Molecular cloning

Rapid transformation protocol of chemically competent *E. coli* cells

Introduction

Chemically competent cell transformation is a fundamental technique in molecular biology that enables the introduction of foreign DNA into *E. coli* cells. In 1884, Theodor Escherich discovered *E. coli* while studying infant gut microbes [1,2]. Its fast growth, adaptability, and lack of pathogenicity quickly made it a staple microorganism in microbiology laboratories.

Ground-laying work for the chemical bacterial cell transformation method was performed by Mandel and Higa in 1970 [3] and Stanley N. Cohen and colleagues in 1972 [4], who discovered that calcium chloride ($CaCl_2$) treatment makes the *E. coli* membrane more permeable and allows for exogenous linear (phage) or circular DNA molecules to be introduced. Following this discovery, transformation efficiency was further increased with improvements such as addition of a heat-shock step and more optimized media and growth conditions [3–5]. Invitrogen™ competent cells are prepared using a modified Hanahan procedure [5] to achieve high transformation efficiency (>10^9 CFU/µg plasmid DNA).

Invitrogen™ One Shot™ TOP10 chemically competent cells are supplied with two transformation protocols (Figure 1):

- **Standard protocol:** For complex cloning projects (e.g., large-fragment cloning) or when high colony numbers are required (e.g., library construction), for low DNA quantity or quality, or for site-directed mutagenesis. Following this protocol, heat shock and outgrowth of transformed cells is performed.
- **Rapid protocol:** Balancing speed, efficiency, and transformation yield makes this protocol ideal for routine cloning tasks, when only a few colonies are necessary to verify construct (e.g., PCR cloning, subcloning, or working with constructs and plasmids of moderate size).

Not all cloning applications require the highest transformation efficiency. For routine cloning experiments, the simplified rapid transformation protocol can be used to obtain the desired construct faster. Here we show how this rapid protocol can be applied for One Shot TOP10 competent cells to transform supercoiled plasmid DNA or constructs assembled by:

- Blunt end ligation
- Golden Gate cloning
- Gibson Assembly™ cloning

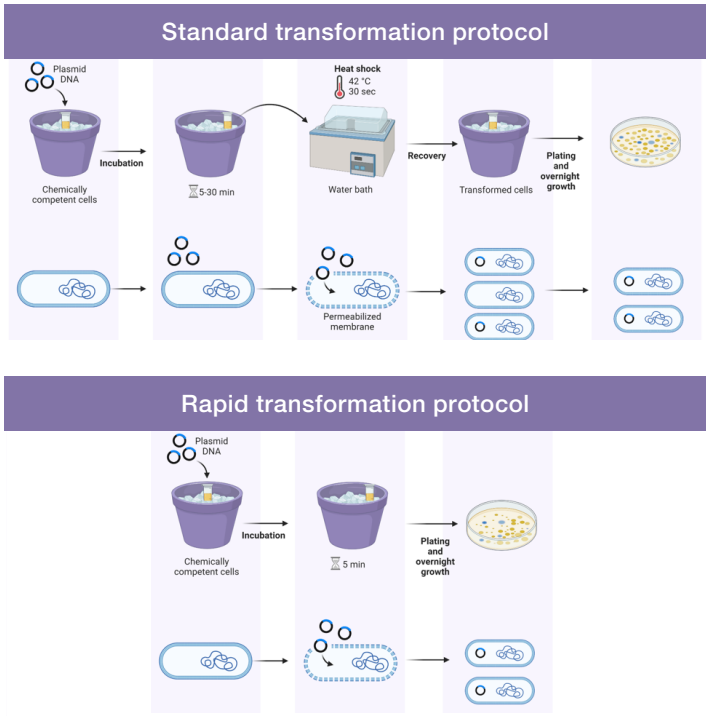

Figure 1. Overview of transformation protocols for One Shot TOP10 chemically competent cells.

The results are compared to the standard heat shock–based transformation method. In Table 1, transformation protocols are compared step by step.

Table 1. Comparison of transformation protocols.

