Supplementary Data

Supplementary figures

A

-38 -12 -3

TAT**TTGACA**TCAGGAAAATTTTT**TG**G**TATAATAGAT**

ATA**AACTGT**AGTCCTTTTAAAAA**AC**C**A**

B

Figure S1. Stabilities of transcription initiation complexes of *T. aquaticus and E. coli* RNAPs. (A) Sequence of a fork junction DNA competitor. (B) Relative efficiencies of abortive transcription initiation reactions by *E. coli* and *T. aquaticus* RNAP holoenzymes at N25cons and T5N25 promoter DNA fragments were measured at optimal temperatures temperatures 37 °C (for *E. coli* RNAP) or at 55 °C (for *Taq* RNAP) as in Fig.3, but a fork junction derivative of N25cons was used as a competitor instead of heparin. The concentration of the fork junction competitor was 0.5 μM.

A

	-58 UP element	-35 element	-14

[-58/-14] GCTAAAATTTTTTTTAAAAGTAT**TTGACA**TCAGGAAAATTTTT**TG**

CGATTTTAAAAAAAATTTTCATA**AACTGT**AGTCCTTTTAAAAA**AC**

B

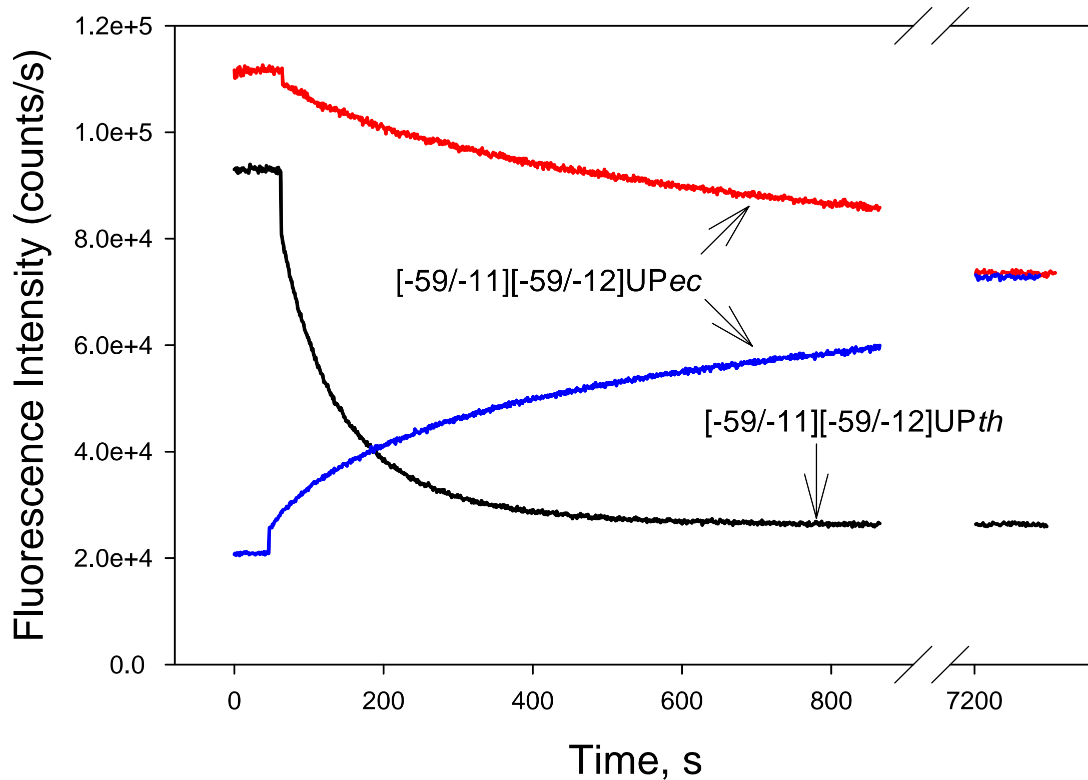


Figure S2. Competition binding assay for *E. coli* RNAP binding to upstream fork junction probes. (A) Sequence of a reference competitor probe [-58/-14]. (B) The panel shows the kinetics of decrease of beacon signal of *Ec* Eσ^{70} beacon complexes with fork junction probes resulted from competitive displacement of the probes by [-58/-14] upon addition of 2 nM [-58/-14] to the samples containing 1 nM *Ec* Eσ^{70} beacon and 2 nM either 12 (red curve) or 13 (black curve) probe at 25 °C. An additional order of addition experiment in which probe 12 was added to the preformed (*Ec* Eσ^{70} - [-58/-14]) complex (blue curve) was carried out to verify that competition assay for RNAP binding to probe 12 reached equilibrium.

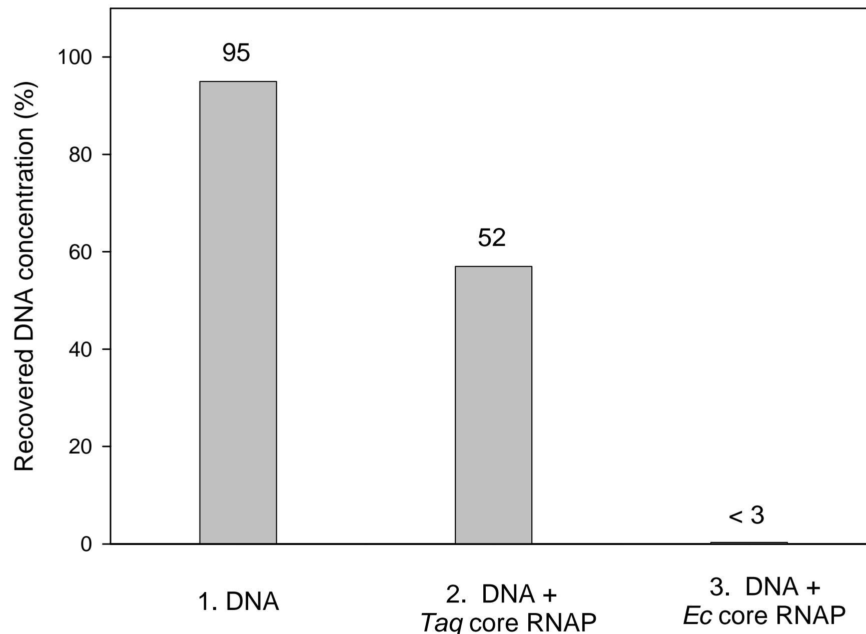
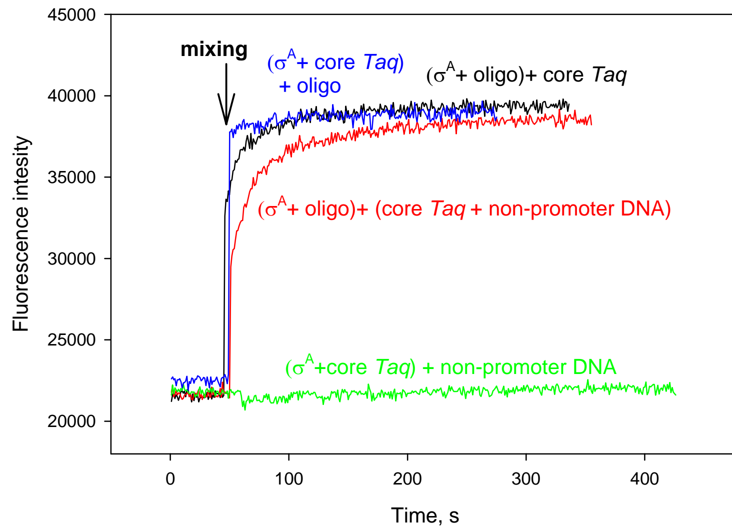
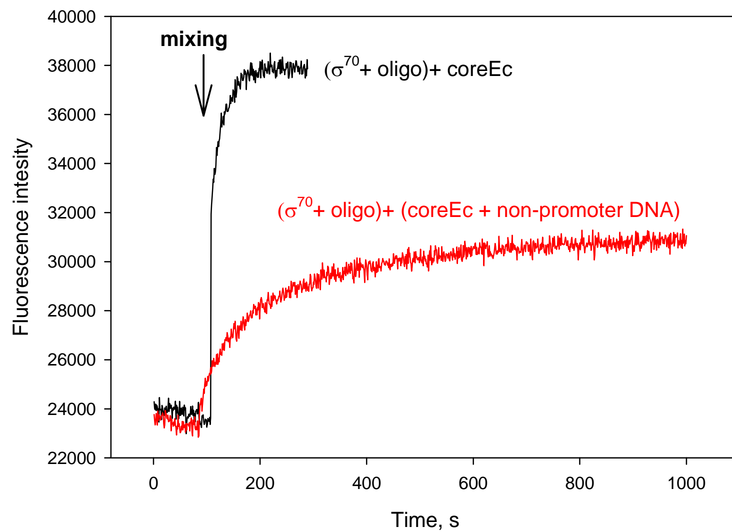


