Cat. # 6021

For Research Use

TaKaRa

DNA Ligation Kit Ver.1

Product Manual

v201811Da

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I. Description

The DNA Ligation Kit Ver.1 is a simple system that allows very rapid DNA ligation reactions. The kit uses T4 DNA ligase and an optimized buffer system. The high efficiency of the ligation reaction eliminates the need for conventional overnight incubation. This kit provides good ligation efficiency in only three minutes for general ligation reactions, and most other types of ligations can be completed within 30 minutes. The reaction product (i.e., ligated DNA solution) can be directly used for bacterial transformation without further DNA purification.

Comparison of reaction volume between DNA Ligation kits Ver. 1 (Cat. #6021) and Ver. 2.1 (Cat. #6022)

	Ver. 1	Ver. 2.1
	volume ratio	volume ratio
In a reaction producing circular DNA ・Ligation of DNA fragment with plasmid vector ・Ligation of Linker DNA with plasmid vector ・Self-circularization	DNA solution : 1 Solution A : 4 - 8 Solution B : 1	DNA solution : 1 Solution I : 1
In a reaction producing linear DNA ・Ligation of DNA fragment with λ phage vector* ・Linker, Adaptor ligation to cDNA	DNA solution : 1 (300 mM NaCl) Solution B : 1	DNA solution : 1 Solution II : 1 Solution I : 2

* TaKaRa DNA Ligation Kit Ver.1 (Cat. #6021) is recommended for this purpose.

II. Components

Solution A : Reaction Buffer 1,000 μl x 3
Solution B : Enzyme Solution 187.5 μl x 2

* Sufficient components for 50 reactions when 60 μl of Solution A and 7.5 μl of Solution B are used per reaction.

III. Storage

-20℃

IV. Protocols and Examples

1. Ligation of a DNA fragment with a plasmid vector

(1) Combine the linearized plasmid vector and insert DNA fragment in a total volume of 5 - 10 μl*1. The recommended amounts of DNA are vector : insert = 0.03 pmol : 0.1 - 0.3 pmol (0.03 pmol of pUC18 DNA corresponds to about 50 ng).
(2) Add 4 - 8 volumes*2 of Solution A to the DNA solution and mix thoroughly.
(3) Add one volume of Solution B (5 - 10 μl) and mix thoroughly.
(4) Incubate at 16℃ for 30 minutes.*3
(5) Following incubation, the DNA ligation mixture can be used directly for transformation into bacterial competent cells (i.e., without further DNA purification) by adding 10 μl of the ligation mixture to 100 μl of competent cells.*4

*1 We recommend 100 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$ for dissolving DNA, however TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) can also be used.
*2 Usually, 4 volumes of Solution A is sufficient for DNA suspended in 100 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$ buffer. If other buffers are used or if the concentration of DNA is high, use 8 volumes of Solution A to obtain optimal efficiency.
*3 The reaction should be performed at 16℃. Higher temperatures (>26℃) will inhibit the formation of circular DNA. If good results are not obtained, the reaction time can be extended overnight. If good ligation efficiency is not obtained, the DNA should be purified by phenol extraction and ethanol precipitation. When ligating PCR products with T-vectors, the reaction time should be restricted to no more than one hour. Longer reaction times may result in lower yields of ligated DNA.
*4 Higher amounts (i.e., ratio) of the ligation mixture may result in a decreased transformation efficiency. The ligation mixture should not be directly used for transformation by electroporation due to the high salt concentration of this mixture. For electroporation, the ligation mixture should first be precipitated with ethanol and then redissolved in a low salt buffer, such as TE, prior to transformation.

Example:

50 ng of *Eco*R I-digested pUC118 vector (25 fmol) was mixed with 2.5 - 250 ng (2.5 - 250 fmol) of a 1.5 kb *Eco*R I-digested DNA fragment at insert/vector ratios ranging from 0.1 to 10.0, in a total volume of 5 μl. 25 μl of Solution A and 5 μl of Solution B were added to the DNA solution. The combined solution was then incubated at 16℃ for 30 minutes. A portion of the solution was used to directly transform JM109 competent cells, and colonies were formed on an LB-Amp plate containing X-Gal and IPTG. (The transformation efficiency of *E. coli* JM109 competent cells was 6.3×10^7 cfu/μg pUC118 DNA). Transformation efficiencies were obtained by counting the number of white colonies (Table 1). For comparison, the results using T4 DNA Ligase (350 U) in standard ligation buffer (incubated at 16℃ for 16 hours) are also shown.

