz

# "CRYOPRESERVATION"

Cryoprotective additive

~ Glycerol

~ DMSO

~ ve birçok diğeri...

Doğa kendi koruyucu maddelerini de oluşturmuş — Hücrenin dondurulmaya başlamasıyla bunların sentezi indüklenebilir

Soğuma stresi ile başa çıkmada OSMOREGÜLATÖR maddeler !

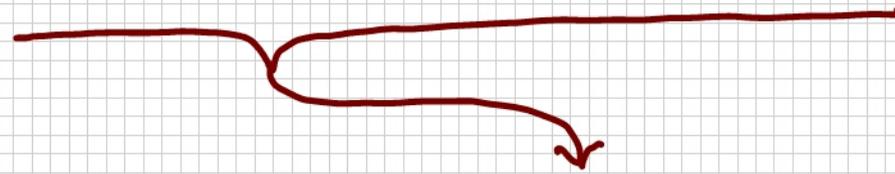
# Konsantrasyon etkisi

# Mekanik stres

# SOĞUTMA HIZI

Cryoprotectant  $\times$  Cooling rate  $\rightsquigarrow$  SUCCESS <sup>°°</sup>

yavaş soğuma  $\rightarrow$  hücrede büzülme  $\leftarrow$   
hücre dışında buz oluşumu  $\rightarrow$  hücreden su kaybı



Membran kaybı



Sitoplazma kaybı

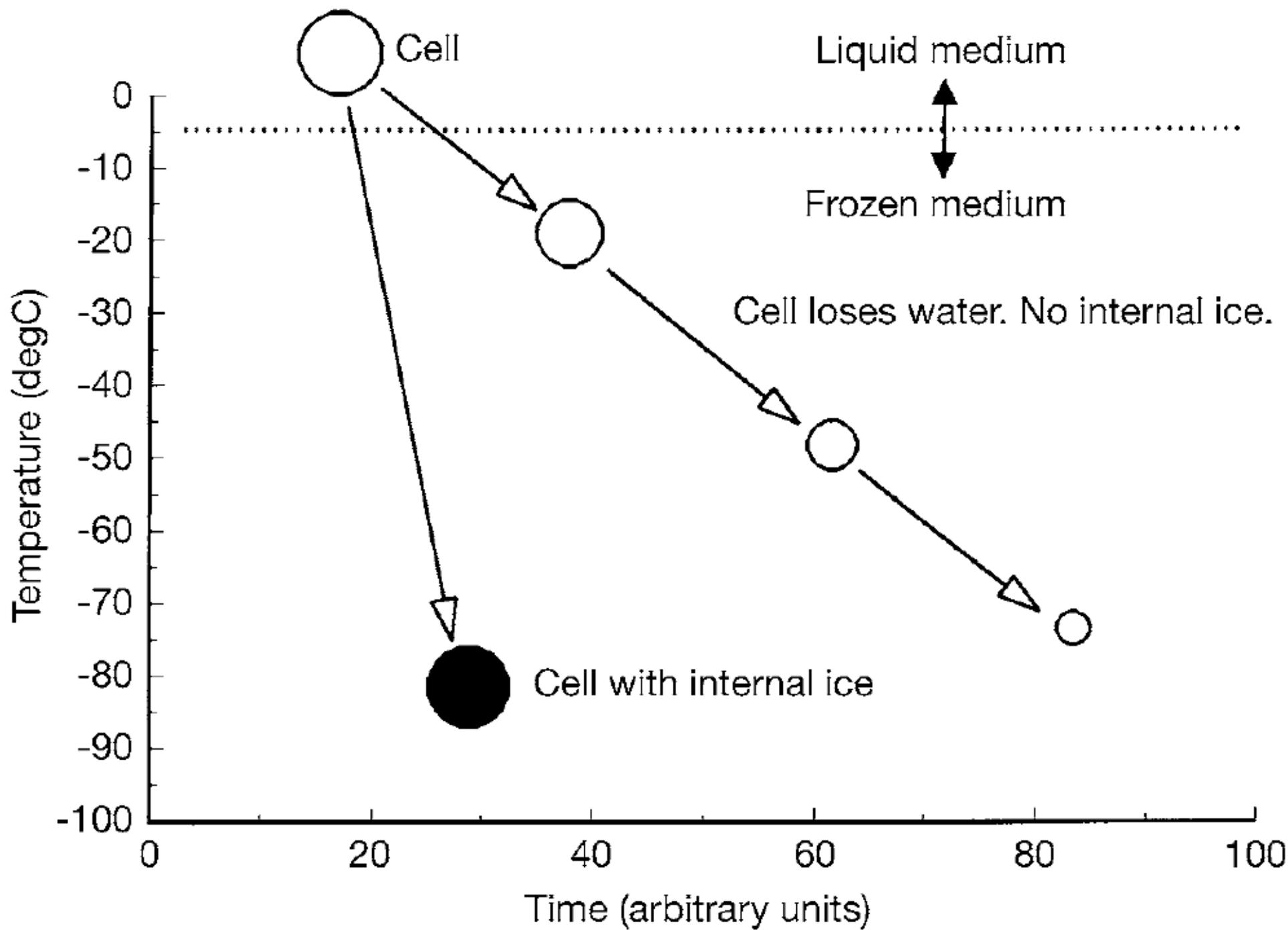
Çözündürülen hücreler kaldıkları yerden devam edebilmek için hasarları onarmalı

