

# Two-step construction of 27 kb plasmids from 51 fragments



## I. Introduction

OriCiro technology has accurate cell-free assembly and amplification ability of circular DNA molecules. Fifty-one fragments of 0.6 kb DNA having 60 bp overlapping ends were designed to construct a 27 kb plasmid DNA.

## II. Experimental Design

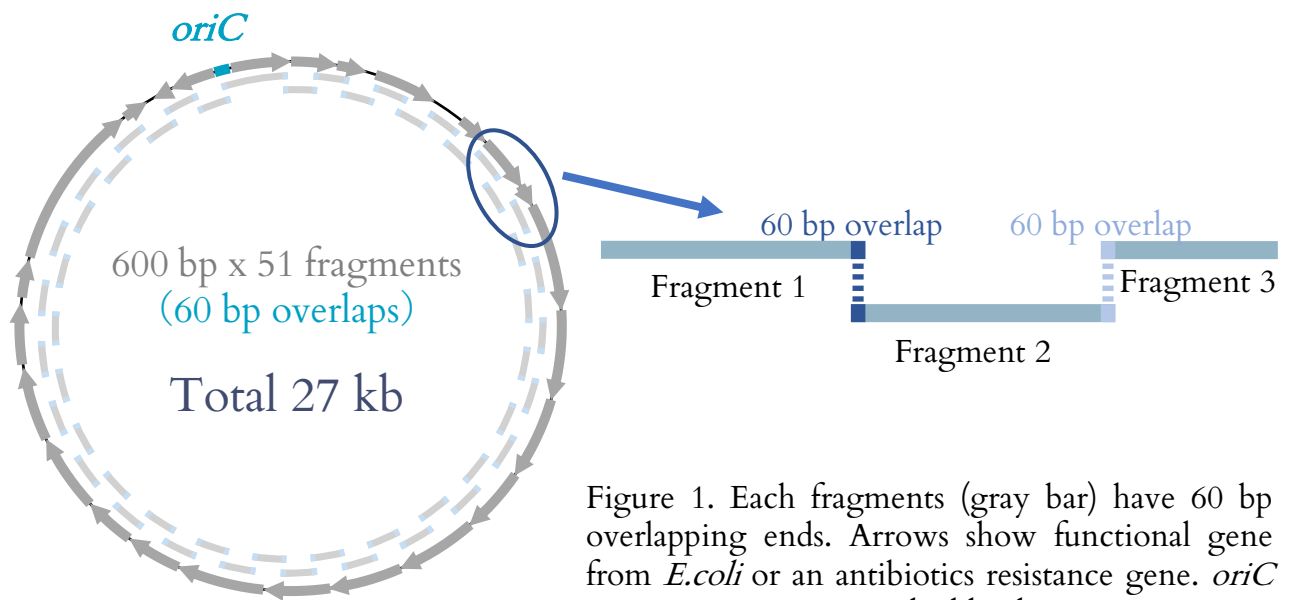


Figure 1. Each fragments (gray bar) have 60 bp overlapping ends. Arrows show functional gene from *E.coli* or an antibiotics resistance gene. *oriC* Cassette is represent in the blue bar.