Table 1. Transformation efficiencies (white colonies/ μg insert DNA)

	Vector	insert/vector (molar ratio)				
		0.1	0.3	1.0	3.0	10.0
DNA Ligation Kit (30 minutes)	dephosphorylated	1.7×10^6	5.0×10^6	1.7×10^7	2.3×10^7	2.1×10^7
	phosphorylated	7.8×10^5	2.5×10^6	8.2×10^6	1.7×10^7	2.3×10^7
T4 DNA Ligase (16 hours)	dephosphorylated	1.6×10^5	2.0×10^5	1.8×10^6	3.1×10^6	1.9×10^6
	phosphorylated	4.6×10^5	1.0×10^6	1.9×10^6	5.0×10^6	1.2×10^7

2. Ligation of DNA with a λ phage vector

(1) Combine 250 ng (0.01 pmol) of λ phage vector DNA (digested with an appropriate restriction enzyme and, if preferred, dephosphorylated by treatment with alkaline phosphatase) and the DNA to be inserted (0.03 - 0.1 pmol) in a total volume of 5 - 10 μl. Best results are obtained in buffer containing 100 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$ and 300 mM NaCl. This salt concentration is important for producing good yields of concatemeric λ DNA. Therefore, if TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) is used, the solution should be adjusted to obtain a final concentration of 300 mM NaCl.
(2) Add one volume (5 - 10 μl) of Solution B to the DNA solution and mix well.
(3) Incubate at 26℃ *1 for 5 - 10 minutes *2.
(4) The ligation reaction mixture (up to 5 μl) can be used directly in λ *in vitro* packaging reactions *3.

*1 Ligations involving λ phage vector DNA work better when performed at 26℃ than at 16℃.
*2 Ligation reactions are usually complete after 5 - 10 minutes of incubation. Longer reaction time will not increase ligation efficiency.
*3 Components of the ligation reaction mixture will not inhibit λ *in vitro* packaging as long as the ratio of the ligation reaction mixture to the total packaging reaction mixture is kept to <10% . If more of the ligation reaction mixture (i.e., more DNA) is to be packaged in a single reaction, then the DNA should be further concentrated by ethanol precipitation and redissolved in TE buffer, such that a volume ratio of <10% in the packaging reaction mixture can be maintained.

Example:

25 ng of *Eco*R I-digested pBR322 plasmid DNA (4,361 bp) and 250 ng of *Eco*R I-digested, dephosphorylated λ gt11 arms were combined in a total volume of 7.5 μl. One volume of Solution B was added and the mixture was incubated at 26℃ for 10 minutes to 16 hours. Ligated DNA was packaged using a commercially available λ *in vitro* packaging kit and was used to transfect *E. coli* Y1090 (r-). Transfection efficiencies are shown in Table 2. For comparison, efficiencies obtained when using T4 DNA Ligase (350 U) in standard ligation buffer are shown.

Table 2. Transfection efficiencies (white plaques/ μg λ gt11 DNA)

Incubation time	DNA Ligation Kit	T4 DNA Ligase
10 minutes	8.5×10^6	1.8×10^6
16 hours	—	3.1×10^6

3. Self-circularization of linear DNA (Intramolecular ligation)

The procedure for self-circularization of linear DNA is essentially the same as for "IV-1. Ligation of a DNA fragment with a plasmid vector" (see page 4). However, it is important to use low concentrations of DNA in the ligation reaction to maximize intramolecular ligation as well as to keep the volume of the DNA solution low for higher bacterial transformation efficiency.

Example:

Sca I-digested pBR322 plasmid DNA (350 ng : 10 μl) was prepared. Solution A (40 μl) and Solution B (10 μl) were added, and the mixture was incubated at 16℃ for 30 minutes. 1 μl of the reaction solution was used to transform *E. coli* HB101 competent cells (100 μl). HB101 competent cells had an efficiency of at least 1 x 10^8 cfu/μg pBR322 DNA. Results are shown in Table 3. For comparison, the results using conventional T4 DNA ligase reactions (350 U of T4 DNA Ligase, in standard ligation buffer, incubated at 16℃ for 16 hours) are also shown.