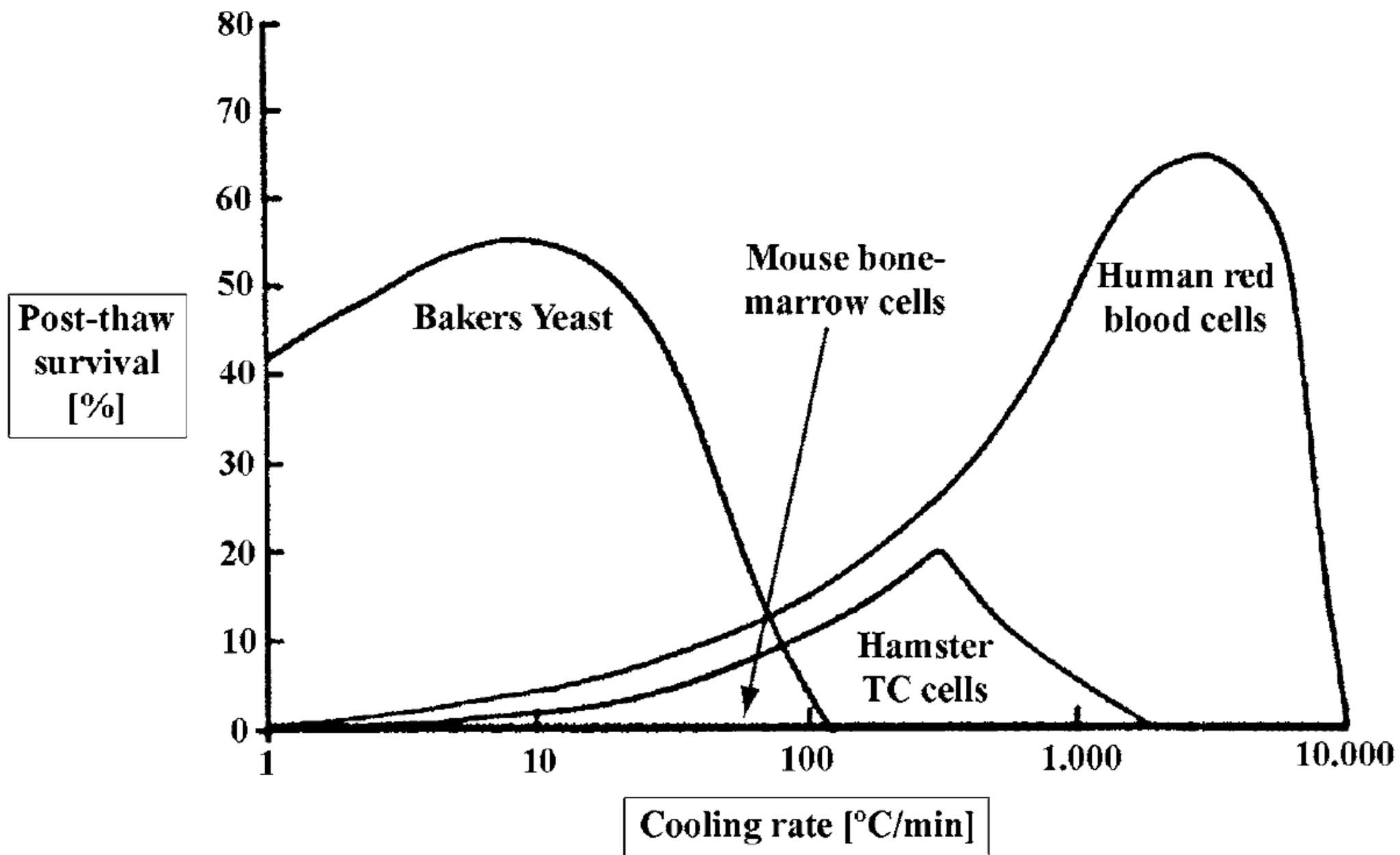
Dondurma işleminden doğabilecek hasarları en aza indirmek için :

