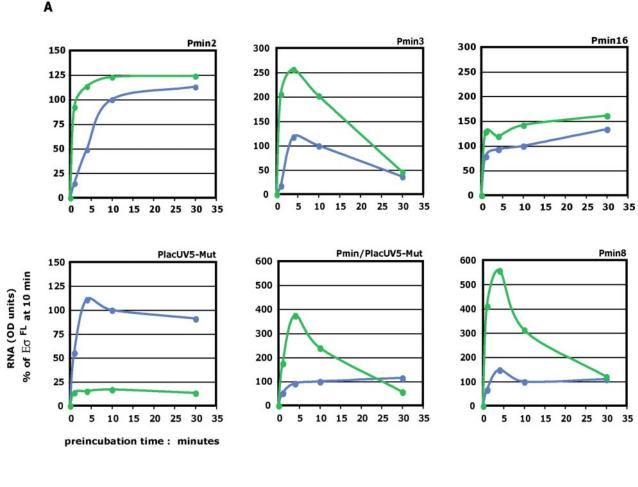
Supporting Information

Hook-Barnard and Hinton 10.1073/pnas.0808133106



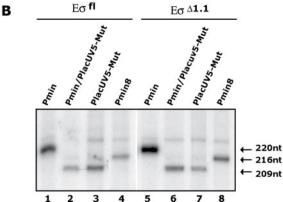


Fig. S1. Region 1.1 inhibition of P_{min} is not determined by the promoter recognition elements, sequences downstream of the -10 element, or a short polymerase/promoter half-life. (A) Plots showing the relative amount of RNA versus the length of incubation of polymerase $[E\sigma^{fl}]$ (blue) or $E\sigma^{\Delta 1.1}$ (green)] with the DNA (in min) before the addition of rNTPs and heparin. The amount of RNA obtained with $E\sigma^{fl}$ at 10 min is set to 100. Note that scales vary, depending on the activity of promoter with $E\sigma^{fl}$. (B) Denaturing acrylamide gel showing the products of single-round transcription reactions using $E\sigma^{fl}$ (lanes 1–4) or $E\sigma^{\Delta 1.1}$ (lanes 5–8) and the indicated promoters. Reactions were incubated for 10 min before the addition of rNTPs and heparin. The P_{min8} mutation eliminates the stutter start seen with P_{min} . Thus, P_{min8} RNA migrates slightly faster than RNA from P_{min} . The $P_{lacUVS-Mut}$ template yields an even shorter transcript of 209 nt.

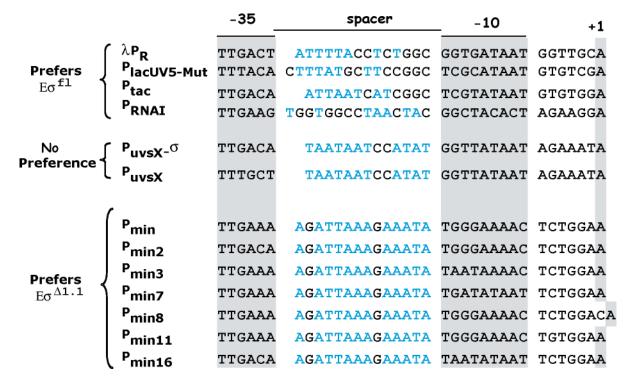


Fig. S2. Sequences of promoters and their preference for $E\sigma^{fl}$ or $E\sigma^{\Delta 1.1}$. The σ^{70} -dependent -35 elements, TGn, -10 elements, and transcription start sites (+1) are shaded in gray. The spacer region, with A or T bases shown in blue, is indicated. Sigma preference as determined in this work and refs. 1 and 2 is indicated.

^{1.} Vuthoori S, Bowers CW, McCracken A, Dombroski AJ, Hinton DM (2001) Domain 1.1 of the sigma(70) subunit of Escherichia coli RNA polymerase modulates the formation of stable polymerase/promoter complexes. J Mol Biol 309:561–572.

^{2.} Wilson C, Dombroski AJ (1997) Region 1 of sigma 70 is required for efficient isomerization and initiation of transcription by Escherichia coli RNA polymerase. J Mol Biol 267:60-74.

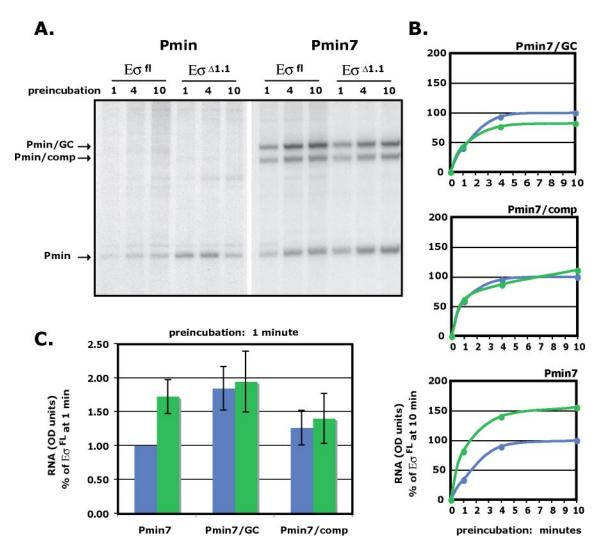


Fig. S3. Effect of the P_{minor} , GC, or comp spacer regions on promoter activity and stable complex formation. Single-round in vitro transcription reactions were carried out as described in Fig. 3. P_{min} , $P_{min/GG}$, $P_{min/GGP}$, or the P_{min7} set templates were prepared to produce transcripts of three different lengths, allowing the promoters to be assayed in the same reaction. (A) Denaturing acrylamide gel showing the products of transcription reactions with the indicated promoters. (B) Plots showing the relative amount of RNA versus the length of incubation of polymerase $[E\sigma^{fl}$ (blue) or $E\sigma^{\Delta 1.1}$ (green)] with the DNA (in min) before the addition of NTPs and heparin. The amount of RNA obtained with $E\sigma^{fl}$ at 10 min is set to 100 for each promoter. (C) Comparison of transcriptional levels for P_{min7} , $P_{min7/GG}$, and $P_{min7/Comp}$. The amount of RNA seen with $E\sigma^{fl}$ at the P_{min7} promoter was set to 1. Values and standard deviations were determined from 3 or more independent single-round in vitro transcription reactions, as described in Fig. 3. Polymerase was incubated with the template DNA for 1 min before the addition of rNTPs and heparin.