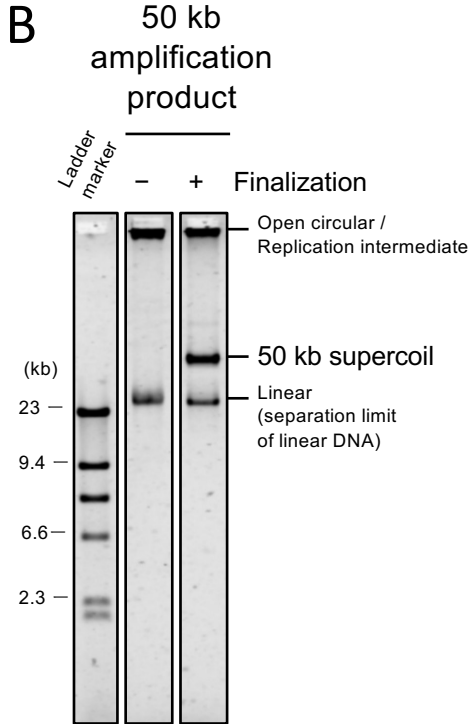
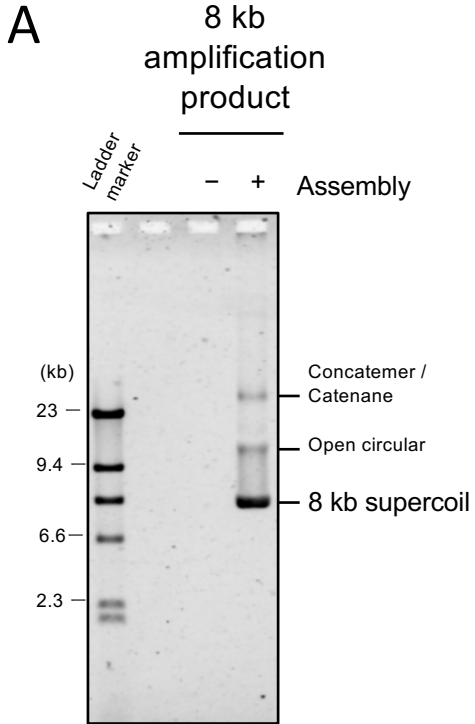
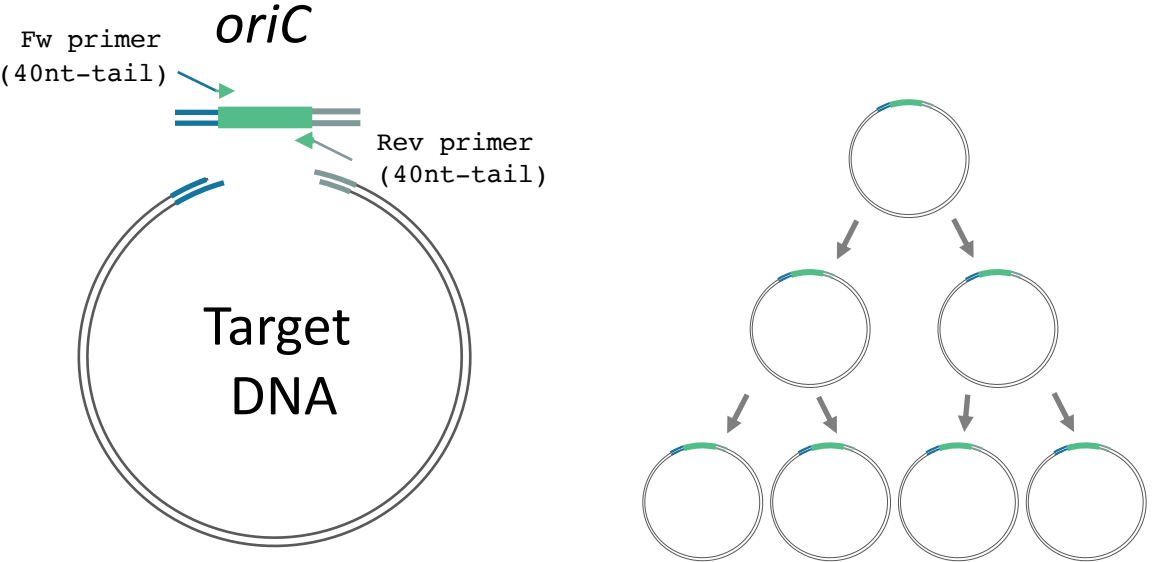


Amplification of 8 kb or 50 kb circular DNA



A) Amplification of 8 kb DNA as a supercoiled form. A 7.5 kb DNA fragment (1ng)^{*1} and a 0.4 kb oriC cassette having 40 bp overlapping ends (50 pg) ^{*1} were mixed in the OriCiro Assembly reaction (5 μL) and incubated at 42°C for 30 min. An aliquot (1 μL) of the assembly reaction (+) or the same amount of DNAs without the assembly reaction (-) was added to the OriCiro Amp reaction (10 μL) and further incubated at 33°C for 6 hr. The products were analyzed in agarose gel electrophoresis.

B) Amplification of 50 kb DNA. 50 kb circular DNA containing *oriC* (5 ng as circular DNA) was incubated in the OriCiro Amp reaction (10 μL) at 33°C for 6 hr. Although the amplification yields replication intermediates but not supercoiled molecules (-), Further incubation after 2-fold dilution with the OriCiro Amp buffer at 33°C for 30 min (Finalization option) generates the supercoiled molecules of 50 kb circular DNA whose size can be separated in normal agarose gel electrophoresis (+).

^{*1}: These DNA were included in the kit as “Control Fragment” and “oriC Cassette”