動物細胞之品質管制

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Importance of Animal Cell Culture

- Study biochemical, physiological and morphological question
- Production of biopharmaceuticals
- Cell therapy
- Screening of bioactive compounds
- Alternative safety assay
- Alternative functional assay

優良的細胞-品質管制重點

- 具有實驗(生產)所需之特性
- 沒有微生物的污染
- 細胞的純淨度
- 良好的細胞保存和供應系統

微生物污染檢測

- 細菌
- 真菌
- 黴漿菌
- 特定病毒

微生物污染

- 種類：細菌、黴菌、酵母菌、病毒、黴漿菌
- 途徑：培養基、血清、空氣、接觸
- 原因：
  - 無菌操作技術不當（細菌）
  - 污染之細胞培養(黴漿菌)
  - 血清（黴漿菌, 病毒）
  - 環境（黴菌）
- 嚴格之無菌操作技術與清潔之環境是減低污染之最好方法

細菌與黴菌污染

- 細菌污染：
  - 徵兆：培養液突然變黃，混濁，細胞脫落
  - 顯微鏡觀察大量微小細菌（細菌已大量產生）
- 酵母菌與黴菌污染
  - 徵兆：培養液突然變黃，混濁，細胞脫落
  - 鏡檢觀察：
    - 酵母菌：串狀卵圓形
    - 黴菌：絲狀、絮狀
  - 常見之污染菌：
    - Penicillium, Aspergillus and Candida等
  - 較長時間才出現污染徵兆，小心 spore 已大量產生!

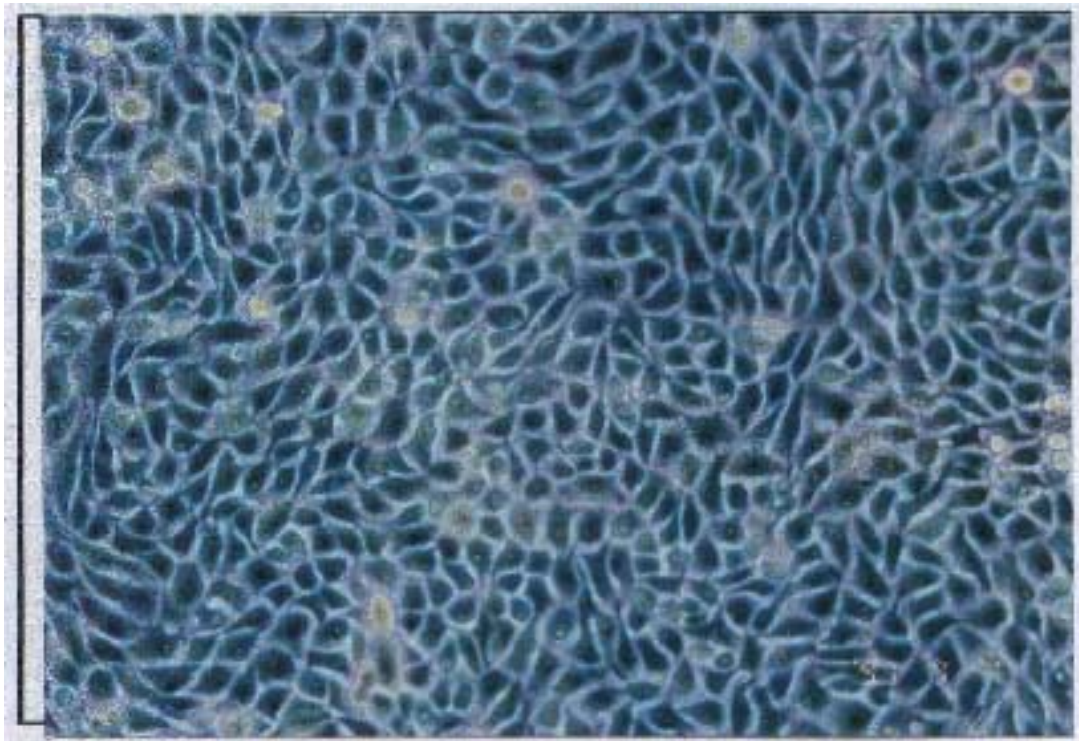
細菌與黴菌之污染偵測

- 測試樣品：
  - 培養中之細胞、培養基、培養液、冷凍細胞等
  - 測試樣品應不含抗生素
- 方法：細菌或黴菌培養基培養2-3 星期
- 結果判讀：agar plate 上有菌落生長或液體培養基混濁

細菌/酵母菌/黴菌之檢測

培 養 基	測試微生物	溫 度	（厭）好氧性	培養時間（天）
Blood agar plate	細菌/厭氧菌	37ºC 37 ºC	Aerobic Anaerobic	14 14
Thioglycollate broth	厭氧菌	37 ºC 26 ºC	Anaerobic	14 14
Tryticase soy broth	細菌	37 ºC 26 ºC	Aerobic	14 14
Brain heart infusion broth	細菌	37 ºC 26 ºC	Aerobic	14 14
Sabouraud broth	酵母菌/黴菌	37 ºC 26 ºC	Aerobic	21 21
YM broth	酵母菌/黴菌	37 ºC 26 ºC	Aerobic	21 21
Nutrient broth w/ 2% yeast extract	細菌/酵母菌/黴菌	37 ºC 26 ºC	Aerobic	21 21

黴漿菌檢測



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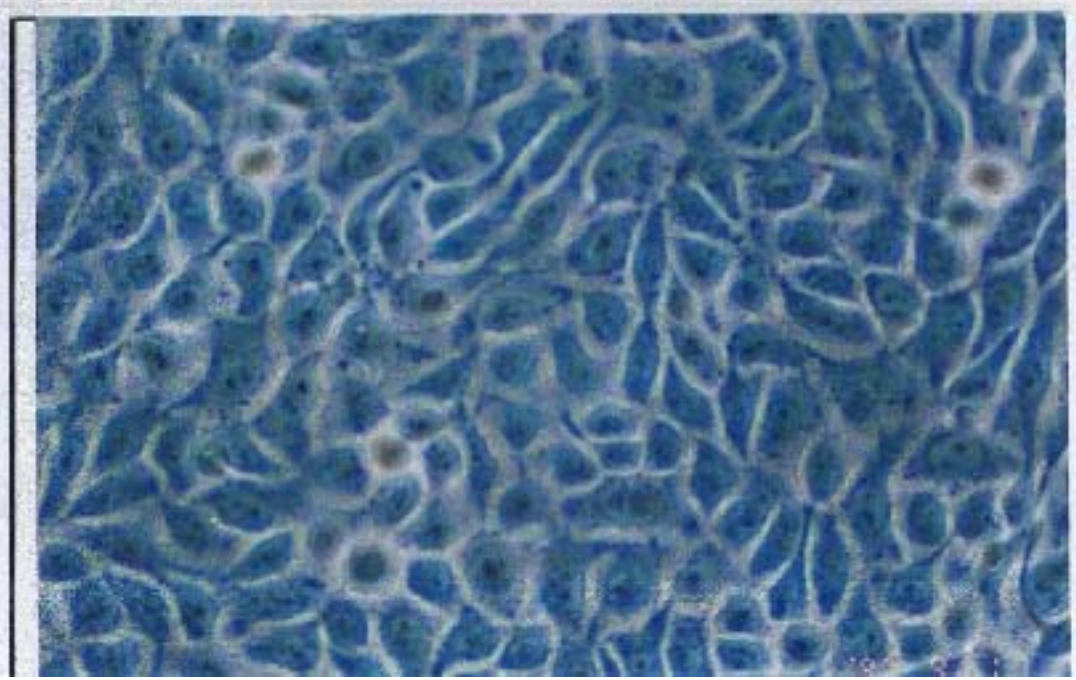
培養天數 / 情形：