Table 3. Transformation efficiencies (colonies/μg of DNA added)

DNA added	DNA Ligation Kit (30 minutes)	T4 DNA Ligase (16 hours)
17 ng	7.2 x 10^6	5.0 x 10^5

4. Linker, Adaptor ligation

● **Ligation of a linker with a plasmid vector**

Conditions for linker ligation (8 bases or longer) are essentially the same as for "IV-1. Ligation of a DNA fragment with a plasmid vector" (see page 4).
Recommended vector/linker molar ratios are:

- phosphorylated linker : dephosphorylated vector = 10 - 100 : 1
- phosphorylated linker : phosphorylated vector = >100 : 1

● **Linker, Adaptor ligation to both termini of a DNA fragment (ex. Linker ligation of cDNA)**

1) Prepare a 5 - 10 μl solution containing the DNA fragment to be ligated (0.01 - 0.1 pmol) and the linker (or adaptor). Recommended DNA fragment/linker, adaptor molar ratio is:
   - DNA fragment : linker [adaptor] = 1 : >100
2) Add one volume (5 - 10 μl) of Solution B and mix well.
3) Incubate at 16℃ for 30 min. However, if the linker is shorter than 8 bp or if the linker has a low GC-content, the ligation reaction should be performed at <10℃ for 1 to 2 hours.
4) Inactivate T4 DNA Ligase by heating at 70℃ for 10 minutes.
5) If the ligated DNA is to be further subjected to restriction enzyme digestion, then ethanol precipitate and resuspend the DNA in an appropriate buffer prior to digestion.

Example:

100 ng of dephosphorylated vector, pUC118 *Hin*c II/BAP DNA (50 fmol) and 2.6 - 130 ng (0.5 - 25 pmol) of phosphorylated *Bgl* II linkers (5′-CAGATCTG-3′) were combined in a total volume of 5 μl. Solution A (25 μl) and Solution B (5 μl) were added, and the mixture was incubated at 16℃ for 30 minutes. A portion of the solution was used directly to transform *E. coli* JM109 competent cells and colonies were formed on an LB-Amp plate containing X-Gal and IPTG. (the transformation efficiency of *E. coli* JM109 competent cells was 1.5 x 10^8 cfu/ μg pUC118 DNA). Transformation efficiencies were obtained by counting the number of white colonies are shown in Table 4. For comparison, the results using conventional T4 DNA Ligase reaction (350 U of T4 DNA ligase and standard ligation buffer incubated at 16℃ for 16 hours) are also shown.

Table 4. Transformation efficiencies (colonies/ μg of pUC118 DNA)

	linker/vector (molar ratio)			
	10	50	100	500
DNA Ligation Kit (30 minutes)	2.0 x 10^6	8.0 x 10^6	3.0 x 10^7	2.5 x 10^7
T4 DNA Ligase (16 hours)	1.2 x 10^6	3.2 x 10^6	2.3 x 10^6	2.4 x 10^6

V. Troubleshooting

Q1 : Ligation efficiency is low.

A1 : • Extend the reaction time to overnight.
• Repeat the ligation reaction, but adjust the final NaCl concentration of the ligation reaction mixture to 500 mM.

If ligation efficiency is not improved after performing all of the above suggestions, then repurification of the DNA is recommended.

Q2 : Can the ligation mixture be directly used for electroporation-based transformation?

A2 : Transformation efficiency may decrease when the ligation mixture is used directly for electroporation-based transformation due to the salt concentration of the ligation reaction mixture. For use with electroporation methods, the DNA should be precipitated with ethanol and redissolved in an appropriate buffer prior to use.

Q3 : How should I perform a ligation reaction when using cosmid DNA?

A3 : To perform a ligation reaction using cosmid DNA, follow the protocol "IV-1. Ligation of a DNA fragment with a plasmid vector" (page 4). However, for *in vitro* packaging, follow the protocol "IV-2. Ligation of DNA with a λ phage vector" (page 5).

Q4 : Is it possible to use a portion of a restriction enzyme digest directly as the DNA solution for use with the DNA Ligation Kit?

