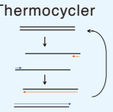
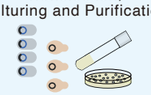
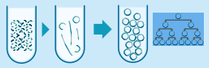
Comparison ／ Distinguished advantages of OriCiro amplification technology

	PCR	*E. coli* cloning	OriCiro Technology
DNA size	+ < 10 kb	++ < 300 kb	+++ < 1 Mb ※1
Operation	Thermocycler 	Transfection, Culturing and Purification 	Isothermal incubation 
Biosafety	Cell-free	Recombinant DNA experiment	Cell-free
Fidelity	10^{-4}~10^{-6} error / base / cycle	10^{-10} error / base / generation	10^{-8} error / base / cycle ※2
Sequence applicability	Not applicable to GC-rich and repeat	Not applicable to cell-toxic sequences	Applicable to any sequence
Product	Linear DNA	Circular DNA	Circular DNA

※1 Our products (ver.1.0) are optimized for up to 50 kb.
※2 Technology under development to mach *E. coli* cloning.

Specifications ／ Price

Product Name	OriCiro™ Cell-Free Cloning System 1.0
Size	1 Kit (Assembly: 5 reactions, Amp: 10 reactions)
Component	OriCiro™ Assembly Kit and OriCiro™ Amp Kit
Storage	below -70°C
Price ＊	$550
Cat. No.	MS0011-A

＊ Shipping costs and taxes are not included.

OriCiro™ Assembly Kit
(5 reactions, 5 µl / reaction)

Component	2×RA Mix *oriC* cassette Control fragment

OriCiro™ Amp Kit
(10 reactions, 10 µl / reaction)

Component	10X RE Mix 5X Buffer I 5X Buffer II

In consideration of the biosecurity and biosafety risks involved in the construction of long chain DNA, we ask our customers to submit a consent form at the time of purchase of this product.

OriCiro™ Cell-Free Cloning System 1.0

Application

- Artificially designed viral vector and plasmid construction.
- Amplification of long DNA and sequences difficult to amplify by PCR.
- Alternative to time-consuming and laborious *E. coli* cloning.
- Recombinant phage production
- Efficient cloning of any DNA sequence, including cytotoxic and GC-rich sequences.
- The amplification products can be used directly for *E. coli* transformation, etc.

