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Cell Line Banks and Their Role in Cancer Research

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Abstract The utility of centralized cell banks in providing reference cultures for cancer research is reviewed. Procedures applied at The American Type Culture Collection in development, maintenance and expansion of such a resource are discussed for example, with emphasis on human tumor cell lines. The various categories of cell-line holdings are explained, and status with regard both to the numbers of lines available and distribution experienced are documented. The locations of other national cell repositories plus contact data are provided. © 1996 Wiley-Liss, Inc.

Key words: cell bank, authentication, characterization, mycoplasma, virus, contamination, DNA profiling, identification, cell culture collections

The development of a public bank of cell lines is usually the result of an extensive collaboration involving administrators, scientists, and laboratory technical personnel. Cell lines are made available to cell banking institutions through the generosity of originating scientists, most of whom supply the initial culture without charge and with minimal restrictions on distribution. Advisors to the bank give willingly of their time and expertise to ensure that collections are complete and that appropriate characterizations and quality control tests are applied. Collection curators, scientists, and support personnel within the cell banking institutions contribute, not only by expanding, authenticating, cryopreserving, and distributing the cell lines, but also in the compilation and dissemination both of historical information and substantial additional data relating to the seed and distribution stocks. With some programs collection staff are also involved in the development of new cell lines. Enlightened administrators within numerous granting agencies, as well as individuals elsewhere, have recognized the advantages in centralized banks for specific lines, and have garnered together funds to ensure development and maintenance of appropriate collections. Thus the responsibility for both the achievements and the problems associated with function of public reference cell line collections are indeed shared by a great many individuals from within the scientific community.

Numerous occasions where cell lines exchanged among cooperating laboratories have been contaminated with cells of other species have been detailed, documented, and described elsewhere [1]. Similarly, the problem of intraspecies cross contamination among cultured human cell lines has been recognized for over 25 years, and detailed reviews are available on the subject [1,2]. An incidence of cell cross contamination as high as 35% has been reported [3]. The loss of time and research dollars as a result of these problems can never be determined but is certainly extensive.

Bacterial and fungal contaminations represent an added concern. In most, but unfortunately not all instances, they are overt and easily observed. The inclusion of a series of culture tests will permit detection of most common species that will thrive in cell culture systems [4]. Contamination by mycoplasma is a much more serious consequence due to the insidious nature of such infection. The presence of some mycoplasma species may produce cytopathic effects but others may metabolize and proliferate extensively without inducing noticeable morphological change in the host cell culture. Still the unrecognized introduction of such mycoplasmal infection interferes with studies on metabolism, receptors, virus-host interactions, cell division, and so forth, virtually negating research findings entirely. The problem has been emphasized repeatedly over the years [5,6] with test data indicating infection frequencies of 5 to 16% of all

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lines tested [6,7]. Thus the difficulties of detection and prevalence of contaminated cultures in the research community suggest that restatements are warranted.

With regard to cancer research in particular, banking agencies need to consider applying not only standard quality assurance tests such as those for the contaminations mentioned above but also characterizations required somewhat more specifically by the oncologist. Some examples include ability of cancer cells to form colonies in soft agar, tumorigenicity tests, occurrences of specific oncogenes and their chromosomal location(s), and so forth. As always, decisions in this regard will depend upon funds available and the needs of particular cell line recipients.

This article will summarize activities and problems in collection development from the perspective of a national cell bank such as that maintained at The American Type Culture Collection (ATCC, Rockville, MD), with some emphasis on the collection of human lung cancer cell lines. Reference is made to cell line banks in the United States and elsewhere. Their general procedures may vary somewhat from those applied at the ATCC.

MATERIALS AND METHODS

Step-by-step descriptions for most of the methods used at the ATCC for cell banking and characterizations have been published in detail [4,8]. The steps can be summarized under the general headings of acquisition, preservation, authentication, and distribution (ATCC-APAD functions). There are slight modifications in procedure depending upon program requirements, and these are pointed out in the presentation.

Acquisition

Cell lines pertinent for accessioning are selected by ATCC scientists and advisors during regular reviews of the literature. The originators themselves also frequently offer the lines directly for consideration. Expansion, authentication, and distribution of cell lines are very costly processes requiring considerable support from the government and other sources. For this reason evidence of utility and demand for each particular line is needed before it can be accepted. The stringency of this varies somewhat depending upon the program supporting addition of the cell line(s) in question. Required data for submission of lines varies somewhat among banking categories. In general, however, a statement of exact species, strain, source tissue, and precise original and subsequent dissociation procedures, plus indication of the growth medium and supplements chosen, are required. A statement of the specific functional or other characteristic that would make appropriate strains of unique value is also requested. In addition, preference is indicated for inclusion of detailed histories of each strain: information concerning date or origin, sex, race, and age of donor; isolation procedures; inoculation densities used and number of passages undergone as well as indication of the average number of cell generations accrued. Information on karyological analyses and survival potential of the cell lines is also solicited. For human and other animal cancer lines, information on the tumorigenicity, host range, and presence and location of specific oncogenes is of special interest.

Due to the potential danger in the spreading of animal pathogens via cells or culture fluids, the United States Department of Agriculture (USDA) has imposed restrictions on their importation. Cell cultures can only be brought into the United States legally after issuance of an import permit to the importer. In general, there are no restrictions on distribution of cell lines within the United States after legal importation.

It should be emphasized at this point that in those cases where investigators wish to apply for patents associated with cell lines, the forms, steps, and information required are quite different from those for the general or other supported accessioning programs. Individuals or organizations interested specifically in patent deposits should contact the ATCC for further information in that regard.

For all cell cultures except those submitted for patents, decisions are made to proceed with initial authentication after review of the cell line credentials and consultation with advisors (see below).

Preservation

Generally, starter cultures or ampules are obtained from the donor, and progeny are propagated according to instructions to yield the first "token" freeze. Procedures used for the cryopreservation of cell lines have been described in detail elsewhere [4,8]. Briefly, aliquots in 1 ml of the usual growth medium plus 5% dimethyl

sulphoxide as cryopreservative are dispensed to glass or plastic vials. In cases where the line is grown in medium without serum, addition of 10% fetal bovine serum to the freeze medium usually improves recoverability. The vials are sealed well and cooled at 1°C per minute to −40°C in a programmable freezer. Thereafter they are placed directly into liquid nitrogen refrigerators for storage either in the vapor or liquid phase.

Complete information concerning the production of media at the ATCC and testing procedures for serum supplements have also been published [4]. The quality control in this case includes tests for both adventitious organisms and for growth promoting properties of fluids used.

Authentication and Characterization

Cultures derived from the "token freeze" are subjected to critical authentications and if these tests suggest that further efforts are warranted the material is expanded to produce seed and distribution stocks. The major authentication and characterization efforts are applied to cell populations in this seed stock of ampules. The distribution stock consists of ampules that are distributed on request to investigators. The reference seed stock, on the other hand, is retained to generate further distribution stocks as the initial stock becomes depleted. Authentication is considered the act of confirming or verifying the species and identity of a particular cell line. Characterization is the definition of the many traits of the line, some of which may be unique and may thus also serve to identify or authenticate specifically. Characterizations may be performed at the cell bank and by the originating investigators.

Microbial contamination. Bacterial and fungal contamination of cell lines is detected by inoculation of pooled aliquots from about 5% of the stock produced to a series of 7 different media shown to detect most organisms that would be expected to contaminate human and other cell lines. Included are blood agar, thioglycollate broth, trypticase soy broth, brain heart infusion broth, sabouraud broth, YM broth, and nutrient broth with 2% yeast extract. Incubations (14–21 days) are both aerobic and anaerobic at 37° and 26°C [4,8].

Testing for mycoplasma infection routinely includes both direct culture in mycoplasma broth and on mycoplasma agar in an anaerobic jar and application of the "indirect" Hoechst staining procedure followed by examination under a fluorescent microscope. This latter test is performed using an indicator cell line to increase sensitivity. Detailed protocols and rationale have been provided elsewhere [4,8,9]. In equivocal cases the GenProbe kit or PCR [10] tests have been utilized.

Examination of cell lines for inadvertant infection with viruses has included careful scrutiny for overt cytopathogenic effects; hemadsorption and co-cultivation tests; inoculation to chick embryonated eggs; biochemical testing for reverse transcriptase activity and/or a series of screens for specific viruses using fluorescent antibody labelling or hybridization with nucleic acid probes [4,8,11].

Verification of species. Species of origin is verified by cytogenetics and/or isoenzymology for all of the lines referred to in this paper. The Authentikit [12] was used for isoenzyme development in most cases. G- and Q-banding procedures were used for detailed karyotype constructions, and 50 metaphases from conventionally stained preparations were used to determine modal chromosome numbers and to score structural abnormalities. Detailed protocols have been published [4,8].

Cell line individualization. DNA profiling is now being utilized to identify human tumor lines [4,8,13] in addition to karyology which had been performed previously on many of the lines.

1. Single-locus probes and DNA extraction. The human single-locus hypervariable probes, pYNH24 (D2S44) [14], pCMM86 (D17S74) [15], and p79-2-23 (D16S7) [16] are obtained from the ATCC. Genomic DNA is extracted from human cell lines according to standard procedures [17].
2. Southern analysis. Five micrograms of high molecular weight DNAs from human cell lines are digested with *HinfI*. The resulting fragments are separated in a 1% agarose gel, denatured and transferred to a nylon membrane (BioTrace HP). The membrane is washed in 0.1 × SSC for 10 min and prehybridized for 5 h at 65°C in 0.5 M sodium phosphate, pH 7.2; 7% SDS; 1 mM EDTA, pH 8.0; 1% BSA [18] and hybridized 12–17 h at 65°C in the same buffer with the addition of a "cocktail" of three

random-primed ^{32}P-labeled single-locus probes at 1×10^6 cpm/ml. Membranes are washed twice in 2 × SSC, 0.1% SDS at room temperature for 10 min and twice in 0.1 × SSC; 0.1% SDS at 65°C for 15 min and finally rinsed in 2 × SSC at room temperature for 10 min [13] and then exposed to Amersham Hyperfilm-MP (Amersham, Arlington Heights, IL). Image analysis is performed as described below.

