

Supplementary Figure Legends

Figure S1. SDS polyacrylamide gel of final gel filtration (GE Healthcare SD200) step. Peak 1 eluted at the void volume (aggregated protein). Peak 2 eluted at a volume consistent with monomeric *Mbo* RNAP.

Figure S2. Full con bubble promoter trap used as competitor.

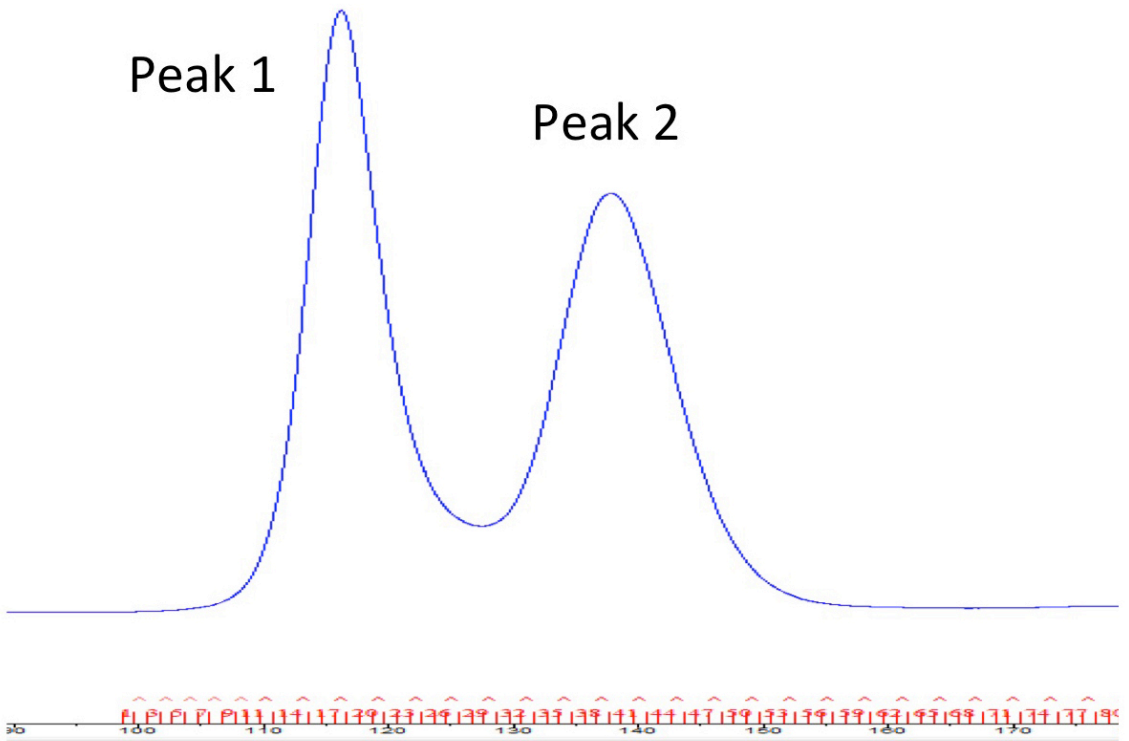
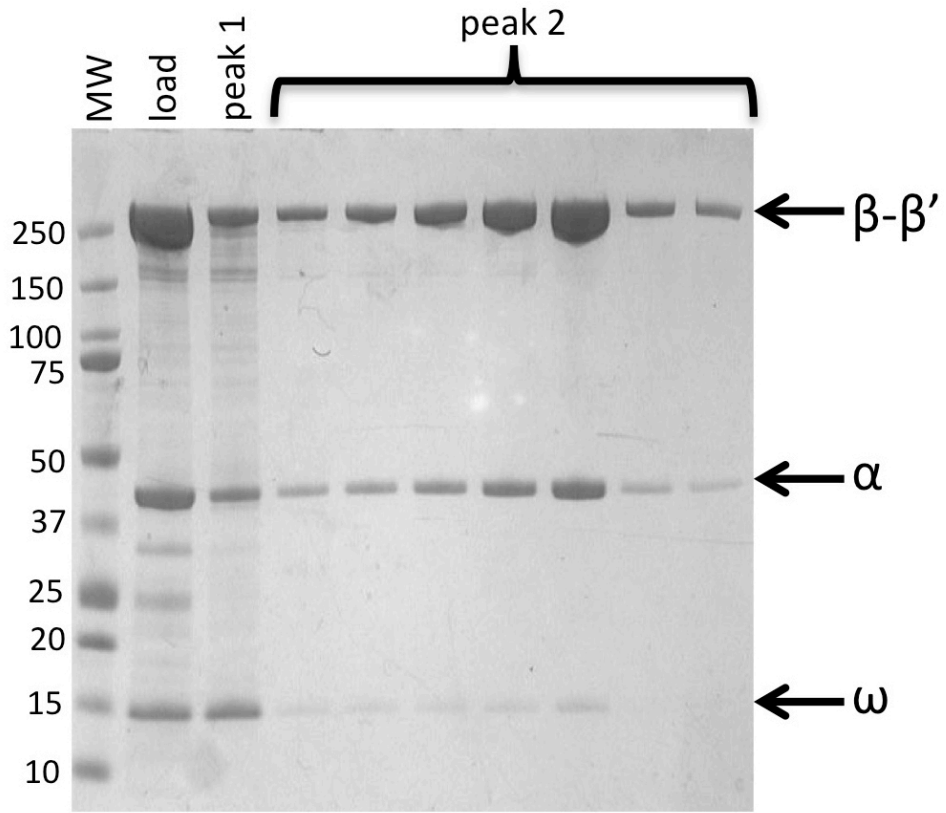
(A) Sequence of the full con bubble promoter trap. The promoter sequence is derived from the *fullcon* promoter [selected for optimal binding; (1)] but with an engineered noncomplementary transcription bubble (-11 to +1).

