

## Bromovirus Isolation and RNA Extraction

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### 1. Introduction

Bromoviruses are a group of plant single-stranded RNA viruses that belong to the genus *Bromoviridae*. Their polyhedral particles of ca. 26 nm in diameter have the icosahedral T-3 surface lattice symmetry (1), with 180 identical polypeptides used to build their virion particles. Because of the tripartite nature of their genomic RNA, three different particles exist: one containing one molecule of RNA-1, one containing one molecule of RNA-2, and one containing one molecule each of RNA-3 and RNA-4 (see Fig. 1).

The physicochemical properties of all bromoviruses are similar. Their virions are in a stable, compact form at pH between 3 and 6, but swell when the pH is raised above 6.5 (2). They also swell reversibly in presence of  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , with concomitant changes in capsid conformation (3). Besides coat protein (CP), no lipids or carbohydrates are reported to be contained within bromovirus particles.

Three type members: brome mosaic virus (BMV), cowpea chlorotic mottle virus (CCMV), and broad bean mottle virus (BBMV), as well as melandrium yellow fleck virus and cassia yellow blotch virus, appear to have the characteristics of bromoviruses (4). The bromoviruses have restricted host range: BMV infects mostly *Graminae*, whereas BBMV and CCMV infect a few species in the *Leguminosae*. All three bromoviruses can infect *Nicotiana benthamiana*, a useful virus purification host. In all these hosts, bromoviruses cause systemic mottle or mosaic symptoms, in which they reach and maintain levels of 0.3–3 mg/g of leaf tissue. BMV, BBMV, and CCMV give local lesions in some *Chenopodium* species.

The nucleotide sequences of the genomic RNAs of BMV, BBMV, and CCMV are known (5). The BMV replicase proteins 1a and 2a are encoded by