3. Digitizing and analysis of films. Exposed autoradiograph films are digitized using a 1,024 × 1,024 pixel camera attached to a SUN SparcStation 2 running BioImage software (Millipore Corp., Bedford, MA). The BioImage software is used to analyze the digitized image, locate bands and calculate fragment sizes. The cell line identifiers, band R_f, and size data are exported from the SparcStation and imported into a database (Paradox 4.5, Borland International, Scotts Valley, CA) on an IBM compatible personal computer (PC). Analysis of the fingerprints is done on this PC with custom software using the data in Paradox tables.

Tumorigenicity. When determined at the ATCC, 10^7 cells of the test line are inoculated at separate sites subcutaneously and intraperitoneally into nude mice. The animals are fed ad libitum and examined biweekly for tumor formation. The occurrence date and size of tumor is recorded or after 2 months the test is considered negative if no growths are observed.

Miscellaneous Tests

Other authentication or characterization steps such as clone forming efficiencies, immunological assays, histopathology, reverse transcriptase quantitation, and so forth are performed as described elsewhere [4,8].

These various steps in the overall accessioning scheme are summarized in Figure 1. The fully characterized seed stock serves as a cryopreserved "reservoir" for production of distribution stocks over the years. Because we return to seed stock ampules to generate new distribution material, we are able to ensure ordering investigators that all the cultures obtained closely resemble those ordered 2, 5, 10, or more years previously. This is a most critical consideration for design of cell banking procedures.

Distribution

Advisory committees and review. The ATCC consistently relies on advice from ad hoc consultants and more formally organized com-

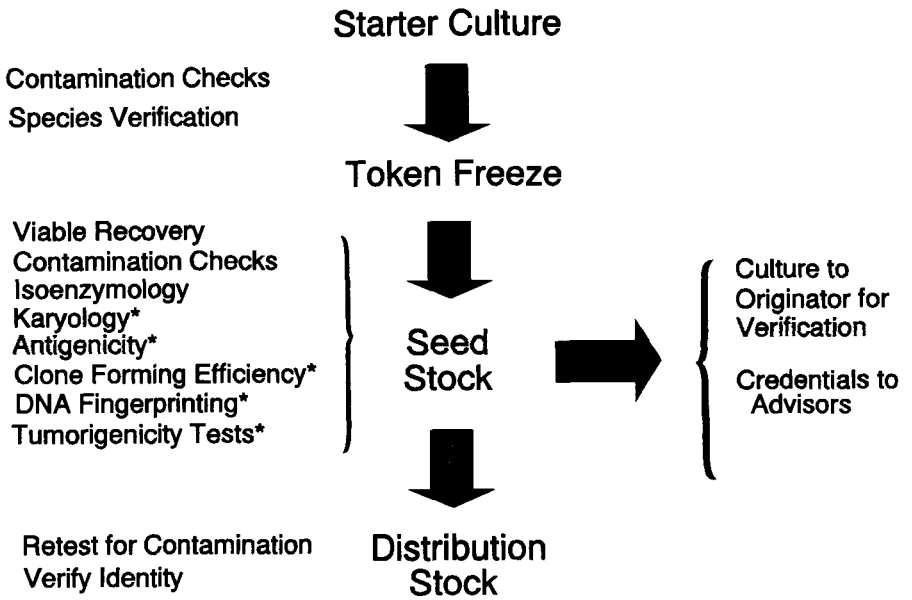

Fig. 1. A scheme illustrating steps recommended for addition of a cell line to a repository. The characterization and authentication steps asterisked on the left hand side will vary depending on the cell lines being added and program support.

mittees. The degree and mode of interaction vary somewhat depending upon the department, disciplines, and source of support for differing accessioning programs. In certification of cell lines, for example, input is requested concerning lines to be added, characterizations to be applied, and descriptions on the cellular material eventually banked. Committee members are often consulted at points during the accessioning procedures and especially for the final certification process. In this case, complete descriptions and pertinent data are circulated for critical review. The cell lines and specific descriptions are considered acceptable if a majority of the committee members indicate concurrence.

A policy of returning both a typical distribution ampule or live culture plus the proposed catalog description and data description on each cell line to its respective donor is followed at the ATCC. The donating scientist is thus given the opportunity to examine material prepared at the ATCC to verify that the essential characteristics are retained. Descriptions on cell cultures are also returned to the donor for suggested additions or revisions.

Cataloging and related means for information dissemination. The ATCC Catalogue of Cell Lines and Hybridomas [19] contains descriptions and data on over 3,200 cell lines from about 80 species. Information is entered on a text editing computer system and is updated periodically. Since 1981, the ATCC has distributed its catalogues and updates to requestors without charge.

A mailing list comprised of 40,000 names of members of the scientific societies represented on the ATCC board of trustees is utilized to communicate directly with members of ATCC's supporting organizations. A quarterly newsletter was initiated in 1981 which contains updated information on new ATCC accessions as well as relevant articles and data. This project has continued and the newsletter is circulated to all individuals indicating interest.

In addition to our printed catalogue, information about all ATCC cell lines is now available through several electronic media. Searchable versions of the catalogue are produced for distribution via diskette, CD-ROM, and various information services.

ATCC databases, updated monthly with data on new cell lines, are searchable from the ATCC home page (http://www.atcc.org). ATCC is now also a node on the Internet through a special arrangement with NIH. A T1 line has been installed connecting ATCC to NIH's NUnet. This enables us to make our databases more widely available to academia and other Internet users.

A CD-ROM version of the catalogue has been published by the Hitachi Corporation (Brisbane, CA). In addition, the ATCC catalogue is also available on computer diskettes for both the MS-DOS and Macintosh operating systems. The diskettes are produced by ATCC staff incorporating a very fast text and easy-to-use search engine (Folio).

Two information specialists are currently retained in the Cell Culture Department to respond to telephone inquires on cell lines available through the ATCC. Problems relating to recovery, characterization, and propagation are discussed directly and appropriate solutions are recommended.

Shipping. Cell cultures are distributed either frozen in 1 ml sealed ampules or as growing populations in 25 cm^2 flasks. Most frozen shipments are sent early in the week to insure arrival before the weekend. Frozen ampules are removed from the distribution stocks and left in a liquid nitrogen refrigerator until ready for packaging. All shipments going by one carrier are then packaged within a short period of time to minimize the exposure to dry ice temperatures. For domestic shipments, cell cultures are shipped in a 2 pound insulated container with a capacity for 6 pounds of dry ice. For foreign shipments insulated containers with 15 pounds of dry ice and a holding time of 5 days are utilized. The shipping of frozen material eliminates quality control problems associated with the preparation of actively growing cultures. The latter involves greater risk of contamination, both from microorganisms and in the cell culture laboratory, and it puts greater pressure on recipients to coordinate their own laboratory work with the suppliers' shipping schedules. Detailed instructions on the handling of frozen cells are provided with each shipment and in the ATCC catalogue.

Actively growing cell cultures in 25 cm^2 flasks are provided by the ATCC when practical, warranted, or requested. These cultures are shipped in the flasks filled with growth medium, well sealed and packaged in sturdy styrofoam shipping containers with liberal cushioning for physical protection and thermal insulation. Flask cul-

tures are generally shipped by Express carrier, and are sent early in the work week to prevent arrival on the weekend.

Cost recovery. The functions required for acquisition, cryopreservation, and authentication of cell lines for distribution (APAD) are complex and costly. The ATCC, like most of the other existing national cell banks, is a non-profit organization which derives its support for these activities from grants and contracts obtained competitively from government and other sources, testing and production services performed for the scientific community, and fees from cultures shipped. In 1992 most ampules of cryopreserved cells ranged in price from sixty-four dollars to one hundred and fifteen dollars. Flask cultures were provided for an additional thirty-five dollars, such surcharge being necessary to offset the added expense of establishing the culture and filling the flask with medium in preparation for shipping.

With very few programs the granting agency supporting APAD functions has elected to provide cultures to the scientific community without charge, and costs are borne by the grant or contract. This is advantageous in that it encourages maximum use of the resource and discourages exchange of cultures which may not be authentic. Some believe, however, that this practice withholds realization from the investigator of the true cost of cell banking and distribution.

RESULTS

Component Collections

The ATCC Cell Repository was developed through support from a number of different sources. This is reflected in part by the prefix given to the various groups of cell lines included in the ATCC catalogue and as listed in Table I. All lines were added as certified cell lines (CCL) during the first eight years of development of the Repository [20]. Final stocks of cells in this category are examined for mycoplasma [4,8,9], bacteria, fungi, protozoa, and cytopathic viruses [4,8]. Recovery is documented by quantitating initial viable cell yields, clone forming efficiencies and propagability over a given period of time which is usually 1 week. The morphology of representative living or stained progeny cultures is recorded and photographed. Detailed karyotypes are constructed [4] for each CCL. The cell line species can also be verified by immunological tests [4] and/or by isoenzyme analyses [4,8]. Other specific characterizations such as tests for surface antigens, tumorigenicity, biochemical traits, drug susceptibility, definition of fine structure, etc., are applied where indicated to verify identities or cell line properties.

The category termed Cell Repository Lines (CRL) includes human fibroblast-like cells from

TABLE I. Categories of Lines in the ATCC Cell Repository (9/93)*

Designation	Description	Comments
CCL[a]	Certified cell lines	Most thoroughly characterized and committee certified
CRL[a]	Cell repository lines	Includes human skin fibroblasts (HSF), human and animal cancer cell lines plus special collections. Determined to be free of microbial contaminants and species verified
HB[b]	Hybridoma	Characterized as for CRL plus isotype of monoclonal released; donor verifies specificity
TIB[c]	Cell lines in tumor immunology bank	Human, murine, and other cell lines; hybridomas characterized as for CRL plus identification and quantitation of immunoglobulins released; various other traits are assessed
HTB[d]	Cell lines in human tumor cell bank	Lines from human tumors and normal tissues; characterized as for CRL plus various other traits (karyology, isoenzymology, tumorigenicity, etc.)

