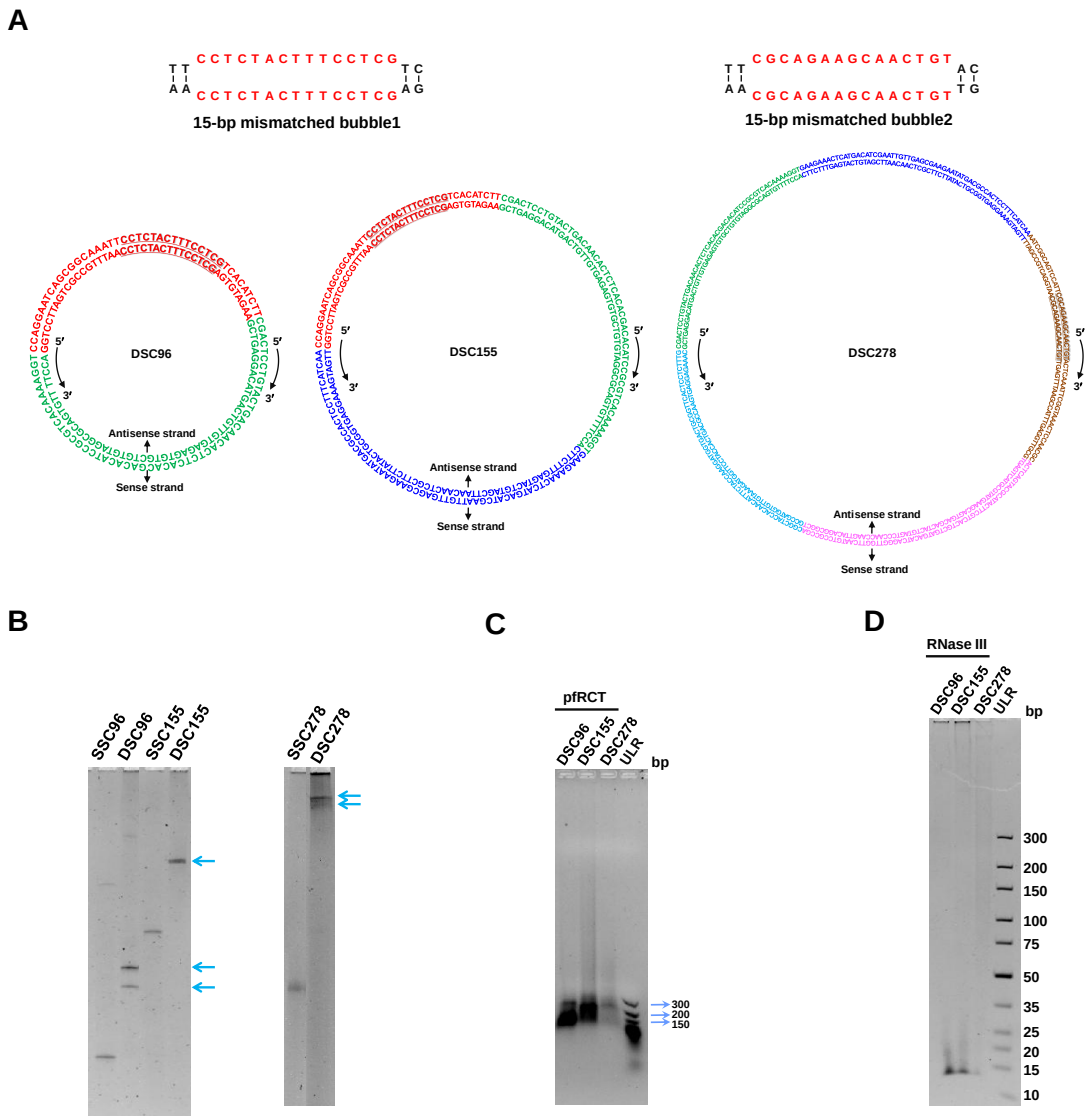
Supplemental_figure_S11



Highly efficient preparation of siRNAs by pfRCT with circular templates with various lengths. A) Sequence design of circular dsDNA templates (DSC96, DSC155 and DSC278). For DSC96 and DSC155, the sequence of 15-bp mismatched bubble1 was used; For DSC278, the sequence of 15-bp mismatched bubble2 was used (the bubble sequences are underlined). B) Preparation of circular ssDNA (SSC96, SSC155 and SSC278) and circular dsDNA (DSC96, DSC155 and DSC278), analyzed by 8% dPAGE. SSC96 was prepared by "One-step strategy" (Sui et al. 2019) using two strands (the red part and green part). SSC155 and SSC278 were prepared by "Stepwise strategy" (Sui et al. 2021). SSC155 and SSC278 were prepared by using 3 or 5 substrates, respectively. Preparation procedures are depicted in Methods. DSC96, DSC155 and DSC278 were all prepared under 65^{o}C by *Taq* Dnl and were purified with Exo I and Exo III. C) Transcription results (1%

agarose). Conditions: [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. Total volume was 20 μL, 37°C for 4 h. D) Treating the products in C) with ShortCut RNase III (6% PAGE). Conditions: [ShortCut RNase III] = 0.2 U/μL, 1× $MnCl_2$ (20 mM), 0.5× ShortCut RNase III buffer, incubated at 37°C for 1 h.

Sui Z, Liu MQ, Wang WN, Chen H, Wang GQ, An R, Liang XG, Komiyama M. 2019. Efficient preparation of large-sized rings of single-stranded DNA through one-pot ligation of multiple fragments. *Chem Asian J* **14:** 3251–3254. doi:10.1002/asia.201900963

Sui Z, An R, Komiyama M, Liang XG. 2021. Stepwise strategy for one-pot synthesis of single-stranded DNA rings from multiple short fragments. *ChemBioChem* **22:** 1005–1011. doi:10.1002/cbic.202000738