Step	Standard protocol	Rapid protocol
Thaw competent cells	On ice	On ice
Add DNA (plasmid)	1 µL (up to 5 µL), not more than 10% of total volume	1 µL (up to 5 µL), not more than 10% of total volume
Incubate on ice	30 min	5 min
Heat shock (42°C)	30 sec	–
Post-shock incubation on ice	2 min	–
Recovery in SOC medium	60 min at 37°C with shaking	–
Plate on selective agar	Flexible (incubation step may be extended)	Immediate
Incubation (growth of colonies)	Overnight (12–16 hr)	Overnight (12–16 hr)
Hands-on time	~1.5–2 hours	~10–15 min

Note: The high-efficiency standard transformation protocol is described in detail in the user guide for One Shot TOP10 competent cells.

Materials and methods

Rapid transformation protocol using One Shot TOP10 competent cells

Note: This protocol is only recommended for transformation using ampicillin selection.

Required materials and equipment

- Invitrogen™ One Shot™ TOP10 Chemically Competent *E. coli*
- Luria-Bertani (LB) plates with appropriate antibiotic (e.g., Invitrogen™ One Shot™ LB Agar Plates with 100 µg/mL Ampicillin)
- 37°C shaking and non-shaking incubator
- pUC19 DNA for the transformation control reaction (optional)

Procedure Important: To obtain the best results, warm selective plates at 37°C until the plates are warm to the touch.

1. Thaw One Shot TOP10 Chemically Competent *E. coli* cells on ice immediately before transformation.

- Thaw one vial for each transformation.
- Do not hold vials in hand or expose to warmth, as higher temperatures significantly decrease cell competency.
- Centrifuge vials briefly after thawing to collect cell suspension droplets.

2. Add 1–5 µL of DNA (e.g., ligation reaction) directly into the vial of One Shot TOP10 Chemically Competent *E. coli* cells and mix gently by stirring with a clean pipette tip.

Important: Do not mix by pipetting up and down or vortexing.

Optional: If you are performing a transformation control, add 2.5 µL of pUC19 control DNA into a separate vial.

3. Incubate the transformation mix on ice for 5 min.
4. Spread 50 µL from each transformation mix on a prewarmed selective LB agar plate with amplicillin.

Optional: For the positive control, plate 50 µL of transformation mix onto LB agar plates containing 100 µg/mL ampicillin.

5. Incubate the plates overnight at 37°C.
6. The next day, pick individual colonies and screen for the presence of the insert by colony PCR. Alternatively, start overnight cultures for plasmid DNA isolation followed by restriction enzyme or sequencing analysis.

Results

Comparing efficiency of different cloning methods

The rapid transformation protocol for One Shot TOP10 Chemically Competent *E. coli* works well with various DNA cloning and propagation methods: simple supercoiled plasmid propagation, blunt end cloning, Golden Gate cloning, and Gibson Assembly cloning. Here we compare standard and rapid transformation protocols by cloning constructs obtained by the aforementioned techniques.

Supercoiled plasmid propagation

Propagation of supercoiled plasmid in *E. coli* is a common technique among scientists. This procedure can become tedious with many plasmids, so the rapid transformation protocol can be used to help reduce hands-on time compared to the standard protocol.

Supercoiled pUC19 plasmid was transformed into One Shot TOP10 Chemically Competent *E. coli* at different quantities (10 pg, 100 pg, or 1 ng) following either the rapid or standard protocol. The results show that the rapid transformation protocol is suitable for simple plasmid propagation when a few hundred colonies or more are sufficient (Figure 2).

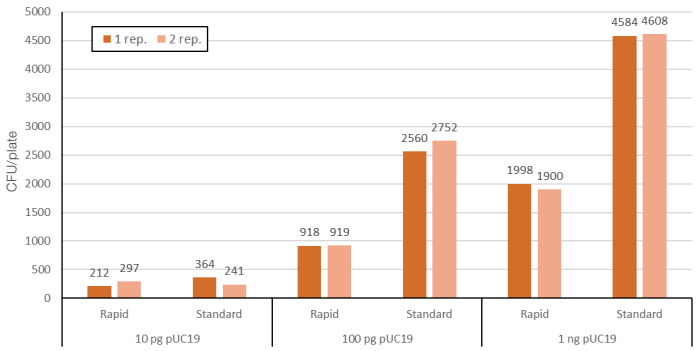

Figure 2. Comparison of rapid and standard transformation protocols for supercoiled plasmid propagation. Rep. = replicate. CFU = colony-forming unit.

