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Evaluation of the efficiency and utility of recombinant enzyme-free seamless DNA cloning methods

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ABSTRACT

Simple and low-cost recombinant enzyme-free seamless DNA cloning methods have recently become available. *In vivo Escherichia coli* cloning (iVEC) can directly transform a mixture of insert and vector DNA fragments into *E. coli*, which are ligated by endogenous homologous recombination activity in the cells. Seamless ligation cloning extract (SLiCE) cloning uses the endogenous recombination activity of *E. coli* cellular extracts *in vitro* to ligate insert and vector DNA fragments. An evaluation of the efficiency and utility of these methods is important in deciding the adoption of a seamless cloning method as a useful tool. In this study, both seamless cloning methods incorporated inserting DNA fragments into linearized DNA vectors through short (15–39 bp) end homology regions. However, colony formation was 30–60-fold higher with SLiCE cloning in end homology regions between 15 and 29 bp than with the iVEC method using DH5α competent cells. *E. coli* AQ3625 strains, which harbor a *sbc*A gene mutation that activates the RecE homologous recombination pathway, can be used to efficiently ligate insert and vector DNA fragments with short-end homology regions *in vivo*. Using AQ3625 competent cells in the iVEC method improved the rate of colony formation, but the efficiency and accuracy of SLiCE cloning were still higher. In addition, the efficiency of seamless cloning methods depends on the intrinsic competency of *E. coli* cells. The competency of chemically competent AQ3625 cells was lower than that of competent DH5α cells, in all cases of chemically competent cell preparations using the three different methods. Moreover, SLiCE cloning permits the use of both homemade and commercially available competent cells because it can use general *E. coli recA*$^-$ strains such as DH5α as host cells for transformation. Therefore, between the two methods, SLiCE cloning provides both higher efficiency and better utility than the iVEC method for seamless DNA plasmid engineering.

1. Introduction

Seamless DNA cloning methods are useful for plasmid engineering because DNA fragments can be ligated in a restriction enzyme site-independent manner. In the past decade, several purified-enzyme-dependent seamless DNA cloning methods have been developed [1–3]. Seamless cloning methods generally rely on short (~15 bp) end homology regions for ligation of insert and vector DNA fragments. These methods are available through commercial kits, which are widely used [4–14]; however, seamless cloning kits are cost-prohibitive. Recently, several simple and recombinant enzyme-free seamless DNA cloning methods have been described [15–18], which utilize the endogenous homologous recombination activity of laboratory *Escherichia coli* strains.

The most simple method is the *in vivo E. coli* cloning (iVEC) system [16–18]. This method directly introduces only DNA fragments containing insert and vector DNA molecules into *E. coli* competent cells. The introduced DNA molecules can be combined through short (30–50 bp) end homology regions using the endogenous *in vivo* homologous recombination activity of *E. coli* [18]. The iVEC system was originally reported by two groups more than 20 years ago [19,20], but longer end homology regions were required for efficient cloning. Jacobus et al. and Kostylev et al. recently reported that several DNA fragments can be simultaneously incorporated into a common linearized vector using the iVEC method with *E. coli* DH5α [17,18]. More recently, the National BioResource Project (NIG, Japan) has characterized and distributed a

Abbreviations: CFU, colony-forming units; G6PDH1, glucose-6-phosphate dehydrogenase 1; iVEC, *in vivo Escherichia coli* cloning; PCR, polymerase chain reaction; Prx IIE, type II peroxiredoxin E; SLiCE, seamless ligation cloning extract; TSS, transformation and storage solution.

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specific *E. coli* strain, AQ3625 (same as JC8679), for efficient iVEC [21]. Oliner *et al.* reported that the efficiency of *in vivo* cloning was higher with AQ3625 than with DH5α, likely because AQ3625 harbors a mutation in *sbcA23*, which activates the RecE homologous recombination pathway [20].

Seamless ligation cloning extract (SLiCE) cloning uses the endogenous homologous recombination activity of cellular extracts from laboratory *E. coli* strains, to ligate DNA fragments *in vitro* [15,22,23]. The homologous recombination activity of *E. coli* cellular extracts is preserved by using specific detergent buffers during lysis [15,22,24]. PCR-amplified fragments with short (15–19 bp) end homology regions can be efficiently ligated into a vector *in vitro* using SLiCE cloning with cellular extracts of various laboratory *E. coli* strains including JM109, DH5α, DH10B, and XL10-Gold [15,23]. SLiCE prepared from *E. coli* JM109 can be used in place of a commercial kit [22], such as the In-Fusion HD Cloning Kit from Clontech Laboratories. Moreover, SLiCE cloning can be used to simultaneously ligate two unpurified PCR fragments into a common vector [15,25], and to assemble various DNA fragments of small (90 bp) to large (13.5 kbp) size [26].

