Read the instruction manual thoroughly before you use the product / For Research Use

Ver. 1.0

OriCiro

OriCiro® Cell-Free Switching System

OriCiro® Cell-Free Switching System allows you to switch the plasmid DNA amplification from using living cells to cell-free system. SS OriC cassette is inserted into your plasmid DNA via homology arms designed to target a conserved sequence flanking replication origin of popular vector plasmids*. The resultant *oriC*-containing plasmid is then amplified in our unique cell-free reaction as a supercoiled form without the need of cumbersome cloning relying on *E. coli.*

**In vivo* function of the original plasmid is not interfered by the *oriC*-insertion.

Figure 1. Integration of the *oriC* sequence in a target plasmid DNA and amplification with cell-free system.

To use Switching System, your target plasmid DNA should have the same sequence with both ends of SS OriC Cassette. Most of the general plasmid DNAs which are used for molecular cloning experiments have these sequences (Table 1).

Table.1 Compatibility of target plasmid DNAs and Switching System.

Target plasmid DNAs	Switching System
pUC vectors	OK
pBluescript® vectors	OK
pGEM® vectors	OK
pTZ vectors	OK
pBR322 and derivatives	OK
pETcoco vectors	NG
pACYC and derivatives	NG
pSC101 and derivatives	NG

I. Components

(1)	2X SS Mix	12.5 µl
(2)	SS OriC Cassette *1 (40 pM, 14 pg/µl)	5 µl
(3)	10X RE Mix	5 µl
(4)	5X Buffer I	20 µl
(5)	5X Buffer II	20 µl

*1: SS OriC Cassette is a 546 bp DNA fragment containing the *oriC* (*E. coli* chromosomal origin) sequence. Both ends of the SS OriC Cassette have 60 bp homologous sequences against the target plasmid DNA (Figure 2). See Appendix for sequence information.

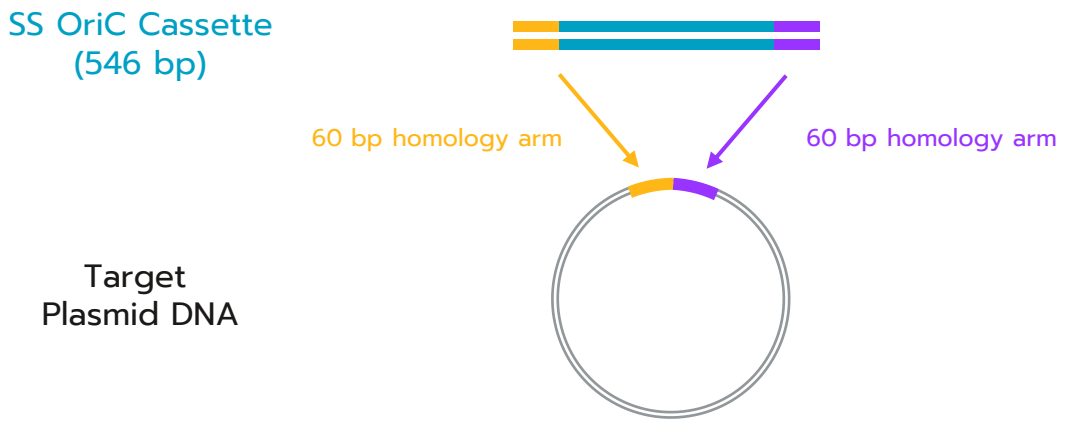

Figure 2. Target plasmid DNA and SS OriC Cassette

II. Equipment and materials required but not included

- Nuclease-Free Water
- Vortex mixer
- Microcentrifuge
- Thermal cycler or thermo block
- 0.2 ml microtubes (PCR tubes)
- Micropipettes and tips

III. Storage

OriCiro® Cell-Free Switching System is shipped on dry ice. Upon receipt, the kit must be immediately stored at **below -70°C**. 2X SS Mix and 10X RE Mix contains enzymes, and repeated freeze-thaw cycles must be avoided, although at least three freeze-thaw cycles are possible without the loss of function. 10X RE Mix must be frozen quickly using liquid nitrogen or dry ice ethanol before storing again at -70°C. Although, 2X SS Mix, SS OriC Cassette, 5X Buffer I and 5X Buffer II can be stored at below -20°C; all components including 10X RE Mix can be stored together at below -70°C for convenience.

