Animal Cells

LIVING RESOURCES FOR BIOTECHNOLOGY

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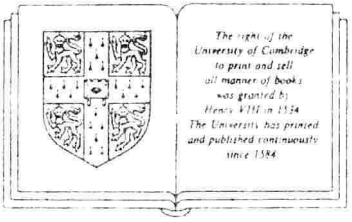
Edited by

A. Doyle, R. Hay and B. E. Kirsop



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SERIES INTRODUCTION

The rapid advances taking place in biotechnology have introduced large numbers of scientists and engineers to the need for handling microorganisms, often for the first time. Questions are frequently raised concerning sources of cultures, location of strains with particular properties, requirements for handling the cultures, preservation and identification methods, regulations for shipping, or the deposit of strains for patent purposes. For those in industry, research institutes or universities with little experience in these areas, resolving such difficulties may seem overwhelming. The purpose of the World Federation for Culture Collections' (WFCC) series, Living Resources for Biotechnology, is to provide answers to these questions.

Living Resources for Biotechnology is a series of practical books that provide primary data and guides to sources for further information on matters relating to the location and use of different kinds of biological material of interest to biotechnologists. A deliberate decision was taken to produce separate volumes for each group of microorganism rather than a combined compendium, since our enquiries suggested that inexpensive specialised books would be of more general value than a larger volume containing information irrelevant to workers with interests in one particular type of organism. As a result each volume contains specialised information together with material on general matters (information centres, patents, consumer services, the international coordination of culture collection activities) that is common to each.

The WFCC is an international organisation concerned with the establishment of microbial resource centres and the promotion of their activities. In addition to its primary role of coordinating the work of culture collections through the world, the committees of the WFCC are

active in a number of areas of particular relevance to biotechnology, such as patents, microbial information centres, postal and quarantine regulations, educational and conservation matters (see Chapter 8). The Education Committee of the WFCC proposed the preparation of the current volumes.

The WFCC is concerned that this series of books is of value to biotechnologists internationally, and the authors have been drawn from specialists throughout the world. The close collaboration that exists between culture collections in every continent has made the compilation of material for the books a simple and pleasurable process, since the authors and contributors are for the most part colleagues. The Federation hopes that the result of their labours has produced valuable source books that will not only accelerate the progress of biotechnology, but will also increase communication between culture collections and their users to the benefit of both.

Barbara Kirsop
President, World Federation for Culture Collections

PREFACE

Until recently, the exploitation of animal cell cultures in biotechnological manufacturing has lagged behind the use of microorganisms such as bacteria, filamentous fungi and yeasts. One reason for this is the fastidious growth requirements of animal cells, which require complex and expensive growth media and elaborate culture systems. Further reasons for this slow progress are the slow growth characteristics of animal cells with doubling times of 24 hours (compared with as little as 30 minutes for some bacteria) and the generally low productivity levels.

Before the development of hybridomas (producing monoclonal antibodies) and genetically manipulated cell lines, the major industrial use for cell lines was as a substrate for virus production in viral vaccine manufacture (e.g. poliomyelitis, measles and rubella). New developments have radically altered the picture. The potential for monoclonal antibodies in diagnosis and therapy, together with biochemical manufacturing purification processes, is enormous and has become the basis for new industrial enterprises.

Because of the difficulty of obtaining secretion of some mammalian proteins in an active form from genetically engineered bacteria, another developing area is the engineering of animal cells for the production of therapeutic products. There is also growth in the use of cell lines for the production of immunoregulatory proteins, including interferon and interleukins, and the search is still continuing for suitable cell substrates. Interest in animal cells as tools for biotechnology has, therefore, never been greater.

The aim of this volume is to bring together as much useful information as possible for those who may be newcomers to the field of animal

cell technology. The result is an international collaborative effort, with the strengths and weaknesses associated with such initiatives. The speed of developments in this fast moving field inevitably leads to source books becoming out of date before publication; the authors therefore apologise for any omissions which will be rectified in future editions.

Alan Doyle
European Collection of Animal Cell Cultures

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I would also like to pay a special tribute to one of our contributors, Mr Keith Allner, a friend and colleague at PHLS CAMR, who sadly died before publication of this volume. He contributed a wealth of experience to works such as this and he will be greatly missed.

