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RNA Analysis

Size and 3' End Group Determination

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1. Introduction

The most commonly used method to analyze the quality of RNA is electrophoresis in agarose gels. The distance an RNA molecule moves in a gel is dependent both on its mol wt and its conformation. Hence, to accurately compare RNAs across virus groups or to determine their size, it is crucial to completely denature the sample. Formaldehyde was the first denaturant to be used for this purpose ***(1)*** and is still a popular choice. Other reagents that denature but do not degrade RNA include formamide, which destroys base pairing, and glyoxal (ethanedial). Glyoxalation introduces an additional ring into guanosine residues, which then sterically hinders GC-pair formation. The first method described here incorporates formaldehyde in the gel and is adapted from Lehrach et al. ***(2)***. The second approach, which is based on the method of McMaster and Carmichael ***(3)***, uses glyoxal to denature the sample prior to loading it onto a nondenaturing gel. The latter technique has the advantage of allowing native (nondenatured) RNA to be run on the same gel as denatured RNA.

The last part of this chapter deals with the identification of the 3'-terminal base of an RNA molecule. This is important, since many cloning techniques result in the addition of extra (nonviral) nucleotides at the 3' end of the viral sequence. It may, therefore, not be possible to deduce the true 3' end of a viral RNA by sequence analysis of cloned cDNA. The technique employed makes use of T4 RNA ligase to add cytidine 3',5'-*bis*(phosphate) (pCp) to the 3'-OH terminus of the RNA of interest. By using [5'-^{32}P]pCp, the product of the reaction has a ^{32}P phosphate group in the last phosphodiester linkage, effectively labeling the RNA at its 3' terminus ***(4)***. Complete alkaline hydrolysis of the

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