

Lambda DNA Assembly and Amplification

I. Introduction

OriCiro technology possess accurate cell-free assembly and amplification ability of circular DNA molecules. Especially, OriCiro technology has an advantage in long circular DNA such as 50 kb length. Here we describe the use of OriCiro technology in reconstitution of Lambda genome DNA.

II. DNA construct



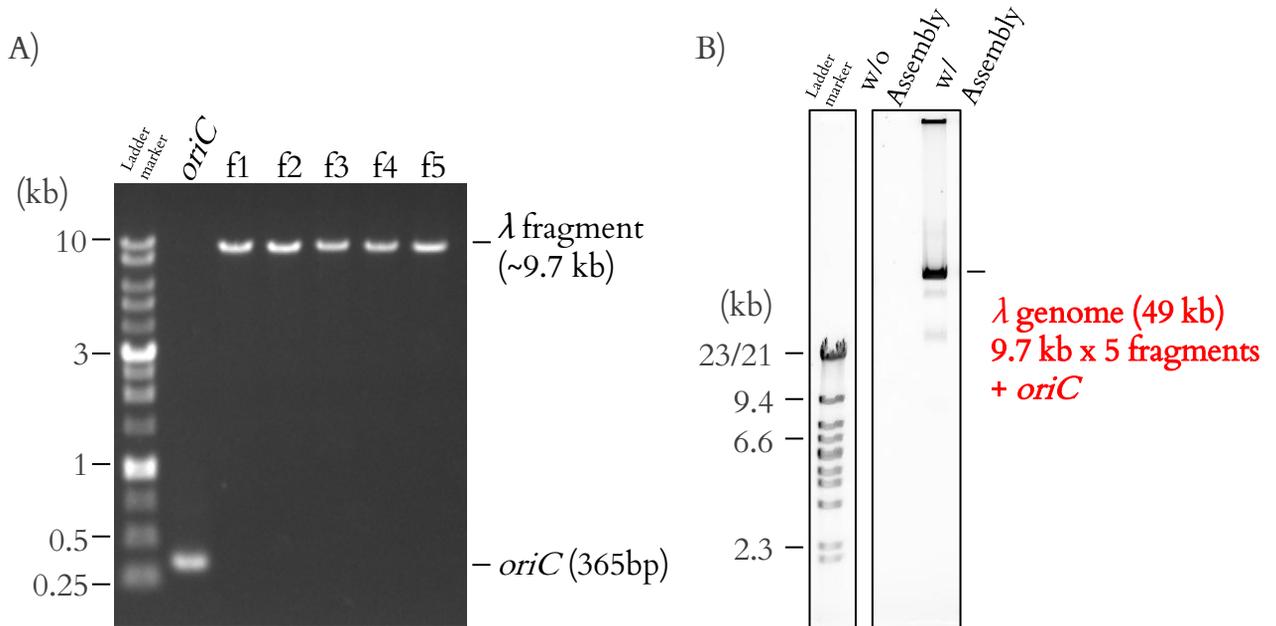
Figure 1. 9.7 kb DNA fragments were designed to divide the Lambda genome DNA as 5 fragments. *oriC* cassette was assembled with these 5 Lambda DNA fragments.

III. Materials & Methods

1. KOD FX Neo and KOD Plus Neo (Toyobo) were used for PCR of lambda DNA fragments and *oriC* cassette, respectively.
2. Assembly reaction was carried out at 42°C for 60 minutes and the mixture was treated at 65°C for 2 minutes to repress misannealed products.
3. Using an aliquot of the assembly reaction, amplification of 49 kb circular DNA was performed at 30°C for 15 hours.
4. The products were diluted two-fold with the OriCiro Amp buffer and further incubated at 30 °C for 30 minutes.
5. Amplified DNA was used for in vitro packaging system of lambda phage. *Escherichia coli* strain (MG1655 Δ*hsdR* Δ*endA*) was infected with packaging extract only or lambda phage which contains the amplified DNA.

IV. Result & Conclusion

- A) **PCR of Lambda DNA fragments.** Estimated size of *oriC* cassette and Lambda DNA fragments were amplified as a single PCR product.
- B) **Assembly and Amplification of 49 kb Lambda DNA with *oriC*.** The efficient production of 49 kb supercoiled DNA was detected.
- C) **Plaque Assay.** Only amplified DNA-packaged Lambda phage infected *E. coli* created a plaque. No plaques were shown in the culture plate which only Packaging Extract was mixed with *E. coli*.



- C)
- Negative Control
(Packaging Extract only)
- λ genome (49 kb) 9.7 kb x 5 fragments
+ *oriC* - packaged λ phage