*Patent cultures have been added as CCL, CRL, or HB lines depending somewhat upon time of deposit and nature of the line. Restricted deposits are banked in strict confidence until the patent is granted. CCL = certified cell lines; CRL = cell repository line; HB = hybridoma bank; TIB = tumor immunology bank; HTB = human tumor cell bank. Cell lines with the prefixes shown have been added and distributed through support from the following government grants and contracts: [a]NO1-RR-2-2105 (NIH); NO1-HR-6-2930 (NHLBI): R26-CA25635 (NCI); [b]NO1-AI 90511 (NIAID); [c]NO1-CB-15533 (NCI); [d]NO1-CB-71014 (NCI).

skin biopsies of normal and abnormal individuals plus a variety of other useful cell types such as human and other animal tumor lines pertinent for cancer research. Special collections of lines such as those developed at the Naval Biosciences Laboratory in California [19] and many of the holdings in the collection of human lung cancer lines developed by A. Gazdar, H. Oie, J. Minna, and associates [21], have been added in this grouping.

The Cell Repository Lines are tested for contaminating microbial organisms [4], the cell line species of origin is verified [4] and recovery from liquid nitrogen storage is documented. We consider these to be the absolute minimum characterizations required before any line should be released from a reputable cell bank for use by the research community. For many lines, as indicated in the catalogue, no additional characterizations are applied. However, in selected cases and where essential, additional tests are performed and results are indicated in the description. For example, ATCC has been performing karyological analyses on the human tumor lines for CRL holdings as time and funding permit. These are considered especially necessary to ensure against intraspecies cross contamination where the karyotype has not been described previously. For similar reasons, the individual identities of lines from the Gazdar-Minna collection have been verified by DNA fingerprinting.

TABLE II. Quantitative Composition of the ATCC Cell Repository (9/93)

Category	No. lines	% of total
Certified cell line (CCL)	249	18.1
Human skin fibroblasts (CRL-HSF)	201	14.5
Cell repository line (CRL)	377[a]	27.1
Tumor immunology bank (TIB)	193	13.8
Human tumor cell bank (HTB)	154	11.1
Hybridoma bank (HB)	215	15.4
Total	1,389[a]	100

[a]Cell lines (over 1,900) in various special collections are not included in this total.

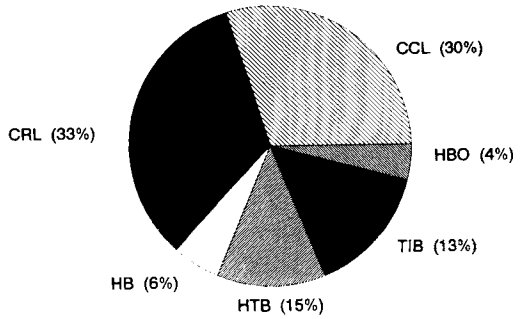

Fig. 3. Distribution by ATCC of cell cultures by category during 1992. A total of 55,156 cell lines were supplied during the year.

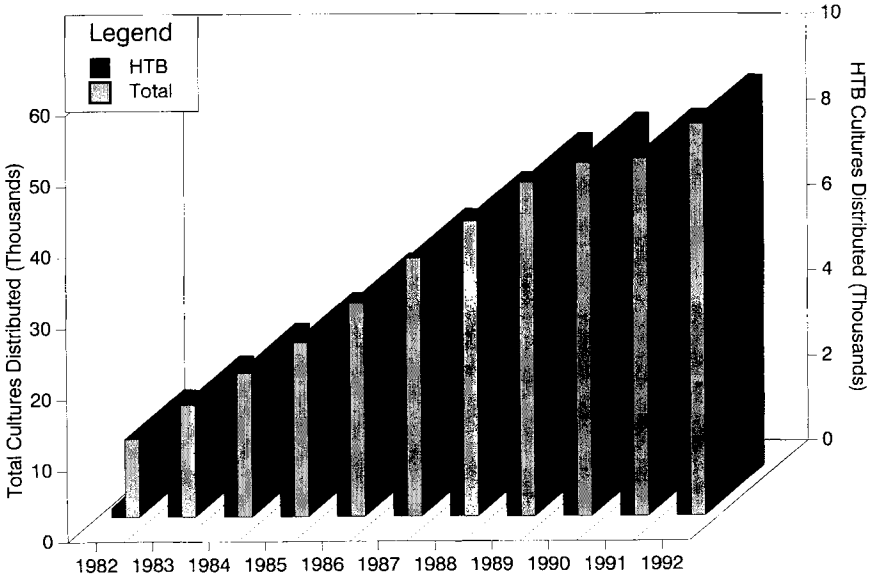

Fig. 2. Distribution of cell lines by The ATCC each year from 1982 through 1992. The dark bars show number of cultures distributed from the Human Tumor Cell Line Bank per year while the dotted bars show total cell cultures distributed per year.

TABLE III. Human Lung Tumor Cell Lines*

Designation	ATCC number	Age	Sex	Race	Diagnosis	Source	Medium[a]	PFP[b]	Comments
A-427	HTB 53	53	M	W	Carcinoma	Primary	EM10	0.00006	Tumorigenic; hypotriploid
A-549	CCL 185	58	M	W	Carcinoma	Primary	F12K/10	ND	Enzymes related to surfactant synthesis studied; lamellar inclusions but sparse
Calu-1	HTB 54	47	M	W	Epidermoid carcinoma, grade III	Metastasis to pleura	MC10	0.0359	Tumorigenic in nude mice and hamsters; hypotriploid
Calu-3	HTB 55	25	M	W	Adenocarcinoma	Metastasis to pleura	EM10	0.0377	Differentiated adenocarcinomas develop in nude mice; hypotriploid
Calu-6	HTB 56	61	F	W	Anaplastic carcinoma	Primary	EM10	0.0031	Poorly differentiated carcinomas in nude mice; hypotriploid
NCI-H69	HTB 119	55	M	W	SCLC	Pleural fluid	R10	0.00006	Tumorigenic and grows in soft agar; sub-tetraploid with 3p deletion; APUD characteristics
NCI-H82	HTB 175	40	M	W	SCLC	Pleural fluid	R10	0.0082	Tumorigenic; near triploid, no Y; high c-myc DNA and RNA, reduced amount and abnormal p53 mRNA (3.7 kb)
NCI-H128	HTB 120	60	M	B	SCLC	Pleural fluid	R10	0.0078	Tumorigenic and grows in soft agar; triploid, APUD characteristics
NCI-H146	HTB 173	59	M	W	SCLC	Pleural	R10	0.0009	Tumorigenic; near triploid; high c-myc mRNA but no gene amp.; elevated biochemical markers for SCLC; keratin and vimentin +'ve
NCI-H209	HTB 172	?	M	W	SCLC	Bone marrow	IMDM 10	0.0624	Tumorigenic; hyperdiploid, no normal B group; elevated biochemical markers; reduced p53 mRNA
NCI-H292	CRL 1848	32	F	B	Pulmonary mucoepidermoid carcinoma	Cervical node	R10	ND	Tumorigenic; near diploid, no N1 or N6; keratin and vimentin +'ve; supports HBV replication
NCI-H345	HTB 180	64	M	W	SCLC	Bone marrow	H1–2	0.0223	Tumorigenic; hypotriploid, no N13; normal levels of p53 mRNA; elevated levels of SCLC biochemical markers

TABLE III continues on next page

TABLE III. Human Lung Tumor Cell Lines (continued)*

Designation	ATCC number	Age	Sex	Race	Diagnosis	Source	Medium[a]	PFP[b]	Comments
NCI-H441	HTB 174	?	M	?	Pap.AC	Pericardial fluid	R10	0.0472	Hyperdiploid; grows in soft agar; SP-A+, Clara and lamellar inclusions
NCI-N446	HTB 171	61	M	W	SCLC	Xenograft	R10	0.1015	Hypertriploid, no N1, N13; high c-myc DNA and RNA
NCI-H460	HTB 177	?	M	?	LCLC	Pleural fluid	R10	0.0472	Tumorigenic and grows in soft agar; hypotriploid, no N9; normal levels of p53 mRNA; keratin and vimentin +'ve
NCI-H520	HTB 182	?	M	?	SqCA	Lung	R10	0.0703	Tumorigenic and grows in soft agar; hypotriploid, no Y or D group; reduced p53 mRNA; keratin and vimentin +'ve
NCI-H596	HTB 178	73	M	W	Asq.LC	Chest wall	R10	0.0362	Tumorigenic; near triploid, no Y; normal levels of p53 mRNA; keratin, vimentin, and involucrin +'ve
NCI-H661	HTB 183	43	M	W	LCLC	Lymph node	R10	0.1015	Hyperhexaploid, no N2, N4 or N9; normal levels of p53 mRNA; keratin and vimentin +'ve
NCI-H676B	HTB 179	63	M	W	AdCA	Pleural fluid	A4	0.000025	Hypodiploid; mucin +; abnormal size p53 mRNA (2.3 kb)
NCI-H820	HTB 181	53	M	W	PapAC	Lymph node	A4	0.0037	Near triploid; produces lamellar bodies and surfactants SP-A, B, and C
SK-LU-1	HTB 57	60	F	W	Adeno-carcinoma	Primary	EM10	0.00003	Tumorigenic in immuno-tolerant rats; hypotetraploid
SK-MES-1	HTB 58	65	M	W	SCLC	Pleural effusion	EM10	0.0132	Hypotriploid with 17–20 markers
SW-900	HTB 59	53	M	W	SCLC	Primary	L15/10	0.0004	Tumorigenic in nude mice; hypotriploid

*See [19] for originators, references, and more detail.

[a]Abbreviations for medium used (on the left-hand side) include: EM, Eagle's minimum essential medium; R, RPMI 1640; DM, Dulbecco's modification of Eagle's medium; F12 and F12K, Ham's medium and Kaighn's modification, respectively; IDM, Iscove's modification of Eagle's medium; L15, Leibovitz medium; MC, McCoys 5A medium; AMEM, The Alpha modification of Eagle's medium [see reference 19 for formulae]. The number on the right-hand side indicates percentage of serum (usually fetal bovine) used. Additional recommended ingredients are indicated by the "+" sign.

[b]PFP = phenotype frequency product. The isoenzyme systems used in this calculation vary somewhat with program. For identical systems the product gives an indication of potential relatedness between cell lines. See [3, 4, 8, 22] for more detail.