Alan Doyle

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Resource Centres

A. DOYLE, R. HAY, T. OHNO and H. SUGAWARA

1.1 Introduction

Public cell culture collections came about in response to a widespread need for well characterised, microbe-free seed stocks derived from cultures supplied by cell line originators. The explosion of research on viruses in the mid to late 1950s, enabled by the extensive use of cell culture, led to exploitation of the techniques, often without awareness of the need for critical, cell line quality control. By 1960 the problems of cellular and microbial contamination of cell lines had become so acute that scientists in the United States banded together to establish a bank of tested cells. Soon they realised that the application of sensitive and extensive quality control tests would be desirable. These efforts coupled with improving preservation technology were carried out internationally (Stevenson, 1963).

Over the past 25 years millions of dollars have been invested in the cell banking programmes over and above the research costs of the developed cell lines. These funds have been willingly provided by granting agencies in the expectation of insuring the investment in research that uses cells as model systems.

While the rationale for development and use of well organised collections is understood by many laboratory scientists, poorly characterised cell stocks for use in research studies are still exchanged all too frequently. Thus it is important to review periodically the potential pitfalls associated with the use of microbial and cell stocks obtained and processed casually, to increase and reinforce awareness of the problem within the scientific community.

Numerous instances of the exchange of cell lines contaminated with cells of other species have been documented, and published by others

(Nelson-Rees & Flandermeyer, 1977; Harris *et al.*, 1981; Nelson-Rees *et al* , 1981a, b). Similarly, the problem of intraspecies cross-contamination among cultured human-cell lines has been recognised for over 15 years and detailed reviews are available on the subject (Gartler, 1968; Nelson-Rees *et al.*, 1974, 1981a, b; Lavappa, 1978). The loss of time and research funds as a result of these problems is incalculable.

Although bacterial and fungal contaminations represent an added concern, in most instances they are overt and easily detected and are therefore of less serious consequence than the more insidious contamination by mycoplasma. That the presence of these microorganisms in cultured cell lines often completely negates research findings has been emphasised over the years by Barile *et al.* (1973), Hopps *et al.* (1973) and McGarrity (1982). Still, the difficulties of detection and prevalence of contaminated cultures in the research community suggest that repeated restatements are warranted.

The methodology for characterisation of cell lines varies somewhat among banking agencies. For example, precise details on procedures used at the American Type Culture Collection (ATCC) for the acquisition, preservation, characterisation, cataloguing and distribution of cell lines have been widely publicised. They are available elsewhere and need only be cited at this point (Scherer, 1962; Stulberg *et al.*, 1970; Hay *et al.*, 1982; Hay, 1984a, 1985, 1986). In addition, techniques used by other institutions with like goals will be described in the forthcoming chapters of this handbook. Therefore, only a general scheme to outline the steps recommended for addition of a new cell line will be presented and discussed (Figure 1.1).

Most investigators submit starter cultures without charge and with minimal restrictions on distribution. For some component lines, recipients must sign a release form indicating that the cultures will not be redistributed or otherwise used for commercial purposes.

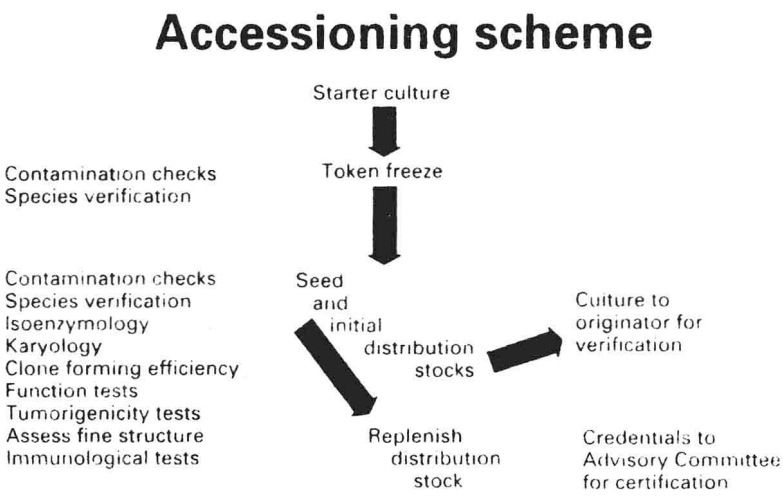
Required data for general submission of lines include a statement of exact species, strains, tissue sources, and precise description of original and subsequent dissociation procedures. Information on the growth medium and supplements should be included. A statement of specific functional characteristics, which would make appropriate strains uniquely valuable, also is required. In addition, preference is indicated for supply of detailed histories on each strain; information concerning date of establishment, sex, race, and age of donor; isolation procedures; inoculation densities; number of passages, and an indication

of the average number of cell generations accrued. Information on karyology and survival potential of the cell lines is also solicited.

