cations; for example, it may be used to add translational start or stop codons, to change the translational context of an existing ATG codon, or simply to add restriction enzyme sites, to facilitate subsequent cloning steps.

2. Materials

2.1. Reaction Components

PCR amplification requires DNA primers, dNTPs, buffer, $MgCl_2$, *Taq* polymerase, and template DNA. The optimal concentrations of these components, particularly $MgCl_2$, are likely to vary between applications, and often have to be determined experimentally. The range of concentrations most frequently used, and general guidelines, are provided below.

1. Primers: PCR primers are most stable when stored as a lyophilized pellet, but for convenience can be stored as a high-concentration stock solution in sterile distilled water (SDW) at –20°C. It is best to avoid use of buffers containing EDTA, since *Taq* polymerase requires Mg^{2+} ions, which will be chelated by EDTA. Primers are generally used at concentrations between 0.2 and 1.0 μ*M* for each primer. High primer concentrations may promote mix-priming and primer-dimer artifacts produced by the interaction of primers alone.
2. dNTPs: A concentrated stock of dNTPs, with each dNTP at 1.25 m*M*, can be stored in aliquots at –20°C to avoid repeated freeze–thaw cycles. PCR reactions generally contain 20–200 μ*M* dNTPs and it is important that all four nucleotides are present at the same concentration to ensure the highest level of fidelity. The specificity and fidelity of PCR is increased by using low dNTP concentrations.
3. *Taq* polymerase: The recommended range of *Taq* polymerase is 1–4 U/100 μL reaction, but requirements will vary with different templates or primers, and a range of concentrations can be tested. If the concentration is too high, a nonspecific background smear can develop; too little produces low yields. A wide range of heat-stable DNA polymerases are now available, including some that have a 3' to 5' exonuclease proofreading activity. Also available is a thermostable reverse transcriptase, which can be used to make both cDNA and DNA amplification products in RT-PCR reactions.
4. PCR buffer: Buffers for PCR reactions, are generally based on 10–50 m*M* Tris-HCl (pH 8.3–8.8) and contain up to 50 m*M* KCl to facilitate primer annealing. Gelatin, BSA, or Tween-20 are often added to help stabilize the enzyme, and cosolvents such as DMSO are sometimes also included to facilitate template denaturation. PCR buffers are usually supplied with the polymerase and vary in composition, depending on the supplier. If a reaction is not working, it is often worth trying either an alternative buffer system or source of polymerase.
5. $MgCl_2$: *Taq* polymerase is a magnesium-dependent enzyme, and free Mg^{2+} concentration also can affect primer annealing and strand desiccation. Generally, reactions contain 1.5 m*M* $MgCl_2$, but it can be beneficial to optimize the magnesium ion concentration, usually within a 0.5- to 4-m*M* range.