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Large Scale Overexpression and Purification of *Taq* DNA Polymerase in *Escherichia Coli*

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Overview

Thermus aquaticus DNA polymerase(*Taq*) is a protein that is commonly used in a polymerase chain reaction(PCR) to create copies of DNA for diagnostic tests. As a result of its utility there was strong incentive to produce purified samples of this protein in large quantities. In order to purify this protein several purification techniques were used to separate *Taq* from various other proteins found in the *Escherichia Coli*(*E.coli*) used to produce the target recombinant protein.

Materials and Methods

Figure 1. 10L Bioreactor. This image shows the 10L bioreactor in operation.

Taq expression in large scale

E.coli carrying the *Taq* expression vector was grown in a 10L bioreactor using TB media, and the growth was monitored by measuring the OD600. During the incubation process, NaOH was used to maintain the pH as a means of counteracting the pH drop observed as *E.coli* is in the exponential growth phase. When the OD600 reached to 3.148, IPTG was added to induce Taq expression. Roughly two days after induction, the cells were harvested via centrifugation.

Taq purification

A sonicator was used to disrupt the cells and the sample was centrifuged to obtain the crude lysate, containing all soluble proteins including *Taq*. Many non-target mesophilic proteins were removed by incubating the crude lysate at 72ºC for 30 minutes(referred to as “heatcut” purification). Following the heatcut-selection step, two chromatography columns were used to further purify *Taq*, these columns included a heparin affinity column and a Q column. The heparin column was used to isolate DNA-binding proteins and the Q column was used to purify *Taq*, which can be negatively charged at a certain pH. The quality of the purification process was evaluated by running SDS-PAGE and performing qPCR monitoring the fluorescence signal with SYBR-Green which binds to the amplification products.

Results

The purity of the *Taq* can be observed visually in the SDS-PAGE gel shown below. Additionally, the purity and volume of the protein collected is summarized in the purification table below.

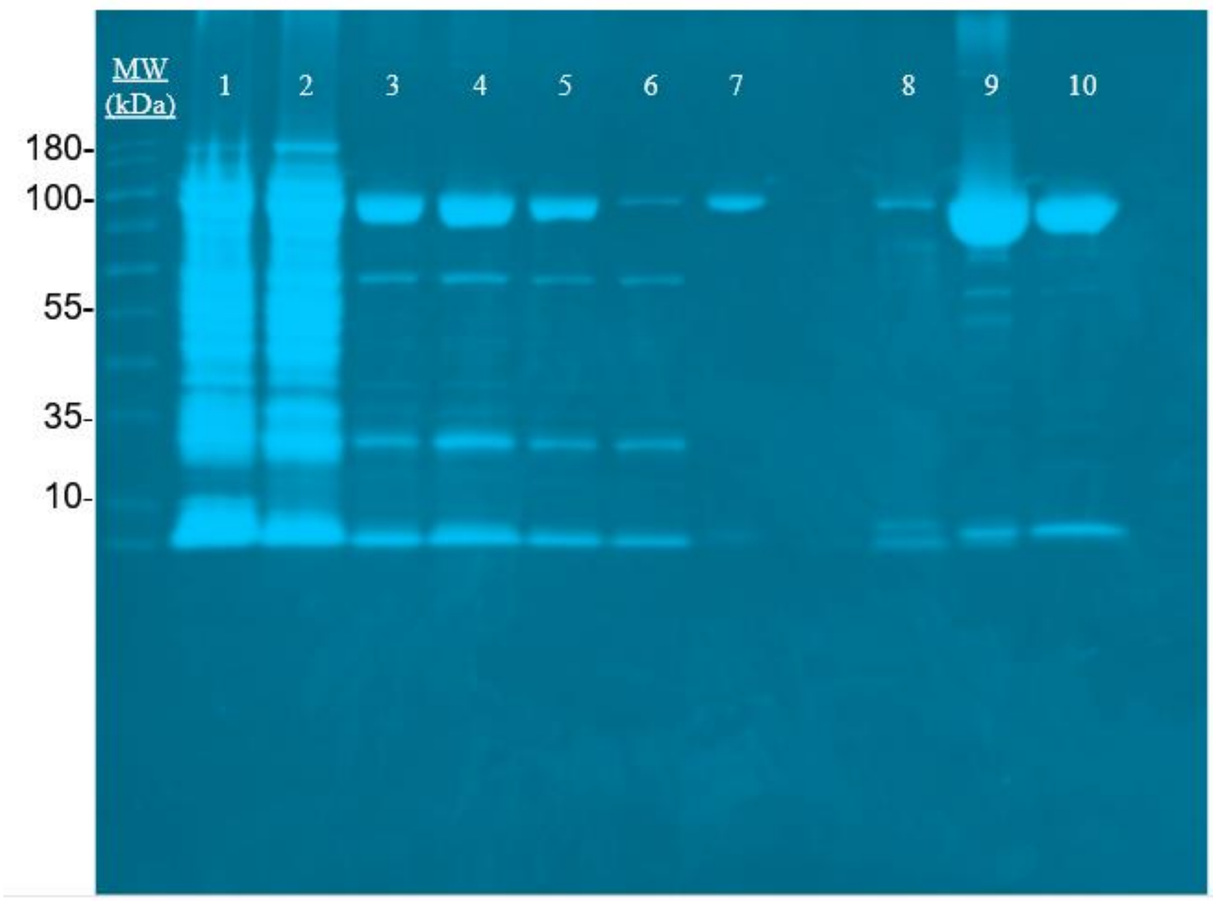

Figure 2. SDS-PAGE analysis of purified *Taq*. Lanes 1 and 2 depict the crude lysate purification step. Lanes 3 and 4 depict the pre-heparin column sample. Lane 5 represents the post-heparin/pre-Q column sample. Lane 6 represents Q column flowthrough. Lane 7 is the post Q-column sample. Lanes 8-10 illustrate that the highest protein concentration was collected and stored.

Purification Step:	Volume:	Quality A260/A280:	Specific Activity (C_T):	Yield:
Crude Lysate	25mL	38.823	N/A	98%
Pre-Heparin Column	20mL	15.908	N/A	78%
Pre-Q Column	3mL	0.240(conc.)	N/A	12%
Pre-Dialysis	10mL	1.805	N/A	10%
Post-Dialysis	8mL	2.111	N/A	8%

Conclusion

By producing *Taq* through large-scale overexpression allowed a greater variety of purification techniques were used, leading to a large volume of purified protein that was obtained in the process. It should also be noted that the Post-Dialysis sample had higher fluorescence relative to the other samples. Through this experiment I found that the *Taq* can be expressed and purified efficiently through intuitive purification techniques.

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