- # Yavaş dondurulmalı — Hızlı dondurma, hücre içinde buz oluşumuna neden olabilir !!
- # Suyu taklit eden ve yerini alabilen bir "cryoprotective" ajan kullanmak

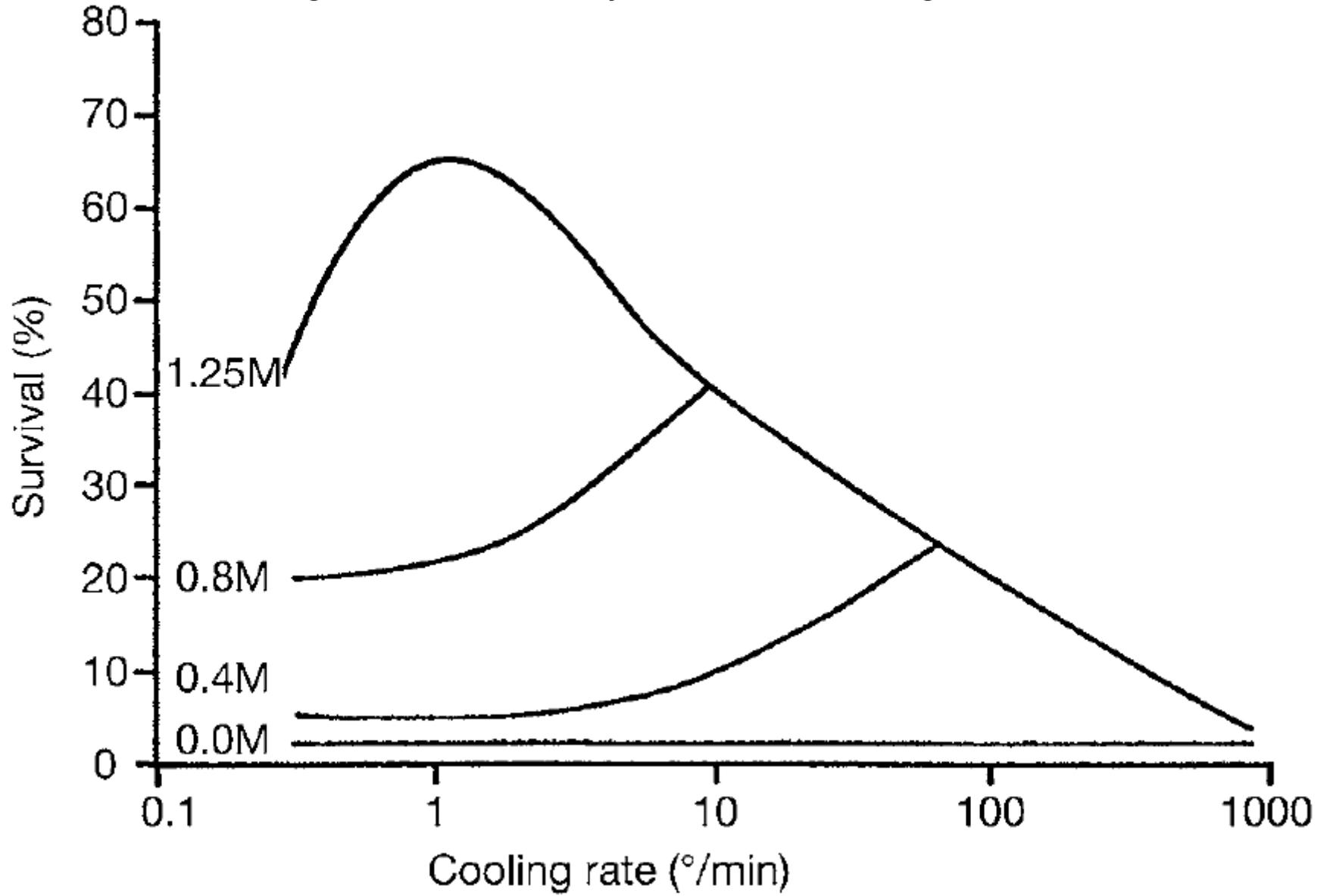
Genel olarak,  
yavaş yavaş dondurmaları  
hızlı hızlı çözmeliyiz ...