IV. Protocol

(1) Prepare 10 µl of 40 pM of target plasmid DNA in PCR tube.*1

(2) Incubate the target plasmid DNA at 75°C for 60 minutes and hold on ice. Avoid evaporation of the reaction during incubation.

(3) Thaw 2X SS Mix on ice, mix it well using a vortex mixer at a maximum speed and spin down with a microcentrifuge.

(4) Prepare the following mixture on ice and mix well by vortex. *2

< Reagent >	
Nuclease-Free Water	0.5 µl
SS OriC Cassette (40 pM)	1 µl
Target plasmid DNA (40 pM)	1 µl
2X SS Mix	2.5 µl
Final Volume	5 µl

*1: • Use a 0.2 ml PCR tube to avoid evaporation. Dilute the target plasmid DNA with TE (pH 8.0).

• This system is suitable for 4.0 kb to 13 kb size of plasmid DNA. Approximate mass concentration of each size of DNA equivalent to 40 pM is:

Size	pg/µl (= 40 pM)
4 kb	106
5 kb	132
6 kb	158
7 kb	185
8 kb	211
9 kb	238
10 kb	265
11 kb	292
12 kb	319
13 kb	346

Referenced URL: https://www.promega.com/resources/tools/biomath/

*2: • Use a 0.2 ml PCR tube to avoid evaporation.

(5) Incubate the mixture at 42°C for 20 minutes and hold on ice until use. *3

*3: Do not freeze the mixture. Follow the next reaction immediately.

(6) Turn on a thermal cycler or an air incubator and preheat at 33°C. Avoid evaporation of the reaction during incubation. If a thermal cycler is used, the lid should be set at 40°C.

(7) Thaw 5X Buffer I and 5X Buffer II on ice, mix well using a vortex mixer and spin down with a microcentrifuge.

(8) Thaw 10X RE Mix on ice, mix gently using a vortex mixer and spin down with the microcentrifuge.

(9) Prepare the following pre-mixture on ice. Mix before and after the addition of 10X RE Mix as indicated.*4

< Reagent >	
Nuclease-Free Water	4 µl
5X Buffer I	2 µl
5X Buffer II	2 µl
→ Vortex mixing	
10X RE Mix	1 µl
→ Pipette mixing*5	
Final Volume	9 µl

*4: Use a 0.2 ml PCR tube to avoid evaporation.
*5: For the pipette mixing, set pipette volume to the total mixture volume, and pipette up and down several times with agitation.

(10) Incubate the pre-mixture at 33°C for 15 minutes.

(11) Add 1 µl of the reaction mixture of step (5) into the pre-mixture and mix with pipetting.

(12) Incubate the mixture at 33°C for 6 hours*6 and keep it at 4°C before use.

*6: Thermal cycler program is useful to hold automatically at 4°C after the 33°C incubation.

(13) Prepare the following buffer on ice and mix well using a vortex mixer.

< Reagent >	
Nuclease-Free Water	6 µl
5X Buffer I	2 µl
5X Buffer II	2 µl
Final Volume	10 µl

(14) Add all the buffer into the reaction mixture of step (12) and further incubate at 33°C for 30 minutes.

(15) Check the products using agarose gel electrophoresis [*7]

*7: • Typical DNA concentration is 50–100 ng/μl.
• The gel-loading buffer should contain SDS etc. to remove proteins from DNA.
• Supercoiled DNA Ladder (New England Biolabs) is recommended as a size maker. Alternatively, analyze it by restriction mapping.
• No need to purify the products in prior to electrophoresis.

V. Appendix

a. SS OriC Cassette (546 bp)

SS OriC Cassette is a 546 bp DNA fragment containing the *oriC* (*E. coli* chromosomal origin) sequence. Both ends of the SS OriC Cassette have 60 bp homologous sequences against the target plasmid DNA. If the backbone of your plasmid DNA has been modified, please ensure these 60 bp homologous sequences (colored letters) remain in the target plasmid DNA. SS OriC Cassette has HindIII site at 169 bp.