## III. Primer Design

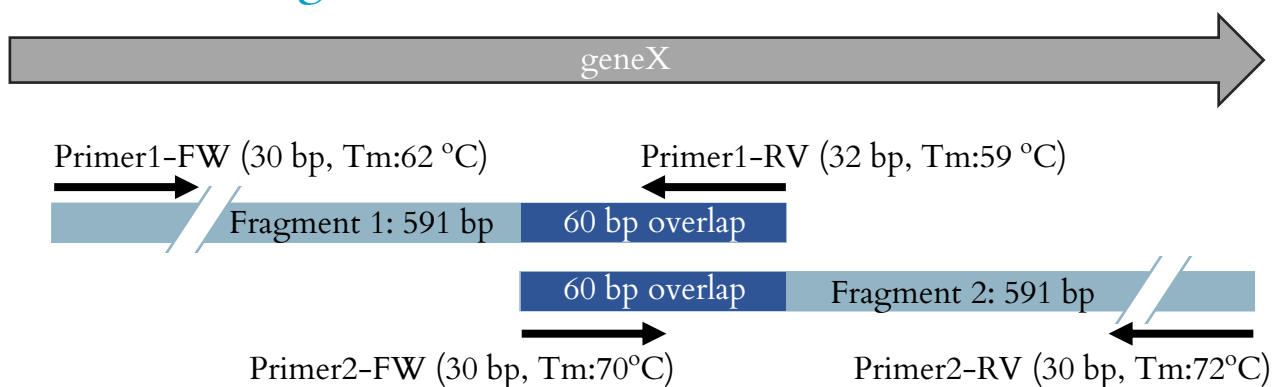


Figure 2. Primer design for two DNA fragments. 3'-end of Fragment 1 and 5'-end of Fragment 2 included same 60 bp sequence.

## IV. Assembly

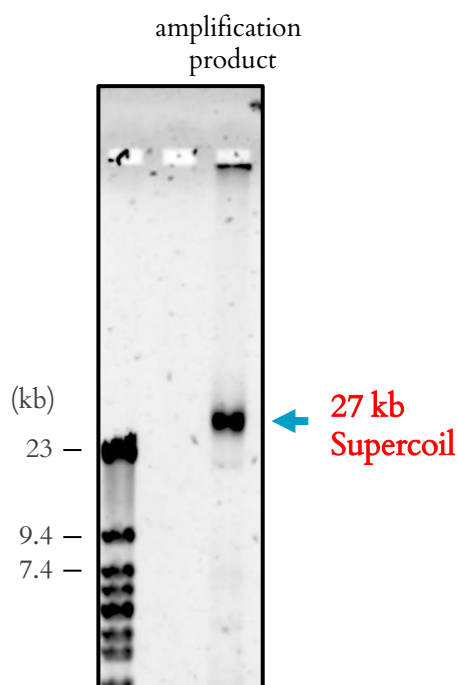
1. PCR products were purified with purification kit (MACHEREY-NAGEL, NucleoSpin Gel and PCR Clean-up)
2. The following reaction mixture was prepared on ice
  - ~1 ng 51 fragments pool
  - 2.5  $\mu$ l 2X RA Mix
  - 5  $\mu$ l total volume
3. Incubated the mixture at 42 °C for 30 min followed by heat treatment at 65 °C for 2 minutes

## V. Amplification

1. Thermal cycler was preheated at 33 °C (Lid was at 40 °C )
2. The following reaction mixture was prepared on ice
  - 4  $\mu$ l Nuclease-Free Water
  - 2  $\mu$ l 5X Buffer I
  - 2  $\mu$ l 5X Buffer II
  - 1  $\mu$ l 10X RE mix
  - 9  $\mu$ l total volume
3. Incubated the mixture at 30 °C for 15 minutes
4. Add 1  $\mu$ L 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

## VI. Result & Conclusion

1. Dilute the 0.5  $\mu$ l of products with 5  $\mu$ l of Loading Buffer (25 mM Tris-HCl pH 8.0, 25 mM EDTA, 0.1% SDS, 5% glycerol, 0.1% bromophenol blue)
2. Used 0.5% Agarose, 0.5X TBE for electrophoresis
3. **The efficient production of 27 kb supercoiled DNA was detected**



Product Name	OriCiro™ Cell-Free Cloning System 1.0	OriCiro™ Amp Kit 1.0
Size	1 Kit (Assembly: 5 RXN, Amp: 10 RXN)	1 Kit (Amp: 10 RXN)
Storage	Below -70°C	Below -70°C
Cat. No.	MS0011-A	MS0021-A

**OriCiro Genomics, Inc.**

318 UTokyo South Clinical Research Bldg.,  
7-3-1 Hongo, Bunkyo, Tokyo 113-8485, JAPAN  
Tel. +81-(0)3-3868-3610  
E-mail. product@oriciro.com  
URL. <https://www.oriciro.com/>



**OriCiro**