These two recombinant enzyme-free seamless DNA cloning methods are simple and greatly reduce the cost of seamless DNA cloning. However, the efficiency and accuracy of these seamless DNA cloning methods have not been directly compared to date. Therefore, in the present study, the efficiency, accuracy, and utility of iVEC and SLiCE cloning were evaluated using DNA fragments with short-end homology lengths (15–39 bp) that were suitable for standard seamless DNA cloning.

2. Materials and methods

2.1. *Escherichia coli* strains

E. coli DH5α [27] and AQ3625 (same as JC8679) [28] were used for transformations. *E. coli* AQ3625 (ME No. ME9276) was provided by the National BioResource Project (NIG, Japan): *E. coli*. *E. coli* JM109 [29] was used to prepare cellular extracts for *in vitro* SLiCE cloning. Genotypes of these strains are listed in Table S1.

2.2. *Preparation of competent* E. coli *cells*

Chemically competent *E. coli* cells were prepared using the modified transformation and storage solution (TSS) method [30]. Glycerol (10% (v/v), final concentration) was added to the original TSS solution [31]. The competency of chemically competent DH5α and AQ3625 cells prepared using the modified TSS method was 1.5×10^6 colony forming units (CFU)/μg pUC19 DNA and 0.78×10^6 CFU/μg pUC19 DNA, respectively. To compare the competency of chemically competent cells between DH5α and AQ3625, Inoue's method [32] and calcium chloride method [33] were also used.

2.3. *Preparation of vector and insert DNA*

DNA sequences encoding *Arabidopsis* type II peroxiredoxin E (*PrxIIE*, 0.5 kbp, AT3G52960) [34,35] and chloroplast glucose-6-phosphate dehydrogenase 1 (*G6PDH1*, 1.6 kbp, AT5G35790) [36] were used as insert DNAs. Two genes were cloned from an *Arabidopsis* cDNA library [37,38]. Insert DNA fragments and linearized pET23a vector DNA were amplified by PCR using Tks Gflex DNA polymerase (Takara-Bio, Otsu, Japan) and the primers listed in Table S2.

2.4. *Preparation of SLiCE from* E. coli *JM109*

The SLiCE from *E. coli* JM109 was prepared as described previously [23]. Briefly, *E. coli* JM109 cells 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 shake flask. The cells were grown at 37 °C in a reciprocal shaker (160 rpm with 25 mm stroke) until the OD_{600} reached a value of 2.0 (late log phase). The cultures were incubated for 5.0 h. The cells were harvested by centrifugation at 5000×*g* for 10 min at 4 °C. The cells were then washed with 50 mL of sterilized water (ice-cold), and centrifuged at 5000×*g* for 5 min at 4 °C. The wet cells were recovered with a yield of 0.37*g*, and gently resuspended in 1.2 mL of CelLytic B Cell Lysis Reagent (Sigma, B7435), which was a commercially available bacterial cell lysis buffer containing 40 mM Tris-HCl (pH 8.0) and zwitterionic detergents. The resuspended cell mixture was left to stand for 10 min at room temperature to allow the lysis reaction to proceed. The cell lysates were then centrifuged at 20,000×g 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 an equal volume of ice-cold 80% (v/v) glycerol was added and mixed gently. Each SLiCE extract (40 μL) was aliquoted into a 0.2-mL 8-strip PCR tube. The SLiCE extracts were snap-frozen in a bath of liquid nitrogen and stored at −80 °C in 40% (v/v, final concentration) glycerol.

