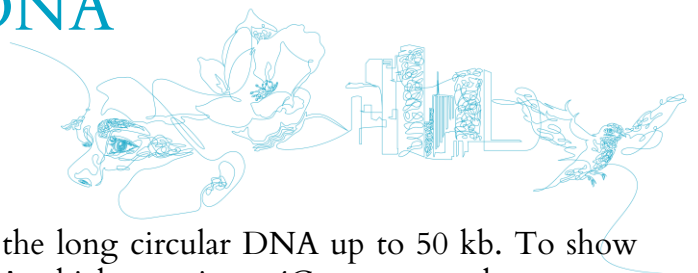


Amplification of 50 kb DNA

I. Introduction

OriCiro Amp Kit has an ability to amplify the long circular DNA up to 50 kb. To show this performance, 50 kb of long circular DNA which contains *oriC* was prepared.



II. DNA construct



Figure 1. Target DNA which contains *oriC* was used for OriCiro Amp reaction.

III. Amplification

1. Thermal cycler was preheated at 33 °C (Lid was at 40 °C)
2. The following reaction mixture was prepared on ice

4 µl	Nuclease-Free Water
2 µl	5X Buffer I
2 µl	5X Buffer II
1 µl	10X RE mix
9 µl	total volume
3. Incubated the mixture at 30 °C for 15 minutes
4. Add 1 µL of assembled products
5. Incubated the mixture at 30 °C for 6 hours
6. The products was diluted two-fold with the OriCiro Amp buffer, and further incubated at 33 °C for 30 minutes (Finalization option)

IV. Result & Conclusion

1. Loading Buffer (25 mM Tris-HCl pH 8.0, 25 mM EDTA, 0.1% SDS, 5% glycerol, 0.1% bromophenol blue) was added to the amplification products
2. Used 0.5% Agarose, 0.5X TBE for electrophoresis
3. **The efficient production of 50 kb supercoiled DNA was detected after Finalization option**

