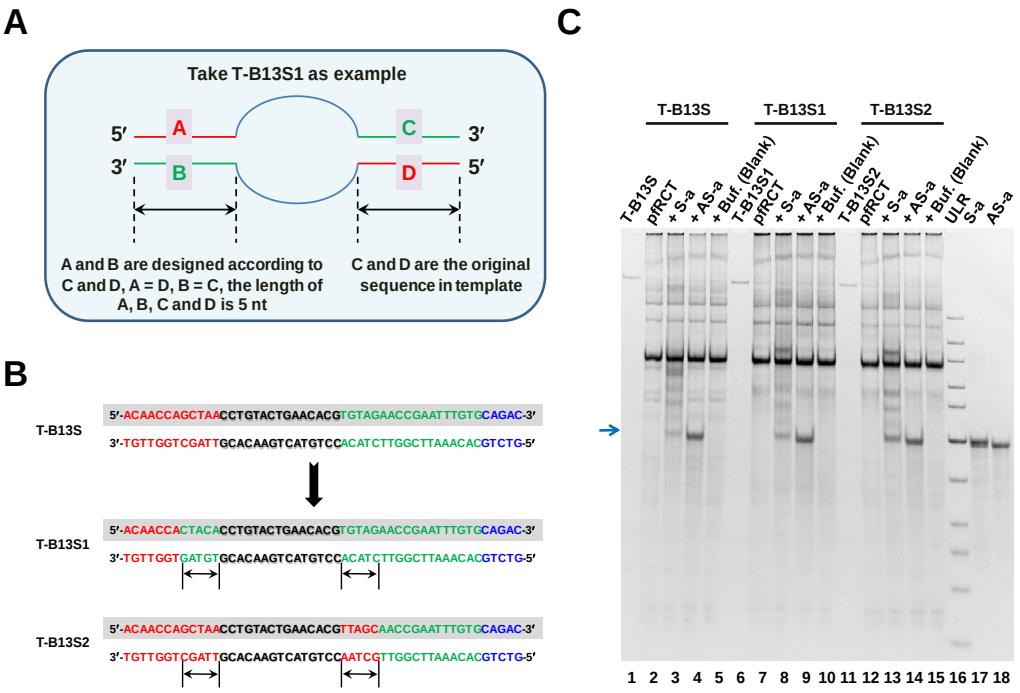
Supplemental_figure_S12



Effects of sequence symmetry flanking the bubble on the direction bias of T7 RNAP. A) The principle of sequence design to increase the symmetry on the both sides of the bubble (Take T-B13S1 as an example). B) Detailed sequences of the templates flanking the bubble. T-B13S1 and T-B13S2 were designed according to T-B13S (the length of palindromic sequence at bubble is 13 bp, including 5-bp mismatches). For T-B13S1, 5-bp sequence on the left of the bubble (green) was changed to the same as the original sequence on right part. For T-B13S2, 5-bp sequence on the right of the bubble (red) was changed to the same as left part. C) Transcription results (10% PAGE). Lanes 1, 6 and 11 are dsDNA templates. Lanes 2, 7 and 12 are pfRCT results (RNase H and Helper-B13 coexist). Lanes 3–5, 8–10 and 13–15, hybridization results of RNA product with 59-nt single-stranded DNA ("S-a" refers to short ssDNA with sense sequence; "AS-a" refers to short ssDNA with antisense sequence). Transcription condition: [Template] = 50 nM, $[NTP]_{each}$ = 2 mM, [T7 RNAP] = 2 U/μL, [RNase Inhibitor] = 2 U/μL, 1× T7 RNAP buffer, [RNase H] = 0.25 U/μL, [Helper-B13] = 1 μM. Total volume was 20 μL, 37°C for 4 h. Hybridization conditions: 5 μL transcript, 0.5× RNase H buffer, [S-a/AS-a] = 1 μM. Exact procedures are shown in Methods.