2.2. Primer Design

It is important to check that the primers are complimentary to the correct template strand and in the appropriate 5' to 3' orientation to produce the desired PCR fragment. In general, PCR primers are designed to be between 20 and 30 bases in length, and to have a 50–60% GC content. Where possible, the 3' end of the primer should ideally be a G or C residue to anchor the primer base to the template for efficient elongation, but runs of several Gs and/or Cs should be avoided, since they can promote mis-priming. The primer sequences should be checked for self-complementarily and primer–primer complementarity, since this will significantly affect the efficiency of amplification by competing with the template.

Degenerate primers can be synthesized containing a mixture of more than one base at any position, and are used when amino acid sequence data is the basis for primer design. Amino acids with the minimum degeneracy should be selected, and it helps to avoid degeneracy at the 3' end. Inosine, which hybridizes equally well to any of the four bases, can also be used, and this reduces the complexity of the final primer mixture. Computer software programs are available to assist primer design and can be used to check for secondary structure and degree of degeneracy.

2.3. Minimizing PCR Contamination

The ability of PCR to produce large amounts of DNA from low quantities of template necessitates that extreme care be taken to avoid false positives caused by contamination; for this reason, a negative control reaction should be included in all experiments. The most likely sources of contamination are the carryover of products from a previous PCR reaction or from one sample to the next. A number of steps can be taken to reduce these problems. Carryover of products from one reaction to another can be avoided by physically separating the operations performed to set up the reaction from those required to handle the products, by using, for example, a separate pipet dedicated to handling PCR products. Aerosol-resistant tips or positive-displacement pipets can be used to prevent contamination caused by aerosols reaching the shaft of conventional pipets. Adding the template DNA last can also help to reduce opportunities for transfer between samples. In addition, aliquoting reagents will limit any contamination to a single experiment.

3. Methods

Two methods are described: the use of RACE-PCR to amplify CP genes using only N-terminal amino acid sequence data (*see* **Subheading 3.1.**), and the addition of an ATG start codon to the 5' end of a CP gene (*see* **Subheading**