2.5. *SLiCE cloning of PCR fragments*

SLiCE buffer (10×, 500 mM Tris-HCl, pH 7.5, 100 mM $MgCl_2$, 10 mM ATP and 10 mM dithiothreitol) was prepared as described previously [15,23]. The standard SLiCE reaction was performed as described previously [23]. Briefly, one microliter of SLiCE and one microliter of SLiCE buffer (10×) were added into the mixture of insert (4–67 ng) and vector (10–50 ng) DNA fragments, and then filled up to total 10 μL with sterilized distilled water, and then SLiCE reactions (10 μL total) were performed at 37 °C for 15 min. Reaction conditions including the quantities of insert and vector DNA fragments are described in detail in the figure and table legends. The mixtures after the SLiCE reaction were transformed into chemically competent DH5α cells using the standard heat-shock procedure [23].

2.6. *iVEC cloning of PCR fragments*

The same amount of insert and vector DNA fragments used in SLiCE cloning were mixed in a total of 10 μL and directly transformed into chemically competent DH5α or AQ3625 cells, using the standard heat-shock procedure [23]. Quantities of insert and vector DNA fragments in the mixture are described in detail in the figure and table legends.

2.7. *Evaluation of cloning efficiency*

The number of colonies formed on agar plates after transformation was counted in each experiment. Cloning efficiency was defined as the fraction of total colonies in which a PCR product of the correct length was amplified by colony PCR amplification. In particular, cloning efficiencies were represented as "the number of colonies with the correct length of insert DNA confirmed by colony-PCR/the number of colonies subjected to colony-PCR" [15]. Cloning accuracy was expressed as the fraction of correctly cloned expression vectors in colony-PCR-positive clones. In particular, cloning accuracies were represented as "the number of correct clones confirmed by DNA sequencing/the number of colony-PCR positive clones". DNA sequences were determined by Sanger DNA sequencing [39].

2.8. *Insert-check by colony-PCR in transformed* E. coli

Colony PCR amplification was performed as described previously [25,38]. Briefly, each colony was picked with a sterile toothpick, and put into the bottom of a 0.2-mL 8-strip PCR tube or a 96-well PCR plate. After the toothpicks were removed from the PCR-tube, 10 μL of KAPATaq Extra DNA polymerase (KAPA Biosystems, Wilmington, MA)

PCR mix was added to each sample; this mixture included the T7P and T7T primers corresponding to the T7 promoter and T7 terminator sequences of the pET vectors, respectively (Table S2, and [15]). PCR reactions were performed following the KAPATaq Extra standard protocol. For target DNAs > 1.5 kbp, Tks Gflex DNA polymerase was used in place of KAPATaq Extra DNA polymerase.

3. Results and Discussion

3.1. Evaluation of the cloning efficiency of iVEC (DH5α) and SLiCE using purified PCR fragments

The iVEC method using *E. coli* DH5α (iVEC-DH5α)) [17,18] and the SLiCE method using cellular extracts prepared from the *E. coli* JM109 strain [15,22–24] are recombinant enzyme-free seamless cloning methods, and these methods do not require any purified recombinant enzymes or special *E. coli* strains. To determine which of the two recombinant enzyme-free seamless DNA cloning methods provided a potential advantage, the cloning ability of both methods was compared by measuring the rate of colony formation (*i.e.*, number of colonies formed after transformation) and cloning efficiency (*i.e.*, the fraction of colonies in which a PCR product of the correct size could be amplified by colony PCR amplification) (Fig. 1). These two indices are important for evaluating cloning methods in general [15]. The colony formation rate was 30–60-fold higher for purified PCR fragments with short (15–29 bp) end homology regions using the SLiCE method compared to that using the iVEC-DH5α method (Fig. 2). Even when purified PCR fragments with longer (39 bp) end homology regions were used, which is an optimal length for the iVEC-DH5α method [17,18], the colony formation rate was still 5-fold higher using the SLiCE method than the iVEC-DH5α method (Fig. 2). The cloning efficiency of the SLiCE method using purified PCR fragments with short (15, 19, or 29 bp) end homology regions was also higher than that of the iVEC-DH5α method, although the cloning accuracy was the same between the two methods (Table 1). These results clearly indicate that the SLiCE method had more efficient cloning ability than the iVEC-DH5α method, with short (15, 19, or 29 bp) end homology regions. Using purified PCR fragments with longer (39 bp) end homology regions, the cloning efficiency of the iVEC-DH5α method was the same as that of the SLiCE method. This result is consistent with the conclusion that longer end homology regions (30–50 bp) are optimal for the iVEC-DH5α method [18]. In contrast, the cloning efficiency of SLiCE was high at 63–94% (Table 1, cloning efficiency), irrespective of the length of the end homology regions (15,19,29, or 39 bp). These results indicate that SLiCE cloning has higher flexibility and robustness as a seamless DNA cloning method than the iVEC-DH5α method.