Örneğin, tüpü doğrudan  
37°C'ye atmalıyız,  
yeniden canlandırırken





Farklı gliserol konsantrasyonlarının hücre sağ kalımı üzerindeki etkisi



# Ne kadar sıcak ne kadar soğuk ?

-10°C ~ -196°C

Buzdolabı üstü dondurucular

-20 - -35°C derin dondurucular

-80°C derin dondurucular

-140°C ultra derin dondurucular

Sıvı azot

Buralarda bir yerlerde  
güvenli zon başlıyor

Evrenli dondurma sıcaklığı ?  
? ? ? ? ?

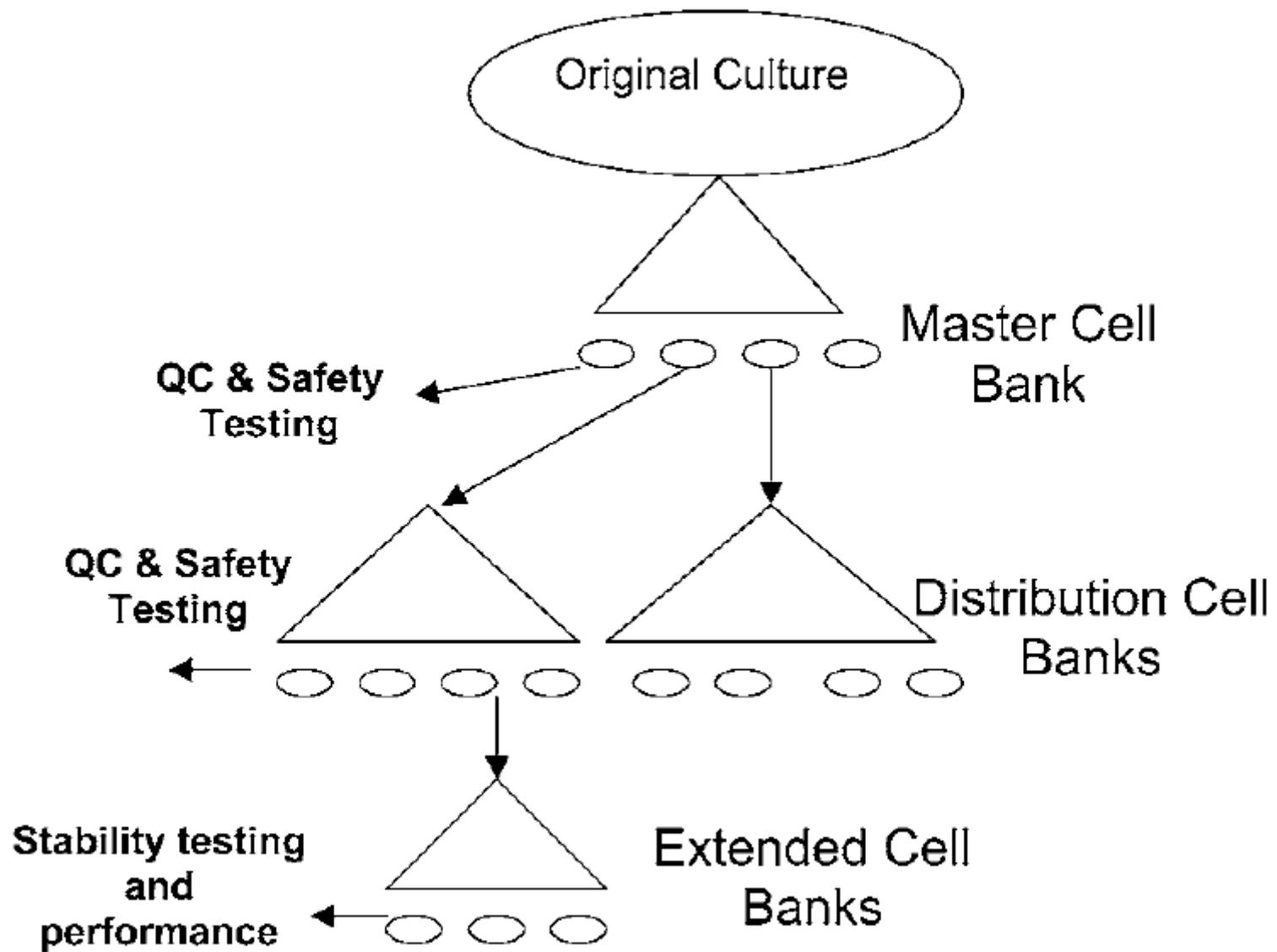
? Su aktivitesi ? ? ? ? ?