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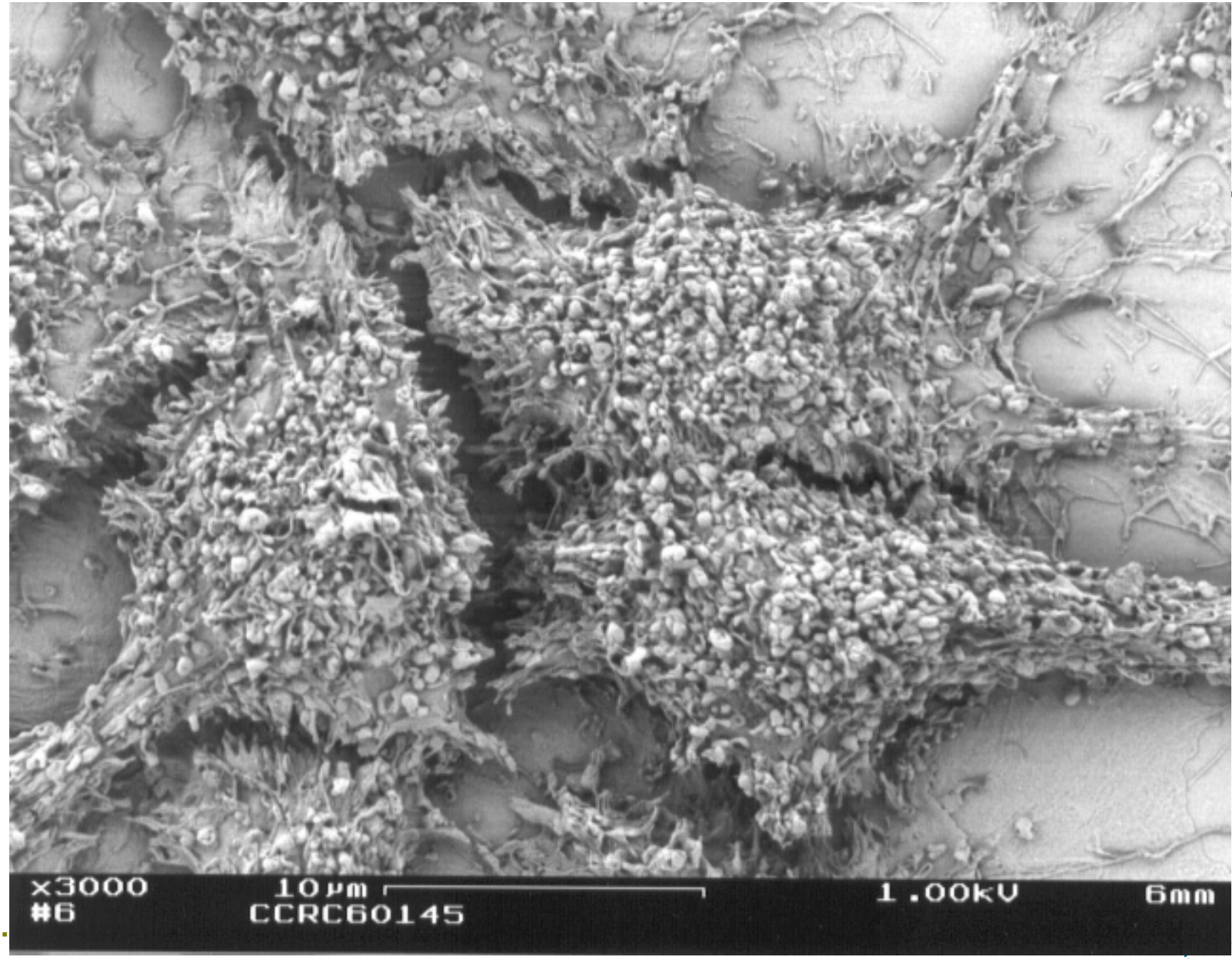

x3000
#6
10μm
CCRC60145
1.00kV
6mm

黴漿菌污染之統計表

	受黴漿菌污染（%）	報告年度
USA--FDA	15	1993(past 30 yr)
USA--ATCC	15-20	1992
Japan—(IFO, RIKEN, JCRB)	80 ~30 22	1981 1987-1993 1998
Germany--DSM	36	1990-1994
Argentina	65	1987
Israel	32	1986-1993
China	95	1990
Taiwan	44	2001