A4 : It is recommended that digested DNA first be precipitated with ethanol and then dissolved in an appropriate buffer before use with the DNA Ligation Kit. Likewise, if restriction enzyme digestion of ligated DNA is desired following the ligation reaction, then the ligated DNA should also be ethanol precipitated and resuspended in an appropriate buffer prior to digestion.

Q5 : Should salt (e.g. NaCl) be added to the ligation reaction mixture before ethanol precipitation?

A5 : Yes, salt should be added directly to the ligation reaction mixture (a final concentration of 150 mM NaCl, 2 M ammonium acetate, or 300 mM sodium acetate) prior to precipitation with ethanol.

VI. Experimental Examples

Circularization ligation were performed at 25℃for 3 minutes or at 16℃for 30 minutes, and the ligation efficiencies were compared. The following results indicate that 3-minute ligation time provides as good performance as ligation using conventional conditions.

•Example 1 : Self ligation of linearized DNA (sticky- and blunt-end ligation)

200 ng (10 μl) of pUC118 DNA, digested with *Eco*R I or *Hin*c II respectively, was prepared. Using DNA Ligation Kit, ligation was performed at 25℃for 3 minutes or at 16℃for 30 minutes. 4.8 μl (16 ng) of ligation reaction solution was used to transform *E. coli* JM109 competent cells (1.3 x 10^8 transformants/μg pUC118 DNA). The results are shown in Table 5.

Table 5

End type	Ligation at 25℃ for 3 min	Ligation at 16℃ for 30 min
Sticky-end (*Eco*R I)	1.0 x 10^8	8.2 x 10^7
Blunt-end (*Hin*c II)	3.0 x 10^6	2.5 x 10^7

•Example 2 : Linker ligation

Using DNA Ligation Kit, p*Bgl* II linker d[pCAGAATCTG] 260 ng was ligated to 100 ng of pUC118 DNA digested with *Hin*c II, dephosphorylated with alkaline phosphatase, at 25℃for 3 minutes or at 16℃for 30 minutes. Part of ligation reaction solution was used to transform competent *E. coli* JM109 competent cells (1.3 x 10^8 transformants/μg pUC118 DNA). The results are shown in Table 6.

Table 6

	Ligation at 25℃ for 3 min	Ligation at 16℃ for 30 min
Ver.1	1.8 x 10^7	2.0 x 10^7

VII. Notes

1. Solution A may be thawed and mixed at room temperature. Solution B, which contains the T4 DNA Ligase, should be thawed on ice and gently mixed before use. The solutions can be thawed and frozen repeatedly.
2. DNA ligation mixtures can be loaded directly onto agarose gels for gel electrophoresis. Ethanol precipitation* is recommended for concentrating DNA samples that will be loaded onto polyacrylamide gels. Do not directly extract the ligation mixture with phenol.

   * Ethanol precipitation;
     1) Add one-tenth volume of 3 M sodium acetate (pH 5.2) or one-twentieth volume of 5 M NaCl, and 2 - 2.5 volume of ethanol into the reactant.
     2) Leave at -20℃ for 20 minutes, or at -80℃ for 10 minutes.
     3) Collect the DNA by centrifugation at 4℃. When a small amount of DNA is to be collected, carrier may be useful for ethanol precipitation.

VIII. Reference

Hayashi, K, Nakazawa, M., Ishizaki, Y., Hiraoka, N. and Obayashi, A.
Nucleic Acids Res. (1986) **14**: 7617-7631.

IX. Related Products

T4 DNA Ligase (Cat. #2011A/B)
DNA Ligation Kit Ver. 2.1 (Cat. #6022)
DNA Ligation Kit <Mighty Mix> (Cat. #6023)
TaKaRa DNA Ligation Kit LONG (Cat. #6024)
pUC118 *Hin*c II/BAP (Cat. #3322)
pUC118 *Eco*R I/BAP (Cat. #3320)
pUC118 *Bam*H I/BAP (Cat. #3321)
pUC118 *Pst* I/BAP (Cat. #3323)
pUC118 *Hin*d III/BAP (Cat. #3324)
PrimeGel™ Agarose LE 1-20K GAT (Cat. #5801)
E. coli HST08 Premium Competent Cells (Cat. #9128)
E. coli HB101 Competent Cells (Cat. #9051)
E. coli JM109 Competent Cells (Cat. #9052)

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