



Fig. 1. Molecular organization of the brome mosaic virus genome. The ORFs are boxed and labeled. The domains within ORFs 1 and 2 are marked by double-sided horizontal arrows. The noncoding sequences are marked as solid lines. The 5' CAP and 3' tRNA-like structures are marked, respectively, at the 5' and 3' RNA ends. The size of BMV CP is 27 kDa.

RNA-1 and RNA-2, respectively. Protein 1a has at least two domains: one for a putative helicase, and one for putative capping enzyme (guanylyl- and/or methyltransferases); 2a represents the catalytic unit (**Fig. 1**). BMV RNA-3 component encodes the nonstructural movement (3a) protein and the CP. Minus-strand synthesis promoters are located within the 3' noncoding tRNA-like structure region (6). Other sequences responsible for BMV RNA3 replication are within the intercistronic region and at the 5' end. The 5' noncoding region also contains internal regulatory motifs. The intercistronic region has the subgenomic RNA-4 promoter (7), as well as signals involved in asymmetric RNA synthesis (8).

## 2. Materials

1. Virus inoculation buffer: 0.01M NaH<sub>2</sub>PO<sub>4</sub>, 0.01M MgCl<sub>2</sub>, pH 6.0 (with NaOH).
2. Virus extraction buffer: 0.5M sodium acetate, 0.3M acetic acid, 0.01M MgCl<sub>2</sub>, 0.1M ascorbic acid (only for BBMV).
3. Virus storage buffer: 0.05M sodium acetate, 0.01M acetic acid, 1 mM Na<sub>2</sub>EDTA, 1 mM MgCl<sub>2</sub>.
4. 10X RNA extraction buffer: 0.5M glycine, 0.5M sodium chloride, 0.1M EDTA, pH 9.0.
5. RNA loading solution: 0.5% bromophenol blue, 0.5% xylene-cyanol, 15% Ficoll dissolved in DEPC-treated water.