Figure S3. Filter binding assay for *Taq* and *E. coli* core RNAP enzymes interaction with DNA. Samples 1-3 contained 1 nM N25cons promoter fragment in transcription buffer [40 mM Tris–HCl (pH 8.0), 100 mM NaCl, 5% glycerol, 1 mM DTT, and 10 mM MgCl2]. *Ta*q or *E. coli* core RNAPs were added to samples 2 and 3, respectively; final core RNAP concentrations were 10 nM. After incubation of samples 2 and 3 for 5 min at 37° C and 50° C, respectively, the samples were passed through Micropure-EZ Enzyme Remover filter (Amicon, Inc) which removed both RNAP enzymes and RNAP-bound DNA. Relative DNA concentrations (shown in the panel) were determined using a fluorometric PicoGreen assay kit (Invitrogen). The measurements were repeated two or three times, the experimental variation of DNA recovery among replicate measurements did not exceed 15% of the average value.

A



B



C

-60 **+30**

CAGATAGCTCTTTTCCGTTTCCTCCGCTGCGGCCGGAATAACTCCCGGCATCGCGCCACCACTGACACGGAACAACGGCAAACACGCCGC
GCGGCGTGTTTGCCGTTGTTCCGTGTCAGTGGTGGCGCGATGCCGGGAGTTATTCCGGCCGCAGCGGAGGAAACGGAAAAGAGCTATCTG

Figure S4. The effect of non-promoter DNA on assembling of the *Taq* and *Ec* RNAP holoenzymes. (a) Time dependence of the fluorescence intensity upon the addition of oligo or non-promoter DNA to samples containing σ^A and *Taq* core RNAP (blue and green curves, respectively); time dependence of the

fluorescence intensity upon the addition of *Taq* core RNAP or *Taq* core RNAP preincubated for 5 min with non-promoter DNA to samples containing σ^A and oligo (black and red curves, respectively). (b) Time dependence of the fluorescence intensity upon the addition of *Ec* core RNAP or *Ec* core RNAP preincubated with non-promoter DNA to samples containing σ^{70} and oligo (black and red curves, respectively). (c) Shown is the sequence of non-promoter DNA probe, loosely based on the sequence of the *E. coli* rrnB P1 promoter.

The samples contained 1 nM labeled σ^A or σ^{70}, 400 nM -12/+2 oligo, 1.5 nM *Taq* core RNAP or *Ec* core RNAP, and 5 nM non-promoter DNA probe, final concentrations. The measurements were carried out in transcription buffer at 25° C, the mixing time was 15 s.