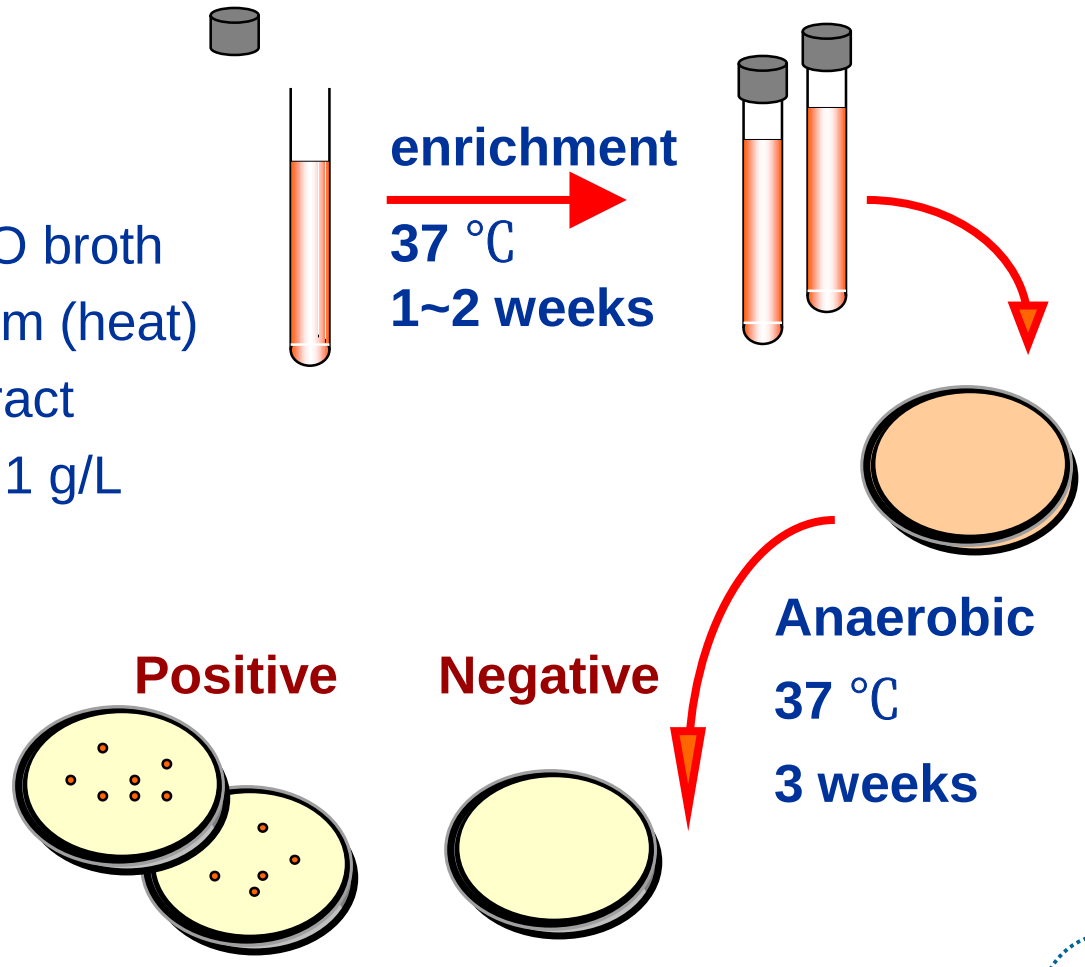
1999-2001 contamination test

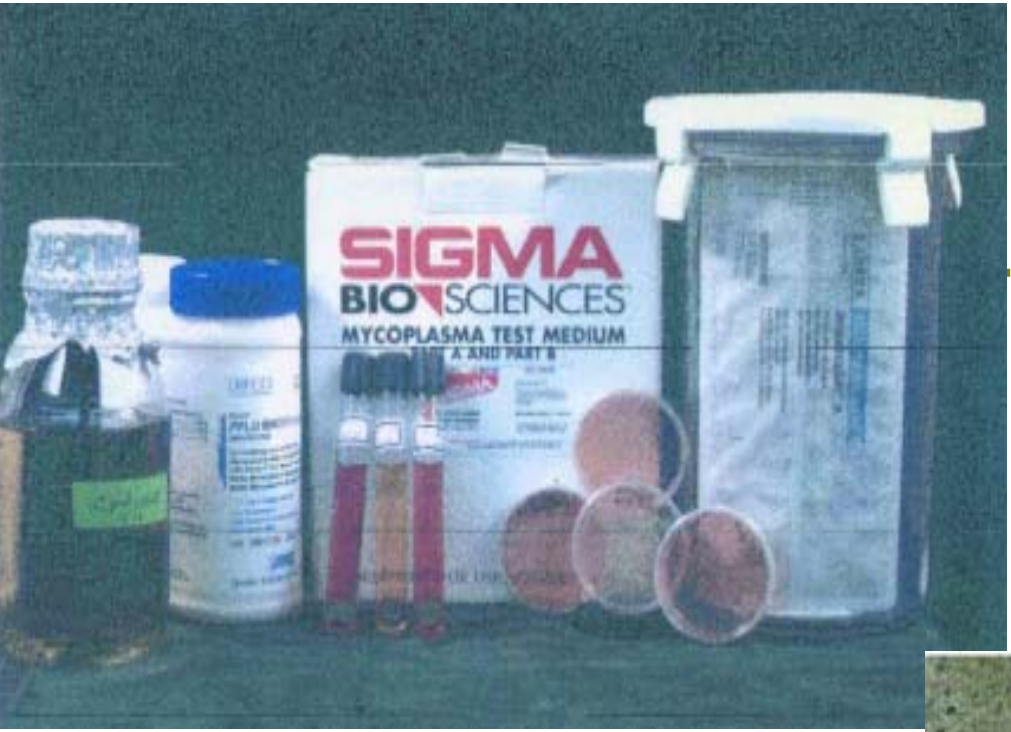
	Number	%
Clean	84	55.6
Mycoplasma (+)	67	44.4
Total	151	100

CCRC 09/2001

Microbiological Culture Method

- Broth culture
  - **supplements:**
    - 60% Difco PPLO broth
    - 20% horse serum (heat)
    - 1.5% Yeast extract
    - L-Arginine.HCl: 1 g/L
    - Dextrose: 5 g/L
    - phenol red
- Agar culture
  - **supplements**
  - **1.5% agar**