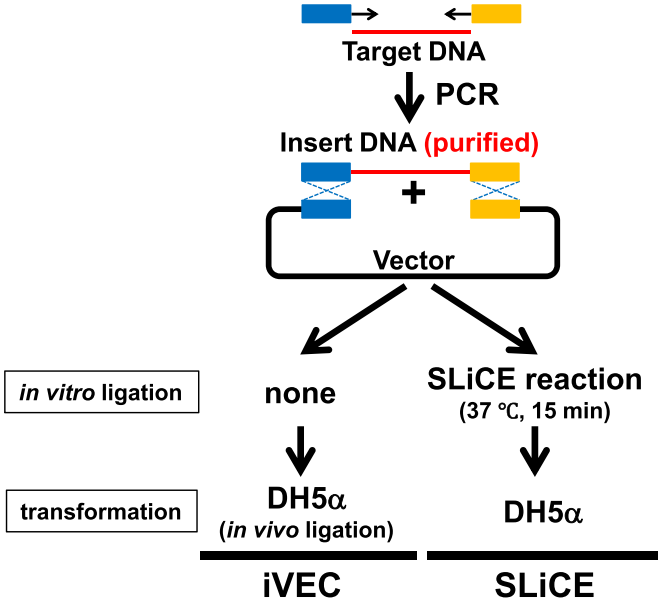

Fig. 1. Workflow of iVEC-DH5α and SLiCE cloning with purified PCR fragments. Insert DNA fragments were PCR-amplified, purified, and mixed with linearized vector DNA. The mixture of insert and vector DNAs was directly transformed into DH5α cells in the iVEC method (*in vivo* ligation). The mixture ligated using SLiCE (*in vitro* ligation) was transformed into DH5α cells in the SLiCE method. Chemically competent DH5α cells were prepared by the modified TSS method (DH5α, 1.5×10^6 CFU/μg pUC19 DNA)).

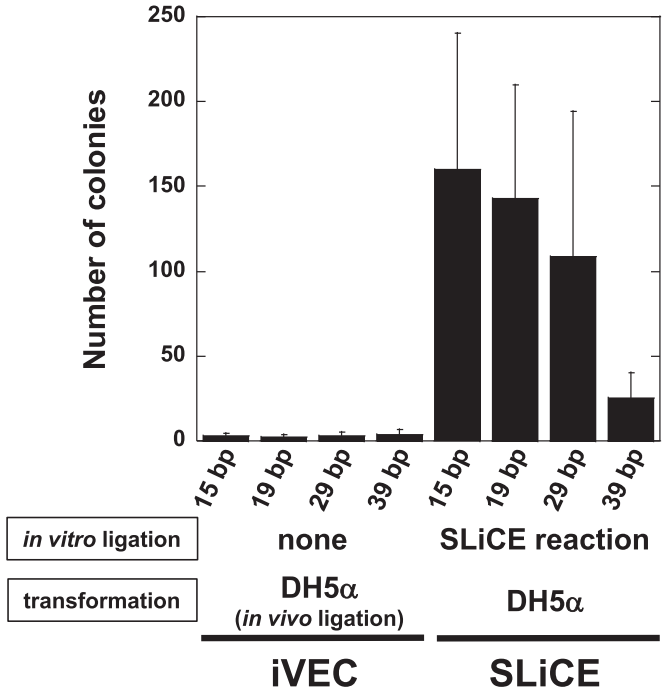

Fig. 2. Cloning efficiency of iVEC-DH5α and SLiCE using purified PCR fragments. The number of colonies formed (*i.e.*, colony formation rate) with purified PCR fragments of variable end homology region lengths (15,19,29, or 39 bp) using the iVEC-DH5α method [17,18] or the SLiCE method [15,23]. Number of colonies indicates the number of colonies that formed when 3 ng of vector DNA was transformed. Purified insert DNA fragments of *PrxIIE* (4 ng) and linearized pET23a vector (10 ng) were mixed in 10 μL. iVEC was directly transformed to DH5α competent cells using 3 μL in 10 μL. The SLiCE sample was reacted in a total volume of 10 μL, and then 3 μL of the 10 μL was used for transformation to DH5α. Each value for the number of colonies is the mean ± standard deviation of three independent experiments. DH5α chemically competent cells for both the iVEC-DH5α method and the SLiCE method were prepared with a competency of 1.5×10^6 (CFU/μg pUC19 DNA) by the modified TSS method.

