Methods in Molecular Biology

Volume 2

NUCLEIC ACIDS

Edited by

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Preface

In recent years there has been a tremendous increase in our understanding of the functioning of the cell at the molecular level. This has been achieved in the main by the invention and development of new methodology, particularly in that area generally referred to as "genetic engineering". While this revolution has been taking place in the field of nucleic acids research, the protein chemist has at the same time developed fresh methodology to keep pace with the requirements of present day molecular biology. Today's molecular biologist can no longer be content with being an expert in one particular area alone. He/she needs to be equally competent in the laboratory at handling DNA, RNA, and proteins, moving from one area to another as required by the problem he/she is trying to solve. Although many of the new techniques in molecular biology are relatively easy to master, it is often difficult for a researcher to obtain all the relevant information necessary for setting up and successfully applying a new technique. Information is of course available in the research literature, but this often lacks the depth of description that the new user requires. This requirement for in-depth practical details has become apparent by the considerable demand for places on our Molecular Biology Workshops held at Hatfield each summer. This book is therefore an attempt to provide detailed protocols for many of the basic techniques necessary for working with DNA, RNA, and proteins. This volume gives practical procedures for a wide range of nucleic acid techniques. A companion volume (Volume 1) provides coverage for protein tech-

niques. Each method is described by an author who has regularly used the technique in his or her own laboratory. Not all the techniques described necessarily represent the state-of-the-art. They are, however, dependable methods that achieve the desired result.

Each chapter starts with a description of the basic theory behind the method being described. However, the main aim of this book is to describe the practical steps necessary for carrying out the method successfully. The Methods section therefore contains a detailed step-by-step description of a protocol that will result in the successful execution of the method. The Notes section complements the Methods section by indicating any major problems or faults that can occur with the technique, and any possible modifications or alterations.

This book should be particularly useful to those with no previous experience of a technique, and, as such, should appeal to undergraduates (especially project students), postgraduates, and research workers who wish to try a technique for the first time.

John M. Walker

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Contents

Chapter 1

The Burton Assay for DNA

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Introduction

The Burton assay for DNA is a colorimetric procedure for measuring the deoxyribose moiety of DNA. It is reasonably specific for deoxyribose, although very high concentrations of ribose (from RNA) or sucrose must be avoided. The method can be used on relatively crude extracts and in other circumstances where direct measurement of ultraviolet absorbance of denatured DNA is not practical. The assay has been widely used.

Materials

1. Diphenylamine reagent: Dissolve 1.5 g of diphenylamine in 100 mL glacial acetic acid. Add 1.5 mL of concentrated (98–100%) H_2SO_4 and mix well. Store this reagent in the dark. Just before use, add 0.5 mL of acetaldehyde stock solution.

2. Acetaldehyde stock: 2 mL acetaldehyde in 100 mL distilled water. Store at 4°C where it is stable for a few months.
3. 1*N* perchloric acid (PCA).
4. *Standards*: Dilute a DNA stock solution with distilled water as follows:

DNA stock, μL	0	10	20	50	100	200
Water, mL	1.0	0.990	0.980	0.950	0.900	0.800
DNA concentration, μg/mL	0	10	20	50	100	200

5. DNA stock: 1 mg/mL in distilled water. Store frozen at −20°C where it is stable for a few months.

Method

1. Extract the sample as required (*see* Notes).
2. Add 0.5 mL of 1*N* PCA to 0.5 mL of sample or standard. Hydrolyze for 70 min at 70°C.
3. Cool the hydrolyzed samples on ice for 5 min. Centrifuge (1500*g*; 5 min; 4°C) and decant the supernatants into marked tubes.
4. Add 1 mL of 0.5*N* PCA to each pellet, vortex, repeat step 3, and carry the combined supernatants forward to step 5. (This step is optional; *see* Note 3)
5. Add 2 vol. of diphenylamine reagent to 1 vol of the supernatants (0.5*N* PCA hydrolyzates from step 3). Mix and incubate at 30°C for 18 hr.
6. Read the absorbance at both 595 and 650 nm, using the 0 μg/mL standard as a blank.
7. Plot a standard curve of absorbance at 595 nm minus absorbance at 650 nm as a function of initial DNA concentration and then use the curve to read off unknown DNA concentrations.

Notes

1. Extraction conditions may have to be optimized for particular applications. The following procedures are routinely used in our laboratory.

(a) This extraction is required if the sample contains mercaptoethanol, dithiothreitol, or other interfering low molecular weight substances. It is also required as a preliminary if extraction (b), which is for whole cells or organelles that contain lipids, is to be used. Add 1 vol. of 0.2*N* PCA in 50% ethanol:50% distilled water and mix by vortexing. Cool on ice for 15 min and then centrifuge (5 min, 1500*g*, 4°C). Discard the supernatant.

(b) To the pellet add 1 mL of ethanol–ether (3:1, v/v). Incubate for 10 min at 70°C. Centrifuge (5 min, 1500*g*) and discard the supernatant. To the pellet add 1 mL of ethanol (96%), vortex and centrifuge (5 min, 1500*g*). Discard the supernatant.

2. If the sample is a pellet, at step 2 add 1 mL of 0.5*N* PCA to the pellet and proceed with the hydrolysis at 70°C. If the sample is too dilute (< 10 µg/mL) and is available in a volume larger than 0.5 mL, then it may be concentrated by precipitation, as described in Note 1, extraction (a), or by precipitation with 0.5*N* PCA.
3. Step 4 is optional. It provides a more quantitative recovery of nucleic acid in the supernatant, but reduces the sensitivity of the overall assay.
4. The diphenylamine reagent is not water soluble. Rinse out glassware with ethanol before washing in water. Take care to use a dry spectrophotometer cuvet and clean it with ethanol.
5. It is recommended to run a standard curve with each group of assays, preferably in duplicate. Duplicate or triplicate unknowns are recommended.

References

1. Burton, K. (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochem. J.* **62,** 315–323.