After review of the credentials of each given line and consultation with advisors, decisions are made to proceed with initial characterisations. Generally, starter cultures or ampoules are obtained from the donor, and progeny are propagated according to instructions to yield the first 'token' freeze. Cultures derived from such token material are then subjected to the minimal, but critical, characterisations. These include a series of tests for microbial contamination, including mycoplasma, plus fluorescent antibody staining and/or isoenzyme analysis to verify species (see Chapter 5).

If these tests suggest that the cell line is acceptable, it is expanded to produce seed and distribution stocks. Antibiotics are not used in the culture media at this stage so as not to mask infection. Note especially that the major characterisation efforts are applied to cell populations in the initial seed stock of ampoules. The distribution stock consists of ampoules that are distributed on request; reference seed stock, on the other hand, is retained to generate further distribution stocks as the initial stock becomes depleted.

Fig. 1.1. Scheme illustrating accessioning procedures used at the ATCC. Note that some or all of the characterisations listed on the *left-hand side* are performed depending on the category to which the line is assigned.



Although this procedure was designed to suit the needs of a large central repository, it is also applicable to smaller laboratories. Even where the number of cell lines and users may be limited, it is important to separate 'seed stock' from 'working or distribution stock'. If this is not done the frequent replacement of cultured material, recommended to prevent phenotypic drift or senescence, may deplete valuable seed stock which may be difficult and expensive to replace or, indeed, may be no longer available.

It is important to recognise that the characterised seed stock serves as a frozen 'reservoir' for production of distribution stocks over the years. Because seed stock ampoules are used to generate new distribution material, recipients can be assured that the cultures obtained closely resemble those received 2, 5, 10 or even more years previously. This is a most critical consideration for design of cell banking procedures. The seed stock is the most valuable of collection material, and records on all of the characterisations performed should relate directly back to the immediate progeny from these stocks.

The extent and types of characterisations performed vary depending upon the cell line, the category under which it is banked and the purpose for which the stock is intended. A description of the various components within the ATCC cell line Repository has been published elsewhere (Hay, 1984b). It is strongly recommended that a laboratory distribute, or accept for use from any source, only those cell lines that have both been thoroughly tested for microbial contaminants and had their species of origin verified.

Ideally the karyology of the cell line should also be determined and compared with that of other lines having similar characteristics. This latter step can significantly decrease the likelihood of intraspecies cellular cross-contamination (Nelson-Rees *et al.*, 1981b).

Cell banks rely on advice from *ad hoc* consultants and more formally organised committees. The degree and mode of interaction vary somewhat depending upon the department, disciplines, and source of support for differing accessioning programmes. In certification of cell lines, for example, input is requested concerning lines to be added, characterisations to be applied, and descriptions of the cellular material eventually banked. Specialists 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 often circulated for critical review. The cell lines and specific descriptions are considered acceptable if a majority of the experts indicate concurrence.

A policy of returning both a typical distribution ampoule or live culture plus the proposed catalogue description and data description on each cell line to its respective donor has been adopted by major banks. The donating scientist is thus given the opportunity to examine material prepared at the bank to verify that the essential characteristics are retained. Descriptions on cell cultures are also returned to the donor for suggested additions or revisions.

Above are summarised the key considerations for producing and certification of cell line stocks to be used in research or distributed for scientific study. The chapters which follow give additional information not only on the many international sources for cells but also on the characterisation of the material, culture maintenance and scale-up, safety in culture handling, information data banks, patenting of cell lines and international coordinating organisations.

1.2 Resource centres

With the increased biotechnological use of animal cells, there is a parallel increase in the number of culture collections and other resource centres. Some are listed in the World Data Center's *Directory of Collections of Cultures of Microorganisms* (V. F. McGowan & V. B. D. Skerman, 1986) available from the United Nations Environment Programme, Information Service, PO Box 30553, Nairobi, Kenya, or from the World Data Center (WDC), see Chapter 2. Other information can be sought from the data bases now being developed, for example the Committee on Data for Science and Technology's (CODATA) Hybridoma Data Bank (see Chapter 2).

Animal cell technology is now used in a multitude of disciplines in the biological sciences. As with culture collections of microorganisms, the activities of animal cell banks stretch across medical, veterinary, agricultural and biotechnological interests. As fields related to this technology are constantly developing, it is increasingly important to ensure that the information available is as comprehensive as possible and the use of electronic systems is essential for the effective distribution of data (see Chapter 2).

As well as the traditional culture collections exemplified by the ATCC, the needs of animal cell technologists have led to the development in many institutes of resource centres built around specific areas of interest, such as human genetics. Increasingly, funds are becoming available to establish resource centres and new developments are occurring throughout the world.