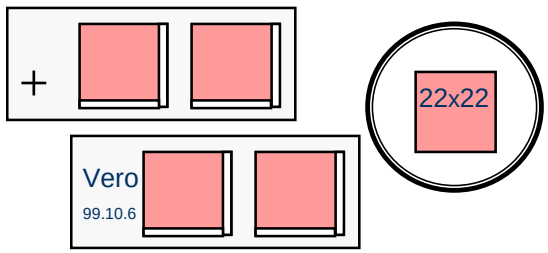

SIGMA
BIOSCIENCES
MYCOPLASMA TEST MEDIUM


Fried-egg

Hoechst DNA Staining

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

Day 1



Indicator cell : **Vero**

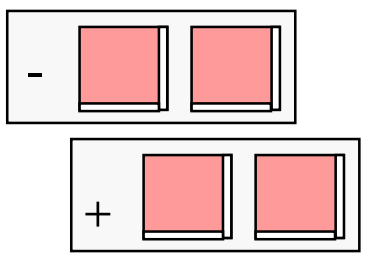
10(4) / well

Test Sample

37 ℃, 5% CO_2

Culture 5 days

Day 6

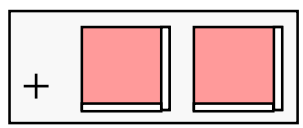


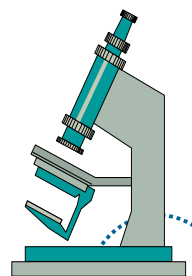
→ Fixation → Stain →

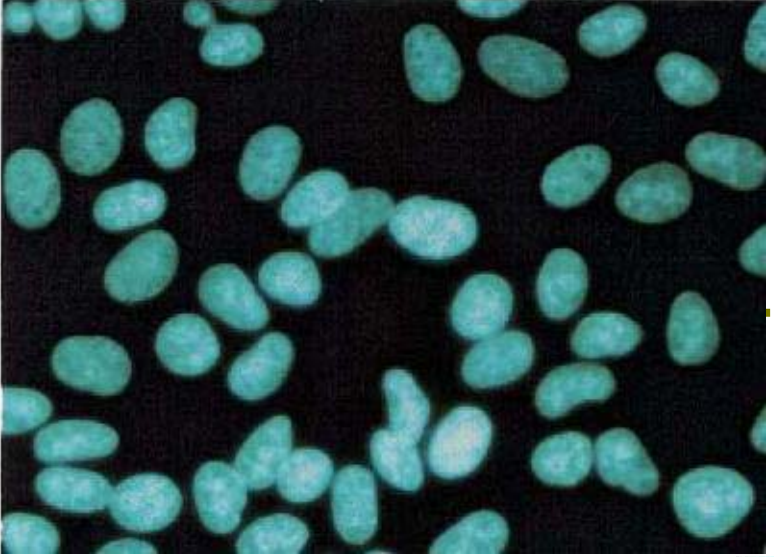
Fluorescent microscope

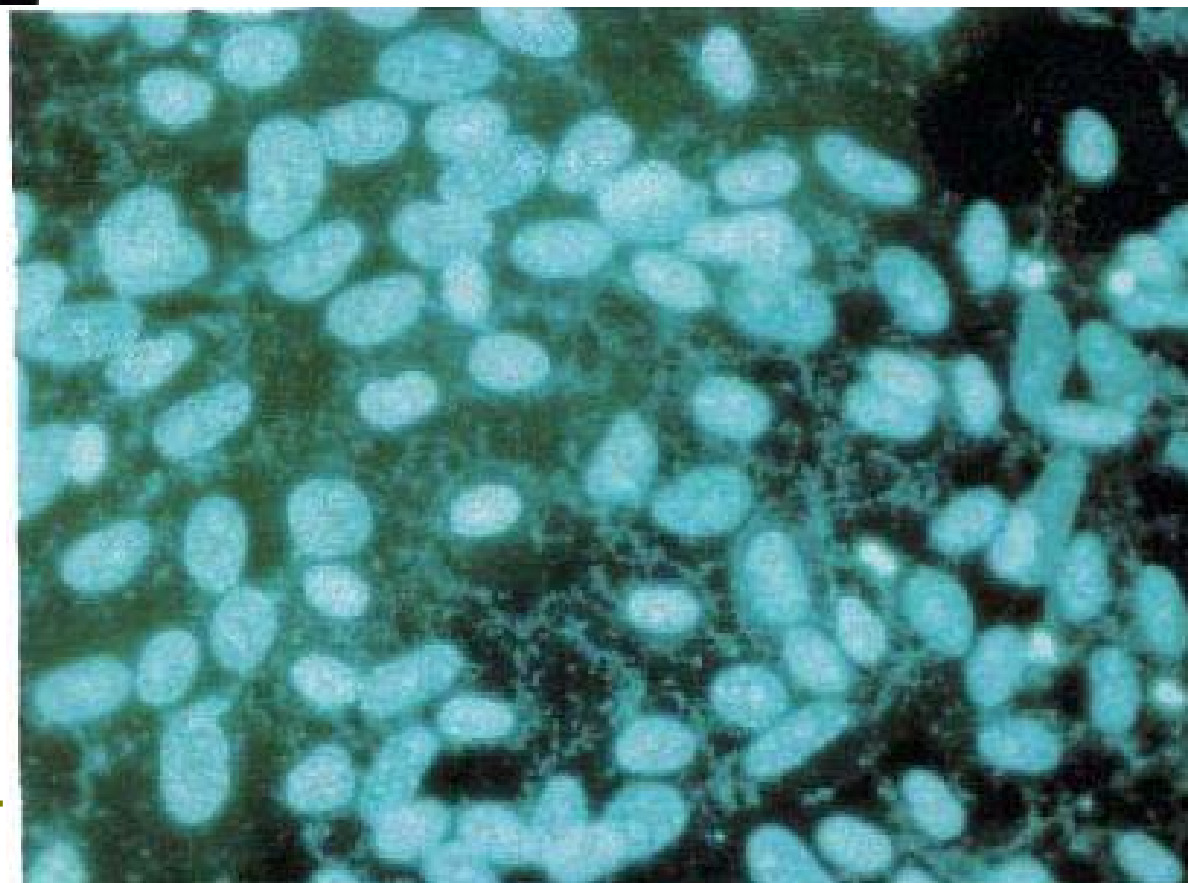
UV 330~380 nm

blue color



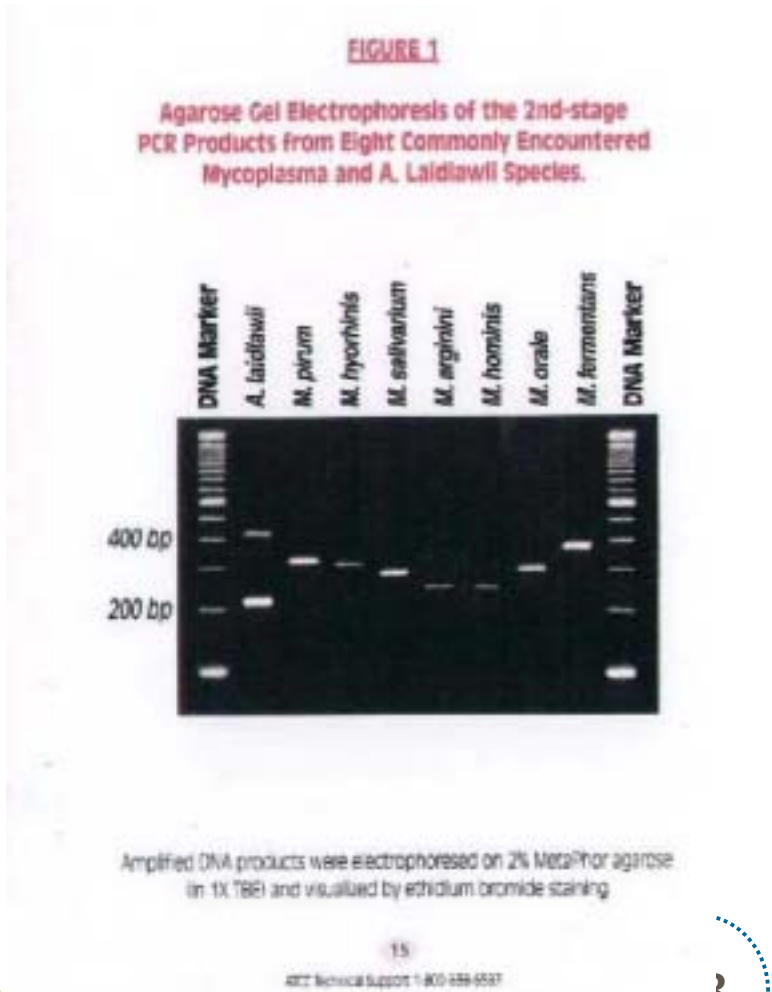


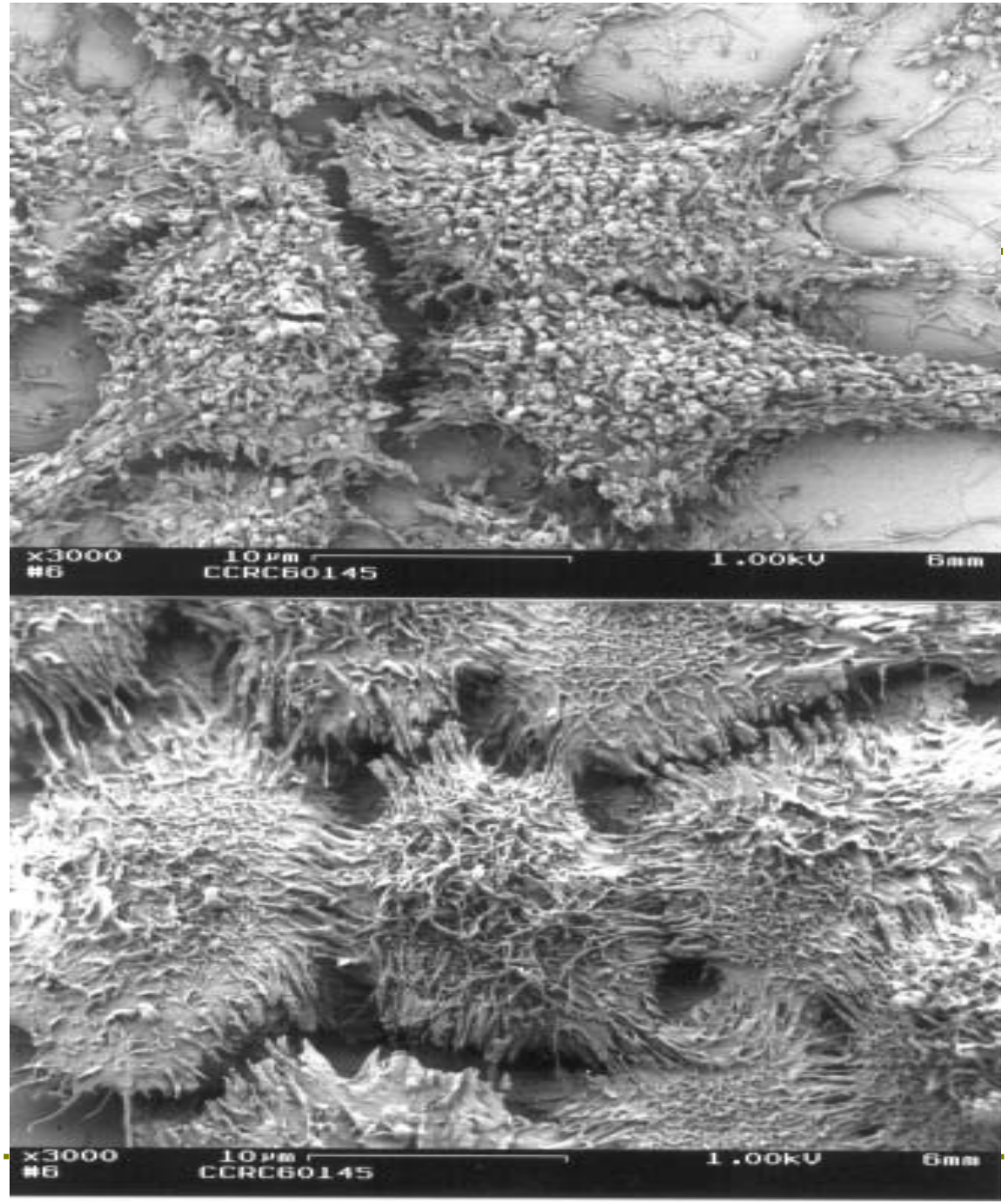




PCR method

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




x3000
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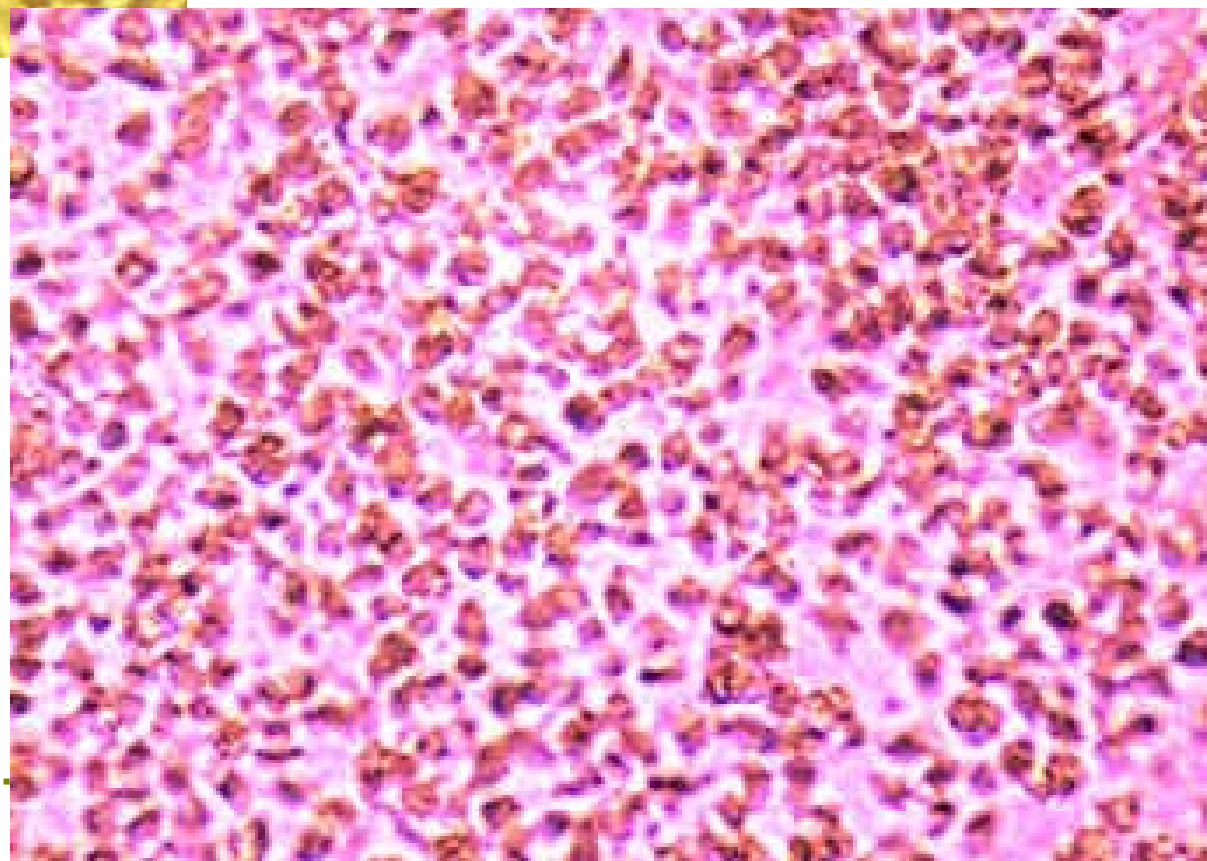
10μm
CCRC60145
1.00kV
6mm
x3000
#6
10μm
CCRC60145
1.00kV
6mm

