Building transfection-ready DNA by OriCiro's cell-free approach

Enzymatic DNA synthesis from fragments or vector to 10 µg plasmid DNA in the tube reaction

1. Introduction

The industry still relies heavily on PCR and cell-based cloning for the manipulation of genetic information at early and late stages of development. Both are decades old methodologies and are deeply rooted in effectively all aspects of biological research and drug development. As paradigmatic as PCR and cell-based cloning are, they are not without their limitations and drawbacks and more efficient and flexible alternatives could greatly accelerate development timelines at a variety of steps in the drug development process and for a wide variety of modalities and indications.

PCR provides an efficient method of synthesizing linear DNA in vitro, but is limited in DNA strand length and sequence composition and can lead to sequence biases introduced during each amplification cycle. Cell-based cloning is often used when trying to amplify larger sequences, however, this approach also comes with various challenges from time-consuming cloning and unpredictable cytotoxicity issues at the bench and iterative experimentation for fine-tuning conditions for viable cell banks for manufacture. In the context Cell-based cloning is widely used today for amplifying large DNA as an essential part of biotech research and development. However, it is inefficient, cumbersome, and time-consuming process.

2. Address the challenge by Cell-free DNA synthesis

OriCiro technology possess accurate cell-free assembly and amplification ability of circular DNA molecules and solves these problems.
The core technology is based on OriCiro Genomics' optimized in vitro propagation process based on more than 20 proteins which are essential for E. coli genome propagation.

Cell-Free Cloning System
consists just of two-step reactions. OriCiro Assembly ligates up to 50 DNA fragments seamlessly. It also has an advantage in long circular DNA such as 50 kb length, and it takes just a day to get the enough amount of DNA for research. Not only fast and simple, but this technology also brings you accurate and functional DNA products even with a large size of DNA

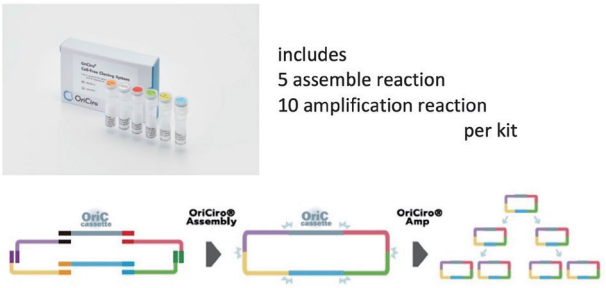

Figure 1: OriCiro®Cell-Free Cloning system

Cell-Free Switching System
allows you to quickly switch the plasmid DNA amplification from using living cells to our easy-to-use cell-free, enzymatic amplification system. SS OriC Cassettes can be inserted by a two-step isothermal enzymatic reaction to enable cell-free amplification within hours. It is applicable to pUC-, pET- and pGEM-based vectors of 4-13 kb

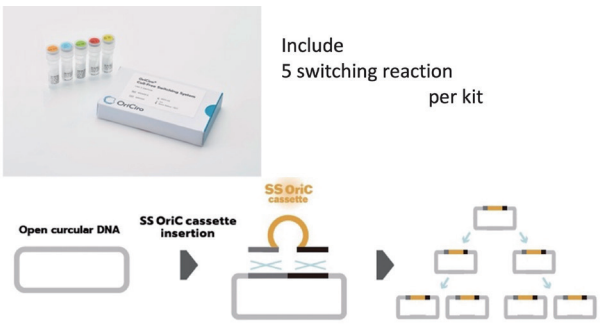

Figure2: OriCiro®Cell-Free Switching system

3. Getting an enough amount of DNA for transfection in the tube reaction

Circular DNA having oriC can be made by using Cloning system or Switching System. It allows you to assemble multiple DNA fragment and oriC cassette or insertion oriC cassette into compatible vectors. OriCiro® Amp Kit contains more than 20 kinds of purified enzymes that are essential for the E. coli chromosome replication cycle. This enables to amplify exponentially circular DNA having oriC (E. coli chromosomal origin) and up to 100 ng DNA/ µL reaction from a single DNA molecule within 6 hours of incubation. To obtain around 10 µg plasmid DNA for cell transfection, we perform 100 µL scale