Table 1
Cloning efficiency and cloning accuracy of iVEC-DH5α and SLiCE cloning methods using purified PCR fragments (*PrxIIE*).

Method[a]	Homology length (bp)	Cloning efficiency[b]	Cloning accuracy[c]
iVEC (DH5α)	15	6/11 (54.5%)	4/6 (66.7%)
	19	2/7 (28.6%)	2/2 (100%)
	29	7/11 (63.6%)	6/7 (85.7%)
	39	10/14 (71.4%)	10/10 (100%)
SLiCE	15	10/16 (62.5%)	9/10 (90.0%)
	19	15/16 (93.8%)	12/15 (80.0%)
	29	15/16 (93.8%)	13/15 (86.7%)
	39	11/16 (68.8%)	10/11 (90.9%)

[a] Insert DNA fragments of the *PrxIIE* gene (0.5 kbp) and linearized pET23a vector DNA were amplified by PCR, and purified by agarose gel electrophoresis and a Gel/PCR Extraction Kit (FastGene). Purified insert DNA fragments (4 ng) and linearized pET23a vector DNA (10 ng) were used at an insert:vector molar ratio of 3:1. Part (3 μL) of the total 10 μL solution was used to transform DH5α competent cells (1.5×10^6 CFU/μg pUC19 DNA) prepared by the modified TSS method [30].

[b] Cloning efficiency is defined as the fraction of total colonies in which a PCR product of the correct expected size was amplified by colony PCR amplification.

[c] Cloning accuracy is defined as the fraction of clones correctly confirmed by DNA sequencing among colony-PCR positive clones.

3.2. Evaluation of the cloning efficiency of iVEC (AQ3625) and SLiCE cloning using unpurified PCR fragments

Seamless DNA cloning methods can also successfully ligate unpurified PCR-amplified fragments into vectors because of their high cloning efficiency. Gel-band purification of PCR-amplified DNA fragments is a time consuming step for DNA cloning, as it takes approximately one hour. Recently, it has become possible to skip DNA purification by agarose gel electrophoresis because high-fidelity thermostable DNA polymerases can specifically amplify the target DNA fragments without amplification of nonspecific DNA fragments. However, DNA cloning of unpurified PCR products requires high efficiency. In the present study, the cloning efficiencies of unpurified PCR fragments into vectors by iVEC and SLiCE were evaluated next. The colony formation rate was low with the iVEC-DH5α method using purified PCR fragments of the *PrxIIE* gene, compared that of SLiCE cloning using the same DNA (Fig. 2). As a result, colony formation was not expected with the iVEC-DH5α method using unpurified PCR fragments because of the 1/10–1/100 colony formation rate for seamless cloning of unpurified PCR fragments [15]. Therefore, *E. coli* AQ3625 was used as a host strain to ligate unpurified PCR fragments with the iVEC method (Fig. 3). *E. coli* AQ3625 harbors a mutation in the *sbcA23* gene, which activates the RecE homologous recombination pathway. The efficiency of the iVEC method with AQ3625 was higher than that with DH5α [20]. The National BioResource Project (NIG, Japan) started to distribute a specific *E. coli* AQ3625 strain for efficient iVEC in April 2016 [21]. Use of *E. coli* AQ3625 in the present study improved the rate of colony formation of the iVEC method (Table 2). In fact, the number of colonies that formed with unpurified PCR fragments was higher with the iVEC-AQ3265 method than with the SLiCE method using DH5α cells (Table 2). In addition to the rate of colony formation, both cloning efficiency and cloning accuracy are important indices of the utility of DNA cloning methods [15]. In the present study, with unpurified PCR fragments of *G6PDH1* gene, it was not possible to obtain any correct clones by 16-colony screening, and only one correct clone was obtained with that of *PrxIIE* gene (Table 2, iVEC (AQ3625)). In contrast, the cloning efficiency of the SLiCE method was 15/16 clones (for *PrxIIE*) and 10/16 clones (for *G6PDH1*), and the cloning accuracy of the SLiCE method was >85% (Table 2, SLiCE). These results show that the SLiCE method is a more efficient recombinant enzyme-free seamless DNA cloning method than iVEC-AQ3625, even though the competency of the AQ3625 and DH5α strains is the same. The higher cloning efficiency and cloning accuracy of SLiCE (*in vitro* cloning) when compared to iVEC-AQ3625 (*in vivo* cloning) might be explained by a difference in transformation efficiency between circular DNA and linear DNA. As another possible explanation, the cell lysis buffer might specifically extract the homologous recombination activity required for seamless cloning, but not nuclease activity in *E. coli* cells.

