

## 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