Novel synthetic biology tool

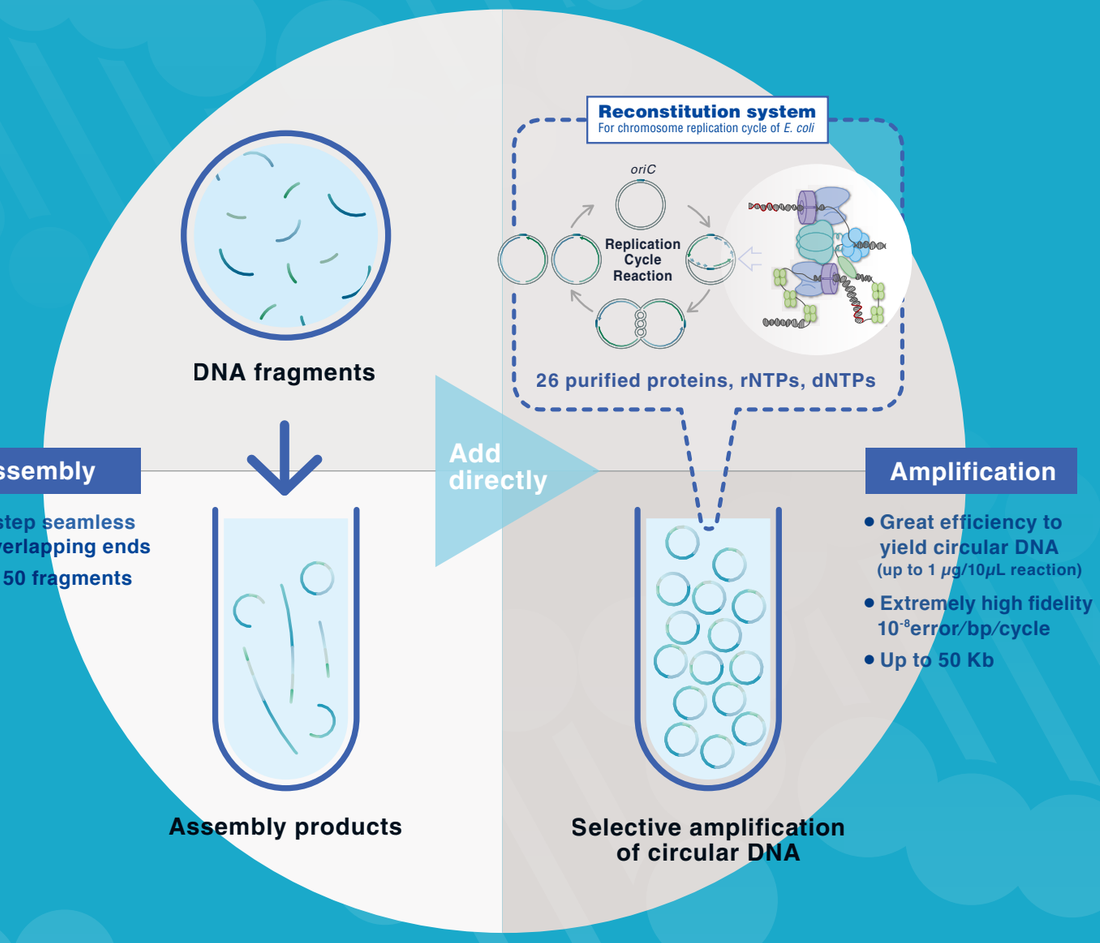
OriCiro

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/

AP2020.10.1000

CELL-FREE LARGE CIRCULAR DNA CONSTRUCTION TOOL

"Rapid, Easy and Efficient" method beyond conventional *E. coli* cloning



Now released

OriCiro™ CELL-FREE CLONING SYSTEM

OriCiro

OriCiro™ CELL-FREE CLONING SYSTEM

LARGE CIRCULAR DNA CONSTRUCTION TOOL

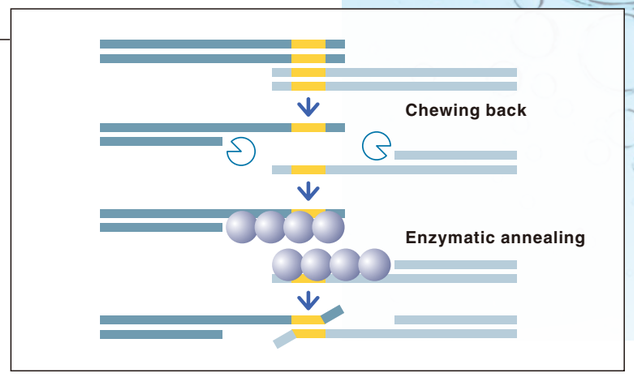
The OriCiro™ Cell-Free Cloning System is a rapid and powerful synthetic biology tool replacing cumbersome DNA cloning (plasmid construction) process relying on *E. coli*. The system consists just of two-step reactions. OriCiro Assembly ligates multiple DNA fragments seamlessly. The assembly product is then added directly to OriCiro™ Amp to get selective amplification of circular DNA. The amplified product is supercoiled DNA topologically identical to plasmid DNA isolated from *E. coli*.

Novel Synthetic Biology Tool

Assembly × Amplification

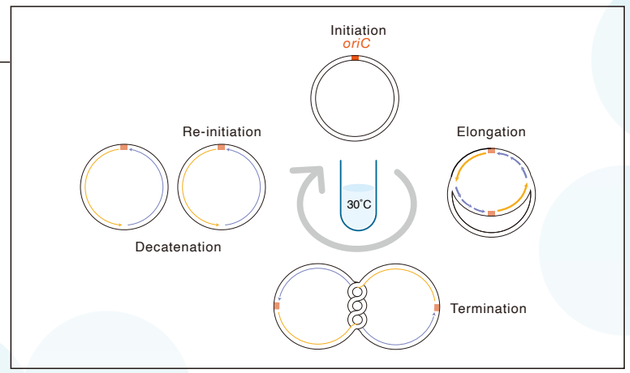
OriCiro Assembly kit

In OriCiro Assembly kit, multiple DNA fragments are assembled together seamlessly at 42˚C for 30 min via 40 bp overlapping ends. DNA fragments generated by PCR or restriction enzyme digestion are available. Our unique enzyme-based annealing mechanism allows powerful assembly up to 50 fragments simultaneously.



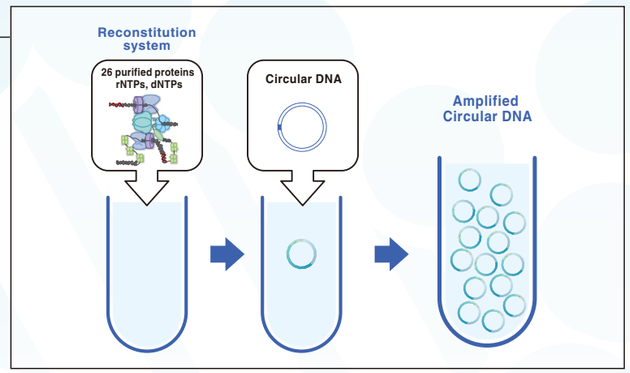
Synergy of Assembly & Amplification

The assembly product is added directly to the amplification reaction. Circularized DNA generated in the assembly reaction are selectively amplified even from single molecule level. Assembly intermediates, which are linear form, are shaken off during the amplification process. Only circular DNA molecules (supercoiled form) are thus obtained (see Experimental data).



