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cDNA Library Construction for the Lambda ZAP®-Based Vectors

Marjory A. Snead, Michelle A. Alting-Mees, and Jay M. Short

1. Introduction

Because the vast majority of plant viruses have a positive-sense RNA genome, which acts as the viral mRNA, the RNA must first be converted into cDNA before cloning, amplification, and subsequent manipulation. Successful cDNA synthesis should yield full-length copies of the original population of mRNA molecules. Hence, the quality of the cDNA library can be only as good as the quality of the mRNA. Pure, undegraded mRNA is essential for the construction of large, representative cDNA libraries ***(1)***. Secondary structure of mRNA molecules can cause the synthesis of truncated cDNA fragments. In this case, treatment of the mRNA with a denaturant, such as methylmercuric hydroxide, prior to synthesis may be necessary ***(2)***. Other potential difficulties include DNA molecules contaminating the mRNA sample. DNA can clone efficiently and their introns can confuse results. RNase-free DNase treatment of the sample is recommended.

After synthesis, the cDNA is inserted into an *Escherichia coli*-based vector (plasmid or λ) and the library is screened for clones of interest. Since 1980, λ has been the vector system of choice for cDNA cloning ***(3–10)***. The fundamental reasons are that in vitro packaging of λ generally has a higher efficency than plasmid transformation and λ libraries are easier to handle (amplify, plate, screen, and store) than plasmid libraries. But, most λ vectors have the disadvantage of being poorer templates for DNA sequencing, site specific mutagenesis, and restriction fragment shuffling, although this trend is reversing to some degree with the continued development of PCR techniques.

The development of excisable λ vectors, such as those based on restriction enzyme digestion ***(11)***, site-specific recombination ***(12)***, or filamentous phage

From: *Methods in Molecular Biology, Vol. 81: Plant Virology Protocols: From Virus Isolation to Transgenic Resistance*
Edited by: G. D. Foster and S. C. Taylor