病毒污染

- 徵兆：
  - cytopathic effect (CPE, 有明顯之形態變化): cell rounding, syncitium formation, vacuolation
  - no cytopathic effect (無明顯之形態變化): 須特殊方法偵測

- 偵測方法：
  - CPE observation
  - Hemadsorption
  - Co-cultivation
    - BHK-21, WI-38, Hela, Vero, MDCK
  - RT assays
  - PCR
  - EM fine structure

CPE of virus infection

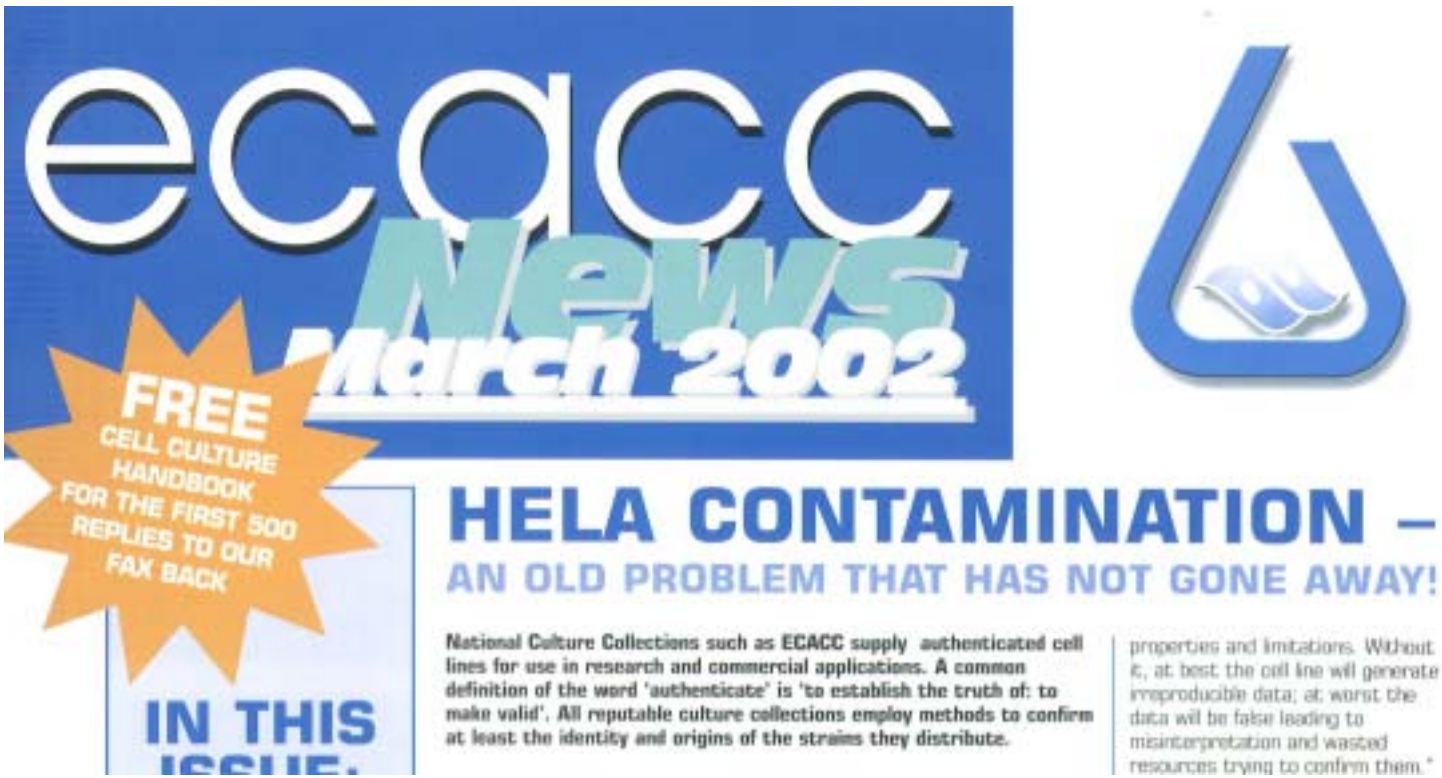


細胞株種間和種內污染報告

Reported cell species	Cultures received	Interspecies	Intraspecies	% Total
Human	160	40(25%)	18(11%)	36
Mouse	27	2	-	8
Cat	24	1	-	4
Others	64	35	-	54
Total	275	78	18	35

from: Eucaryotic cell culture, basics and applications (New York: Plenum Press) pp13-31 (1984)

HeLa 細胞污染


ecacc
News
March 2002
FREE
CELL CULTURE
HANDBOOK
FOR THE FIRST 500
REPLIES TO OUR
FAX BACK
IN THIS
HELA CONTAMINATION –
AN OLD PROBLEM THAT HAS NOT GONE AWAY!
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 limtations. 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."

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.

- Inappropriated Y
  - OV-1063 CRL-2183
  - CHP-234 CRL-2272
  - NCI-H738 CRL-5839
  - NCI-H1514 CRL-5873
  - NCI-H1622 CRL-5880
  - HBL-100 HTB-124
- Stop distribution

細胞株錯誤 (III)

Data from ATCC web.

