(BAM N-ter) designed from the amino acid sequence of the N-terminus of the BaMMV CP ***(4)***.

Adaptor primer: 5'-GACTCGAGTCGACATCGA-3'

		H	E	E	P	D	(P)
BAM N-ter:	5'-CAC	GAA	GAA	CCA	GAC		CC -3'
		T	G	G	C	T	
					G		
					T		

BAM N-ter contains mixed bases at five positions, to allow for variation in codon usage. The adaptor and BAM N-ter primers are estimated to have T_ms of 56°C and 55°C, respectively (**Note 3**). The reaction contains 2.5 µL of 10X PCR buffer (10X stock is 200 m*M* $(NH_4)_2SO_4$, 750 m*M* Tris-HCl, pH 9.0, at 25°C, 0.1% [w/v] Tween, supplied by Advanced Biotechnologies, Surrey, UK), 4 µL of 1.25 m*M* dNTPs, 2.5 µL of 25 µg/mL BAM N-ter, 2.5 µL of 25 µg/mL adaptor primer, 1.5 µL of 25 m*M* $MgCl_2$, 0.2 µL of *Taq* polymerase (5 U/µL, Advanced Biotechnologies), 6.8 µL SDW, and 1 µL of first-strand cDNA reaction (**Note 4**). A negative control reaction is also set up using SDW, instead of the cDNA. 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. Forty cycles of PCR amplification are carried out (94°C for 30 s, 55°C for 1 min, 72°C for 2.5 min), followed by a 10-min extension at 72°C (**Note 5**) in a thermocycler (OmniGene, Hybaid, Middlesex, UK).

4. 5 µL of each PCR reaction are electrophoresed on a 1.3% TAE agarose gel (40 m*M* Tris acetate, 1 m*M* EDTA, pH 8.0) containing 0.5 µg/mL ethidium bromide in both the gel and the electrophoresis buffer; the products are visualized under UV light. An ~1100-bp product, corresponding to the expected size for the BaMMV CP gene, plus 3' untranslated region, is produced in the reaction containing viral cDNA (**Fig. 2A**, lane 1), but not in any of the negative-control reactions (**Fig. 2A**, lanes 2–4). To verify that this PCR product contains the BaMMV CP gene, 5-µL aliquots are digested with either *Hin*dIII or *Dra*I, which produced fragments of the expected size (**Fig. 2B**, lanes 1 and 2).

The method described can also be adapted to clone CP genes for which two specific primers are available, by substituting the second specific primer for the adaptor primer in the PCR reaction. The cDNA can be synthesized using either the dT_{17} adaptor or a shorter oligo(dT) primer.

3.2. Addition of Methionine Start Codon to BaMMV CP Gene

The BaMMV CP gene is expressed via proteolytic cleavage of a larger precursor, and therefore an ATG start codon must be added to the 5' end of the CP gene for expression in transgenic plants. This was achieved using primer BAM-3,