Nucleic Acid Removal from Taq Polymerase Preparations Using an Aqueous/Organic Biphasic System

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The polymerase chain reaction (PCR) has revolutionized the molecular biology industry, with profound implications for medical, forensic and research sciences. Prerequisites for this technology to work include the presence of deoxynucleotriphosphates (dNTPs), a relevant buffering system, magnesium chloride, primers and template DNA and finally, the DNA polymerase. Taq polymerase, a thermostable DNA polymerase isolated from Thermus aquaticus (1,4) is still the most universally used polymerase for PCR, although others exist and are continuing to emerge onto the market.

The cloning, expression and purification of Taq polymerase has been studied extensively (5,6). However, one of the most difficult issues surrounding the purification of (thermostable) DNA polymerase remains the contaminating DNA, present in crude extracts of the enzyme. Clearly, when using high copy number plasmid constructs in an attempt to increase expression of the polymerase gene, the ensuing amount of contaminating DNA can become very high, from both genomic and plasmid sources. Furthermore, the enzyme itself has, not surprisingly, a high affinity for its substrate (DNA), resulting in the fact that the removal of all the contaminating DNA becomes an arduous task. Even the use of strong anion exchangers do not remove all the contaminating DNA. However, total removal is not necessary in most cases, as evidenced by the vast majority of commercial Taq and other DNA polymerase preparations. Nevertheless, if a lot of DNA is present, it might be visible on an ethidium bromide-stained gel, interfering with the analysis of amplified products. DNA is also an effective magnesium ion chelator, and if high amounts are present in the PCR, they can deleteriously affect the PCR in terms of yield and specificity. Generally, the issue is most important when the Taq preparation includes sequences of sufficient homology to the sequences being studied. Situations in which false positives can arise include amplifications carried out on host (Escherichia coli) DNA, or when universal primers, designed to amplify specific conserved sequences that share significant homology with the host DNA, are used. An example of this would include amplifications of the conserved regions of ribosomal RNA coding DNA (8). Hence, the purity of the Taq preparation must be sufficient not to interfere with the vast majority of assays. Methods exist that have been used to remove the DNA, although these protocols have a number of drawbacks. Anion exchange columns can be used (2), however, the DNA-containing solution is typically so viscous and binds the resins so strongly that flow through the column can rapidly become restricted, thereby increasing pressures and requiring regular column cleaning. When purifying large (production size) volumes of crude extracts, this can simply preclude the method altogether, unless supplementary DNA removal protocols are used before the chromatography. Such methods can include the rather laborious and lengthy use of neutralized polyethyleneimine (PEI), centrifugation and dialysis (3). Furthermore, PEI binds DNA tightly, and problems have been associated with the batch-to-batch reproducibility of this precipitation technique (3). Apart from being time-consuming, this method has the additional drawback that PEI inactivates the DNA polymerase enzyme when present in too high a concentration. Ultrafiltration can also be used for DNA removal, in which 300,000 Da molecular weight cut-off filters are used to separate the majority of the DNA from the enzyme. Unfortunately, this method is extremely time-consuming, and still results in significant DNA contamination in the polymerase preparation.

A patent-pending technique has recently been developed and used to eliminate the type of problems illustrated above through the construction of an aqueous/organic biphasic system. In principle, this system is formed through the addition of a normally 100% water-miscible organic solvent (ethanol in this case) to a fractionally-saturated salt solution in water. Although a variety of water-miscible organic solvents can be used to create two-phase systems, the use of different solvents can result in systems exhibiting different partitioning characteristics. Differential protein partitioning has already been demonstrated using this technology (7), and it has been found to be extremely useful in the removal of nucleic acids from a crude cell-free extract of Taq DNA polymerase, heat-treated to remove most of the host contaminating protein, and essentially prepared as previously described (3). Two simple stages were carried out as follows: (i) The crude cell-free extract was made up to 10% saturated sterile dibasic potassium phosphate (pH 10.3) and mixed well for several seconds, (ii) An equal volume of ethanol was added to the 10% salt solution and mixed vigorously for several seconds. After settling out of the two phases (which can be accelerated by using centrifugation if necessary, 1000–2000× g for 2 min), the top (organic) phase, containing the Taq is removed. The bottom (aqueous) phase, containing the nucleic acids is discarded.

Figure 1. An ethidium bromide-stained agarose gel (1%), showing the absence or presence of contaminating nucleic acids. A crude Taq extract was made as described (5). The original crude extract and extracts after biphasic partitioning treatment were desalted to facilitate gel loading. Lane 1, Lambda HindIII marker; lane 2, crude Taq extract (starting material) before treatment; lane 3, crude Taq extract after biphasic treatment; organic (upper) phase; lane 4, crude Taq extract after biphasic treatment; aqueous (lower) phase.
Figure 1 illustrates the results of this procedure. It is clear that the (ethidium bromide-stained) nucleic acids have partitioned into the lower, aqueous phase (Figure 1, lane 4) from the starting solution (Figure 1, lane 2). All the Taq activity remained in the upper, organic phase (Figure 1, lane 3). In contrast, Figure 2 shows the distribution of the proteins (including Taq) after partitioning. Figure 2, lane 3 shows the crude Taq preparation before partitioning, and Figure 2, lanes 4 and 5 show the upper (organic) and lower (aqueous) phases, respectively. All the protein, both the contaminating and the Taq itself, were retained in the upper, organic phase. The purified Taq solution can be used directly for PCR or desalted and stored. If further purification is required, the Taq solution can be loaded onto an anion exchange column directly, without experiencing the problems associated with the DNA-containing solution. Despite the dilution due to partitioning, the total activity (100%) of the Taq is maintained (assayed by the PCR effectiveness of amplifying a 200-bp product from the human β-actin gene). The solution itself is stable in ethanol for 4 days at 4°C without any loss of activity, although for prolonged storage, desalting followed by freezing in 50% glycerol is recommended. Furthermore, as the Taq solution contains ethanol (ca. 50%), the solution is inherently anti-microbial, and no further additions of anti-microbial agents need to be made to ensure sterility. This method for nucleic acid removal is very rapid (a few minutes) and highly selective for nucleic acid removal.

REFERENCES


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