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Rapid purification of high-activity *Taq* DNA polymerase

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The method described here is derived from that of Engelke *et al.* (1), and uses the same cloned form of *Thermus aquaticus* (*Taq*) DNA polymerase to produce this enzyme in *E.coli*. The modified purification method described here is quite simple, however it is important to note that factors such as the bacterial strain used, induction time and protein concentration during isolation have been optimized. Deviation from the established parameters can have large effects on enzyme yield and activity.

E.coli strain INV1alphaF' (Invitrogen Corp., San Diego, California) was transformed with the pTaq plasmid, which contains the *Taq* gene expressed under control of the *tac* promoter (1). Large-scale cultures of *E.coli* containing the pTaq plasmid were initiated by adding 500 µl of an overnight culture to one litre of LB broth with ampicillin (80 mg/l). These cultures were grown at 37°C for 11 hours to an OD_{600} of approx. 0.8, and then IPTG was added to a concentration of 125 mg/l. After 12 hours of induction the cells were harvested by centrifugation and washed in 100 ml per litre of original culture volume of buffer A (50 mM Tris–HCl pH 7.9, 50 mM dextrose, 1 mM EDTA; all reagents used in the purification were molecular biology grade, and care was taken to avoid contamination with biological material or metal ions). Cells were recovered by centrifugation and resuspended in 50 ml per litre of original culture volume of pre-lysis buffer (buffer A plus 4 mg/ml lysozyme). After 15 minutes at room temperature an equal volume of lysis buffer was added (10 mM Tris–HCl pH 7.9, 50 mM KCl, 1 mM EDTA, 1 mM PMSF, 0.5% Tween 20, 0.5% Nonidet P40) and the lysis mixture was incubated in 200 ml aliquots in pyrex flasks at 75°C for 1 hour. The lysis mixture was then transferred to plastic bottles for centrifugation at 15,000 rpm for 10 minutes at 4°C, and the clarified lysate was transferred to a clean pyrex flask.

Taq polymerase was recovered from the clarified lysate by adding 30 g of powdered $(NH_4)_2SO_4$ per 100 ml of lysate while stirring rapidly at room temperature. The solution was then centrifuged at 15000 rpm for 10 minutes and the protein precipitate was harvested (both in pellets and as surface precipitate) and resuspended in 20 ml of A buffer per 100 ml of original cleared lysate. The resuspended protein was dialyzed against 2 changes of at least 12 hours each of storage buffer (50 mM Tris–HCl pH 7.9, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 50% glycerol) at 4°C. After dialysis the resulting protein was diluted 1:1 with sterilized storage buffer and stored at −70°C.

Standard purification was done with 8 l batches of *E.coli* culture, which produced average yields well in excess of 10^6 Units of *Taq* polymerase per litre of starting culture. The final product of the purification showed a 90 kD band on SDS–PAGE (Figure 1), and few contaminating protein bands were evident, even after silver staining. The enzyme was highly concentrated and had to be diluted (usually by a factor of 100) before use, since *Taq* polymerase shows a decrease in activity at high concentrations (1).

The activity of the purified *Taq* polymerase was determined by titration against commercial preparations in a DNA amplification assay (1), and was found to be on the order of 1 unit/100 ng of protein, with the typical concentration of activity of the final purification product being 100 units/µl (Figure 2). Exonuclease activity was tested for by incubating working concentrations of enzyme with cut plasmid DNA at 37°C overnight, and no evidence of nuclease activity was seen when the DNA was run on an agarose gel. Stocks of undiluted enzyme stored at −70°C have shown no decrease in activity over 18 months (the polymerase concentration of 10 mg/ml is sufficient to stabilize the enzyme without the addition of other protein), and 1/10 diluted stocks have remained stable at −20°C for over 12 months.

It was found during the development of this purification protocol that two of the most important factors in determining the yield of enzyme were the strain used and the induction time. Other strains of *E.coli* can be used as hosts for the pTaq plasmid (eg. DH1, DH5), however the protein yield is generally less than for the strain used here, which is optimized for the production of protein driven by the *tac* promoter.

Whichever strain is used to produce the enzyme, it is very important to optimize the length of induction time with IPTG, since over-long induction results in the proteolytic degradation

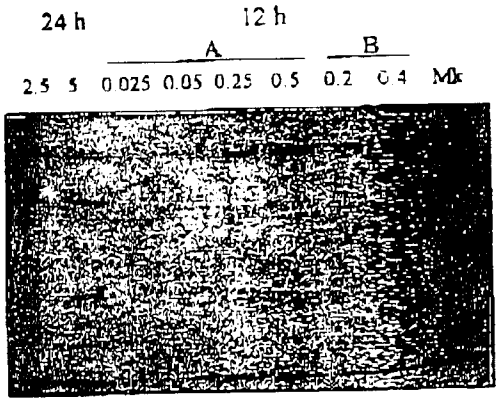

Figure 1. 12% SDS–PAGE gel stained with Coomassie Blue showing *Taq* polymerase purified from cells induced with IPTG for 24 h and 12 h (two batches, A and B). Numbers above lanes indicate µl of final purified product run on gel. Intact enzyme has a MW = 90 kD (largest marker band shown is 97 kD), and protein from the 24 h induced batch shows smaller size bands due to proteolysis.

of the *Taq* polymerase. Figure 1 shows the results of purification from cells induced for 24 and 12 hours, and most of the enzyme recovered from cells induced for 24 hours is degraded, with the major large protein running slightly below the native *Taq* polymerase band and other proteolysis products visible as smaller protein bands. Induction times shorter than 10 hours showed a drop-off in enzyme production.

The author requests that those wishing to obtain the pTaq plasmid contact the original sources cited below.

REFERENCE

1. Engelke,D.R., Krikos,A., Bruck,M.E. and Ginsburg,D. (1990) *Anal. Biochem.* 191, 396–400.

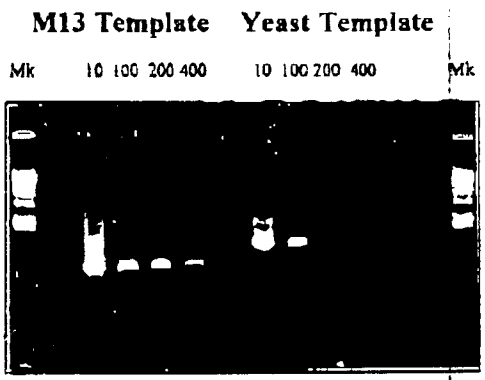

Figure 2. Comparison of purified Taq polymerase in PCR amplification of 50 ng M13 DNA using universal primers (left) and 1 μg yeast genomic DNA using a single-copy gene primer set. 1 μl of enzyme diluted by the factor given above each lane was used per 35 cycle reaction under standard conditions. The 100× dilution showed similar activity to 1 unit of commercially-available enzyme (note that this represents 0.01 μl of purified enzyme, which is less than half the amount run in the faintest band in the gel shown in Figure 1).