Blunt end cloning

Most proofreading polymerases (e.g., Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerase) leave blunt ends on PCR products. Blunt end fragments can easily be cloned into the pJET1.2/blunt vector included in the Thermo Scientific™ CloneJET™ PCR Cloning Kit (**Cat. No. K1231**). The rapid transformation protocol can be used to further simplify the process.

A 1.5 kb DNA insert was amplified by PCR using Invitrogen™ Platinum™ SuperFi™ II PCR Master Mix (**Cat. No. 12369010**), which includes a high-fidelity, proofreading polymerase. The PCR product was column purified and cloned into the linearized pJET1.2/blunt cloning vector using the CloneJET PCR Cloning Kit (Table 2).

Table 2. Reaction setup for blunt end cloning.

Component	Quantity
Linearized vector (pJET1.2/blunt)	50 ng
Insert (1.5 kb)	75 ng
T4 DNA ligase (5 U/µL)	1 µL
2X reaction buffer	10 µL
Water	Up to 20 µL

The ligation mixture was incubated for 5 min at room temperature (22°C). After incubation, 1 µL of the ligation mix was used for transformation of One Shot TOP10 Chemically Competent *E. coli* using the standard or rapid protocol. Transformed cells were plated onto LB agar plates with 100 µg/mL ampicillin. After incubation, sufficient colony output was obtained with each method (Figure 3).

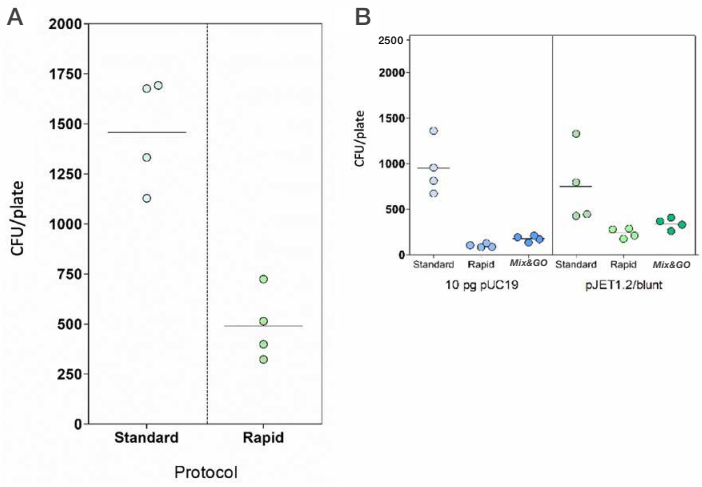

Figure 3. Comparison of protocols for blunt end cloning. **(A)** Standard and rapid protocols were used for transformation after blunt end ligation of insert and vector. **(B)** Protocols were also compared to the *Mix & Go!*™ Transformation Kit from Zymo Research.

Golden Gate cloning

Golden Gate cloning was tested using Thermo Scientific™ FastDigest™ Eco31I (BsaI) (**Cat. No. FD0293**) restriction enzyme to evaluate transformation efficiency following rapid and standard protocols. Golden Gate cloning is a one-step, single-tube reaction in which DNA fragments are cut outside the recognition site using a Type IIS restriction enzyme, and T4 DNA ligase joins homologous ends, leaving the final product without restriction enzyme recognition sites [6]. In this experiment, a 1.5 kb fragment with incorporated Eco31I (BsaI) recognition sites at each end was cloned into a target vector (Table 3).

Table 3. Reaction setup for Golden Gate cloning.

Component	Quantity
Vector	75 ng
Insert (1.5 kb)	80 ng
T4 DNA ligase (5 U/µL)	1 µL
FastDigest Eco31I (BsaI)	1 µL
10X reaction buffer	2 µL
Water	Up to 20 µL

The reaction mix was incubated in a thermal cycler for 15 min at 37°C and chilled at 4°C for 15 min. After the incubation, 1 µL of the reaction mixture was transformed into One Shot TOP10 Chemically Competent *E. coli.* Cells were plated on LB agar plates containing 100 µg/mL ampicillin for selection and incubated overnight at 37°C to allow colony growth.

The results show that the rapid protocol works well for Golden Gate cloning, allowing clones to be obtained in less than 20 min of hands-on time without a significant loss in efficiency (Figure 4).