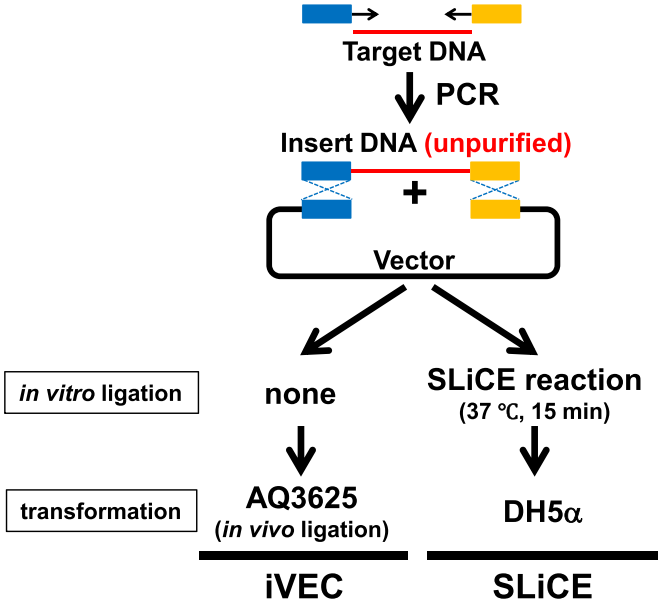

Fig. 3. Workflow of iVEC-AQ3625 and SLiCE cloning with unpurified DNA fragments. Insert DNA fragments were PCR-amplified and mixed with linearized vector DNA, without purification. The mixture of insert and vector DNAs was directly transformed into AQ3625 cells in the iVEC method (*in vivo* ligation). The mixture ligated using SLiCE (*in vitro* ligation) was transformed into DH5α cells in the SLiCE method. Chemically competent DH5α (1.5×10^6 CFU/μg pUC19 DNA) and AQ3625 (0.78×10^6 CFU/μg pUC19 DNA) cells were prepared by the modified TSS method [30]. Short (19 bp) end homology regions between insert and vector DNAs were used.

Table 2
Colony formation rate, cloning efficiency, and cloning accuracy of iVEC-AQ3625 and SLiCE cloning methods using unpurified PCR fragments (*PrxIIE* and *G6PDH1*).

Method[a]	Insert DNA	Number ofcolonies[b]	Cloning efficiency[c]	Cloning accuracy[d]
iVEC(AQ3625)	*PrxIIE*	58 ± 8	3/16 (18.8%)	1/3 (33.3%)
	G6PDH1	43 ± 23	0/16 (0.00%)	–
SLiCE	*PrxIIE*	27 ± 11	15/16 (93.8%)	13/15 (86.7%)
	G6PDH1	25 ± 11	10/16 (62.5%)	9/10 (90.0%)

[a] Insert DNA fragments of *PrxIIE* (0.5 kbp) and *G6PDH1* (1.6 kbp) genes, which have short (19 bp) end homology regions, were amplified by PCR, and treated by *Dpn*I. Unpurified insert DNA fragments of *PrxIIE* (21 ng) or *G6PDH1* (67 ng), and linearized pET23a vector DNA (purified, 50 ng) were used at an insert:vector molar ratio of 3:1. Part (3 μL) of the total 10 μL solution was used to transform chemically competent cells prepared by the modified TSS method [30]: AQ3625 (0.78×10^6 CFU/μg pUC19 DNA) or DH5α (1.5×10^6 CFU/μg pUC19 DNA).
[b] Number of colonies indicates the number of colonies that formed when 15 ng of vector DNA was transformed. Each value for the number of colonies is the mean ± standard deviation of three independent experiments.
[c] Cloning efficiency is defined as the fraction of total colonies in which a PCR product of the correct expected size was amplified by colony PCR amplification.
[d] Cloning accuracy is defined as the fraction of clones correctly confirmed by DNA sequencing among colony-PCR positive clones.

