

Collections of tissue cultured cell lines suspended by freezing

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It is now possible and financially feasible to maintain collections of living cells from a wide variety of mammalian species. Primary cell cultures are established from a variety of tissues (skin, lung, embryo, etc.), and grown under standard culture techniques. Cell lines can then either be studied or frozen for future studies. Frozen cell lines are best maintained in liquid nitrogen (-210°C) but short term storage may be accomplished in an electric ultracold freezer (-90°C). Standard procedures for establishment and maintenance of mammalian cell lines in research collections are described.

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1. Introduction

Tissue cultured cells offer scientists a wide range of experimental designs for detailed biological investigation. Tissue culture cells circumvent a major problem of maintenance of live animals in the laboratory. Now that techniques have been developed for freezing such cell lines without killing them, it is logical that collection of cell lines suspended by freezing would become established in museums and laboratories where tissue culture cells are being used for systematic investigation. Just as is the case in any other properly maintained systematic collection, an extensive collection of cell lines would be an important resource for the development of a strong research program.

2. Methods

Primary tissue cultures can be established for most mammalian species from biopsies from a wide variety of tissues including skin, lung, heart, and connective tissue. Such biopsies are taken under sterile conditions. In the field biopsies are taken from freshly killed specimens; however, skin biopsies can be taken from live specimens without any significant damage to the individual. Biopsied material is brought to the laboratory, minced finely and set up in 75 cm³ culture flasks with growth medium. Usually within two weeks, cells begin to grow and after adequate time and with proper manipulation, a cell line distributed among 8–10 75 cm³ flasks is established. These flasks serve as the source of initial studies of a given species (such as chromosomal work) and such cell lines can be repeatedly tapped until desired quality of preparation is obtained.

Soon after the cell line is established, a portion of the cells are removed from actively growing cultures, to be suspended by freezing. Two procedures can be used (Smith 1952, Scherer & Hoogasian 1954). The cells can be put in a 10 % glycerine-growth medium, distributed into Nunc tubes (tubes designed for use in liquid

nitrogen), placed in the refrigerator overnight, and then placed in liquid nitrogen. Or, instead of the 10 % glycerine-growth medium, the cells can be placed in Nunch tubes with a 10 % DMSO-growth medium. These tubes are placed in a standard freezer until frozen and then dropped into liquid nitrogen. Once in the liquid nitrogen, the cells remain viable indefinitely regardless of which method is used.

3. Collection storage techniques

To record the critical data for each specimen added to the tissue culture collection, we have designed specially printed, hardcloth bound books. For each specimen the following data are recorded: species' name, sex, date collected, general and specific locality, catalogue number of the person who prepares the voucher, museum that houses the voucher specimen, the voucher specimen's museum number, type of tissue used for the primary culture, cytogenetic procedures performed on the cell lines and on the live animal, and location in the freezer of the suspended cell line. If the animal is sacrificed, it is imperative that the voucher specimen be preserved as a museum specimen. However, in some cases, such as those involving endangered species and zoo animals, the specimen cannot be sacrificed, and in such cases, a photograph of the diagnostic features of the specimen should be preserved.

Cells can be stored in an ultracold freezer, on dry ice, or in liquid nitrogen. It has been our experience that cells in an ultracold freezer have a half-life of less than 5 years. Dry ice and liquid nitrogen are colder and the cells remain viable for longer periods and, theoretically, liquid nitrogen storage should be essentially indefinite. The primary problem with dry ice is that it evaporates

relatively quickly and the supply must be replenished weekly. Some of our liquid nitrogen refrigerators will hold liquid nitrogen for over 90 days, so replenishing the supply is much less frequent. One problem is that liquid nitrogen refrigerators are expensive and the larger units with numbered positions for over 1000 vials, have a much faster evaporative rate than the smaller units which can hold only 150 or so vials of cells per dewar. We use liquid nitrogen tanks which are numbered and the cells stored in lettered canisters in each tank. An example of the information included in a listing would be as follows: *Pteropus neohibernicus*, TK 20117, Tank III, canister A, cane 2. A system like this is easily computerized for storage and easy access.

Our collection is computerized and the computer is used to print three catalogues that are used for day to day work. The three catalogues are: 1) numerically by specimen number, 2) taxonomically in alphabetical order by genus, and 3) by freezer location.

4. Discussion

Comparative cytogenetics is one of several approaches to the study of animal systematics that has had a marked impact on this field of biology in the past 10 years and this field is the reason we started our cell line collection. Initially, comparative cytogenetics was restricted to the characterization of chromosomal complements within species according to diploid number (2n) and gross morphology (reflected by FN) of the chromosomes. From these data, relationships could be postulated but not convincingly proved. Technological advances in the

early 1970's (Seabright 1971, Hsu 1973), however, gave rise to differential staining techniques which allowed a more detailed analysis of chromosomal homologies, and facilitated the study of chromosomal relationships among taxa. The single most important technique is G-banding, described by Seabright (1971), because from G-banded preparations, chromosomes are usually well characterized by their banding patterns. These chromosomes (or portions of them) can then be recognized in other species and after adequate numbers of species have been analyzed, relationships based on chromosomal trends can be proposed (Patton & Baker 1978, and Haiduk et al. 1981).

The full importance of living cell collections can only be speculated upon at present. As new cytogenetic techniques are developed, cells can be thawed, grown up, and used in new studies without the expense of additional field work. It may be especially critical to preserve cell lines of rare and endangered species. Also, no matter how effective new *in vivo* oriented techniques are, some groups such as whales and elephants will probably have to be studied by tissue culture methods.

Also, advances in other areas of biology may provide new tools for systematics and evolutionary studies which can be used on cultured cells. Some comparative studies that are now potentially feasible are: 1) surface proteins using immunological methods; 2) DNA sequencing; 3) biochemistry of cellular metabolism; and 4) chromosomal rearrangements in response to a variety of mutagens. Living cell collections represent an important resource with the potential to contribute much to the advancement of the science.

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