[23] Cloning of Viral Double-Stranded RNA Genomes by Single Primer Amplification

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Introduction

Several cloning strategies have been devised for viruses that possess segmented double-stranded (ds)RNA genomes (1–3). However, these procedures have relied on the availability of large amounts of dsRNA extracted from purified virus preparations. Of necessity, these procedures depend on a renewable source of viral RNA, either by propagation in cell culture or in a suitable experimental host. There are many situations in which a renewable resource of virus is not available. One such circumstance occurred in our laboratory during the characterization of a virus obtained from a fatal case of gastroenteritis in Bristol, England: the causative agent was later identified as a group C rotavirus (4).

Several groups of rotaviruses are currently recognized based on the antigenic differences in the major inner shell protein and on the characteristic banding pattern of their 11 dsRNA genome segments. Rotaviruses from only groups A, B, and C are associated with human disease. Group A rotaviruses are the major cause of acute gastroenteritis in children under 5 years of age. The group A viruses can be propagated in cell culture, and a number of sequences for all genome segments are available for direct reverse transcription and polymerase chain reaction (PCR) amplification. Group B rotaviruses have been responsible for major outbreaks of adult gastroenteritis in China; group C rotaviruses cause sporadic outbreaks in both adults and children throughout the world. No cell culture systems are available for the "atypical" group B and C human rotaviruses; hence, the only source material for the molecular characterization of these viruses is human fecal specimens. Thus, single-primer amplification of viral dsRNA (5) was developed for the express purpose of cloning atypical rotaviruses of unknown sequence directly from very small quantities of human fecal specimens. The human stool sample is an extremely hostile environment from which to extract undegraded RNA, but the successful cloning of all 11 genome segments of group C rotavirus RNA suggests that this method may be generally applicable to other dsRNA viruses from a variety of biological sources.

The success of the procedure is critically dependent on the preparation of highly purified dsRNA, and it is strongly recommended that enzymes and reagents are obtained from the suppliers stated in this protocol. The procedure can be divided into four sections: (*a*) purification and analysis of dsRNA from fecal samples; (*b*) radiolabeling and purification of primer 1; (*c*) ligation of primer 1 to dsRNA; and (*d*) cDNA synthesis and amplification by PCR.

Methods in Molecular Genetics, Volume 7