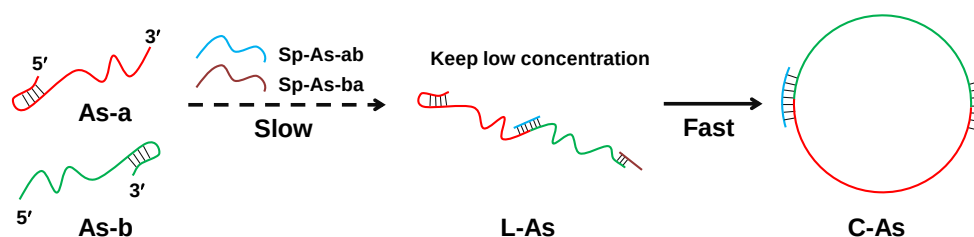


## Supplemental\_figure\_S2



### Schematic illustration of “One-step strategy” for preparation of circular ssDNA.

Two short ssDNA fragments (As-a and As-b) and corresponding splints (Sp-As-ab and Sp-As-ba) are mixed for a one-step circularization. Due to the difference in concentration effect between intramolecular and intermolecular ligation reactions, the intermediate (L-As) could immediately perform intramolecular circularization and converted to the final product (C-As) as soon as it is produced, so the concentration of which could be kept at a low level (like a process of slow-release). Thus, the formation of polymers (produced by intermolecular ligation) can be greatly decreased (even inhibited), and the desired single-stranded circular DNA (C-As) could be obtained in a high yield.