

**CLONING AND PURIFICATION OF TWO SIMILAR SSB-LIKE
PROTEINS FROM THE THERMOPHILIC BACTERIA *THERMUS*
AQATICUS AND *THERMUS THERMOPHILUS***

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Single-stranded DNA-binding proteins (SSBs) are indispensable elements in the cells of all living organisms. SSBs play essential roles in DNA replication, recombination, and repair. Here, we present the cloning and purification process for two similar SSB-like proteins from the thermophilic bacteria *Thermus aqaticus* and *Thermus thermophilus*.

Based on the known SSB-like gene sequence from *Thermus thermophilus* VK-1, the specific primers for PCR amplification were synthesized. Specific, approximately 900 bp, PCR products were obtained. The purified fragments were ligated into pET-30LIC. *E. coli* TOP10F' cells were transformed with the ligation mixture. One plasmid from each cloning was selected, and used for the expression and purification procedure.

The *E. coli* BL21(DE3)pLysS strain transformed with pETSSBTth2 or pETSSBTaq2 was grown at 37°C in 500 ml LB containing 50 µg/ml chloramphenicol and 34 µg/ml kanamycin to an A₆₀₀ of 0.3. IPTG was then added to give a final concentration of 1 mM. The cells were harvested after 8 h by centrifugation, and the pellet was resuspended in 30 ml of buffer A₀ (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 0.1 % Triton X-100). The cells were disrupted by sonication, and the insoluble debris was removed by centrifugation. The supernatant (about 28 ml) was then heat-treated at 75° for 15 min, and denatured host proteins were removed by centrifugation. The clarified supernatant was applied directly onto a QAE-Cellulose column pre-equilibrated with 4 vol of buffer A₁ (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA). The column was washed with 60 ml buffer A₁, and the SSB-like proteins were eluted with linear gradient (100 ml) of 0.05-1 M NaCl in buffer A₁. Proteins eluted at about 0.2 M NaCl with a purity of about 80%. These fractions were combined (20 ml) and loaded onto a ssDNA-cellulose column (10 ml) equilibrated with buffer B (20 mM Tris-HCl, pH 7.5, 0.5 M NaCl). The column was washed with 6 vol of buffer B, then with a buffer B containing 0.8 M NaCl, and finally eluted with buffer C (20 mM Tris-HCl, pH 7.5, 1.5 M NaCl, 50% ethylene glycol). The protein-containing fractions were combined and dialyzed against buffer A₁ with PMSF and stored at 4°C until use.