



A simple and ultra-low cost homemade seamless ligation cloning extract (SLiCE) as an alternative to a commercially available seamless DNA cloning kit

Yuki Okegawa, Ken Motohashi*

Department of Bioresource and Environmental Sciences, Faculty of Life Sciences, Kyoto Sangyo University, Kamigamo Motoyama, Kita-ku, Kyoto 603-8555, Japan



ARTICLE INFO

Article history:

Received 12 August 2015

Received in revised form

8 September 2015

Accepted 9 September 2015

Available online 11 September 2015

Keywords:

Homologous recombination

Seamless DNA cloning

SLiCE

ABSTRACT

The seamless ligation cloning extract (SLiCE) method is a novel seamless DNA cloning tool that utilizes homologous recombination activities in *Escherichia coli* cell lysates to assemble DNA fragments into a vector. Several laboratory *E. coli* strains can be used as a source for the SLiCE extract; therefore, the SLiCE-method is highly cost-effective. The SLiCE has sufficient cloning ability to support conventional DNA cloning, and can simultaneously incorporate two unpurified DNA fragments into vector. Recently, many seamless DNA cloning kits have become commercially available; these are generally very convenient, but expensive. In this study, we evaluated the cloning efficiencies between a simple and highly cost-effective SLiCE-method and a commercial kit under various molar ratios of insert DNA fragments to vector DNA. This assessment identified that the SLiCE from a laboratory *E. coli* strain yielded 30–85% of the colony formation rate of a commercially available seamless DNA cloning kit. The cloning efficiencies of both methods were highly effective, exhibiting over 80% success rate under all conditions examined. These results suggest that SLiCE from a laboratory *E. coli* strain can efficiently function as an effective alternative to commercially available seamless DNA cloning kits.

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1. Introduction

Traditional DNA cloning methods using restriction enzymes and DNA ligases are time consuming because the limited availability of restriction endonuclease sites often interferes with generation of the desired DNA constructs. To avoid such limitations and complicated procedures, various seamless DNA cloning methods have been developed over the last decade [1–4]. Of these, the seamless ligation cloning extract (SLiCE) method is a novel homemade protocol that utilizes *in vitro* homologous recombination activities in cell lysates prepared from *Escherichia coli* [5,6]. Zhang et al. originally demonstrated that the cell lysates prepared from an *E. coli* DH10B-derivative modified to express a λ prophage Red/ET recombination system, which was termed the PPY strain, had high DNA cloning activity using short end homology regions between insert and vector DNA fragments [5]. *E. coli* laboratory strains also contain two endogenous RecA-dependent and -independent pathways of

homologous recombination [7–11]; however, the *E. coli* RecA[−] laboratory strain cell lysates did not exhibit high cloning efficiency when utilizing short end homology regions (15–20 bp) [5].

Recently, we reported that the cloning efficiency of cell lysates from *E. coli* RecA[−] laboratory strains was improved by harvesting *E. coli* cells at late log phase and extracting the lysates carefully on ice [6]. This demonstrates that the endogenous RecA-independent recombination activities in *E. coli* RecA[−] laboratory strains can function efficiently for the SLiCE-method using short homology lengths (approximately 15–19 bp), without the requirement of exogenously expressing a λ prophage Red/ET recombination system. In addition, SLiCE prepared from an *E. coli* RecA[−] laboratory strain could simultaneously incorporate two unpurified insert DNA fragments into vector, indicating highly efficient cloning activity [6]. SLiCE from *E. coli* laboratory strains is also cost-effective for seamless DNA cloning; however, the use of a commercially available cell lysis reagent, CellLytic B Cell Lysis Reagent (Sigma B7435) has been required for the preparation of SLiCE [5,6]. Thus, the need for the commercial cell lysis reagent increases the cost of the SLiCE method.

More recently, we found that SLiCE can instead be prepared with buffers containing Triton X-100 [12], which is a commonly available nonionic detergent that is generally used for protein

Abbreviations: CFU, colony-forming units; G6PDH1, glucose-6-phosphate dehydrogenase 1; PCR, polymerase chain reaction; Prx IIE, type II peroxiredoxin E; SLiCE, seamless ligation cloning extract

* Corresponding author. Fax: +81 75 705 1914.

E-mail address: motohas@cc.kyoto-su.ac.jp (K. Motohashi).

preparations [13–16]. By using the Triton X-100 buffer, the SLiCE-method becomes an ultra-low cost homemade seamless DNA cloning method [12]. On the other hand, commercially available kits for seamless DNA cloning have been widely used [17–24]. Although commercial kits are associated with a high cost per reaction, they are generally accepted to be easy to use and efficient. However, the differences in seamless DNA cloning efficiency between a homemade method and commercial kits are not well characterized. Therefore, in this study, we evaluated the efficiency of these methods under various molar ratios of insert DNA fragments to vector DNA.

2. Materials and methods

2.1. Preparation of SLiCE from an *E. coli* RecA[−] laboratory strain

SLiCE was prepared from *E. coli* JM109 using a buffer containing 3% (w/v) Triton X-100 [12]. *E. coli* JM109 pre-cultured in LB Miller medium (1 mL) at 37 °C were transferred to 2 × YT medium (50 mL) in a 100-mL round-bottom, long-neck Sakaguchi shaking flask. The cells were grown at 37 °C in a reciprocal shaker (160 rpm) until the OD₆₀₀ reached a value of 3.0 (late log phase). The cells were harvested by centrifugation at 5000g for 10 min at 4 °C. The cells were then washed with 50 mL sterilized water (ice-cold), and centrifuged at 5000g for 5 min at 4 °C. The washed cells were recovered with a yield of 0.40 g, and gently resuspended in 1.2 mL 3% (w/v) Triton X-100 in 50 mM Tris–HCl (pH 8.0) and incubated for 10 min at room temperature. The cell lysates were then centrifuged at 20,000g for 2 min at 4 °C. All subsequent procedures were performed on ice. The supernatants were carefully transferred into 1.5-mL microtubes to remove the insoluble materials, and determined the protein concentration as ~0.9 mg/mL by the BCA assay method. An equal volume of ice-cold 80% (v/v) glycerol was added to the supernatant, and mixed gently. The produced SLiCE extracts were snap-frozen in a bath of liquid nitrogen and stored at −80 °C in 40% (v/v, final concentration) glycerol.

