Supplementary Material

Cell-Free Protein Synthesis by Diversifying Bacterial Transcription Machinery

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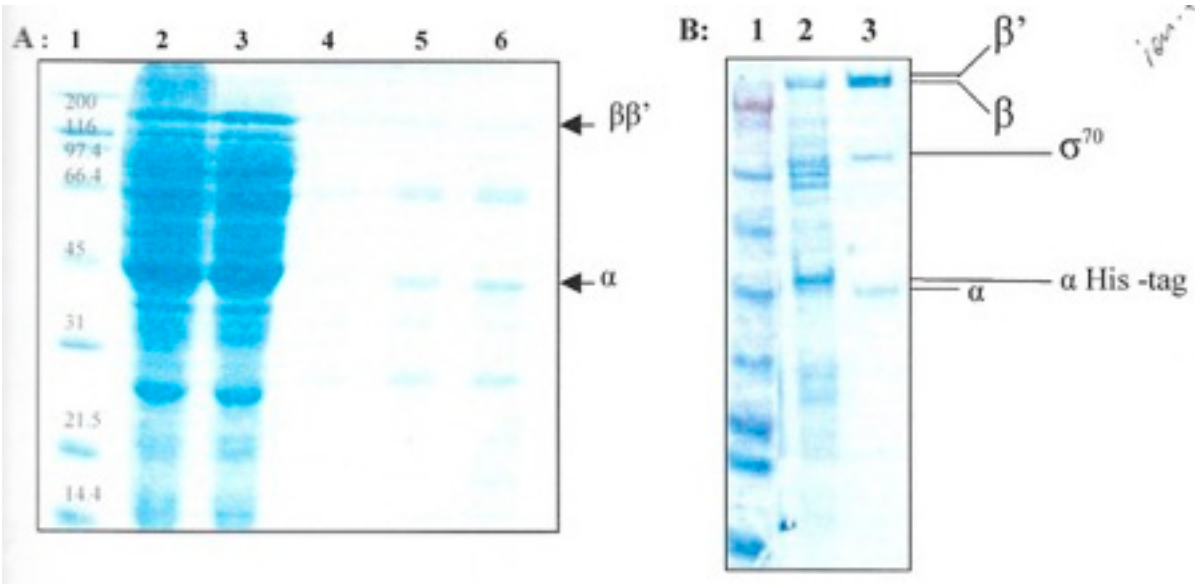
Table S1. Oligonucleotide primers used for PCR amplification of *T. maritima* genes

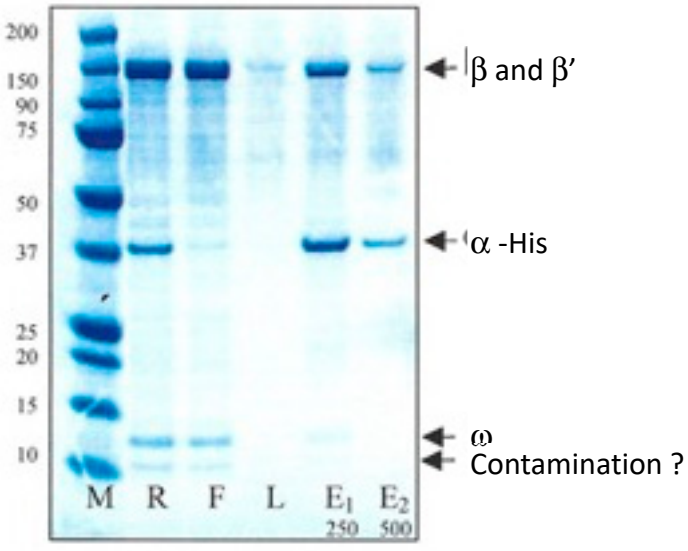
Oligonucleotide primer	Sequence starting from 5′ to 3′
Tm Xyl 0808-His	ATGCATCATCATCATCATCATGGAGGGACCCTTTTGAACAT
Tm Xyl 0808-down	CTCGTGATCGGAACTGATGAG
Tm LacI 1856-His	ATGCATCATCATCATCATCATCCAACAATAGAAGATGTCG
Tm LacI 1856-down	GACCACTCGATCTGAACATCC
Tm GntR 0439-His	ATGCATCATCATCATCATCATAAAAAAATCGAAGTGGACCTC
Tm0439-GntR-down	GAACGAAACACCCTCCGCC

Table S2. Oligonucleotide primers used for PCR amplification of promoter-operator regions

Oligonucleotide	Operator DNA	DNA size	Sequence from 5′-extremity
ArgRTn-Up	*T. neapolitana argR*	148 bp	IRD700-TGTTACTCTTGAGTTACCAAAAC
ArgRTn-down			TTATGAGTTCCTGTCTTC
XylRo-104UP	*E. coli xylFo*	104 bp	IRD800-GGTCATAAATCAAGAAATAAA
XylRo-down			CACCGCGATAAACGTAACC
LacIo-68UP	*E. coli lacIo*	68 bp	IRD700-GCTTCCGGCTCGTATGT
LacIo-down			GGTCATAGCTGTTTCCTGTG
GntRo-68UP	*E. coli gntKo*	68 bp	IRD800-GTCCGGCTGGACAATGTT
GntRo-down			GTGGTGCCCCCACAATAC
ArgCo-UP	*B. stearothermophilus argCo*	100 bp	CTTAGGGAGGGGCAAGAA
ArgCo-down			CCCGTATGCCTCATGTAG



Supplementary Fig. S1. Purification of the core enzyme RNA polymerase from the soluble fraction of *E. coli* extracts. A: 1 - molecular weight marker; 2 and 3 - eluate from a NiNTA column; 4, 5, 6 - various fractions remaining on the column after elution with 40 mM, 65 mM, and 90 mM imidazole. B: 1 - molecular weight marker; 2 - sample containing pooled eluted fractions; 3 - commercial RNA polymerase.



Supplementary Fig. S2. Subunits α, β, β′ and ω of *E. coli* RNA polymerase in inclusion bodies after purification using the His-tagged α subunit. M - molecular mass marker; R - renatured proteins after purification; F - filtrate of proteins not bound on the Ni-NTA column; L - sample washed with 5 mM imidazole; E1 - elution with 250 mM imidazole; E2 - elution with 500 mM imidazole.