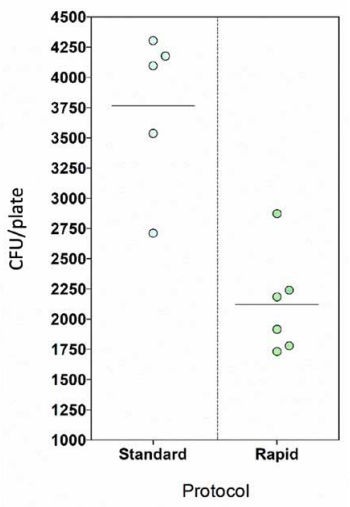

Figure 4. Comparison of standard and rapid transformation protocols for Golden Gate cloning.

Gibson Assembly cloning

Finally, Gibson Assembly cloning was tested using rapid and standard transformation protocols. This cloning method enables seamless, one-step, single-tube assembly of multiple overlapping DNA fragments in an isothermal reaction utilizing complementary overlaps [7]. Hands-on time for Gibson Assembly cloning is typically around 15–30 min; the time required to acquire positive clones can be further reduced by using the rapid transformation protocol. A total of 2, 3, or 5 DNA fragments were assembled with Invitrogen™ GeneArt™ Gibson Assembly™ HiFi Master Mix (**Cat. No. A46628**) and transformed into competent cells using rapid or standard protocols. The DNA fragments were PCR amplified using Platinum SuperFi II PCR Master Mix, column purified, and used for assembly following recommendations in the user guide. The final reaction mixture contained 0.08 pmol of each fragment varying from 300 bp to 2.6 kb in length (Table 4).

Table 4. Reaction setup for Gibson Assembly cloning.

Component	Quantity
Vector	0.08 pmol
Inserts (300 bp to 2.6 kb)	0.08 pmol each
2X GeneArt Gibson Assembly HiFi Master Mix	10 µL
Water	Up to 20 µL

The assembly reaction was performed at 50°C for 15 min for 2 and 3 fragments and for 60 min for 5 fragments. Prior to transformation, the reaction mixtures were diluted 5-fold with nuclease-free water, and 1 µL of the resulting assembly reaction was transformed into One Shot TOP10 Chemically Competent *E. coli* according to the rapid or standard protocol.

Results suggest that One Shot TOP10 Chemically Competent *E. coli* cells are suitable for transformation of assembly reactions. Relatively high CFU output can bereached (Figure 5) without a significant drop in fidelity.

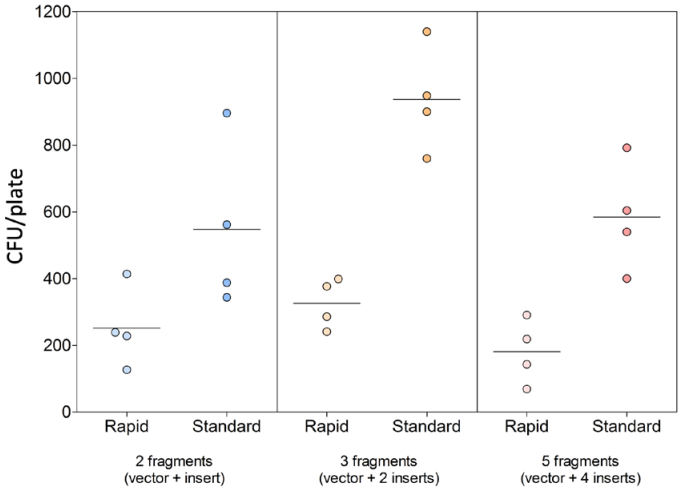

Figure 5. Comparison of rapid and standard transformation protocols for Gibson Assembly cloning.

The most complex construct (5-fragment assembly) out of all tested was evaluated for fidelity by fluorescence imaging (the assembled plasmid contained ORFs of YGFP and tdTomato proteins). Clones were randomly selected, streaked on a fresh plate, incubated at 37°C overnight, and visualized using a Typhoon™ biomolecular imager under Cy®2 and Cy®3 filters (Figure 6). A positive clone was counted if both YGFP and tdTomato fluorescence was observed.