(B) Control experiments demonstrating the effectiveness of the full con bubble promoter trap as a competitor. For the left lane of each pair (+ competitor), the full con bubble promoter trap (2 μ M) and the transcription template (either the AC50 or AP3 promoters as indicated, 10 nM) were added to RNAP holoenzyme (50 nM) and transcription substrates simultaneously, then incubated in an abortive initiation reaction for 10 min. No competitor (- competitor) was added to control reactions under identical conditions. The absence of transcription for the '+ competitor' trials indicates that all the RNAP binds the full con bubble promoter trap before binding and transcribing the promoter template, and does not dissociate over the time course of the experiment. Filter binding experiments (not shown) indicate that RNAP does not dissociate from the full con bubble promoter trap over the course of several hours. Assays were performed in 10 mM KGlu for the AP3 promoter and 150 mM KGlu for the AC50 promoter as described in materials and methods.

Figure S3. Double exponential fit for promoter AP3 promoter lifetime assay. (*Mbo* RNAP; \pm CarD). Assays were performed as described in Figure 3. Transcription assays were performed in transcription buffer (see materials and methods) in 10 mM KGlu.

References

1. Gaal, T., Ross, W., Estrem, S.T., Nguyen, L.H., Burgess, R.R. and Gourse, R.L. (2001) Promoter recognition and discrimination by EsigmaS RNA polymerase. *Molecular Microbiology*, **42**, 939–954.



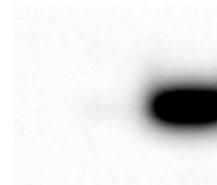
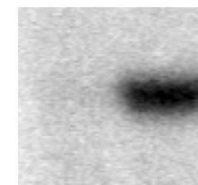
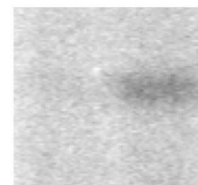
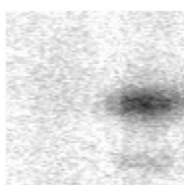
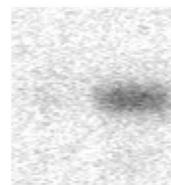
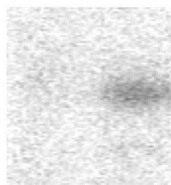
Full con bubble promoter trap**A****-35
element****-10
element**

GGCTC**TTGACA**AAAGTGTTAAATTGTGC**TATACT**GGGATGGTATGGATGACAGAATTCGG
 CCGAG**AACTGT**TTTCACAATTTAACACGA**A**ATACCTACTGTCTTAAGCC
 ATACTGGGATGG

B

AC50

AP3

*Mbo**Mbo + CarD**Eco**Mbo**Mbo + CarD**Eco*

competitor: + - + - + -

+ - + - + -

