

# 黃 效 民 國衛院 細胞庫 生物資源保存及研究中心





•

"The winner of the "greatest technology" award for the past 25 years has got to be <u>Cell Culture</u>, which predates the biotech industry by many decades..."

Angelo DePalma, 25th Anniversary issue of Genetic Engineering News GENETIC August, 2005





# Cell culture is much like cooking...

It requires

- A lot of liquid handling
  - Measuring
  - Mixing
  - Transfer
- Careful observations and control of the environment
- Good ingredients
- Good aseptic technique
- Sometimes, more "art" than science





# How do I culture cells?

#### BCRC 60004 MDCK



Start with good cells



"Optimize" their culture environment



"Optimize" my culture technique



5

# What are good cells?

- Healthy growing, no mycoplasma or other contaminants
- Maintain their key characteristics and functions



- Come with a history and clear instructions
- Come from a reputable source, like BCRC cell bank





## How to "Optimize" the environment?

### **Check the basics:**

- Medium
- Serum
- Gas
- ▶ pH
- Temperature
- Surface



#### Happy cells perform better!



# How to "Optimize" my technique?

- 3 key areas:
  - Harvesting
  - Feeding
  - Storing



### **Plus Aseptic procedures**







🙋 BCRC Strain	Administration System - Micros	oft Internet Explorer			<u>_ 8 ×</u>					
檔案 ④ 編輯 ④ 檢視 ♡ 我的最愛 ④ 工具 ① 説明 ⊞										
←上一頁 →										
網址DD @ http://strain.bcrc.firdi.org.tw/BSAS/index.jsp										
<b>Y!</b> - <i>Q</i> -		搜尋   - □ □ - ◆ -	🖂 信箱 👻 👂	知識+ • 🎦 拍賣 • 🖓 交友 • 🔗 新聞 • 🔤 股市 • 👸	〕購物 ▼ 🎇 家族 🔹 🛛 >>					
Strain	BCRC Administration System			User: Pa	FIRDI Home   BCRC Home 🔺					
BSAS Home					Online member : 6					
Searcl	1									
Searc	h Strain Collection (	Catalog ·····	🚰 BCRC Strain Adn	ninistration System - Microsoft Internet Explorer	- Traditional Chinaga	_[@]×				
			檔案(E) 編輯(E)	検視(V) 我的最愛(▲) 工具(T) 説明(出)						
	Enter BCRC Number:		④ 上一貝 ▼ ⇒ 網址(11) ▲ bttp://b	- O P G G UUUUUUUUUUUUUUUUUUUUUUUUUUUUUUU	3 😼 🖄					
		OR		uani icic:huitoig.w/BSX//coniuolar 按章 → 〒→ ◆→ - □ 云 信箱 → ℘ 知	職+ • 🏏 拍賣 • 🎾 交友 • 🛷 新	□ (* 19 ± / 2014) 間 • ₩ 股市 • 🖓 購物 • 🎎 家族 • >>				
	Entor kouword:									
	Enter Reyworu: Inver cells The search engine found 21 entries for liver cells Entries 1 to 15 are currently being displayed.									
		Display 15 🗸 entries on	next [	Last [3]						
			BCRC Number	Name	De	esignation				
Se	arch for References	5	<u>60025</u>	Hep G2	Obtained from ATCC; ATCC number: HB-80	65				
1			<u>60051</u>	Hepa 1-6	Obtained from ATCC; ATCC number: CRL-1	830				
	Enter Keyword:		<u>60104</u>	Hepa-1c1c7	Obtained from ATCC; ATCC number: CRL-2	026				
		Submit Search Cle	<u>60143</u>	MH1C1	Obtained from ATCC; ATCC number: CCL-1	44				
			<u>60168</u>	HA 22T/VGH						
② Date 2006/11/13 15:54:48 ~welcome to BCRC Strain Administration System ③ 開始 □ @ ③ 2 2 2 BioMail ○ 94NHRI □ Microso			<u>60169</u>	HA 59 T/VGH	Obtained from Dr. Cheng-Po Hu, Veterans general hospital-Taipei, Taiwan					
			<u>60177</u>	C3A (HepG2/C3A)	Obtained from ATCC; ATCC number: CRL-10741					
			<u>60180</u>	BNL CL.2	Obtained from ATCC; ATCC number: TIB-7:	3				
			<u>60211</u>	H4-II-E-C3	Obtained from ATCC; ATCC number: CRL-1	600				
NIH			60215	BNL 1MEA.7R.1	Obtained from ATCC; ATCC number: TIB-7	5				
k			60216	BNL ING A.2	Obtained from ATCC; ATCC number: TIB-7	6				
			② Date 2006/11/13	15:55:37 -welcome to BCRC Strain Administration System	生物资 GIDCDC III.mate sum					
			🥵 😭	] 👩 🧿 » 🛛 🖉 BioMail 🔄 94NHRI 🛛 🖪 Microso 🖉 FIRDI 🦃	生物資 / BCRC   Type to searc	h 🖸 - 🔽 🌾 🖓 😓 😂 🍘 🖽 下午 03:55				



