Methods in Cell Biology

Edited by

DAVID M. PRESCOTT

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DAVID M. PRESCOTT

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PREFACE

Volume XX 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.

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CONTENTS

Chapter 1

Measuring Spontaneous Mutation Rates in Yeast

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I. Introduction

Luria and Delbrück (1943) devised the first reasonably accurate method for measuring spontaneous mutation rates in microorganisms. Their fluctuation test is based on variance in mutant number from clone to clone, which is caused by rare mutations early in the growth of a clone yielding

more mutants in the clone than the more frequent mutations occurring late in clonal growth. They devised the fluctuation test to determine whether phage-resistant strains of *Escherichia coli* arise as random spontaneous mutations or as an adaptive response, such as the depletion of phage receptors among bacterial progeny by a massive phage attack on parental phage receptors (Luria, 1966). The fluctuation test came to be regarded as the preeminent quantitative method which provided respectability to the budding science of bacterial genetics.

Lea and Coulson (1949) developed mathematical expressions to deal more effectively with the boundary conditions of fluctuation experiments, introducing, for example, the method of the median. Ryan (cf. 1963) made many careful comparisons of theoretical distributions with observed distributions of mutants in clones, taking into account such phenomena as delayed nuclear segregation (Witkin, 1951) and phenotypic lag (Ryan, 1955). The validity and usefulness of the fluctuation test is now established, and some technical variant of this test is used for most measurements of spontaneous mutation rates in microorganisms.

The fluctuation test has been modified in order to measure the spontaneous mutation rates in a variety of strains and species of yeast. Yet another method was developed especially for yeast by Ogur and his collaborators (1959); it depends upon the selective elimination of new mutants in a population so that the mutants present when the population is examined represent those that arose in the last cell generation. These methods are described in this chapter.

An independent method for accurately measuring spontaneous mutation rates was introduced by Novick and Szilard (1950), stemming from their work on the continuous cultivation of bacteria in a chemostat. In a chemostat, the bacteria cannot grow at a maximal rate because they are prevented from doing so by the introduction, at a slow rate, of a limiting requirement into the medium while an equal volume overflows. The density of the bacterial culture remains in a steady state.

There is a large literature on the growth and mutation of *E. coli* in chemostat experiments (cf. Kubitschek, 1970). Suffice it to say that the chemostat has been utilized for studying selection (Francis and Hansche, 1972, 1973) and mutation (McAthey and Kilbey, 1976, 1977) in yeast. The basic discoveries in chemostat experiments with *E. coli*, such as the periodic selection of strains more adapted to the chemostat environment and mutations arising as a function of time or cell generation, depending upon the growth-limiting factor, have been demonstrated in yeast. Experimental methods for research on yeast in the chemostat are not dealt with here.

II. Measuring the Spontaneous Mutation Rate in Mitotic Cells by Limiting a Required Nutrient

A. The 1000-Compartment Fluctuation Test

The most accurate method for measuring the spontaneous mutation rate in yeast is based on the continued growth of prototrophic revertants after a required nutrient in the medium has been exhausted. A synthetic complete medium is used in which the relevant nutrient is low enough in concentration so that the titer of cells in the medium is kept well below the saturation level. This nutrient medium is called the limiting medium. For the method described here (von Borstel *et al.*, 1971), the relevant titer of cells is usually limited to somewhere between 10^5 and 10^7 cells/ml. The titer is regulated by altering the concentration of the limiting nutrient so that a reasonable number of revertants can be scored.

Limiting medium is inoculated with 2000 to 5000 cells/ml, and the medium is then distributed in 1-ml aliquots by a Brewer Model 60453 automatic pipetting machine (Baltimore Biological Laboratory) into 10 compartmented culture boxes each having 100 compartments. (The boxes were developed by F. J. de Serres, unpublished, and made on special order by Falcon Plastics). The boxes are then sealed and incubated. The yeast cells settle out on the bottom of each compartment, and revertant colonies begin to appear in about 3 days when incubation is at 26°C. The boxes are incubated for 12 days before colonies are counted, picked, and analyzed.

At the time the colonies are counted, the cell number is determined by hemocytometer counts from compartments without colonies. Two compartments per box are sampled. Since cell growth has ceased, cell counts at this time contribute to the extreme accuracy of this method. That is, residual cell growth, the limiting factor in the accuracy of some methods for measuring the spontaneous mutation rate, is not a limiting factor for accuracy with the 1000-compartment fluctuation test.

Special care must be taken with two items in order to ensure maximum accuracy: First, evaporation from the compartments must be minimal. This can be achieved either by tightly sealing the boxes with masking tape or by incubating them in a chamber where the humidity is kept very high. Second, small variations in temperature can alter measurably the spontaneous mutation rate; with some strains a threefold depression in rate takes place with each 10°C decrease in temperature (R. C. von Borstel and C. M. Steinberg, unpublished data).

Examples of the appearance of the revertants in the boxes can be seen in Fig. 1. The yeast strain in the box on Fig. 1B has a greatly enhanced spon-