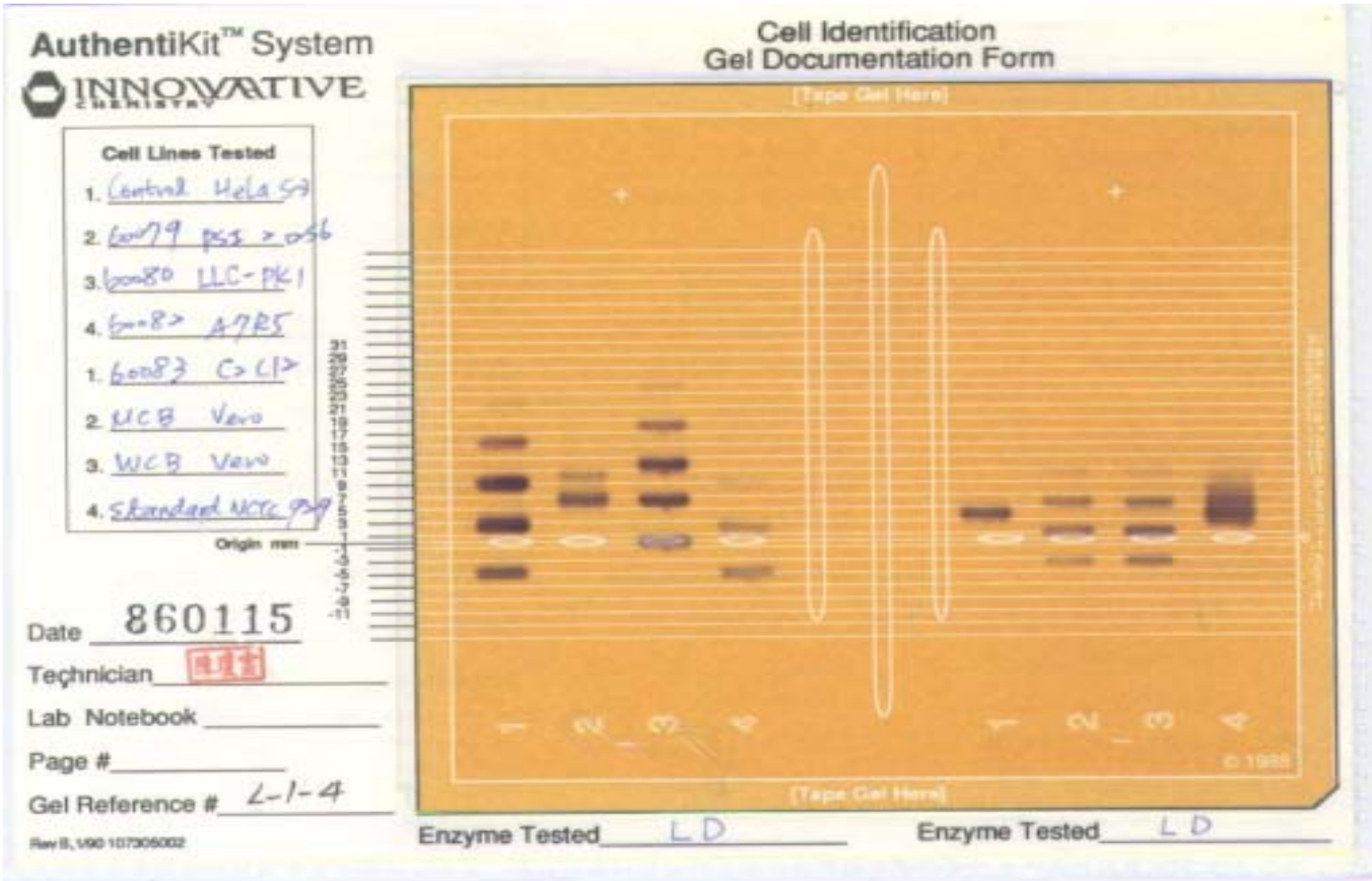
- Identities in question
  - ECV 304 (=T24) endothelium → bladder
  - KSY-1 (=T24) Kaposi's sarcoma → bladder
  - U-373 MG (=U251) glioblastoma
  - U-118 MG (=U138 MG) glioblastoma
  - SNB-19 (=U251) glioblastoma
- Stop distribution, except KSY-1 under patent law

C. 細胞株鑑定與複核

1. *同功酵素電泳分析 (isozyme analysis)*

- 同功酵素: 一群催化同一化學反應，但在結構上或物理特性上有差異的酵素，此種差異一般可藉由電泳加以分離及做活性染色而偵測，可作為種源鑑定或分類之依據

Isozyme analysis

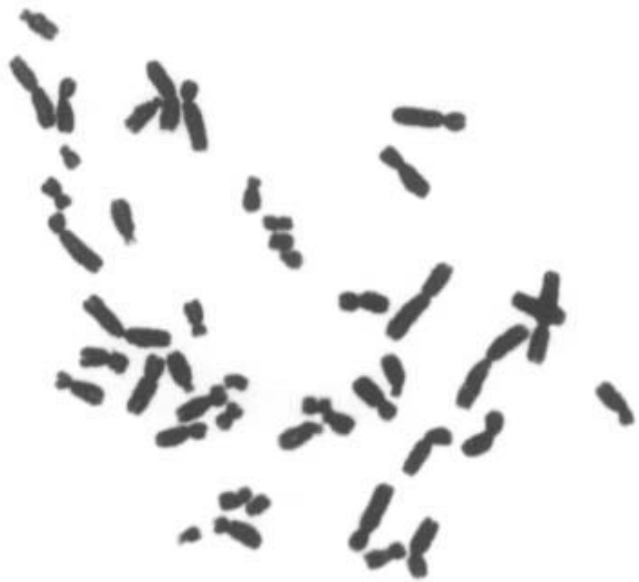


C. 細胞株鑑定與複核

2.染色體核型分析 (chromosome karyotype)

- 以化學藥劑(colchicine/colcemid) 抑制細胞分裂時，紡錘絲之形成而使染色體停留在有絲分裂的中期狀態，經染劑作用，在顯微鏡下觀察，可將染色體依數目、形態、大小等加以區分之技術
- 不同種間細胞的染色體核型一般均有極大之差異
- 同種或類緣關係相近的細胞或腫瘤細胞則須詳細比對染色體的條紋帶(banding)

Simple Giemsa staining

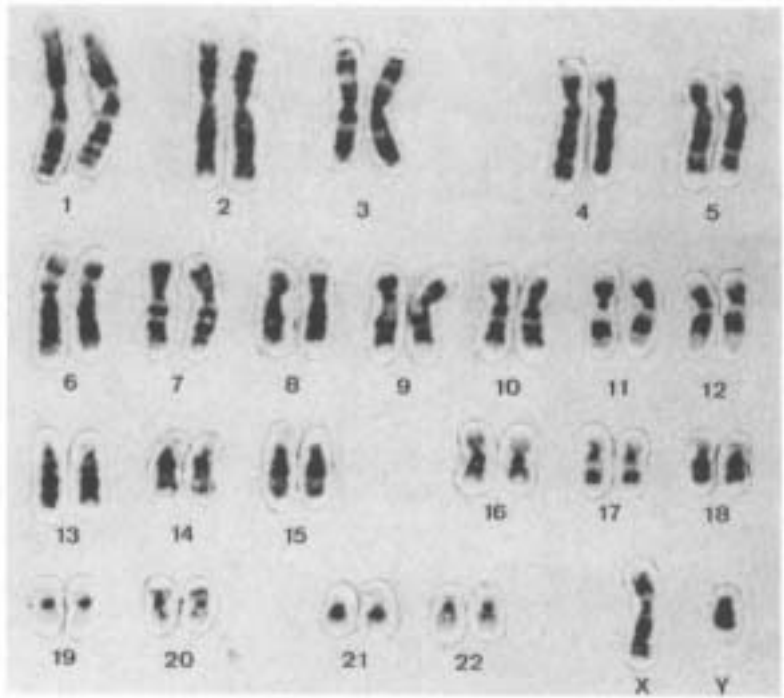


圖四　A.簡易Giemsa染色：人類雙倍體細胞

圖四　B.簡易Giemsa染色：老鼠雙倍體細胞

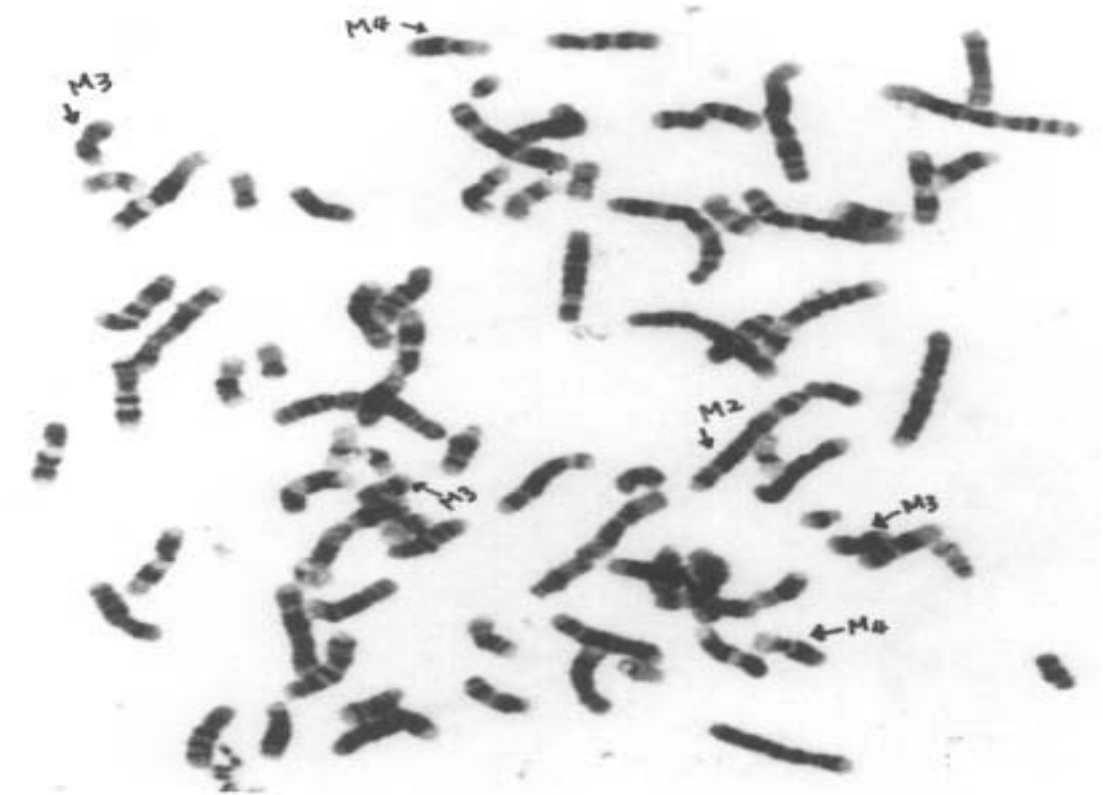
G-banding

人類細胞株MRC-5之染色體條紋染色(G-banding)



