

EDITED BY DAVID M. PRESCOTT

VOLUME XIII

METHODS IN CELL BIOLOGY VOLUME XIII

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Methods in Cell Biology

Edited by DAVID M. PRESCOTT

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1.	Large-Scale	Enucleation (of Mamm a	lian Cells	
	George Veomett, .	Ierry Shay, Paul	V. C. Hough,	and David M. F	<i>rescott</i>

I.	Introduction	-1
H.	Cells and Materials	2
III.	Techniques	2
	References	6

2. Reconstruction of Cultured Mammalian Cells from Nuclear and Cytoplasmic Parts George Veomett and David M. Prescott

I.	Introduction	7
II.	Preparation of Components for Cell Reconstruction	8
III.	Criteria for Determination of Reconstructed Cells	10
IV.	Protocol for Cell Reconstruction	11
V .	Conclusions	13
	References	14

3. Recording of Clonal Growth of Mammalian Cells through Many Generations Hiroshi Miyamoto, Leif Rasmussen, and Erik Zeuthen

I.	Introduction	15
II.	Culture Medium	16
111.	Preparation of the Cells	16
IV.	Microphotographic Recording of Clonal Growth	18
	Clonal Growth	19
VI.	Applications	22
	References	26

,	55,00	
I.	Introduction	30
II.	Quality Evaluation	30
III.	Nonenzymatic Methods for Liver Cell Preparation	32
IV.	Enzymatic Treatment of Liver Slices	34
V.	Collagenase Perfusion	35
VI.	Purification of Parenchymal Cells	57
VII.	Incubation of Cell Suspensions	63
VIII.	Properties of Isolated Parenchymal Cells	64
IX.	In Vitro-Culture of Parenchymal Liver Cells	71
Х.	Preparation of Nonparenchymal Liver Cells	73
	References	78

4. Preparation of Isolated Rat Liver Cells Per O. Seglen

5.	Isolation and Subfractionation of Mammalian
	Sperm Heads and Tails
	Harold I. Calvin

I.	Introduction	85
II.	Isolation of Sperm Heads and Tails	89
III.	Subfractionation of the Sperm Head	95
IV.	Subfractionation of the Tail	100
	References	103

6. On the Measurement of Tritium in DNA and Its Applications to the Assay of DNA Polymerase Activity Bruce K. Schrier and Samuel H. Wilson

I.	Introduction	105
II.	Evaluation of Methods for Precipitation, Collection, and Counting	
	of Tritium-Labeled DNA	106
III.	Determination of Optimal Conditions for Collection and Counting	
	of DNA Polymerase Assay Products	114
IV.	Selection of Methods for a Particular System	117
	References	120

7. The Radioiodination of RNA and DNA to High Specific Activities Wolf Prensky

I.	Introduction	121
II.	Radioiodine	122
III.	Commerford's Reaction: General Description	136

vi

IV.	Equipment Needs	138
ν.	Radioiodination Reaction	141
VI.	Comments and Discussion	147
	References	152

8. Density Labeling of Proteins Aloys Hüttermann and Gertrud Wendlberger

I.	Introduction	153
II.	Density Labeling	156
III.	Equilibrium Density Gradient Sedimentation	158
	Ultracentrifugation	163
	Use of Density Markers	164
VI	Combination with Other Biochemical Methods	167
VIL	Conclusions	169
	References	170

9. Techniques for the Autoradiography of Diffusible Compounds Walter E. Stumpf

I.	Introduction	171
II.	Dry-Mount Autoradiography	174
III.	Thaw-Mount Autoradiography	185
IV.	Smear-Mount Autoradiography	186
V.	Touch-Mount Autoradiography	187
	References	192

10. Characterization of Estrogen-Binding Proteins in Sex Steroid Target Cells Growing in Long-Term Culture A. M. Soto, A. L. Rosner, R. Farookhi, and C. Sonnenschein

195
196
207
210

11. Long-Term Amphibian Organ Culture Michael Balls, Dennis Brown, and Norman Fleming

I.	Introduction	214
II.	Animals	214

III.	Methods	215
	Results	224
	Concluding Remarks	235
••	References	236

12. A Method for the Mass Culturing of Large Free-Living Amebas Lester Goldstein and Christine Ko

I.	Introduction	239
II.	The Basic Ameba Culture Procedure	240
III.	The Culturing and Harvesting of the Food Organism	
	Tetrahymena pyriformis	241
IV.	The Cleaning and Harvesting of Ameba Cultures	243
V.	Concluding Remarks	245
	References	246