# **QC** management

**M&M detection:** Microbials and Mycoplasma



#### Mycoplasma EM

Mycoplasma colonies



Virus CPE

Fluorescence detection



# **QA** management

#### Authentication: Origin & Species



# Testing for bacteria, yeast and fungi

The simple approach (and, as a result, more likely to be used, such as checking of serum black dots)



Make a rack of antibiotic-free medium + sera in 15mL tubes & store at 4°C

### Testing for bacteria, yeast and fungi

The simple approach (and, as a result, more likely to be used, such as checking of serum black dots)





Make a rack of antibiotic-free medium + sera in 15mL tubes & store at 4°C

Add 1mL samples from suspicious culture or untested medium to each of 2 tubes

### Testing for bacteria, yeast and fungi

В

С

Negative

control

The simple approach (and, as a result, more likely to be used, such as checking of serum black dots)



Make a rack of antibiotic-free medium + sera in 15mL tubes & store at 4°C

Add 1mL samples from suspicious culture or untested medium to each of 2 tubes Incubate and examine tubes periodically by eye & at 400x

37°C

В

RT

•

( 18 )









### **Microbiological Culture Method**







# **Hoechst DNA Staining**

bisbenzimide, Hoechst #33258: bind A-T rich DNA





#### Positive

### negative





# **PCR method**

- Polymerase Chain Reaction
- Nested PCR : two-stage PCR



Amplified DNA products were electrophoresed on 2% MetaPhor agarose (in 1X TBE) and visualized by ethidium bromide staining

ATCC Technical Support 1-800-538-6597





### HeLa 細胞污染

ICCIIC

#### HeLa-phobia ???



National Culture Collections such as ECACC supply authenticated cell lines for use in research and commercial applications. A common definition of the word 'authenticate' is 'to establish the truth of: to make valid'. All reputable culture collections employ methods to confirm at least the identity and origins of the strains they distribute.

properties and limitations. Without it, at best the cell line will generate irreproducible data; at worst the data will be false leading to misinterpretation and wasted resources trying to confirm them."





·\*\*\*\*\*\*

......

#### Cases of Mistaken Identity

For decades, biologists working with contaminated or misid entified cell lines have wasted time and money and produced spurious results; journals and funding agencies say it's not their job to solve this problem

IN THE 1980S, WHEN HE WAS A postdoctoral fellow at the Scripps Research Institute in San Diego, California, Reinhard Koffer received what was supposed to be a human cancer cell line from a collaborator. "We cultured it, we cloned genes into it," he recalls, then "[we] genotypedit andrealized it was 100%mouse."

After scores of similar experiences with misidentified cells, Kofler and his colleagues at the Tyrolean Cancer Research Institute in Innsbruck, Austria, now authenficate every line as soon as it arrives at the institute. And periodically afterward, they use a simple, che ap, quick, and re liable DNA fingerprinting technique to verify that each cell line continues to be what it should be. "It's an absolute must now," says Kofler. His lab "repeatedly" encounters problems with cell line contamination, and without this constant vigilance, Kofler says, "I wouldn't becon fident about our wok."

Not every biologist is so wary. A 2004 survey of nearly 500 biologists by Gertrade Bachring of the University of California, Berkeley, and her colleagues, showed that less than 50% of researchers regularly verify the identities of their cell lines using any of the standard techniques such as DNA fingerprinting. "Everybody is in denial" about the widespread problem of cell line cross contamination, says Charles Patrick



04 Early warning. HeLa cells have contaminated scores de of cell lines for more than 4 decades. Reynolds of the University of Southern California and the Childrenh Hospital Los Angeles' Institute for Pediatric Clinical Research, who establishes new pediatric cancer cell lines and tests potential cancer drugs on existing lines.