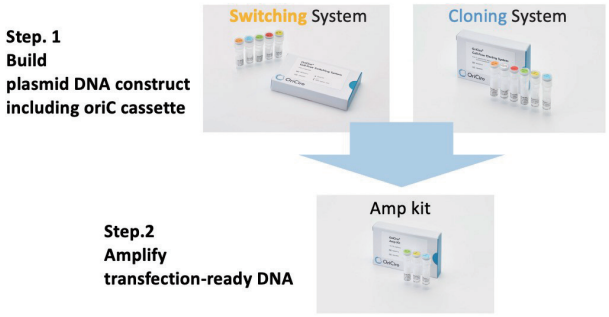

Figure 3: OriCiro Cell-Free DNA synthesis tools portfolio

4. Method

In this experiment, 4 kb and 13 kb circular DNA having oriC cassette were prepared as 10 pg/ µL plasmid. Then, Amplification reaction were performed in the following steps.

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

(µL)	10 µL Standard protocol	100 µL
5 X Buffer I	2	**20**
5 X Buffer Ⅱ	2	**20**
10 X RE Mix	1	**10**
Nuclease-Free Water	4	**40**

3. Incubated the mixture at 33 °C for 15 minutes
4. Add plasmid DNA (10 pg/µL)
   - 1 µL of plasmid DNA into 10 µL scale tube
   - 10 µL of plasmid DNA into 100 µL scale tube

5. Incubated the mixture at 33 °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)

5. Result

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. Used 0.5% Agarose, 0.5X TBE for electrophoresis.
The efficient production both of 4 kb and 13 kb were detected. In addition, DNA concentration was measured by fluorometer. Concentration values correspond to linear correlations in samples comprised 10 µL and 100 µL (Figure 4)

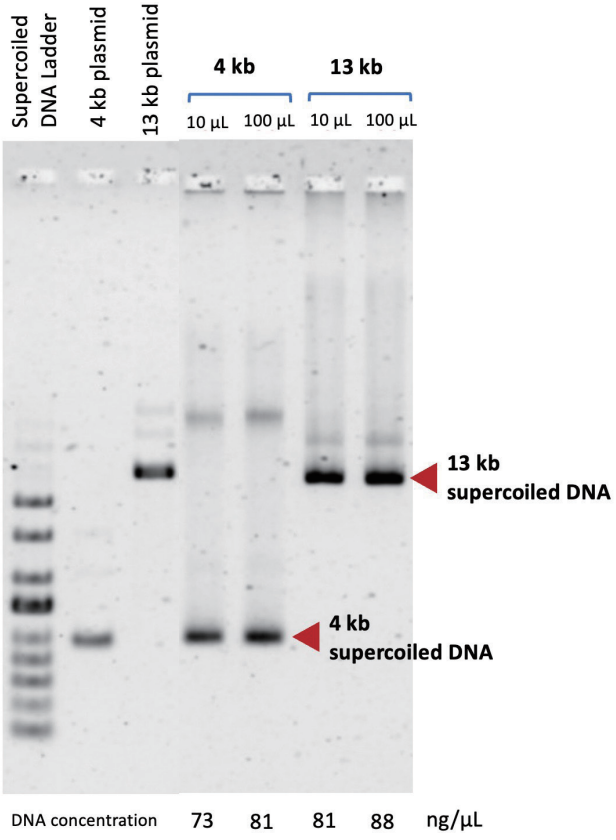

Figure 4: DNA concentration was measured by fluorometer. Concentration values correspond to linear correlations in samples comprised 10 µL and 100 µL which range was 73-81 ng/µL in 4 kb, 71-88 ng/µL in 13 kb.

6. Conclusion

OriCiro's cell-free system shows the capability to amplify transfection-ready DNA. The results in circular DNA amplification that is freed of limitations.

From the safety profile point of view, antibiotic-resistant genes are not necessary and endotoxin-free.
From applications, system allows you to amplify any sequences with high-GC and AT content and cytotoxic sequences.

It also increases throughput greatly and potential as well as streamline and eliminate many steps of growing and purifying DNA from cell-based workflow, OriCiro is game changer for that.

Usage notes

To ensure the successful assembly and amplification, please follow the instruction manual.
https://www.oriciro.com/resource

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