



A. The circular ssDNA substrate described in Materials and Methods. The 5'-end and 3'-end of the substrate were closed by CircLigaseTM ssDNA ligase. To simplify the drawing all 80 dT, making the ssDNA circle, is not outlined in the figure.

B. The radioactively labeled linear ssDNA molecule (100 nt) was circularized by treatment with CircLigaseTM ssDNA ligase (Epicentre). Aliquots of the ligated and unligated oligonucleotides were incubated with Exonuclease I for 1 hour at 37 °C. Equal amounts of the different products were separated on a 10% denaturing polyacrylamide gel and visualized by autoradiography. Exonuclease I could efficiently digest the linear template, but the circular ssDNA molecule was not affected.

C. UTP, ATP, and GTP can all function as a source of energy for DNA synthesis on the bubble template. DNA replication assays were performed using the bubble template (10 fmol), POLy (70 fmol), and TWINKLE (750 fmol). The reactions were incubated for 0, 2, 15, 40, or 70 min at 37 °C. Details of the experimental conditions are described in Materials and Methods.