TABLE IV. New Human Tumor Cell Lines Offered

ATCC designation	Number	Age	Sex	Race[a]	DX[b]	Primary	Site	Medium[c]	Subtype	Comments
H23	CRL 5800	51	M	B	NSCLC	Lung	Lung	R5	Adenocarcinoma	p53 mutation codon 246 K-ras 12 mutation
H125	CRL 5801	61	M	B	NSCLC	Lung	Subcutaneous nodule	R5	Adenosquamous	Expresses multiple markers of squamous differentiation
H157	CRL 5802	59	M	W	NSCLC	Lung	Pleural effusion	R5	Squamous	Expresses multiple markers of squamous differentiation
H1299	CRL 5803	43	M	W	NSCLC	Lung	Lymph node	R5	Large cell	Partial homozygous deletion of p53 gene: lacks protein expression
H187	CRL 5804	47	M	W	SCLC	Lung	Pleural effusion	R5	Classic	Expresses N-myc, but is not amplified
H322	CRL 5806	52	M	W	NSCLC	Lung	Lung	R5	Bronchioloalveolar	Clara cell granules with expression of surfactant protein A. Chemotherapy
H358	CRL 5807	?	M	?	NSCLC	Lung	Lung	R5	Bronchioloalveolar	Complete homozygous deletion of p53 gene: lacks p53 protein; Clara cell granules
H378	CRL 5808	66	F	W	SCLC	Lung	Pleural effusion	R5	Classic	L-myc amplified. Chemotherapy
N417	CRL 5809	?	F	A	SCLC	Lung	Lung	R5	Variant	c-myc amplified. Contains mouse xenotropic retrovirus
H522	CRL 5810	60	M	W	NSCLC	Lung	Lung	R5	Adenocarcinoma	p53 mutation codon 191 K-ras 12 mutation
H526	CRL 5811	55	M	W	SCLC	Lung	Bone marrow	R5	Variant	Variant cell line, N-myc amplified
H660	CRL 5813	63	M	W	EPUSCNE	Prostate	Lymph node	HI-0	ExPulm	

TABLE IV continues on next page

TABLE IV. New Human Tumor Cell Lines Offered (continued)

ATCC designation	Number	Age	Sex	Race[a]	DX[b]	Primary	Site	Medium[c]	Subtype	Comments
H727	CRL 5815	65	F	W	Carcinoid	Lung	Lung	R5	Carcinoid	Best differentiated of available bronchial carcinoid lines
H810	CRL 5816	51	M	B	NSCLC-NE	Lung	Lung	HI-2	Large cell	Non-small cell with neuroendocrine markers
H889	CRL 5817	69	F	W	SCLC	Lung	Lymph node	R10	Classic	p53 mutation codon 242 K-ras 12 mutation
H1155	CRL 5818	36	M	W	NSCLC-NE	Lung	Lymph node	A4	Large cell	Non-small cell with neuroendocrine markers
H1404	CRL 5819	48	M	W	NSCLC	Lung	Lymph node	A4	Papillary adenocarcinoma	Clara cell granules with expression of surfactant proteins A and B
H1688	CCL 257	50	M	W	SCLC	Lung	Liver	R10	Classic SCLC	

[a]Race: A = Asian; B = Black; W = White.
[b]Diagnosis: SCLC = small cell lung carcinoma; NSCLC = non-small cell line carcinoma; NE = neuroendocrine; EPUSC = extrapulmonary small cell carcinoma. No patient was undergoing treatment except where noted under Comments.
[c]Medium: R5 = RPMI 1640, 5% FBS; HI-0 = HITES no serum HI-2 2% FBS; A4 = [See reference 19 for formulae].

There are no restrictions on distribution of cell lines *when intended for research use* but PHS agreements or USDA permits are required in some cases, and requestors of human lines must sign a document indicating acceptance of all risks.

Development of the hybridoma cell bank began in 1980 under support from the National Institute of Allergy and Infectious Diseases (NIAID). Lines added to this component of the collection (prefix HB) are characterized as described above for CRL holdings. In this program the standard ATCC fee which is charged for each culture is used to offset government costs. If the program on recompetition were transferred to another institution, the ATCC might elect, with concurrence of each donor, to retain specific lines for distribution but this cannot be guaranteed.

In addition to the minimal characterization tests the isotypes and quantities of immunoglobulins released by HB lines are determined. Note that hybridomas producing monoclonals outside the realm of interest to NIAID may be included in the CRL category or in the Tumor Immunology Bank (TIB, see below) with similar characterizations. The bank of cell lines for studies on tumor immunology (prefix TIB) and the human tumor cell line bank (prefix HTB) were supported initially by contracts from the National Cancer Institute (NCI) initiated in 1981. In 1986 and 1991, respectively, the two programs were relinquished to the ATCC to be maintained under fee income and general support from NIH. Lines in the HTB category have been characterized to varying extents. In most cases details on patient history and on cell line morphology, karyology, tumorigenicity, and isoenzyme profiles are available. Additional information such as tumor morphology, cell line ultrastructure, and HLA profiles are provided in some cases. Lines in the TIB group include murine or human myelomas, lymphomas, leukemias, fibroblasts, monocyte-macrophages, or hybridomas (mostly mouse-mouse or rat-mouse). A minority are from other species. The lines are character-

TABLE V. Representative Human Tumor Cell Lines (1/93)*

Tissue/tumor (no. available)	Designation	ATCC no.	Age	Sex	Race	Diagnosis	Source	Medium[a]	PFP[b]	Comments
Adrenal (2)	SW-13	CCL 105	55	F	W	SCC	Cortex	L15/10	ND	Grade IV adenocarcinoma, gap junctions
	NCI-H295	CRL 10296	48	F	B	Invasive carcinoma	Cortex	R2+	ND	Produce steroids; tumorigenic
Bladder (9)	RT4	HTB 2	63	M	W	Transitional cell, papilloma	Primary	MC10	0.0050	Tumorigenic; well-differentiated HLA-I and II
	HT-1376	CRL 1472	58	F	W	Transitional cell; Grade III, invasive	Primary	EM10	ND	Tumorigenic; colonies in soft agar; not treated
	UM-UC-3	CRL 1749	?	M	?	Transitional cell	Primary	EM10	ND	Tumorigenic
Bone marrow (5)	IM-9	CCL 159	?	F	W	Multiple myeloma	Bone marrow	R10	0.0077	B-lymphoblastic cell synthesizes IgGk. Receptors for hGH, insulin, and calcitonin
	KG-1	CCL 246	59	M	W	Acute myelogenous leukemia	Bone marrow	IDM20	0.0049	Responds to CSF forming colonies in soft agar; differentiates to macrophages; near diploid
	RS4;11	CRL 1873	32	F	W	Acute leukemia	Bone marrow	AMEM10	ND	Has t (4;11) (q21;q23) and isochromosome q 7; lacks T/B cell markers; strong terminal deoxynucleotide transferase (TdT)
Brain (7)	A-172	CRL 1620	53	M	?	Glioblastoma	Primary	DM10	ND	Non-tumorigenic; poor colony formation in soft agar; inversion (9) (p11q34); translocation (9:19)
	U-87MG	HTB 14	44	F	W	Glioblastoma, Grade III	Primary	EM10	0.0017	Tumorigenic, hypodiploid
Breast (26)	BT-20	HTB 19	74	F	W	Typical Grade II adenocarcinoma	Primary	EM10	0.0115	Tumorigenic, hyperdiploid
	MCF-7	HTB 22	69	F	W	Adenocarcinoma	Pleural effusion	EM10+I	0.0154	Estrogen receptors, bcl-1 mRNA, differentiated, hypertriploid
	SK-BR-3	HTB 30	43	F	W	Adenocarcinoma	Pleural effusion	MC10	0.0044	Tumorigenic, poorly differentiated microvilli, desmosomes
Bronchus (1)	ChaGo K-1	HTB 168	45	M	?	Undifferentiated carcinoma	Subcutaneous metastasis	R10	ND	Secretes α-hCG estradiol and progesterone
Cervix (12)	HeLa	CCL 2	31	F	B	Adenocarcinoma	Primary	EM10	0.0017	First human line; widely studied; G6PD A 4 marker chromosomes
	CaSki	CRL 1550	40	F	W	Epidermoid carcinoma	Metastasis to mesentry	R10	ND	Secretes β-hCG
	ME-180	HTB 33	66	F	W	Invasive SCC	Metastasis to omentum	MC10	0.0098	Tumorigenic; desomomes hypotriploid, XXX
Colon (24)	HT-29	HTB 38	44	F	W	Well-differentiated Grade II adenocarcinoma	Primary	MC10	0.0230	Tumorigenic; hypertriploid 17 marker chromosomes
	Caco-2	HTB 37	72	M	W	Adenocarcinoma	Primary	EM20	0.0187	Hypertetraploid; exhibits enterocyte differentiation; tumorigenic
	SW480	CCL 228	50	M	W	Grade III–IV adenocarcinoma	Primary	L15/10	0.0229	Tumorigenic; K-ras codon 12
Duodenum (1)	HuTu80	HTB 40	53	M	W	Adenocarcinoma	Primary	EM10	0.0017	Tumorigenic; forms well-differentiated papilloma; pseudo-diploid
Embryonal carcinoma (4)	Tera 1	HTB 105	47	M	W	Seminoma	Metastasis to lung	MC10	0.0004	Not tumorigenic; bcl-1 mRNA
	NTERA-2	CRL 1973	22	M	W	Testicular carcinoma	Metastasis to lung	DM10	ND	Clone of Tera-2; pluripotent; differentiates on exposure to RA

