

molecular biology

RapidTransit™: A Convenient Method for Preparing Competent Cells

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Application Notes

- Fast, inexpensive preparation of competent cells
- Eliminates complex buffer preparation
- Suitable for transformation of a variety of *E. coli* strains
- Consistent transformation efficiencies for most cloning needs

Introduction

Efficient uptake of nucleic acid by transformation-competent *E. coli* is an integral step in molecular cloning and requisite for numerous downstream applications such as recombinant protein expression and mutagenesis. Competent cells are typically prepared by laborious methods that require complex buffer preparation and numerous steps. For those requiring ultimate convenience, there are a number of commercially available frozen competent cell strains having high transformation efficiencies. However, the drawbacks of commercially available cells are that they are costly and restricted in strain availability, require ultra-cold storage, and have a limited shelf life. The RapidTransit Transformation Kit (Product Code [R 2653](#)) provides a simple, economical, and reliable alternative to traditional competent cell preparation and commercially available frozen competent strains.

Two rapid methods

The kit incorporates two procedures: the "Direct Addition" method and "Single-Centrifugation" method (Figure 1). With only three contiguous 10-minute incubations and no heat shock during transformation, the Direct Addition method provides minimal hands-on time and transformation efficiencies of $\geq 1 \times 10^5$ colony-forming units per microgram (cfu/ μ g) supercoiled plasmid (Figure 2). Exclusion of reagent preparation and centrifugation steps allows for an automatable procedure where culture growth, cell competence, transformation, and recovery can take place in the same tube or well.

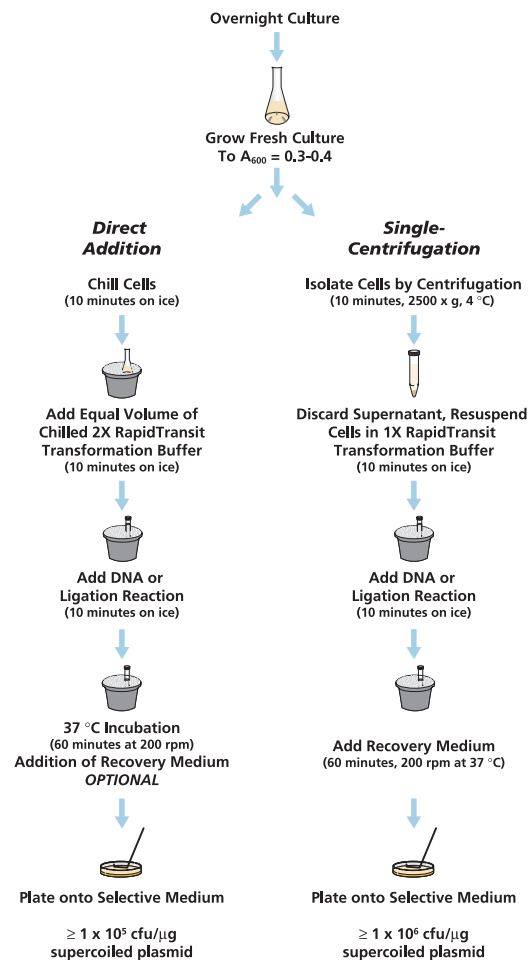


Figure 1. Protocols for preparation of competent *E. coli* using the RapidTransit Transformation Kit.

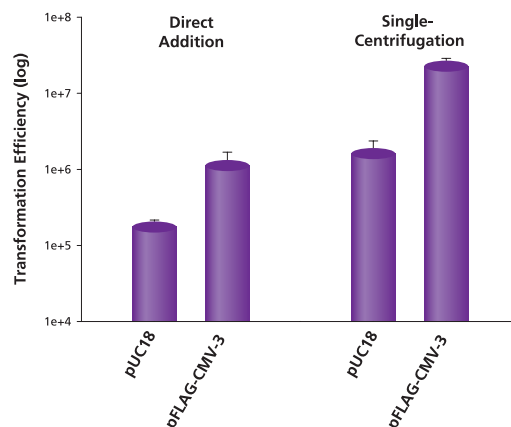


Figure 2. Comparison of transformation efficiencies. Competent NovaBlue™ cells were prepared using both RapidTransit preparative protocols and transformed with one nanogram of the plasmid indicated. Error bars indicate standard deviation calculated for the mean of duplicate reactions. Transformation efficiency is expressed as colony forming units per μ g plasmid. (pUC18 is a 2686-basepair plasmid. pFLAG-CMV-3, a mammalian expression vector, is 6331 base pairs in size.)

