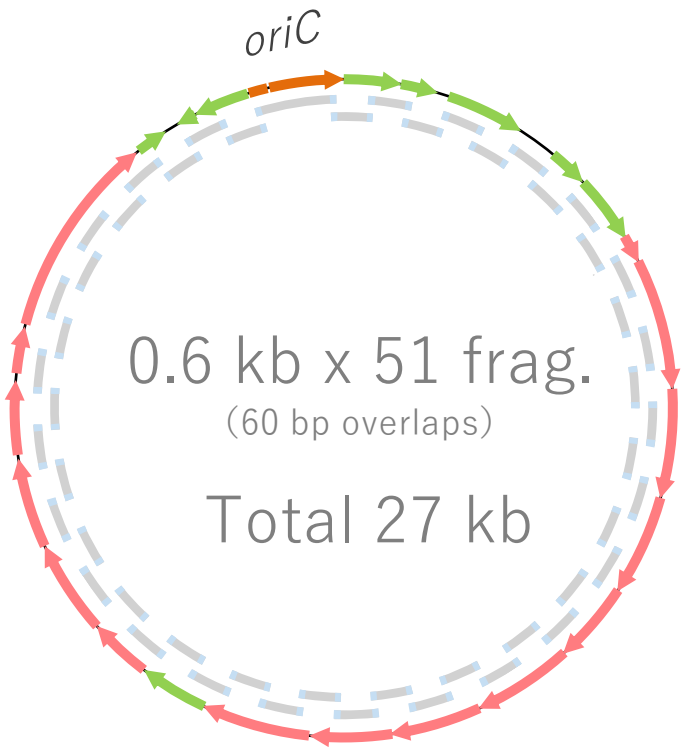
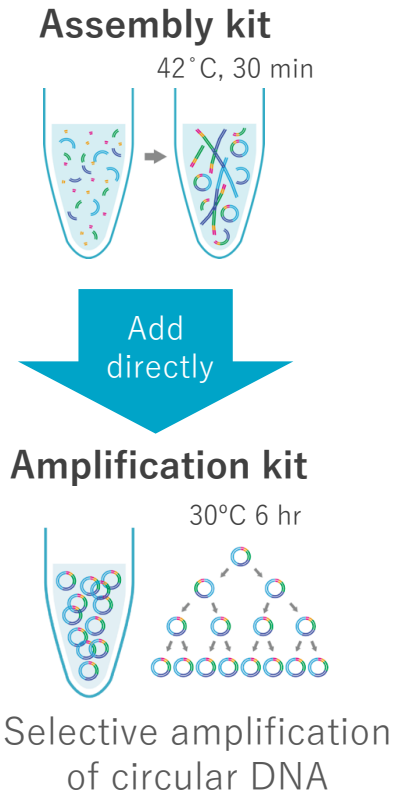
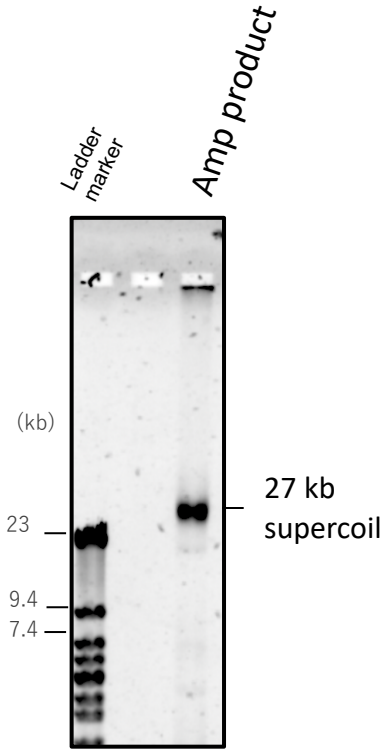
Two-step construction of 27 kb plasmid from 51 fragments







Fifty-one fragments of 0.6 kb DNA having 60 bp overlapping ends were designed to construct a 27 kb plasmid. The fragment pool (total 20 ng) was incubated in the OriCiro Assembly reaction (5 µL) at 42ºC for 30 min followed by heat treatment at 65ºC for 2 min. An aliquot of the reaction (1 µL) was then incubated in the OriCiro Amp reaction (10 µL) at 30ºC for 6 hr. The products was diluted two-fold with the OriCiro Amp buffer and further incubated at 30ºC for 30 min (Finalization option) followed by agarose gel electrophoresis. The result shows efficient production of the 27 kb supercoiled DNA.