TABLE 2: Some commonly used methods used to assess strain stability following cryopreservation.

Method	Test
Anatomical	Microscopical observation of anatomical structures. For example, spores, conidia, flagella, plastids, and hyphal form.
Culture characters	Analysis of culture morphology in plate culture. For example, pigmentation, abundance of sporulation, presence or absence of sectors, or abnormal growth
Growth rate	Measurement of radial growth of fungi and other mycelial organisms in plate culture [9]
Cell density	Cell counts at set time points using microscopical counting methods, flow cytometry or spectrophotometric approaches
Molecular integrity	PCR fingerprinting approaches (ISSR, AFLP) which assess the whole genome [10, 11]
Viability of cells	The use of chromatogenic or fluorogenic viability indicators. Many available, commonly used ones for fungi and bacteria include fluorescein diacetate (FDA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [12] <i>fluorescein isothiocyanate (FITC)</i> , FUN-1 viability staining
Enzymic capacity	APIZYM utilisation of naphthyl-bound substrates that yield a chromatogenic change [13, 14] 4-methylumbelliferone [15–17]
Metabolic stability	High performance liquid chromatography (HPLC) of secondary metabolites [18] Thin layer chromatography (TLC) of secondary metabolites [18, 19]
Pathogenicity	The target organisms are inoculated onto test media with the potential control strain (or metabolite/protein extract from the control strain)/or directly onto a plant or animal, and the extent of pathogenicity and mortality are recorded.

TABLE 3: Validation of fungal cryopreservation protocols.

Criterion	Requirement	Reference
Select optimal growth conditions	To produce healthy material spores, mycelium	[20]
Measure and record baseline data for stability checks	Apply a unique identifier/strain number; select criteria to be measured: Morphological characteristics Sequence ITS region of the genome Growth rates Photomicrographs Metabolic data Genome fingerprinting techniques	[8]
Select the most appropriate preservation protocol	Optimised for organism type	[20]
Select cryoprotectant	Appropriate for the cell type	[8]
Select most appropriate cooling rate	Thermometer calibrated to a standard	[8]
Select most appropriate storage temperature	Temperature below $-140^{\circ}\text{C}$ , monitored and recorded	[21, 22]
Select most appropriate thawing protocol	A rate appropriate to cell type in calibrated and controlled equipment	[8]
Prepare master and distribution stocks	High recovery No contamination Authentic: morphology; phenotypic and molecular integrity	[23]
Method validation		
Performing blind tests	Central laboratory sends unknown organism to collections with limited data and results after above the process compared	Example of such a system is in the public health laboratories for diagnostics
Reproducibility check	Comparing results of the same method at different times Comparison of results obtained with different methods Comparison of results obtained with different operators	[23]
Equipment calibration	All equipment must be regularly serviced and gauges and meters calibrated to recognised standards	[23]
Recording parameters	Daily records of temperature readings of incubators and cryostorage units to ensure that they remain within set parameters	ISO standards



# KAYNAKLAR

1. Smith D, Ryan M. Implementing Best Practices and Validation of Cryopreservation Techniques for Microorganisms. ScientificWorldJournal [Internet]. 2012 May 2 [cited 2014 Apr 1];2012. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3353557/>
2. De Paoli P. Biobanking in microbiology: From sample collection to epidemiology, diagnosis and research. FEMS Microbiology Reviews. 2005 Nov 1;29(5):897–910.
3. Cryopreservation and Freeze-Drying Protocols [Internet]. [cited 2014 Oct 30]. Available from: <http://www.springer.com/life+sciences/biochemistry+%26+biophysics/book/978-1-58829-377-0>
4. Malik KA. Freeze-drying of micro-organism using a simple apparatus. DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1B, D-3300 Braunschweig, Federal Republic of Germany: UNESCO / WFCC - Education Committee; Report No.: 7.