13. Induction and Isolation of Mutants in Tetrahymena Eduardo Orias and Peter J. Bruns

I.	Introduction	248
H.	Elements of Tetrahymena Genetics	249
III.	Strains	253
IV.	Media	256
V .	Routine Methods	257
VI.	Calibration and Suggested Doses of Mutagens	269
VII.	Strategies and Protocols for Mutant Isolation	271
VIII.	Genetic Analysis of the Mutants	275
IX.	Additional Information for Nongeneticists	278
	References	281

14. Isolation of Nuclei from Protozoa and Algae D. E. Buetow

I.	Introduction	284
II.	Monitoring the Preparation	284
III.	Tetrahymena	285
IV.	Paramecium	292
V.	Blepharisma	297
VI.	Didinium	298
VII.	Spirostomum	298
VIII.	Amoeba	299
IX.	Euglena	301
X.	Algae	303
	References	310
ct Inde		313

Subject Index	313
CONTENTS OF PREVIOUS VOLUMES	318

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PREFACE

Volume XIII of this series continues to present techniques and methods in cell research that have not been published or have been published in sources that are not readily available. Much of the information on experimental techniques in modern cell biology is scattered in a fragmentary fashion throughout the research literature. In addition, the general practice of condensing to the most abbreviated form materials and methods sections of journal articles has led to descriptions that are frequently inadequate guides to techniques. The aim of this volume is to bring together into one compilation complete and detailed treatment of a number of widely useful techniques which have not been published in full detail elsewhere in the literature.

In the absence of firsthand personal instruction, researchers are often reluctant to adopt new techniques. This hesitancy probably stems chiefly from the fact that descriptions in the literature do not contain sufficient detail concerning methodology; in addition, the information given may not be sufficient to estimate the difficulties or practicality of the technique or to judge whether the method can actually provide a suitable solution to the problem under consideration. The presentations in this volume are designed to overcome these drawbacks. They are comprehensive to the extent that they may serve not only as a practical introduction to experimental procedures but also to provide, to some extent, an evaluation of the limitations, potentialities, and current applications of the methods. Only those theoretical considerations needed for proper use of the method are included.

Finally, special emphasis has been placed on inclusion of much reference material in order to guide readers to early and current pertinent literature.

DAVID M. PRESCOTT

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Chapter 1

Large-Scale Enucleation of Mammalian Cells

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I.	Introduction										•		1
II.	Cells and Materials									•	•		2
III.	Techniques .				•	•	•		•	•	•	•	2
	A. Without Centrifu	ge In	serts	•		•	•	•	•	•	•		2
	B. With Acrylic Inse	erts		•	•		•	•		•	•	•	3
	References .		•	•		•	•	•	•	•	•		6

I. Introduction

Carter (1967) was the first to realize the potential use of cytochalasin B (CB), a metabolite from *Helminthosporium dematoideum*, for experimental enucleation of cultured mammalian cells. A major step in developing this potential was introduced by Prescott *et al.* (1972) and Wright and Hayflick (1973), who subjected cells to a centrifugal force in the presence of CB. Under these conditions the nucleus is forced into a cytoplasmic stalk which subsequently severs spontaneously.

Prescott *et al.* (1972) and Prescott and Kirkpatrick (1973) used glass and subsequently tissue culture-grade plastic cover slips as a substrate for cell growth. The cover slips are easily handled and can be placed sterilely into centrifuge tubes for the enucleation procedure. The number of enucleated cells obtained is limited by the surface area of the cover slips used for growth. Several other techniques have utilized the same principle of combined

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centrifugation and CB treatment of tissue culture cells, but have varied the surface area for cellular growth. For example, Wright and Hayflick (1973) grow cells on the inner surface of centrifuge tube inserts, while Croce and Koprowski (1973) have described a technique in which the cells are grown directly on the inner surface of the centrifuge tubes. Follett (1974) has described a technique in which 35-mm tissue culture dishes used for cell growth are inverted in the wells of a centrifuge rotor and used for the enucleation procedure. Large numbers (approximately $0.5-1 \times 10^7$) of enucleated cells can be obtained by these procedures. The purity and viability of the cytoplasts (enucleated cells) using mass-enucleation procedures has been shown (Wright and Hayflick, 1973; Croce and Koprowski, 1973; Follett, 1974), but the karyoplasts (nucleated cellular fragments) have not been well characterized.

We describe here an alternate, relatively simple method for obtaining large numbers of enucleated cells and karyoplasts. This method utilizes 25-cm^2 tissue culture flasks both as substrate for cellular growth and as centrifuge tubes. The method is described for mouse L cells, but should be applicable to any cell that grows in monolayers.

II. Cells and Materials

Mouse L cells were grown in 25-cm² tissue culture flasks (Fisher or Falcon brand) in Ham's F12 medium supplemented with 10% fetal bovine serum and antibiotics.