OriCiro Amp kit

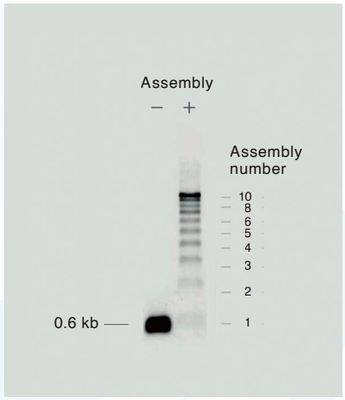
OriCiro Amp kit consists of 26 purified enzymes involved in chromosome replication of *E. coli*. The chromosome replication cycle repeats continuously and autonomously at around 30˚C, enabling exponential amplification of circular DNA having *oriC* (0.3kb chromosome origin, inserted by OriCiro Assembly) with extremely high fidelity (10^{-8} error / base / cycle). The ver. 1.0 kit yields up to 1 µg circular DNA per 10 µL reaction. The maximum amplification size is 50 kb.



Experimental data

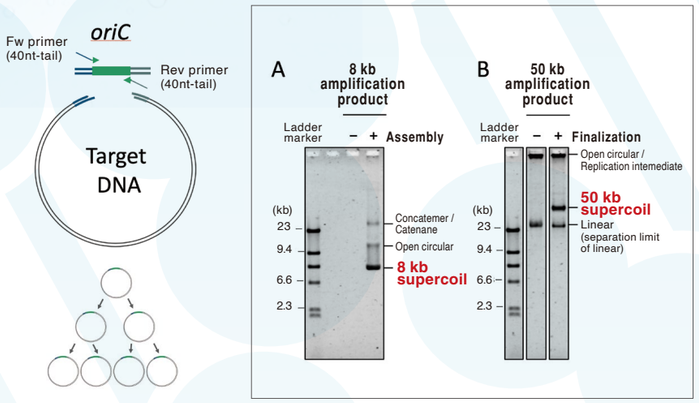
Efficient Assembly of 10 Fragments — Assembly

	Oriciro assembly
Temperature	42°C
Time	30 min



Ten fragments of 0.6 kb DNA having 40 bp overlapping ends were assembled using OriCiro Assembly kit. The kit showed an assembly product of all ten fragments. DNA fragments before (–) or after (+) the assembly reaction were analyzed in agarose gel electrophoresis.

Amplification of 8 kb or 50 kb circular DNA — Amplification

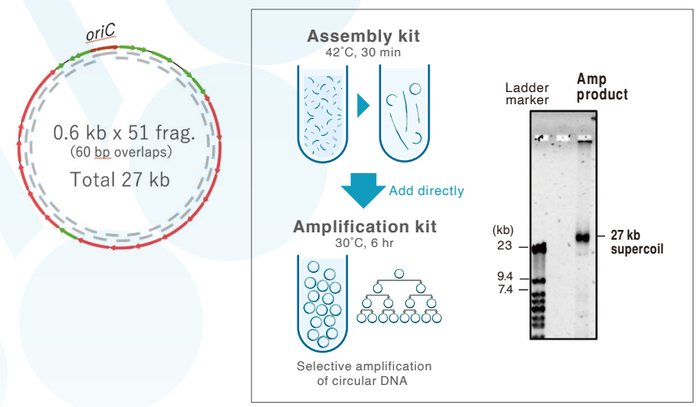


A: 8 kb DNA as a supercoiled form. A 7.5 kb DNA fragment (1ng)*1 and a 0.4 kb *oriC* cassette having 40 bp overlapping ends (50 pg) *1 were mixed in the OriCiro Assembly reaction (5 µL) and incubated at 42°C for 30 min. An aliquot (1 µL) of the assembly reaction (+) or the same amount of DNAs without the assembly reaction (–) was added to the OriCiro Amp reaction (10 µL) and further incubated at 33°C for 6 hr. The products were analyzed in agarose gel electrophoresis.

B: Amplification of 50 kb DNA. 50 kb circular DNA containing *oriC* (5 ng as circular DNA) was incubated in the OriCiro Amp reaction (10 µL) at 33°C for 6 hr. Although the amplification yields replication intermediates but not supercoiled molecules (–), Further incubation after 2-fold dilution with the OriCiro Amp buffer at 33°C for 30 min (Finalization option) generates the supercoiled molecules of 50 kb circular DNA whose size can be separated in normal agarose gel electrophoresis (+).

*1: These DNA were included in the kit as "Control Fragment" and "*oriC* Cassette"

Two-step construction of 27 kb plasmid from 51 fragments — Assembly and Amplification



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 42°C for 30 min followed by heat treatment at 65°C for 2 min. An aliquot of the reaction (1 µL) was then incubated in the OriCiro Amp reaction (10 µL) at 30°C for 6 hr. The products was diluted two-fold with the OriCiro Amp buffer and further incubated at 30°C for 30 min (Finalization option) followed by agarose gel electrophoresis. The result shows efficient production of the 27 kb supercoiled DNA.