Endometrium (5)	AN3 CA	HTB 111	55	F	W	Adenocarcinoma	Metastasis to lymph node	EM10	0.0054	Yields malignant, undifferentiated tumor
	KLE	CRL 1622	64	F	W	Poorly differentiated adenocarcinoma	Primary	DM/F12-10	ND	Tumorigenic; forms microvilli and junctional complexes
	RL95-2	CRL 1671	65	F	W	Moderately differentiated adenosquamous	Primary	DM/F12-10	ND	Estrogen receptors; α-keratin; microvilli
Kidney (9)	Caki-1	HTB 46	49	M	W	Renal carcinoma	Metastasis to skin	MC10	0.0009	Tumorigenic; hypertriploid
	ACHN	CRL 1611	22	M	W	Adenocarcinoma	Pleural effusion	EM10	ND	Tumorigenic; invasive
	769P	CRL 1933	65	F	W	Clear cell adenocarcinoma	Primary	R10	ND	Tumorigenic; colonies in soft agar microvilli and desmosomes; hypodiploid
Leukemia/lymphoma (47)	CCRF-CEM	CCL 119	4	F	W	Acute lymphoblastic leukemia (ALL)	Peripheral blood	R20	0.0004	T-lymphoblast; malignant in newborn hamsters; modal chromosome number 45–47
	Hut 78	TIB 161	50	M	W	Sezary syndrome	Peripheral blood	R10	ND	Tumorigenic; mature T cell line with inducer/helper phenotype; yields and responds to IL-2
	MOLT 4	CRL 1582	19	M	?	ALL	Peripheral blood	R10	ND	Stable T cell; high (TdT); modal chromosome no. 95
	HL-60	CCL 240	36	F	W	Acute promyelocytic leukemia	Peripheral blood	L15/10	0.0188	Neutrophilic promyelocytes differentiate when exposed to RA and others. Surface receptors for Fc; produces myeloid tumors
Liver (3)	Hep-3B	HB-8064	8	M	B	Hepato-cellular carcinoma	Primary	EM10	ND	Tumorigenic; produce haptoglobin α fetoprotein; albumin
	Hep-G2	HB-8065	15	M	W	Hepato-cellular carcinoma	Primary	EM10	ND	α 2-macroglobulin; transferrin; fibrinogen and other liver-specific proteins
	SK-HEP-1	HTB 52	52	?	W	Adenocarcinoma	Ascites	EM10	0.0020	Produces α-antitrypsin; has Weibel Palade bodies and vimentin
Melanoma (21)	C32	CRL 1585	53	M	W	Amelanotic melanoma	Metastasis to ileum	EM10	ND	Tumorigenic; hypodiploid with mode of 45
	Hs294T	HTB 140	56	M	W	Metastatic melanoma	Metastasis to lymph node	DM10	0.0037	NGF and interferon receptors; responsive to RA; tumorigenic and grows in soft agar
	SK-MEL5	HTB 70	24	F	W	Metastatic melanoma	Metastasis to axillary node	EM10	0.0860	Tumorigenic
	COLO 829	CRL 1974	45	M	W	Malignant melanoma	Subcutaneous metastasis	R10	ND	Prior to therapy; some melanin produced; B cell counterpart available as ATCC.CRL 1980
	COLO 829BL	CRL 1980	45	M	W	Malignant melanoma	Peripheral blood	R10	ND	Control B cell line to CRL 1974; DNA fingerprint confirms identity
Myeloma/plasmacytoma (10)	HS-Sultan	CRL 1484	56	M	W	Plasmacytoma, multiple myeloma	Marrow right iliac crest	R10	ND	Produces IgGk; hyperdiploid; EBNA +'ve
	RPMI 8226	CRL 155	61	M	?	Multiple myeloma	Peripheral blood	R20	ND	Produces lambda light chains; no mature plasma cells
	U266BL	TIB 196	53	M	?	Myeloma	Peripheral blood	R15	ND	IgE lambda-secreting
Nasal septum (1)	RPMI 2650	CCL 30	52	M	?	Anaplastic squamous cell	Pleural effusion	EM10	ND	Pseudodiploid with mode 46; keratin +'ve
Neuroblastoma (3)	IMR-32	CCL 127	13 mo	M	W	Neuroblastoma with organoid differentiation	Abdominal mass	EM10	ND	Two cell types present-neuroblasts and large hyaline fibroblasts

TABLE V continues on next page

TABLE V. Representative Human Tumor Cell Lines (1/93) (continued)*

Tissue/tumor (no. available)	Designation	ATCC no.	Age	Sex	Race	Diagnosis	Source	Medium[a]	PFP[b]	Comments
	SK-N-MC	HTB 10	14	F	W	Neuroepithelioma	Metastasis to supra-orbital area	EM10	0.00005	Pseudodiploid; dopamine hydroxylase +
	SK-N-SH	HTB 11	4	F	?	Neuroblastoma	Metastasis to bone marrow	EM10	ND	Hyperdiploid; dopamine hydroxylase +
Ovary (6)	Caov-3	HTB 75	54	F	W	Adenocarcinoma	Primary	EM10	0.0061	Extremely unusual chromosome morphology
	NIH: OVCAR-3	HTB 161	60	F	W	Progressive adenocarcinoma	Ascites	R20+	0.0426	Tumorigenic; grows in soft agar; androgen and estrogen receptors
	PA-1	CRL 1572	12	F	W	Teratocarcinoma	Ascites	EM10	ND	Pseudodiploid, t(15q20q); highly malignant in nude mice
Pancreas (10)	AsPC-1	CRL 1682	62	F	W	Metastatic carcinoma	Ascites	R20	ND	Tumorigenic; CEA, PAA, and PSA+; hyperdiploid
	Capan-1	HTB 79	40	M	W	Metastatic carcinoma	Liver metastasis	R15	0.0311	Tumorigenic; hypotriploid
	PANC-1	CRL 1469	56	M	W	Epitheloid ductal carcinoma	Primary	DM10	ND	Hypertriploid
Pharynx (2)	Detroit 562	CCL 138	?	F	W	Metastatic carcinoma	Pleural fluid	EM10	0.0173	Keratin +'ve
	FaDu	HTB 43	56	M	W	Squamous cell carcinoma	Primary	EM10	0.0003	Tumorigenic; desmosomes; 19 marker chromosomes
Placenta (3)	BeWo	CCL 98	Fetus	M	?	Malignant gestational choriocarcinoma	Hamster xenograft	F12–15	ND	Secretes placental hormones hCG, hPL, estrone, estradiol, estriol, progestrone
	JEG-3	HTB 36	Fetus	?	?	Choriocarcinoma, Erwin-Turner tumor	Hamster xenograft	EM10	ND	Secretes hCG, hC somatomammotrophin and progesterone; tumorigenic
	JAR	HTB 144	Fetus	?	?	Choriocarcinoma	Placenta primary	R10	0.0002	Secretes estrogen, progesterone, gonadotrophin, and lactogen
Prostate (3)	PC3	CRL 1435	62	M	W	Adenocarcinoma, Grade IV	Primary	F12K-7	ND	Tumorigenic and grows in soft agar; low acid phosphatase and steroid reductase
	LNCap.FGC	CRL 1740	50	M	W	Metastatic adenocarcinoma	Metastasis to supraclavicular lymph node	R10	ND	Produces PSA, prostatic acid phosphatase; androgen receptors; tumorigenic
	DU145	HTB 81	69	M	W	Metastatic carcinoma	Metastasis to brain	EM10	0.0041	Grows in soft agar; weak acid phosphatase; triploid; desmosomes
Rectum (2)	SW-837	CCL 235	53	M	W	Adenocarcinoma, Grade IV	Primary	L15-10	0.007	Tumorigenic; hypodiploid
	SW-1463	CCL 234	66	F	W	Adenocarcinoma, Grade II–III	Primary	L15-10	0.00008	Tumorigenic; CEA positive; hypertriploid
Retinoblastoma (2)	WERI-Rb-1	HTB 169	1	F	W	Retinoblastoma	Primary	R10	0.0604	Tumorigenic in rabbits; no colonies in soft agar; near diploid with 15–16 markers.
	Y79	HTB 18	2.5	F	W	Retinoblastoma	Primary	R15	0.1373	Reverse transcriptase positive
Rhabdomyosarcomas (4)	A204	HTB 82	1	F	?	Embryonal rhabdomyosarcoma	Primary	MC10	ND	Tumorigenic; near diploid with abnormality on 22p
	RD	CCL 136	7	F	W	Malignant rhabdomyosarcoma	Pelvic tumor	DM10	0.0038	No myofibrils but myoglobin and myosin ATPase activity; complex hyperdiploid karyology; also designated TE32 and 130T

Sarcoma (26)	HOS	CRL 1543	13	F	W	Osteogenic sarcoma	Primary	EM10	ND	Flat morphology; sensitive to viral and chemical morphological transformation
	MG-63	CRL 1427	14	M	W	Osteogenic sarcoma	Primary	EM10	ND	Yields interferon upon induction; hypotriploid with 18–19 markers
	SK-LMS-1	HTB 88	4	F	W	Leiomyosarcoma	Primary vulva	EM10	0.0027	Tumorigenic; hypertriploid with complex karyotype
	SW 1353	HTB 94	72	F	W	Chondrosarcoma, Grade II	Primary, right humerus	L15/10	0.00009	Hyperdiploid with trisomic N7 only
Skin (20)	A-431	CRL 1555	85	F	?	Epidermoid carcinoma	Primary	DM10	ND	Tumorigenic and grows in soft agar; hypertriploid
Stomach (5)	AGS	CRL 1739	54	M	C	Adenocarcinoma	Primary	F12-10	ND	No prior therapy; tumorigenic; t(13q14q), hyperdiploid
	KATO-III	HTB 103	55	M	Mongoloid	Gastric carcinoma	Pleural effusion	IDM10	0.0742	Tumorigenic; hypotetraploid, t(11;HSR)
	RF-1	CRL 1864	62	M	Hispanic	Metastatic carcinoma	Primary	L15/10	ND	Stains for mucin, CEA+
	RF-48	CRL 1863	62	M	Hispanic	Metastatic carcinoma	Metastasis	L15/10	ND	Metastasis from CRL 1864 (RF-1); mucin and CEA negative
Submaxilla (1)	A-253	HTB 41	54	M	W	Epidermoid carcinoma	Primary	MC10	0.0426	Hypotriploid; 14 marker chromosomes
Testes (3)	Cates1B	HTB 104	34	M	W	Embryonal carcinoma	Metastasis to lymph node	MC10	0.0241	Reportedly hypodiploid to diploid
Thyroid (2)	SW579	HTB 107	59	M	W	Squamous cell carcinoma (SCC)	Primary	L15/10	0.0209	Tumorigenic yielding spindle and giant cell tumors
	TT	CRL 1803	77	F	W	Medullary thyroid carcinoma	Primary	F12K/10	ND	Tumorigenic; neuropeptides, calcitonin and CEA produced
Tongue (4)	SCC-4	CRL 1624	55	M	?	SCC	Primary	F12/DM/10	ND	Tumorigenic; involucrin-ve; hypopentaploid; + for 40kd keratin
	SCC-25	CRL 1628	70	M	?	SCC	Primary	F12/DM/10	ND	Tumorigenic synthesizes low levels of involucrin; epidermal keratin; hypertriploid
Vulva (2)	SW954	HTB 117	86	F	W	SCC Grade II	Primary	L15/10	0.0187	Pseudodiploid; t(3q;11p); der(11)t(11;?)(q13;?) in all cells
	SW962	HTB 118	64	F	W	SCC	Metastasis to lymph node	L15/10	0.0222	Tumorigenic; hypertriploid with at least 15 marker chromosomes

*See [19] for originators, references, and more detail. ND = no data.