The Single-Centrifugation method, which follows an abbreviated traditional protocol for competent cell preparation, provides higher transformation efficiencies of $\geq 1 \times 10^6$ cfu/ μ g (Figure 2). Both methods have been successfully used for transformation of ligation reactions and preparation of recombinant clones (Table 1).

Table 1. Transformation of ligation reactions and clonal isolations.

I. pUC19 Derivative + GC5	Black colonies	White colonies
Control: No DNA	0	0
Control: No Insert, No Ligase	0	0
Control: No Insert, Plus Ligase	0	0
Ligation (total, three reactions)	3	50
II. pACYC184 Derivative + K12 (Wild-type)	Colonies	
Control: No DNA	0	
Control: No Insert, No Ligase	0	
Control: No Insert, Plus Ligase	0	
Ligation (total, four reactions)	487	
III. pUC18 Derivative + CJ236 (Supercoiled Plasmid DNA)	Colonies	
	600	
IV. pBR322 Derivative + GC5	Colonies	
Control: No DNA	0	
Control: No Insert, No Ligase	0	
Control: No Insert, Plus Ligase	0	
Ligation (+ 3 μ l reactions)	401 (one plate)	

Data sets I, II, and III illustrate the utility of the Direct Addition protocol and Single-Centrifugation was used for data set IV. (I) GC5⁺ cells were transformed with a ligation product comprised of a 969-basepair insert in pUC19 (S-Gal⁺ color selection used, in addition to antibiotic). Eighty-six percent of 50 clones tested positively identified as the correct clone; (II) Transformation of a K12 wild-type strain with a pACYC184-derived vector with a 1.9-kb ligation insert: 95% of 40 colonies tested were positively identified as the correct clone; (III) E. coli strain CJ236 transformed with a pUC18 derivative (supercoiled plasmid); (IV) Transformation of GC5⁺ with the ligation product of pBR322 and the 969-basepair fragment described for the first data set. The results for this experiment ("Ligation reaction") represent 1/5 of the transformation reaction. Of 50 clones tested, 100% were positively identified as the correct clone. All positive cloning events were confirmed by restriction analysis.

Efficient transformation across a broad range of E. coli strains

The RapidTransit Transformation Kit has been demonstrated to successfully confer competence to a broad variety of E. coli strains while maintaining high transformation efficiency (Figure 3 and Table 1). This includes several strains possessing wild-type characteristics that are difficult to transform using other preparative methods, including W3110 and CJ236.

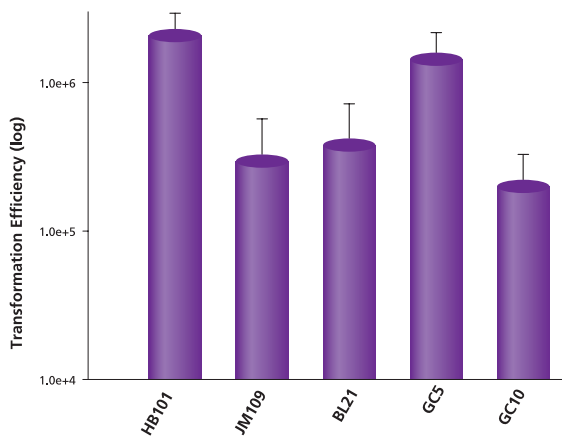


Figure 3. Comparison of transformation efficiency using different E. coli strains. Competent cells were prepared using the Direct Addition method, for the strains shown above. Cells were transformed with one nanogram pUC18. Error bars indicate standard deviation calculated for the mean of triplicate reactions. A similar pattern of efficiency is observed for cells prepared by Single-Centrifugation method, though ~10X higher transformation efficiencies were obtained (data not shown). Transformation efficiency is expressed as colony-forming units per μ g plasmid.

Suitable for cloning and subcloning

The system has been tested with plasmid vectors of diverse size (Figure 1) and possessing an assortment of replicative origins, including pUC18, pBR322 (Figures 2, 3, and Table 1), ACYC184 (Table 1), and p15 (data not shown). The system has been effectively used in conjunction with antibiotic selection (including ampicillin, kanamycin, streptomycin, spectinomycin, and chloramphenicol) and color selection using S-Gal⁺ (Table 1).

Summary

The RapidTransit Transformation Kit, for only 50¢ per reaction, provides a cost-effective alternative to standard labor-intensive preparative methods and commercially available competent cells. Efficient transformation has been demonstrated in a variety of E. coli strains for most cloning and subcloning applications, and the Direct Addition method is readily automatable.

Ordering Information

Product	Description	Unit
R 2653	RapidTransit Transformation Kit	1 kit