

## Cucumovirus Isolation and RNA Extraction

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### 1. Introduction

The *Cucumoviruses* are tripartite (+) sense RNA viruses in the *Bromoviridae* family of plant viruses. The genus includes cucumber mosaic virus (CMV, the type species), peanut stunt virus (PSV), and tomato aspermy virus (TAV) (reviewed in refs. 1 and 2). A fourth member, bean distortion mosaic virus (BDiMV), has also been proposed as a new species (3). CMV is further divided into two subgroups, based on hybridization data and serology (2). PSV probably also contains at least two subgroups, and perhaps as many as six (4). CMV has the broadest host range of any known plant virus, infecting up to 1000 species of plants, and inducing a very wide range of symptoms in infected plants (2). PSV has a narrower host range, infecting predominantly solanaceous plants and legumes, and TAV is predominantly restricted to solanaceous plants and composites (1). Symptoms for all of the cucumoviruses may include stunting, mosaic, and leaf distortion, and may range from mild to severe.

The *Cucumoviruses* encode at least four proteins (outlined in Fig. 1), and possibly a fifth (5). RNA-1 and RNA-2 contain large open reading frames (ORFs) encoding the 1a and 2a proteins, respectively, the viral components of the replicase complex (6,7). A second ORF is found on RNA-2 of all reported cucumoviruses, but the gene product for this ORF has not been shown for all strains of CMV (5). RNA-3 encodes the 3a protein, necessary for virus movement, and recently shown to interact with plant plasmadesmata (8). The 1a, (9), the 2a (10), and the coat protein (CP) (11) have also been implicated in virus movement. The CP is translated from a subgenomic RNA-4, which is collinear with the 3' portion of RNA-3. The genomic RNAs are packaged individually, with the subgenomic RNA-4 packaged along with RNA 3 (12). Some strains of CMV, especially Subgroup II strains, contain additional smaller RNA species