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Rapid purification of recombinant *Taq* DNA polymerase by freezing and high temperature thawing of bacterial expression cultures

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The polymerase chain reaction (PCR) and dideoxy DNA sequencing are frequently used techniques of molecular biology which utilise DNA polymerases. The high temperatures required for PCR necessitate a thermostable enzyme for DNA amplification, and DNA polymerase derived from the thermophilic microorganism, *Thermus aquaticus*, is used most commonly. The high optimal polymerisation temperature of this enzyme also makes it useful for overcoming sequencing artefacts of DNA secondary structure.

Cloning of bacterial expression vectors which produce recombinant *Taq* polymerase has facilitated preparation of the enzyme. Typically, methods for purification of this protein from bacterial cultures involve selective precipitation and ion exchange chromatography (1,2). Here we describe a novel simplified protocol for purifying *Taq* polymerase which is suitable for PCR and DNA sequencing. The method exploits the thermostable properties of *Taq* polymerase. Contaminating macromolecules of the host *E.coli* (strain BL21 used here) are conveniently precipitated after their denaturation by freezing and high temperature thawing. The pTaq expression plasmid we used was originally described by Engelke *et al.* (1) and is derived from the pTTQ18 vector (Amersham) where the inserted *Taq* gene sequence is under the transcriptional control of the *tac* promoter. *E.coli* of the strain BL21 was transformed with pTaq. These bacteria contain the pLysS plasmid (3), which makes them susceptible to lysis by freeze–thawing for the release of recombinant proteins. A colony of transformed BL21 cells was cultured overnight at 37°C in 5 ml of Lauria Bertaini (LB) broth containing chloramphenicol for pLysS selection (35 μg/ml) and ampicillin (100 μg/ml). These bacteria were then used to inoculate a larger volume of LB medium and the culture continued at 37°C until the broth had reached an OD_{600} of 0.4. At this point, IPTG was added to a concentration of 0.5 mM and the culture continued overnight. The bacteria were harvested by centrifugation, washed, and then resuspended in buffer A (50 mM Tris–HCl pH 7.9, 50 mM glucose, 1 mM EDTA) to a twentieth of the culture volume. The suspension was subjected to two cycles of freezing and thawing at temperatures of –70°C and 75°C or room temperature. The cell debris was removed from the lysate by centrifugation (12 000 *g* for 20 min). The enzyme containing supernatant was dialysed against an excess of buffer (20 mM HEPES pH 7.9, 100 mM KCl, 0.1 mM EDTA, 0.5 mM PMSF, 1 mM DTT, 50% glycerol) before storage at –20°C. Recombinant *Taq* polymerase was also prepared by polyethylene imine (PEI) precipitation and ion exchange chromatography according to the procedure described by Engelke *et al.* (1). Protein analysis was by SDS–PAGE with Coomassie blue staining. The activity of the purified enzyme was determined using a PCR amplification reaction with titration against a commercial *Taq* preparation (Promega). The template for the sequencing reaction was double-stranded λgt11 DNA and extension was from the forward primer using the fmol™ sequencing kit (Promega). *Taq* polymerase prepared by bacterial freeze–thawing was substituted for the commercial enzyme, but other conditions were those recommended by the supplier.

By changing the thawing temperature from room temperature to 75°C, most of the host *E.coli* BL21 proteins were denatured, and they were easily removed from the lysate as a precipitate (Fig. 1A, lanes 1 and 2). SDS–PAGE analysis indicates that the 94 kDa *Taq* protein remaining in the lysate is of similar purity to that using PEI precipitation and ion exchange chromatography (1) (Fig. 1A, lanes 2 and 3). There was no significant proteolytic degradation after overnight culture. The yield of enzyme activity was ~20 U/μl in the dialysed lysate or 400 U/ml of culture broth. In our hands, the yield of enzyme prepared according to the procedure described by Engelke *et al.* (1) was ~10-fold lower. The enzyme activity per μg of purified protein was similar with both of the methods. In the PCR assay, which used four different primer combinations to amplify parts of the hepatitis B virus X-gene, the quality of the amplified products was similar to those using *Taq* polymerase prepared by the method of Engelke *et al.* (Fig. 1B). Nuclease activity was not detectable at temperatures from 37 to 72°C after overnight incubation of amplified DNA with *Taq* polymerase added in excess of working concentrations (not shown). The enzyme also enabled sequencing of a double-stranded DNA template (Fig. 1C).

This freeze–thawing method for the purification of *Taq* polymerase is simpler than previously described techniques, and the enzyme yield is high. The suitability of the preparation for PCR and DNA sequencing offers widespread application.

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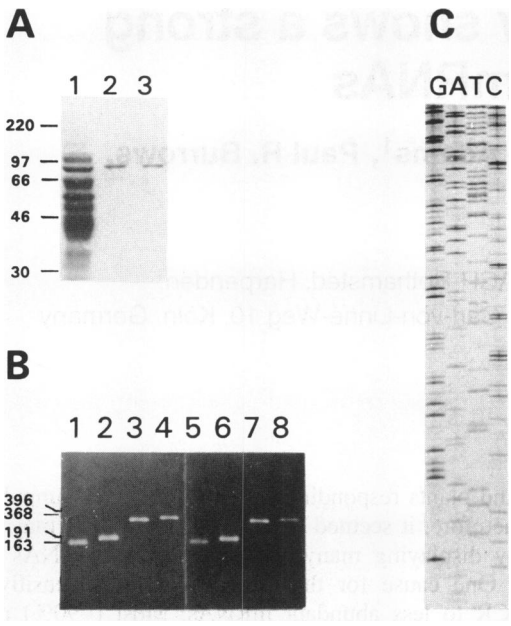

Figure 1. (**A**) Analysis of *Taq* protein purification from BL21 *E.coli* using SDS–PAGE (10%) and Coomassie blue staining. The positions and sizes (in kDa) of the molecular weight markers are indicated on the left. Lane 1 demonstrates the protein in the supernatant prepared from bacteria lysed by two cycles of freezing and thawing at room temperature. Lane 2 shows total protein remaining in the supernatant of similar bacteria after two cycles of freezing then thawing to 75°C. Lane 3 shows protein prepared by PEI precipitation and ion exchange chromatography (1). (**B**) PCR amplification of fragments of the HBV X-gene using four primer combinations. 0.1 ng of template plasmid DNA was used and the primers represent sequences from coordinates of 1420–1443, 1625–1648, 1788–1765 and 1793–1816 with respect to the *adw* subtype of the HBV genome. Lanes 1–4 represent amplification using the freeze–thaw prepared enzyme, and in lanes 5–8, the DNA was amplified with *Taq* prepared by the method according to Engelke *et al.* (1). (**C**) DNA sequencing of double-stranded λgt11 DNA template using *Taq* polymerase prepared by bacterial freezing and thawing to high temperatures.

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