3.2.). Both methods are illustrated using barley mild mosaic virus (BaMMV), but can be adapted for other viruses by using appropriate primers and template. Although the reaction components and conditions provided will amplify many different targets, they are primarily intended as a starting position, and in some cases it will be beneficial to optimize the PCR using the guidelines provided.

3.1. PCR Amplification of BaMMV CP Gene Using a Single Primer

Since the BaMMV CP is located at the 3' end of RNA-1, which is polyadenylated (**Fig. 1A**), the gene can be cloned using the RACE-PCR technique *(3)*. Purified viral RNA or total RNA from infected plants can be reverse transcribed using a dT_{17} adaptor primer, which anneals to the 3' poly(A) tail. This single-stranded cDNA is then PCR-amplified using the adaptor primer and a single gene-specific primer corresponding to the N-terminus of the CP gene (**Fig. 1B**; **Note 1**).

1. Add 10 µL of purified viral RNA (~1–10 ng/µL) to 100 pmol of the dT_{17}-adaptor primer (1.8 µL of 627 µg/mL stock; **Note 2**) and incubate at 70°C for 10 min. The tube should be immediately cooled on ice.

dT_{17}-adaptor primer: 5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3'

   The adaptor sequence contains restriction sites that can be used for subsequent cloning of the RACE PCR product. Three different sites (*Xho*I, *Sal*I, *Cla*I) are included, so that there is likely to be at least one that does not cut within the product itself; if this is not the case, different sites can be used by resynthesizing an alternative primer. Total RNA (~1 µg) from infected plants could also be used as a template.
2. A first-strand cDNA reaction is then performed by addition of 5 µL 5X RT buffer (5X stock is 250 m*M* Tris-HCl, pH 8.3, at room temperature, 375 m*M* KCl, 15 m*M* $MgCl_2$, as supplied by Gibco-BRL), 2.5 µL 100 m*M* DTT, 2.5 µL 10 m*M* dNTPs, 0.5 µL RNasin ribonuclease inhibitor (40 U/µL, Promega, Southampton, UK) 1.7 µL SDW; the contents are mixed and incubated at 37°C for 5 min and then add 1 µL of M-MLV RNase H-reverse: transcriptase (200 U/µL, Gibco-BRL). The tube is further incubated at 37°C for 60 min, followed by 42°C for 30 min, and the reaction is stopped by heating to 94°C for 5 min. For convenience, the reaction is carried out in a DNA thermocycler, but can be performed using a heated water bath. Control reactions are also performed using either no template (SDW instead of RNA) or without the addition of reverse transcriptase. The latter control is to ensure that the PCR products are derived from an RNA template, rather than from contaminating DNA, and is particularly important if total RNA from infected plants is being used, rather than purified viral RNA.
3. A 25-µL PCR reaction is performed using an adaptor primer corresponding to the 5' end of the primer used for cDNA synthesis and a degenerate primer