Indeed, many studies have shown that a surprisingly large number of cell lines have become contaminated, often by older, more well-established cancerous cells. For example, according to a 1999 paper by Roderick MacLeod and his colleagues at the German Cell Bank (DSMZ) in Bnunschweig, 18% of 252 lines donated to the bank were misidentified or contaminated. The extent of the problem "always seems to come as a surprise for people," says John Masters of University College London, president of the European Tissue Culture Society.

And even though biologists read and hear about cross contamination, "people just think that this is not a problem in my lab," says Reynolds. If contaminated cell lines are used merely as "test tubes" to express proteins, a lab's work may not be affected. But, say Masters and others, research with contaminated lines continues to obscure potential drug leads and

### Science (2007) 315:928-931

. . . . . . . . . . . . . . . . . . .



928

16 FEBRUARY 2007 VOL 315 SCIENCE www.sdencemag.org







......

# 細胞株錯誤(I)

#### Data from ATCC web.

### HeLa-contaminated cells

- Chang Liver (liver)
- KB (oral, epidermoid carcinoma)
- Intestine 407 (embryonic intestine)
- HEp-2 (larynx, epidermoid carcinoma)
- WISH (amnion)
- L-132 (embryonic lung)
- still available, noted by HeLa marker



# 細胞株錯誤(II)

Data from ATCC web.

### Identities in question

- ECV 304 (=T24) endothelium  $\rightarrow$  bladder
- ► KSY-1 (=T24) Kaposi's sarcoma → bladder
- U-373 MG (=U251) glioblastoma
- U-118 MG (=U138 MG) glioblastoma
- SNB-19 (=U251) glioblastoma
- Stop distribution, except KSY-1 under patent law













...........

.........

### 低溫庫房之線上監控

-161 -161	L15 L02 L0	01 1.06 1.05	- 		Rad Raz Ras 279 279 279	R03 R07 R06 R0 81 79 79 79	
U1 U2	U3 U4 U5	UE			<mark>U13</mark> U14	U15 U16 U17 U1	n u19
L17 -157 -165	U7 U8 SCO2	5C01 -165	110		U20 U21	U22 U23 U24 U2	5 U26
	-	- C	_				
	U10 U11 U	<sup>112</sup>	148	87 - <b>1</b>	V 🗩	80 U27 U28 U2	29 1130 1131
09 (AC) 液氮菌種保 現在溫度 高溫醫報	U10 U11 U 「」 「」	112       波航氯桶SCO2   低溫警報	148 推帶血庫-種子》	es 🚺		R01 U27 U28 U2	29 030 031

### 液氮槽之線上溫度記錄圖示







38

- Rapid thawing (60 to 90 seconds at 37°C)
- Double check label to make sure it is the right vial!
- Wipe off vial with 70% ethanol before opening
- Grow cells w/ or w/o removing cryoprotectant
- Cell happy, You happy







since 2001

The Quality Manag of ISO 9001 2000/EL

Expiration Date:

Cast 150 9000/2000 Rev

Certificate Issue Date: April 27, 2001

Feb. 14, 2004

ized Signature for BestCERT Quality Registrars Ltd

\*\*\*\*\*\*\*

# **TAF Certificate**

. . . . . . . . . . . . .

Oct. 24, 2007
BCRC accredited as ISO 17025



		Certificate No. : L1865-071024	
	財團法人全國認證基金 Taiwan Accreditation Founda	定會 ation	
Ce	rtificate of Accredi	itation	
	This is to certify that		
Food Indu	stry Research and Develo	pment Institute	
Biore	esource Collection and Resear	rch Center	
	331, Shih-Pin Road, Hsinchu 300, 7	Taiwan	
Ŀ	s accredited in respect of labo	oratory	
Accreditation Criteria	: ISO/IEC 17025:2005		
Accreditation Number	: 1865		
Originally Accredited	: October 24, 2007		1
Effective Period	: October 24, 2007 to October	23, 2010	1
Accredited Scope	: Testing Field, see described in	a the Appendix	
	foy	- San Chen	
	Jay-San Che President, Ta Date : Octol	n aiwan Accreditation Foundation ber 24, 2007	)
District			

The Appendix forms an integral part of this Certificate, which shall be invalid when use without the Appendix



