(0.1 ng/μL) and 10.8 μL of SDW (*see* **Note 7**). A second reaction is set up in parallel, with SDW instead of DNA, as a negative control to check for PCR contamination. The reactions are set up in 0.5-mL tubes and gently overlaid with 25–50 μL of paraffin oil (Fluka) to prevent evaporation during thermocyling.

2. The reaction tubes are placed in a DNA thermocycler (OmniGene, Hybaid) programmed for 25 cycles of amplification, using a step program (94°C for 30 s, 55°C for 1 min, 72°C for 2 min), followed by a 10-min final extension at 72°C (**Note 5**).
3. 10-μL Aliquots of the reaction are loaded onto a 1.3% TAE agarose gel containing 0.5 μg/mL ethidium bromide and products visualized under UV light. Amplification of template DNA produces a ~500-bp product corresponding to the expected size (**Fig. 2C**, lane 1), and no products are visible in the negative-control reaction (**Fig. 2C**, lane 2).
4. 10 μL of the PCR reaction is digested at 37°C for 2 h with 0.5 μL *Bam*HI and 0.5 μL *Mlu*I (each at 10 U/μL, Gibco-BRL), 2 μL 10X react 3 (Gibco-BRL), and 7 μL of SDW. The 280-nt product can be gel purified using the Promega Wizard DNA purification system and an aliquot (~100 ng) ligated overnight at 14°C with ~50 ng of the 3.6 Kb *Bam*HI-*Mlu*I fragment of pBM217 in 1X ligation buffer (50 m*M* Tris-HCl, pH 7.6, 10 m*M* $MgCl_2$, 1 m*M* ATP, 1 m*M* DTT, 5% polyethylene glycol 8000, Gibco-BRL) with 0.5 U T4 DNA ligase (Gibco-BRL). The ligation (0.5 μL) is transformed into *E. coli* strain DH5α (Stratagene) following the manufacturer's instructions. This cloning step replaces the original 5' 270 nt of the coat protein in pBM217 with the 5' end of the PCR product to produce a clone containing the BaMMV CP gene with an ATG start codon in a good translational context (pBMCP-1, **Fig. 1C**). The 5' end of the clone should be sequenced to verify the presence of the ATG start codon and to ensure that no nucleotide changes had been introduced into the CP fragment by misincorporation during the PCR amplification (*see* **Note 8**).

4. Notes

1. The quality of the RNA preparation is vital to the success of this technique, and care must be taken not to contaminate the sample with RNase during extraction and subsequent handling. All tubes and solutions should be autoclaved, and gloves should be worn. The most likely problems are lack of product or generation of false products because of nonspecific amplification. Mismatched annealing may be reduced by increasing the anneal temperature or decreasing the primer concentration. The lack of a product may be caused by RNase contamination or the presence of secondary structure in the RNA template, causing premature termination of the reverse transcription reaction. This can be overcome by using a higher temperature, e.g., 52°C, for the reaction; the temperature can be increased further if a thermostable reverse transcriptase is used. Although RACE-PCR is most frequently used to amplify genes with poly(A) tails, CP genes from nonpolyadenylated viruses could be amplified by using terminal transferase to add a poly(A) tail to the genomic RNA.