5'-ttttgtttgcaagcagcagattacgcgcagaaaaaaggatctcaagaagatcctttga--*oriC* (426 bp)-

-tcttttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtca-3'

b. SS OriC Cassette introduction into the different sizes of plasmid DNAs (Figure 3)

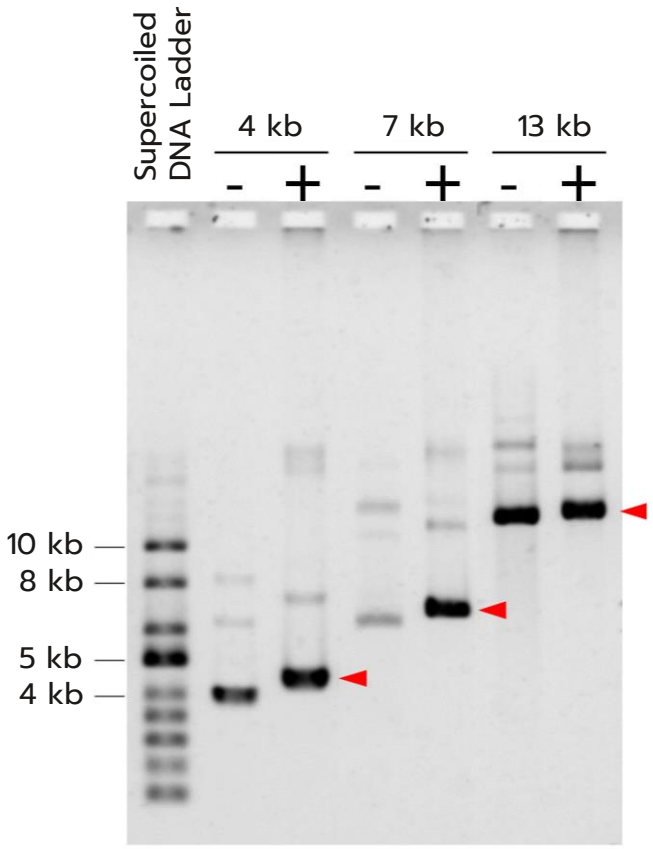

Figure 3. Introduction of SS OriC Cassette into the different sizes of plasmid DNAs using OriCiro Cell-Free Switching System. (-) The target plasmid DNA was extracted from *E.coli* and purified it by a column. These target plasmid DNAs does not possess *oriC* sequence. (+) SS OriC Cassette was introduced in the target plasmid DNA using Switching System. After reaction, the DNA size of products were increased compared with that of the target plasmid DNAs. Concatemer and open circular DNA were also detected. Red arrow shows supercoiled DNA produced by Switching System.

VI. Troubleshooting

a. **No/Less products are observed.**
   1) Increase the DNA amount up to 200 pM.
   2) The target plasmid DNA should have the same 60 bp homologous sequences with both ends of SS OriC Cassette.
   3) This system is suitable for 4.0 kb to 13 kb size of plasmid DNA.
   4) Low amount of the target plasmid DNA reduces the efficiency of the desired plasmid DNA production.
   5) After 20 minutes reaction at 42°C (step 5), do not freeze the mixture. Follow the next reaction immediately.

b. **Byproducts other than the target DNA are observed.**
   1) Open circular plasmid DNA band is detected at upper position than desired plasmid DNA.
   2) High amount of DNA in reaction mixture may lead to production of undesirable plasmid DNA.
   3) Contamination of the target plasmid DNA with other plasmid DNA containing the same 60 bp homologous sequences may lead to production of both plasmid DNAs during the reaction.

c. **Need more amount of products.**
   1) The plasmid DNA which possesses SS OriC Cassette can be re-amplified with OriCiro Amp Kit (Cat#: MS0021-A). Use 20 pg of the plasmid DNA as a template DNA for OriCiro Amp Kit.

References

1. M. Su'etsugu, H. Takada, T. Katayama, H. Tsujimoto, Exponential propagation of large circular DNA by reconstitution of a chromosome-replication cycle, *Nucleic Acids Research*, 2017, 45 (20), 11525–11534

Note :

- This product is for research use only. It is not intended for use this product or its components for any purposes including but not limited to diagnostics, prophylactics, and/or therapeutics or otherwise clinical trials.
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