[a]Abbreviations for medium used (on the left-hand side) include: EM, Eagle's minimum essential medium; R, RPMI 1640; DM, Dulbecco's modification of Eagle's medium; F12 and F12K, Ham's medium and Kaighn's modification, respectively; IDM, Iscove's modification of Eagle's medium; L15, Leibovitz medium; MC, McCoys 5A medium; AMEM, The Alpha modification of Eagle's medium [see reference 19 for formulae]. The number on the right-hand side indicates percentage of serum (usually fetal bovine) used. Additional recommended ingredients are indicated by the "+" sign.

[b]PFP = phenotype frequency product. The isoenzyme systems used in this calculation vary somewhat with program. For identical systems the product gives an indication of potential relatedness between cell lines. See [3, 4, 8, 23] for more detail.

ized when appropriate with regard to immunoglobulins produced, surface or other antigens expressed, and drug sensitivity or resistance. Additional traits such as phagocytotic ability, growth factor production, or cytotoxic activity are documented where pertinent. HTB and TIB cell lines are distributed to qualified investigators for use in their own research. In some cases, as indicated in the ATCC catalogue, before shipment, the government and depositors require investigators to sign an agreement stating that: (1) the line will be used for research purposes only, (2) cell lines and their products shall not be sold or used for commercial purposes, and (3) cells will not be distributed further to third parties for purposes of sale, or producing for sale, cells or their products. A copy of the required agreement is included in the ATCC catalogue.

The ATCC is recognized as an international patent culture repository for cell lines and microorganisms. Cultures deposited on a restricted basis in connection with U.S. or foreign patent applications are held in strict confidence for the owner and the documentation is processed separately from lines in other categories. No characterizations are performed at the ATCC prior to the granting of a patent unless required by the patent circumstance (i.e., deposits under The Budapest Treaty) or requested by the owner. After a patent is issued, the line must be made available to investigators and authentication or characterizations may then be performed by ATCC scientists.

Current Composition

The current quantitative composition of The Repository in terms of the various cell line categories is summarized in Table II. Note that the largest components are those of the Cell Repository and Certified Cell Lines (CRL and CCL, respectively) which are also the most diverse in terms of individual species.

The collection of human skin fibroblasts from genetic variants, individuals with other diseases, and presumptive normal controls is included for information, under the HSF heading in the table. These are also given the prefix CRL in the ATCC catalogue. The appropriate minimal characterizations for this category of lines are applied to the skin fibroblast group.

Distribution

The annual distribution of all cell cultures and those in the Human Tumor Bank (HTB) since 1982 are presented in Figure 2. Immediately apparent is the high rate of increase in distribution. The figure for distribution in 1992 (55,156 cultures) represents an increase of 404% over that experienced in 1982 (10,943 cultures). Interestingly, the rate of increase in distribution of cultures in the HTB roughly parallels that of cultures in the entire collection.

Distribution during 1992 is shown by category in Figure 3. Perhaps not surprisingly, cell lines with the CRL prefix (human skin fibroblasts and others in that category) and those from the most thoroughly characterized group (CCL) were supplied most extensively during the year. Some 8,122 HTB cultures were supplied for cancer research in 1992.

Representative Lines for Cancer Research

Cell lines important for cancer research have been included in virtually all of the categories listed in Table I. It is beyond the scope of this presentation to provide detail on all of these lines but representative data for human lung tumor cell lines are outlined in Tables III and IV. Those listed in Table 3 include 9 small cell carcinomas, a mucoepidermoid carcinoma, 2 large cell carcinomas, 10 adenosquamous and squamous cell carcinomas, and 2 papillary adenocarcinomas. All have been available for some time by standard request as they were acquired by the ATCC during the years 1976 to 1989. More detailed descriptions can be found in reference [19].

The 17 human lung tumor cell lines listed in Table IV represent a part of the collection developed by Gazdar, Oie, Minna, and collaborators described in this special issue. These have been tested at the ATCC to verify absence of microbial contaminants (including mycoplasma) and to confirm human origin, and have been identified by DNA fingerprinting (see below). These lines are available from the ATCC at this point as flask cultures only due to limited inventory holdings. We hope to have all of the lines described in this issue similarly available by time of publication.

Table V is included to illustrate the diversity of human tumor lines available. Some 34 different classes of tissue sources are represented including example lines from all the major risk sites. Additional information including names and laboratory addresses of originating or donating investigators, references describing isolation and characterization, culture requirements, and so forth are provided in reference [19].

Data on Individualization

A typical karyotype for the human lung small cell tumor line NCI-H82 (ATCC-HTB 175) is provided in Figure 4. The line is near-diploid and complex with many structurally altered chromosomes. Additional karyotypic data is included for most of the lung tumor lines elsewhere in this issue.

Approximately 150 human lung tumor and corresponding lymphoblastoid cell lines were fingerprinted at the ATCC. Representative fingerprints are shown in Figures 5 and 6, and the band information is shown in Tables VI and VII. The two figures show patterns obtained after digestion with HinfI and hybridization with a *cocktail of three single-locus probes.*

Figure 5 and Table VI show the patterns and the fragment sizes for a collection of pairs of cell lines where both members of the pair were derived from the same patient. Within the limits of measurement error, no difference was observed between members of a pair. Although this is usually the case, we have observed pairs where one member (usually the one derived from tumor tissue) is lacking a band present in the corresponding lymphoblastoid cell line. We do not yet have a clear explanation for this result.

Figure 6 and Table 7 show patterns and the fragment sizes for DNA from 7 different cell lines after digestion with HinfI and hybridization with single-locus probes pYNH24, p79-2-23, and pCMM86. Although individual matching bands are present (e.g., 5 occurrences of bands at $\approx$1,900 bp, 3 at $\approx$2,500 bp, and 2 at $\approx$3,200 bp, see also Fig. 6), the patterns as a whole are unique.

Each set of fingerprints was compared to each fingerprint in the database using a custom application written in the Paradox Application Language (PAL). For each cell line fingerprint in the database (database fingerprint), each band of the fingerprint being evaluated (test finger-

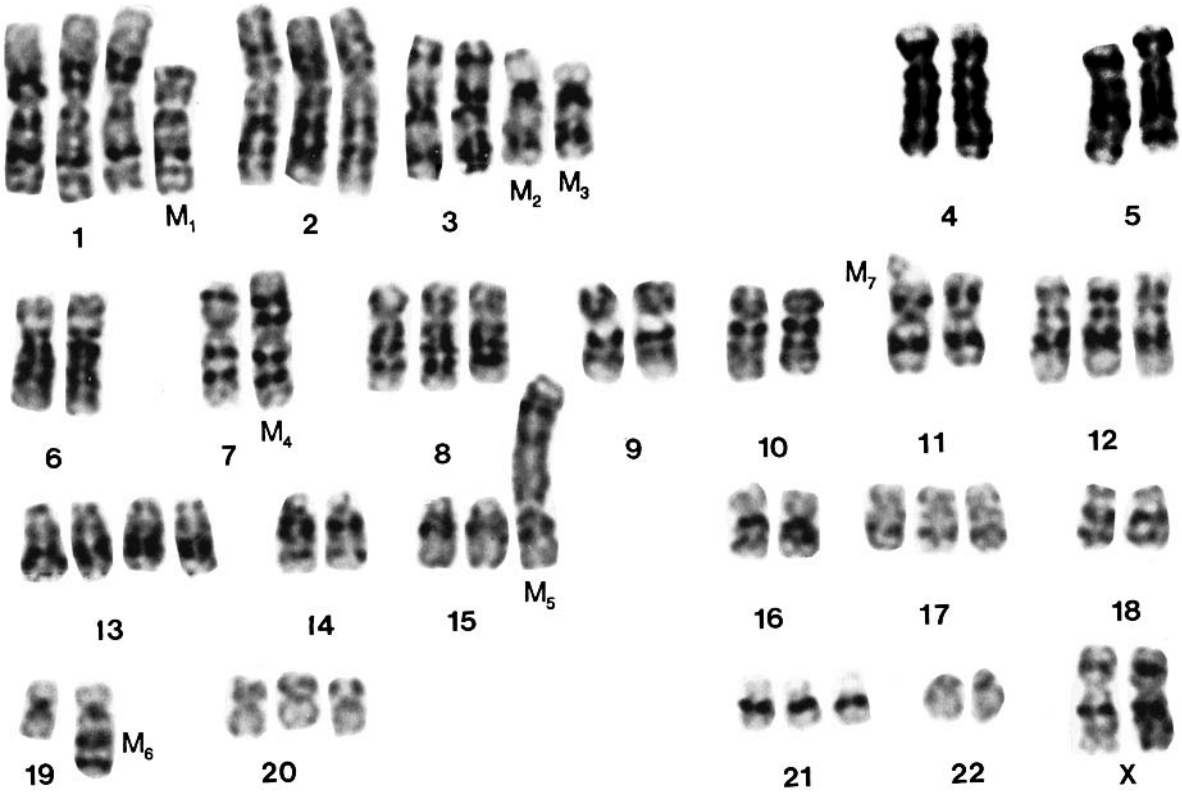

Fig. 4. G-banded karyotype from one of the two coexisting and readily detectable sub-populations of NCI H82 (ATCC HTB 175). The cell line was derived from a small cell lung carcinoma. Both sub-populations had near-triploid chromosome counts with the modal chromosome number of 58. Structurally altered chromosomes common to most cells in the sub-population prese ıted in this figure are shown as follows: M_1 = t(1q7p); M_2 = t(3q19q); M_3 = t(3q19p); M_4 = i(7q); M_5 = t(13q—HSR—C—15 q); M_6 = der (19)t(3;19) (p11.2;q13.3?); and M_7 of a small chromosome of unknown origin. All these chromosomes were present in single copies per cell. The sub-population-specific chromosome pattern includes the presence of M_2, M_3, M_4, and single copies for N7 and N19. The other sub-population lacks those three markers, has paired N7 and N19 and possesses different markers containing N1 and N3 arms.