圖五[65] G-banding（人類雙倍體細胞）

Marker chromosomes



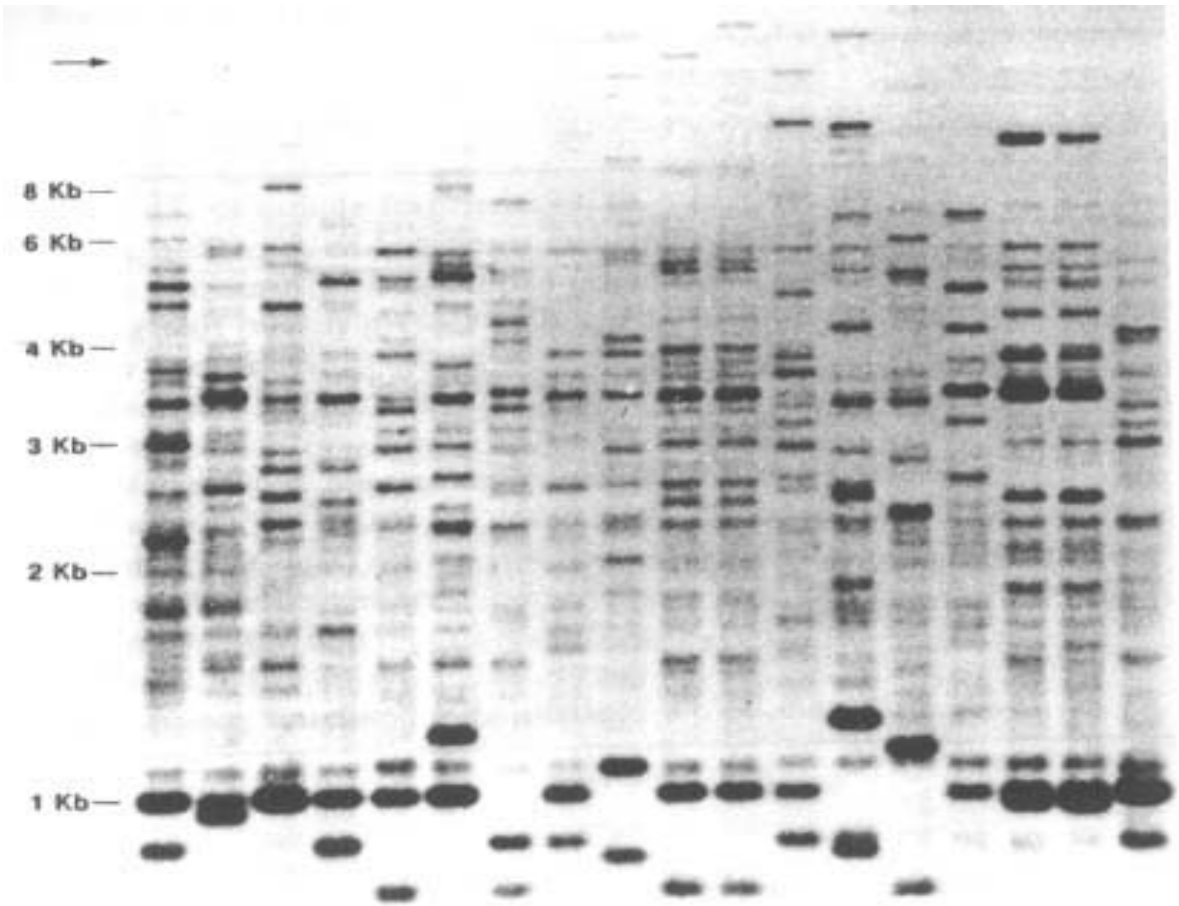
圖六　HeLa細胞之G-banding及marker chromosome

C. 細胞株鑑定與複核

3. DNA指紋分析 (DNA fingerprint)

- 將細胞染色體DNA抽取純化，以特定的核酸剪切酵素作用，經電泳膠體區分並轉漬至nylon membrane，再以特定帶有放射性^{32}P或螢光標記的探針，進行雜交分析，所得到的多樣性條紋圖譜技術