The medium for enucleation consists of growth medium supplemented with $10 \mu g/ml$ of CB (Aldrich Chemical Co., Milwaukee, Wisconsin).

III. Techniques

A. Without Centrifuge Inserts

Cells are grown in the tissue culture flasks for at least 24 hours to allow the cells to attach firmly. The tissue culture medium is then removed, and medium containing CB is added to the flask until it is completely filled. The cap of the flask is then replaced and tightened.

The enucleation procedure is performed in a Sorvall RC-2B centrifuge, utilizing the GSA rotor, both of which are prewarmed to 37°C. Approxi-

mately 125 ml of water at 37° C is added to each rotor well; a filled flask is then added to each well. The rotor, with its cover off, is then spun at approximately 500 rpm, and water is continuously added to the rotor wells until all the wells contain the maximum volume of water, which is apparent when water is ejected from the rotor. The rotor is then stopped, the cover is replaced, and centrifugation is performed at 8500 rpm for 30 minutes.

The percentage of enucleation after one centrifugation is good, generally greater than 95% (Fig. 1). The karyoplasts form a pellet in the corner of the flask and are easily recovered. The trypan blue exclusion test indicates 80-90% viability. With this technique more than 90% of the karyoplasts can be recovered. Approximately $1-1.5 \times 10^6$ enucleated cells can be obtained from a single flask, or $6-9 \times 10^6$ from a single centrifugation run.

The major drawback of this technique is flask breakage, which is about 10%. Fisher flasks tend to crack and shear at the neck, whereas Falcon flasks shear along the side seams. In both cases, flask breakage is minimized by balancing the rotor as described.

B. With Acrylic Inserts

Acrylic inserts were constructed for the GSA rotor and were designed to accommodate Falcon bent-neck tissue culture flasks. The design of these inserts is shown in Fig. 2. They fit very tightly into the rotor wells and may have to be custom-trimmed for some rotors. Approximately 125 ml of water and a filled flask are added to each assembly used. The assemblies are balanced by weight, the tops are screwed on tightly, and centrifugation is performed in the GSA rotor in a Sorvall RC-2B centrifuge at 37°C for 20 minutes at 10,000–11,000 rpm. The results of the enucleation are similar to those obtained by the technique described in Section III,A, i.e., generally greater than 95% enucleation. However, karyoplast recovery is poorer. The reasons for poorer karyoplast recovery are unknown, but may be related to the alterations in centrifugal force and angle at which the flasks are held during centrifugation.

The inserts greatly reduce the breakage of Falcon flasks; only 1-2% of the flasks fail under these conditions, generally by leaking at the neck. The straight-necked Fisher flasks are unsuitable for use with this technique.

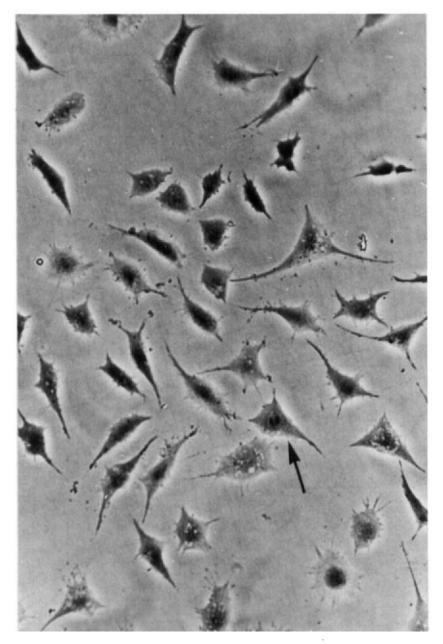


FIG. 1. Phase-contrast micrograph of enucleates from T-flask method of enucleation. (Arrow indicates nucleated cell for reference.) After fixation and staining, this preparation was shown to be 97% enucleated.

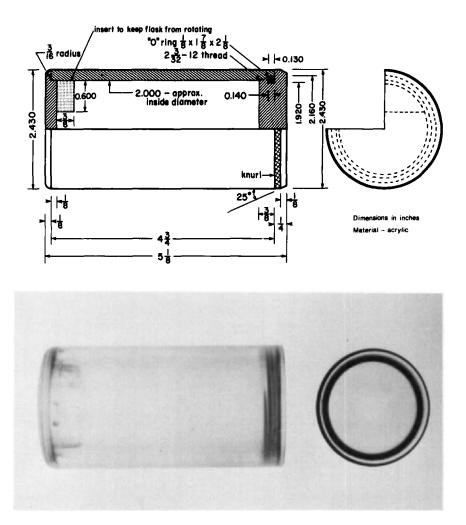


FIG. 2. Diagram and photograph of acrylic inserts for Sorvall model GSA rotor for use with T-flask enucleation procedure.

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