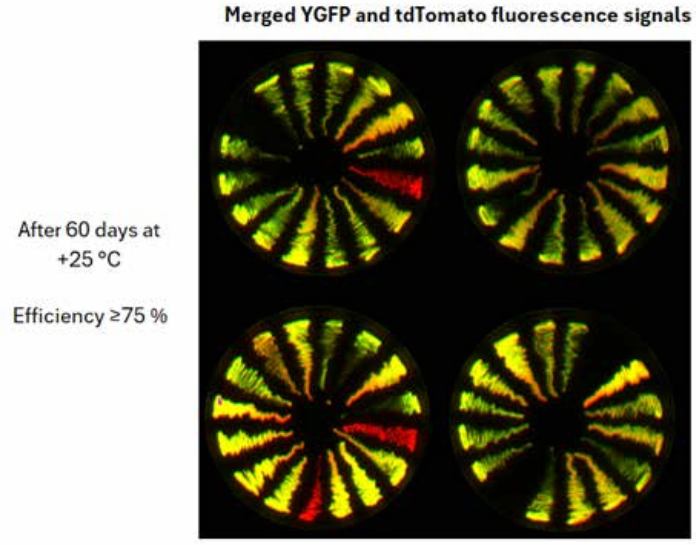

Figure 6. Fluorescence imaging of clones transformed with a 5-fragment construct expressing YGFP and tdTomato.

Conclusions

The rapid transformation protocol with One Shot TOP10 Chemically Competent *E. coli* offers significant advantages for a wide range of cloning methods by streamlining the DNA introduction step with minimal hands-on time and straightforward procedures. While it may not achieve the transformation efficiency of the standard protocol, its speed and simplicity make it ideal for cloning tasks such as plasmid propagation, blunt end cloning, and even Golden Gate or Gibson Assembly cloning. By reducing the time required for transformation, the cloning workflow can be accomplished with faster turnaround from construct design to colony screening. It is a valuable addition to tools for researchers aiming to increase productivity and efficiency in molecular biology applications.

References

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2. Escherich T (1885) Die darmbakterien des neugeborenen und säuglings. *Fortschr Med* 3:515–522.
3. Mandel M, Higa A (1970) Calcium-dependent bacteriophage DNA infection. *J Mol Biol* 53(1):159–162.
4. Cohen SN, Chang AC, Hsu L (1972) Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-Factor DNA. *Proc Natl Acad Sci USA* 69(8):2110–2114.
5. Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166(4):557–580.
6. Engler C, Kandzia R, Marillonnet S. (2008) A one pot, one step, precision cloning method with high throughput capability. *PLOS One* 3(11):e3647.
7. Gibson DG, Young L, Chuang RY et al. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat Methods* 6(5):343–345.

Ordering information

Product	Quantity	Cat. No.
Gibson Assembly cloning		
GeneArt Gibson Assembly HiFi Master Mix	50 reactions	A46628
GeneArt Gibson Assembly EX Master Mix	50 reactions	A46636
Golden Gate cloning		
FastDigest Eco31I (IIs class)	50 µL	FD0293
AarI	125 U	ER1582
FastDigest BpiI	20 reactions	FD1014
T4 DNA Ligase	1,000 U	ER0014
Blunt end cloning		
CloneJET PCR Cloning Kit	40 reactions	K1232
Platinum SuperFi II PCR Master Mix	100 reactions	12369010
Competent cells		
One Shot TOP10 Chemically Competent E. coli	21 x 50 µL	C404003
	42 x 50 µL	C404006
	11 x 50 µL	C404010
MultiShot FlexPlate TOP10 Competent Cells	1 x 96-well plate (20 µL/well)	C4081201
MultiShot TOP10 Chemically Competent E. coli	5 x 96-well plate (15 µL/well)	C40005
One Shot BL21(DE3) Chemically Competent E. coli	21 x 50 µL/tube	C600003
One Shot BL21 Star (DE3) Chemically Competent E. coli	21 x 50 µL/tube	C601003
MultiShot StripWell BL21 Star (DE3) Chemically Competent E. coli	1 x 96 tube rack (50 µL/tube)	C609601
MAX Efficiency DH5α Competent Cells	5 x 200 µL	18258012
MAX Efficiency DH10B Competent Cells	5 x 200 µL	18297010
One Shot Mach1 T1 Phage-Resistant Chemically Competent E. coli	21 x 50 µL/tube	C862003
MultiShot FlexPlate Mach1 T1R Competent Cells	1 x 96-well plate (20 µL/well)	C8681201
Bacterial growth media		
One Shot LB Agar Plates without Antibiotics	2 x 10 plates	A55800
One Shot LB Agar Plates with Ampicilin	2 x 10 plates	A55802
One Shot LB Agar Plates With Kanamycin	2 x 10 plates	A55803

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