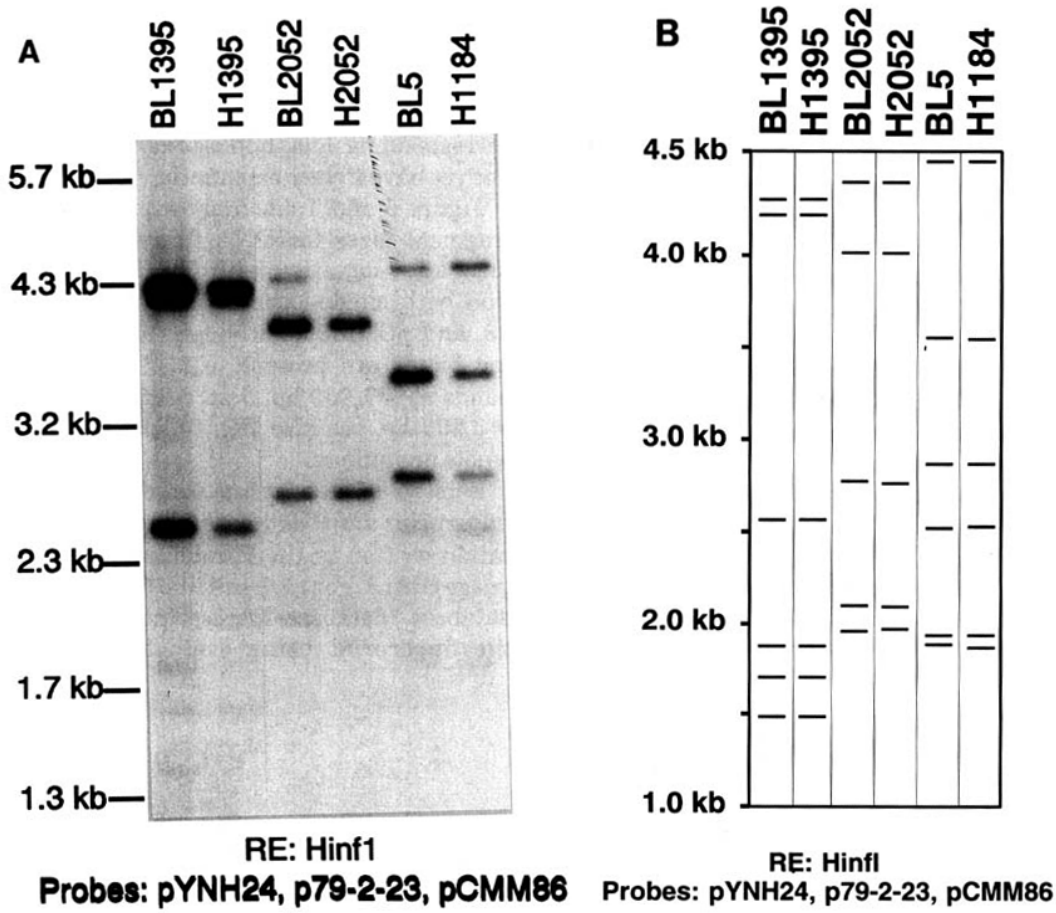

Fig. 5. **A:** DNA profiles of three (3) pairs of human cell lines digested with the restriction endonuclease HinfI and hybridized with the single-locus probes pYNH24, p70-2-23, and pCMM86. **B:** Computerized data from A.

print) was compared to each band in the database fingerprint. In order to score as a match, a band in the test fingerprint was required to be within ±2% of the size of a band in database fingerprint. No band was allowed to match more than one band in the opposite fingerprint (Equ. 1). The 2% factor was chosen based on trial experiments and values used by others [23].

$$\frac{|MW_{database} - MW_{test}|}{(MW_{database} + MW_{test})/2} = x \qquad (1)$$

We obtained an estimate of the number of alleles detected in the population tested (about 150 cell lines) by filtering the database for clusters of non-overlapping band sizes (within the ±2% window). After doing so, there were 72 apparent alleles detected in the cell line population.

DISCUSSION

Banks and distribution centers for reference cell lines have been developed during the past three decades to overcome the problems identified. In the United States there are the Cell Repository with the various components at the ATCC; the NIGMS Human Genetic Mutant Cell Repository and the NIA Aging Cell Repository, maintained at the Coriell Institute for Medical Research in Camden, New Jersey [24]. In Japan there are the Japanese Cell Bank for Cancer Research in Tokyo, the Riken Cell Bank in Tsukuba plus the Cell Bank of the Institute for Fermentation Research in Osaka. In Western Europe there are the European Collection of Animal Cell Cultures (ECACC) in Porton Down, UK, the German Collection of Microorganisms and Animal Cell Cultures (DSM) in Braunsch-

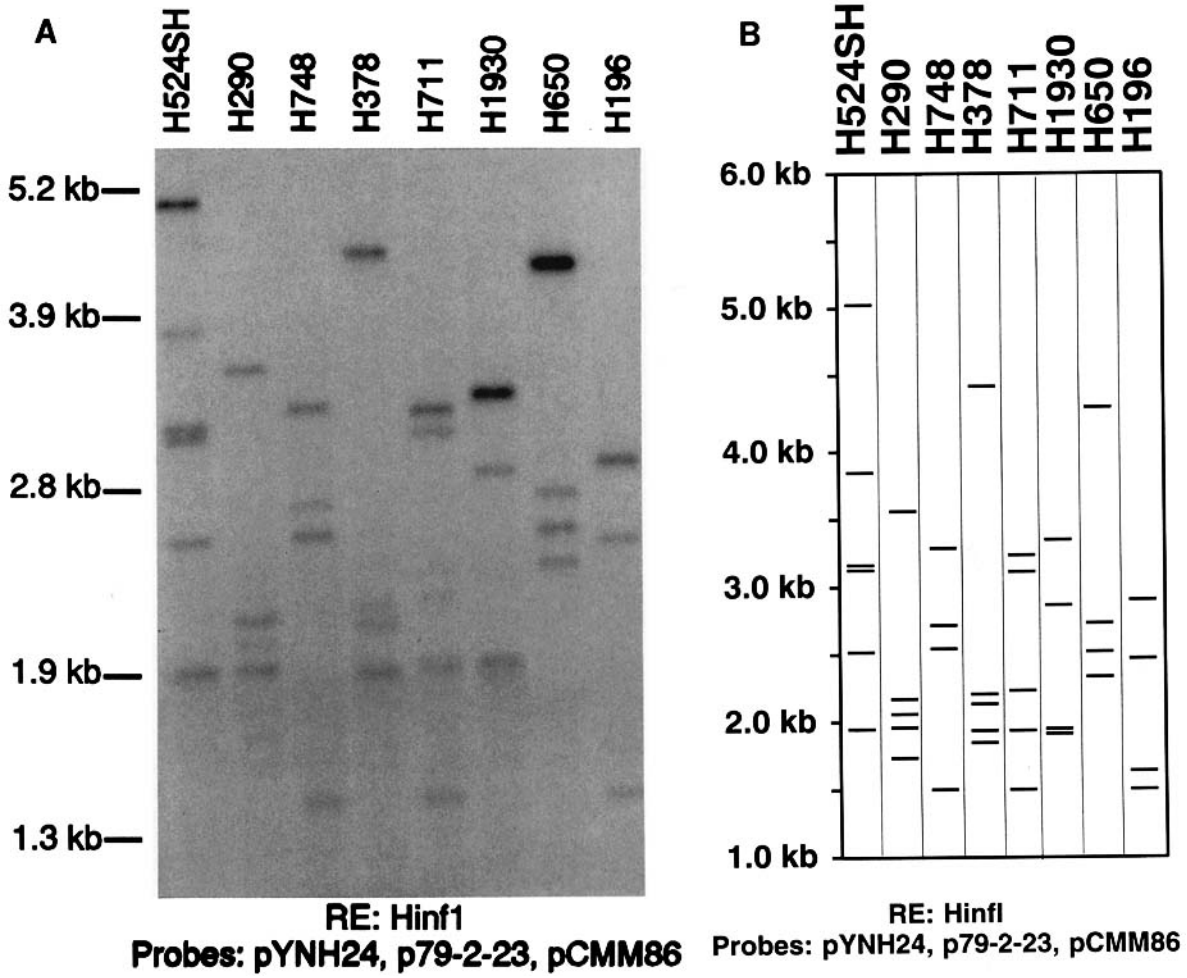

Fig. 6. **A:** DNA profiles of eight (8) unrelated human cell lines digested with the restriction endonuclease HinfI and hybridized with the single-locus probes pYNH24, p79-2-23, pCMM86. **B:** Computerized data from A.

TABLE VI. Band Sizes for Fingerprints of Cell Line Pairs*

BL1395	H1395	BL2052	H2052	BL5	H1184
4,246	4,246	4,338	4,338	4,451	4,451
4,160	4,160	3,936	3,956	3,490	3,498
2,522	2,515	2,717	2,724	2,817	2,817
1,836	1,836	2,053	2,053	2,485	2,478
1,670	1,670	1,929	1,920	1,899	1,899
1,448	1,448			1,832	1,844

*Band sizes shown as number of base pairs (bp).