Jeffrey's probes



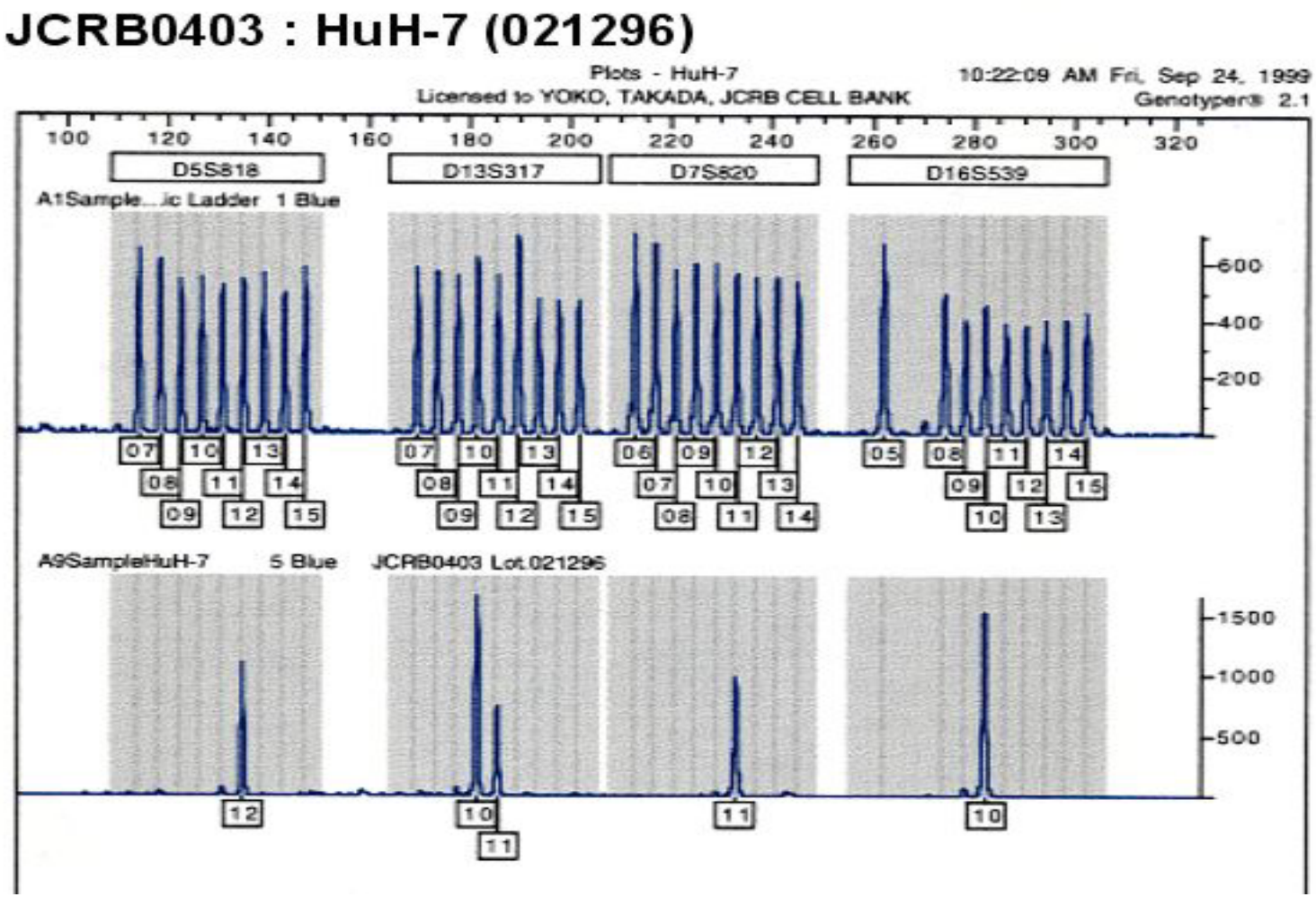
C. 細胞株鑑定與複核

4. PCR指紋分析 (PCR fingerprint)

- 利用數個STR (short tandem repeats)作為多型性指標，進行PCR反應和分析

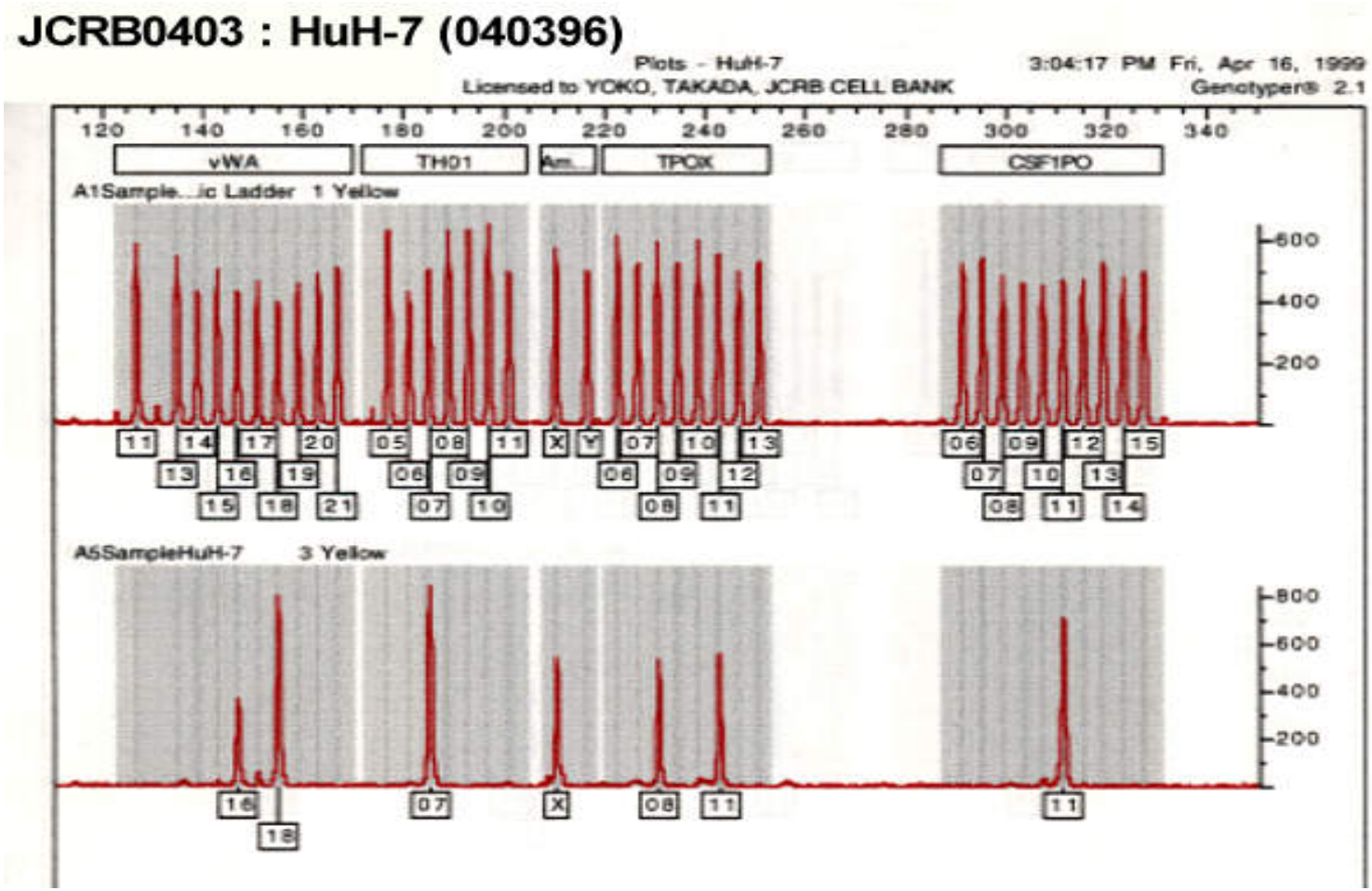
STR PCR analysis (I)

Fig. from JCRB web.

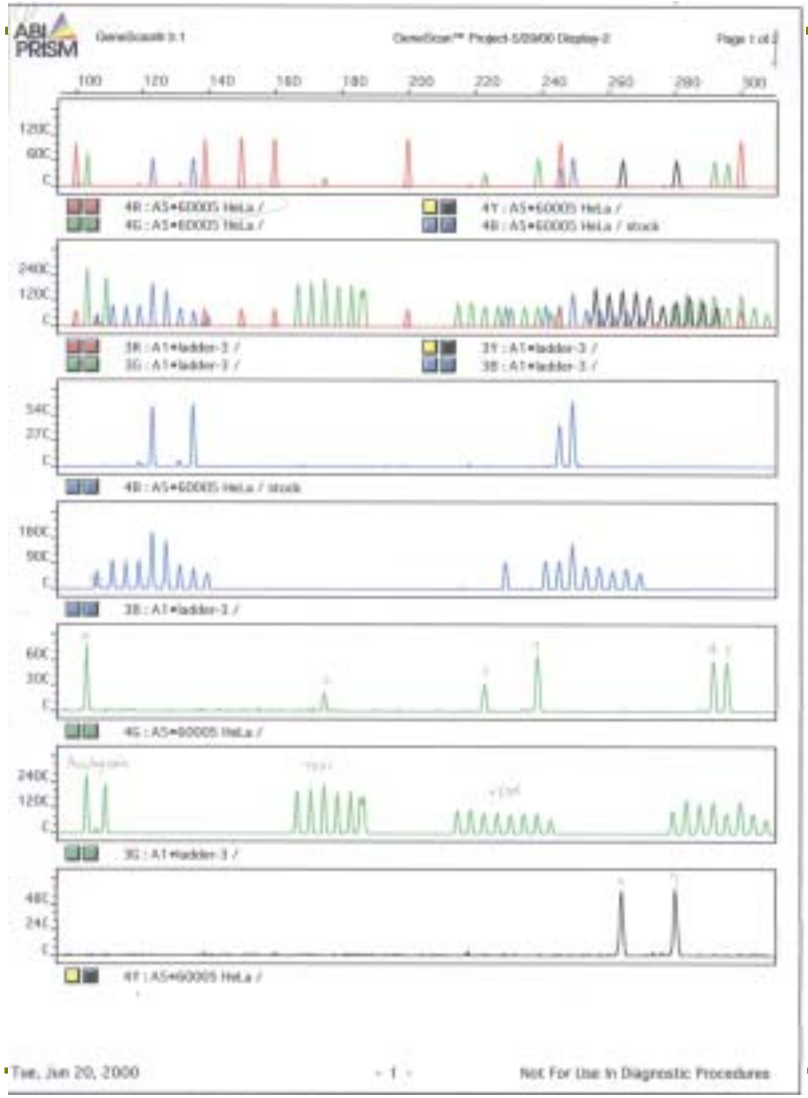


STR PCR analysis (II)

Fig. from JCRB web.



HeLa STR-PCR analysis



CCRC

Issued from BestCERT Quality Registrars Ltd, Fitchburg, MA, 01420, USA

Certificate of Registration

to

ISO 9001:2000(E)

Food Industry Research & Development Institute

No. 331, Shih-Ping Road, Hsinchu City, Taiwan, R.O.C.

The Quality Management System of this company was audited and found to be compliant with the requirements of ISO 9001:2000(E).

Scope of Registration: IAF 39 – (1) Patent-Related Microorganism Depository (2) Contract Microbial Identifications & Tests (3) Cultivation & Preservation of Animal Cells (4) Culture Collection, Preservation & Distribution

Certificate Issue Date: April 27, 2001
Expiration Date: Feb. 14, 2004

Certificate # T2000-416

May 24, 2002

BQR

ANSI-RAB QMS ACCREDITED

Authorized Signature for BestCERT Quality Registrars Ltd.

*Further clarification regarding the scope of this certificate and the applicability of ISO 9001:2000 requirements may be obtained by consulting the organization

Cert ISO 9000/2000 Rev 3-30-01


Thanks for your attention