2.2. Preparation of insert DNA fragments and linearized vector DNA

The *Arabidopsis* type II peroxiredoxin E (*Prx IIE*, 0.6 kilo base pairs (kbp), AT3G52960) [25,26] and chloroplastic glucose-6-phosphate dehydrogenase 1 (*G6PDH1*, 1.6 kbp, AT5G35790) [27] genes were used as insert DNA molecules. pET23a (Merck Millipore, Billerica, MA, USA) was used as a vector DNA template. The insert DNA fragments and linearized pET23a DNA (3.7 kbp) were amplified using the primers listed in Table S1 and TksGflex DNA polymerase (Takara-Bio, Otsu, Japan) by polymerase chain reaction (PCR) [6, 12]. Overlap-regions between insert and vector DNA were designed as 15 bp lengths. PCR-amplified insert and vector DNA were treated with *DpnI* (37 °C, 60 min) to avoid cross-contaminating of methylated-DNA as PCR-template and purified using a FastGene Gel/PCR Extraction Kit (NIPPON Genetics, Tokyo, Japan) following agarose gel electrophoresis.

2.3. SLiCE reaction for seamless DNA cloning

SLiCE buffer (10 × ; 500 mM Tris–HCl, pH 7.5, 100 mM MgCl₂, 10 mM ATP, and 10 mM dithiothreitol) was passed through a 0.2-μm filter, and dispensed in 40 μL aliquots into 0.2 mL 8-strip PCR tubes and stored at −20 °C [6]. The standard SLiCE reaction solution comprised the following components: 10 ng linear vector (PCR amplified), an appropriate amount of insert DNA (1:1–50:1 molar ratio of insert to vector), 1 μL 10 × SLiCE buffer, 1 μL SLiCE extract, and sterilized distilled water to a total volume of

10 μL. The SLiCE reaction mixture was incubated at 37 °C for 15 min. The reaction time was adjusted to compare with that used in the In-Fusion cloning method.

2.4. In-Fusion reaction for seamless DNA cloning

The In-Fusion HD Cloning Kit (Clontech, Mountain View, CA, USA) was purchased from Takara-Bio. The standard In-Fusion reaction solution comprised the following components: 10 ng linear vector (PCR amplified), an appropriate amount of insert DNA (1:1–50:1 molar ratio of insert to vector), 2 μL 5 × In-Fusion HD enzyme premix, and sterilized distilled water to a total volume of 10 μL. The In-Fusion reaction mixture was incubated at 50 °C for 15 min, according to the instruction manual.

2.5. Transformation of competent cells with SLiCE and In-Fusion solutions

Following incubation, heat-shock transformation was conducted by adding 1 μL of SLiCE or In-Fusion reaction solution into 20 μL ECOS X Competent *E. coli* DH5α (Nippon Gene, Tokyo, Japan) according to the instruction manual. The transformation efficiency of the competent cells (20 μL) was approximately 2 × 10⁸ colony-forming units (CFUs)/μg pUC19 DNA. Transformed *E. coli* cells were plated on LB agar plates containing ampicillin and incubated at 37 °C for 12–16 h.

2.6. Efficiency estimation for SLiCE and In-Fusion cloning methods

The efficiency of seamless DNA cloning was evaluated by two parameters, colony-formation rate and cloning efficiency. Colony formation rate was determined as the number of colonies represented as CFUs per nanogram vector. Cloning efficiencies for the insert DNA were given as the ratio of colonies with an insert of the confirmed correct length as estimated by colony-PCR to the total number of colonies tested [6,12].

3. Results and discussion

3.1. Estimation of colony formation rate and cloning efficiency for SLiCE and In-Fusion cloning methods

To date, SLiCE has been prepared according to several methods. SLiCE was originally prepared from the *E. coli* PPY strain, which expresses a λ prophage Red/ET recombination system, using commercially available cell lytic buffer, CellLytic B Cell Lysis Reagent (Sigma B7435) [5]. Recently, we found that SLiCE were able to be prepared from easily available *E. coli* laboratory strains using a cost-effective Triton X-100 buffer as well as with the commercially available cell lytic buffer [6,12]. In this study, we used the simplest and most cost-effective SLiCE identified: the extract prepared from the *E. coli* RecA[−] laboratory strain JM109 with the 3% Triton X-100 buffer, and compared its cloning efficiency with a commercially available seamless DNA cloning kit. The Clontech In-Fusion HD Cloning Kit, which is widely used for seamless DNA cloning, was selected as being representative of commercial kits [17–19,21,23,24]. In this comparative experiment, the cloning abilities of both seamless DNA cloning methods were evaluated under various molar ratios of insert DNA fragments to vector DNA by two parameters: colony formation rate and cloning efficiency. The reaction time of SLiCE-cloning was set as 15 min, which is the same as that recommended in the In-Fusion cloning method (Fig.1).

Firstly, we determined the colony formation rate (the number of colonies after transformation), as the efficiency of SLiCE-cloning

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