TABLE VII. Band Sizes for Fingerprints of Unrelated Cell Lines*

H524SH	H290	H748	H378	H711	H1930	H650	H196
5,057	3,520	3,238	4,440	3,209	3,304	4,297	2,861
3,804	2,133	2,672	2,170	3,081	2,829	2,697	2,426
3,123	2,023	2,506	2,099	2,195	1,904	2,493	1,600
3,081	1,912	1,461	1,888	1,900	1,868	2,314	1,457
2,486	1,688		1,801	1,461			
1,908							

*Band sizes shown as number of base pairs (bp).

weig, and the National Italian Culture Collection which is being developed in Udine. A bank serving scientists in Eastern Europe is maintained at the Institute of Cytology in St. Petersburg by the Russian Academy of Sciences. The Chinese have national cell banks in Wuhan, Kunming, and Shanghai; a new bank for Human Lines of use in cancer research has been set up in Seoul, Korea; and a National Facility for Animal and Tissue Cell Culture (NFATCC) has been established in Poona, India. Argentina has established a cell line bank in Pergamino. Particulars including acronyms, names of contact personnel, addresses, telephone, and telefax numbers are provided in Table VIII.

TABLE VIII. National Cell Line Repositories

Country	Name	Acronym	Location	Contact personnel	Telephone number	Fax number	Status and emphasis
Argentina	Asociacion Banco Argentino de Celulas	ABAC	I.N.E.V.H. C.C. 195 2700 Pergamino Argentina	Dr. Ana Maria Ambrosio	54-477-25700	54-477-33045	Under development
China	Kunming Cell Bank of CAS	CBCAS	Kunming Institute of Zoology Kunming 650107 China	Professor Shi Li-ming	86-871-82661	86-871-82416	Primarily cell lines from exotic and endangered species
	The Cell Bank of China Center for Type Culture Collection	CBCCTCC	School of Life Science Wuhan University, Wuhan, China 430072	Associate Prof. Zheng Congyi	86-027-722157	86-027-713833	Comprehensive
	The Cell Bank of Chinese Academy of Sciences	CBCAS	Shanghai Institute of Cell Biology 320 Yo-Yang Road, 200031 Shanghai, China	Professor Ge Xi-rui	86-4315030 Ext. 41	86-4331090	Comprehensive
England	European Collection of Animal Cell Cultures	ECACC	PHLS Center for Applied Microbiology and Research Division of Biologics Porton Down Salisbury, Wiltshire SP4 OJG England, UK	Dr. Alan Doyle	44-980-610391 Ext. 2512	44-980-611315	Comprehensive
Germany	Deutsche Sammlung von Mikrooganismen und Zellkulturen	DSMZG	DSM Mascheroder Weg 1 B D-3300 Braunschweig Germany	Dr. Hans Drexler	49-531-61870	49-531-618750	Emphasis currently hematopoietic cell lines
India	National Facility for Animal Tissue and Cell Culture	NFATCC	Department of Biotechnology Government of India Jopasana, Paud Road, Kothrud, Pune 411 029 India	Dr. Ulhas V. Wagh	91-212-335928	91-212-369501	Comprehensive
Italy	Dipartimento di Patologia e Medicina Clinica e Sperimentale	NICC[a]	Universita Di Udine P.le S.M. della Misericordia 33100 Udine, Italy	Dr. F.S. Ambesi-Impiombata	39-432-559203	39-432-545526	Under development

Japan	Institute for Fermentation, Osaka	IFO	Institute for Fermentation, Osaka 17-85, Juso-honmachi 2-chome Yodogawa-ku, Osaka 532, Japan	Dr. Masao Takeuchi	06-300-6555	06-300-6814	Comprehensive
	National Institute of Hygienic Sciences	JCRB	Division of Genetics and Mutagenesis 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158, Japan	Dr. Hiroshi Mizusawa	81-3-3700-1141	81-3-3707-6950	Emphasis on lines for cancer research
	Riken Cell Bank	RCB	3-1 Koyadai, Tsukuba Science City Ibaraki 305, Japan	Dr. Tadao Ohno	81-298-363611	81-298-369130	Comprehensive
Korea	Korean Cell Line Research Foundation	KCLRF	Cancer Research Institute Seoul Nat'l Univ. College of Medicine 28 Yongun-dong, Chongno-ku Seoul, 110-744, Korea	Dr. Jae-Gahb Park	82-2-760-3380	82-2-742-4727	Under development. Emphasis on lines for cancer research.
Russia	Russian Cell Culture Collection	RCCC	Institute of Cytology Tikhoretsky avenue 4 194064 S. Petersburg Russia	Dr. George Pinaev	7-812-247-44-20	7-812-247-03-41	Comprehensive
USA	American Type Culture Collection	ATCC	Cell Culture Department 12301 Parklawn Drive Rockville, MD 20852	Dr. Robert J. Hay	301-231-5529	301-231-5551	Comprehensive
	Coriell Institute for Medical Research	CIMR	Coriell Cell Repositories 401 Haddon Avenue Camden, NJ 08103	Dr. Richard A. Mulivor	609-757-4848	609-964-0254	Comprehensive. Emphasis on lines relevant to human genetics and aging

[a]Under development 9/93.

The procedures described for addition of human and other animal cell lines to the ATCC Cell Culture Collection provide standardized stocks for basic and clinical research. The availability of characterized reference cultures stored in liquid nitrogen or its vapor provides the following significant advantages: (1) the investigator can obtain contaminant-free, reference cell lines at the same passage level from time to time over the years for systematic experimentation; (2) cells whose origin, history, and characteristics have been described and documented are thus readily available; (3) a source of types of cells that may normally be difficult to acquire is described; (4) the responsible investigator is released from time-consuming and costly long-term serial subcultivation and supply of cell lines; (5) both individuals and institutions have insurance against the total loss of valuable cells through contamination, alteration, or accident.

The advantages of working with properly identified cells in a system free from contaminating organisms are apparent enough in terms of conservation of time, effort, and research dollars. Additional advantages can be gained in the future by cooperative and coordinated efforts to characterize reference cells more completely over the years.

An added overall benefit to the entire scientific community, is the provision of a common source of reference cells at similar passage levels that will be available for repeated use in perpetuity. It is well known that the characteristics and properties of cells (proliferation rates, structure of chromosomes, product-secretion rates, virus susceptibilities, etc.) sometimes change markedly during cultivation over extended periods in vitro. Thus cell populations developed and maintained in different laboratories may vary widely. The availability of common sources of cells through the ATCC and other, similar banking agencies increases the possibility that meaningful comparative studies can be made both within a given laboratory and among different laboratories throughout the world. The ultimate advantages of working with characterized cells "captured" at a specific stage in their life history cannot be over-emphasized.

Strict adherence to the seed stock concept, which has been neglected or misunderstood by curators of some smaller collections, is essential for provision of the benefits listed above. This should be one major consideration in the development of all central cell banking facilities.

The availability and use of human tumor cell lines has increased substantially in the past decade and with this the risk of cell line cross contamination among human cells has become more prevalent. To test for intraspecies cross contamination among human cell lines, therefore, investigators must rely on cytogenetics to identify specific, normal chromosome patterns, chromosome polymorphisms, and marker chromosomes [1–4,8,19]; DNA profiling methods to identify polymorphic patterns [13]; and analyses of multiple enzymes to construct isoenzyme profiles [3,4,8,12,22].

Cytogenetics without question is the method currently offering the highest degree of characterization. With sufficient skill in interpretation one can thoroughly define each subpopulation within a reference culture. Cells having combinations of different chromosomal aberrations such as inversions, insertions, deletions, dicentrics, double minutes, homogenous staining regions, and so forth can be identified and quantitated [1–4,8,19,25]. Oncogene and suppressor gene sites can eventually be identified, and the possible roles of chromosomal rearrangements in their activation or deactivation can be examined experimentally. These defects individually or in combination also permit thorough definition of each cytogenetically uniform component population within the cell line. One major advantage in such detailed characterization is the ability to specifically identify (authenticate) individual cell lines based on extensive currently published data and on cytogeneticists' past experience. Another is the potential for recognizing mixtures of 2 of more lines consisting in some cases of 1% or lower in the contaminant, depending upon the particular lines involved [1–4,8,25]. A major disadvantage is the time and expense required to produce and evaluate karyotypes.

Isoenzymology by contrast is a rapid and comparatively simple method for authentication. A kit including premeasured reagents is even available for very reliable interspecies identification [12]. If a cell line species is misidentified by the donor this method, like cytogenetics, often permits at least tentative identification immediately. Positive species confirmation can follow after an additional run with the most suitable controls [4,12]. Intra-species identifications are more problematical. In these cases one usually runs 7 or more enzymes. Not infrequently multiple lines may be in use with similar profiles, or individual enzymes will fail to be expressed

[3,4,12,22]. Isoenzymology will not usually detect a minor contamination (about 25% or less on the inappropriate line).

DNA profiling technology is new, and the utility of many different probes for cell line individualization is being explored by National cell banks throughout the world [13,26–29]. The method is rapid and extremely reliable for verification or intra-species identification of human cell lines. This technique will not accomplish the characterization possible with cytogenetics. However, as more data accumulates it will certainly become the method of choice for routine authentication of cell line replenishment distribution stocks which have been thoroughly characterized otherwise. No single probe or set of probes has yet been accepted generally for cell line identification purposes. Jeffreys' 33.6 is the most thoroughly utilized but being a multi-locus probe is more difficult for analyses than single-locus probes such as pYNH24, pCMM86, pCMM101 pYNZ2, and/or others [13,26–29]. We hope that a degree of international consensus on probe use for cell line identification will be reached in the near future.

The remarkable acceleration in demand for reference human tumor and other cell cultures as indicated by the data in Figure 2 probably reflects not only the growth in utility of cell culture techniques for studies on a wide range of scientific problems, but also an increased awareness of the importance of contaminant-free, standardized cultures in research. With regard to the latter, scientists on journal editorial review boards and on government study sections can do much to enhance awareness further. They can strongly recommend that papers submitted for publication and grant applications contain specific information on sources of cell lines and on quality control procedures used in their processing and characterization. Vigilance in this area is needed to avoid recurrence of past errors and the attendant confusion that they generate.

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