3.3. Utility of iVEC and SLiCE seamless DNA cloning

In this study, I evaluated the efficiency of two simple seamless DNA cloning methods under the same conditions. For the purpose, competent cells prepared by modified TSS method [30] were used because these competent cells of the DH5α and AQ3625 strains have similar competency (~10^6 CFU/μg pUC19 DNA) (Table 3). However, as a practical consideration, the intrinsic competency of competent *E. coli* cells is an important determinant of the efficiency of DNA cloning methods. To determine the effect of cell competency on the efficiency of each cloning method, chemically competent cells of both DH5α and AQ3625 strains were prepared by three different methods: the modified TSS method [30], Inoue's method [32], and the $CaCl_2$ method [33]. In all cases, AQ3625 cells were less competent than the corresponding DH5α cells (Table 3), which might be due to the lower

Table 3
Competency of *E. coli* DH5α and AQ3625 chemically competent cells.

methods	strain	competency (CFU /μg pUC19 DNA)
Modified TSS method[a]	DH5α	1.5×10^6
	AQ3625	7.8×10^5
Inoue's method[b]	DH5α	1.8×10^7
	AQ3625	0.5×10^5
Calcium chloride method[c]	DH5α	1.2×10^5
	AQ3625	0.1×10^5

[a] DH5α and AQ3625 were harvested at OD_{600}=0.55 and 0.41, respectively.
[b] DH5α and AQ3625 were harvested at OD_{600}=0.16 and 0.27, respectively.
[c] DH5α and AQ3625 were harvested at OD_{600}=0.46 and 0.56, respectively.

competency of RecA$^+$ strains including *E. coli* AQ3625 and BL21 (DE3). Chemically competent cells of DH5α and other *rec*A$^-$ strains prepared by Inoue's method are generally highly competent [32], and are referred as ultracompetent cells (~10^8 CFU/μg plasmid DNA) [40]. In fact, competent DH5α cells prepared by Inoue's method were also highly competent in this study (1.8×10^7 CFU/μg pUC19 DNA) (Table 3). Transformation of purified PCR fragments ligated *in vitro* with the SLiCE method into competent DH5α cells prepared by Inoue's method [32] resulted in significantly increased colony formation (> 2000 colonies) (Table S3, SLiCE), compared to that (25–160 colonies) of the same reactions but with transformation into DH5α cells prepared by the modified TSS method (Fig. 2). Use of unpurified PCR fragments also provided similar results (Table 2 and Table S4). In contrast, few colonies were observed with the iVEC method using *E. coli* AQ3625 competent cells prepared by Inoue's method (Table S3). More efficient AQ3625 competent cells (> 10^7 (CFU/μg pUC19 DNA)) could not be prepared by Inoue's method, although 7.8×10^5 (CFU/μg pUC19 DNA) AQ3625 competent cells were prepared by the modified TSS method (Table 3). Preparation of AQ3625 competent cells might require a specific method. Thus, the competency of *E. coli* cells is also a significant determinant of the efficiency and utility of seamless DNA cloning.

4. Conclusion

Both iVEC and SLiCE cloning offer simple and low-cost recombinant enzyme-free seamless DNA cloning. Here, the efficiency and utility of each method were evaluated in terms of cloning efficiency and accuracy. The colony formation rate, cloning efficiency, and cloning accuracy of the SLiCE method were high for a wide range of end homology region lengths (Fig. 2 and Table 1), and increasing the intrinsic competency of the host cells greatly improved the colony formation rate of SLiCE cloning (Fig. 2 and Table S3). The colony formation rate and cloning efficiency were lower with the iVEC-DH5α method than with the SLiCE method at short end homology regions (15, 19, or 29 bp), although the colony formation rate of the iVEC method was improved by using the AQ3625 strain. Furthermore, the SLiCE method had higher cloning efficiency and cloning accuracy than iVEC-AQ3625, even when DH5α and AQ3625 cells having similar competency were used (Table 2). In addition, AQ3625 cells were less competent than DH5α cells in all three different preparation methods for chemically competent cells. In future work, the cloning efficiency of AQ3625 cells and the competency of cells in the AQ3625 strain should be further improved. Both improvements will contribute to the development of efficient recombinant enzyme-free seamless DNA cloning.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2017.01.010.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/:10.1016/j.bbrep.2017.01.010.

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