Cat. # 6023

For Research Use

TaKaRa

DNA Ligation Kit
<Mighty Mix>

Product Manual

v201811Da

Table of content

I. Description

Ligation of DNA fragments using T4 DNA Ligase is performed frequently in various molecular biology experiments. Because its reaction rate varies depending upon the structure of the DNA terminus, the enzyme volume and reaction time should be adjusted according to the type of DNA ends. Takara's DNA Ligation Kit series are simple systems that allow very rapid DNA ligation reactions. The DNA Ligation Kit, <Mighty Mix> was developed by modification of the Ver.2.1 kit and offers the features as described below :

- Single component : "Ligation Mix"
  This kit is composed of a single solution containing all required ligation reagents (i.e. enzyme and Buffer). All types of ligation can be performed by simply mixing the Ligation Mix and your DNA. This reaction mix can then be directly used for transformations or for *in vitro* packaging. The Ligation Mix is stable even after repeated freeze-thaw cycles.* (* up to 50 cycles).
- Highly suitable for ligation of blunt-ended DNA and for TA cloning.
  Good efficiency is obtained not only with sticky-end DNA but also in cloning of blunt-end DNA or in TA cloning where it is sometimes difficult to get higher efficiency.
- Five-minute ligations are possible
  A 5-minute ligation at 25℃ offers the same efficiency as a 30 min ligation at 16℃ for vector ligations, thus saving time.

Comparison of reactant volume of DNA Ligation Kit <Mighty Mix>

	DNA solution : Ligation Mix (volume ratio)	Reaction conditions
(1) Insertion of DNA fragment into plasmid vector [Standard Protocol] [Rapid Protocol]	1 : 1	16℃, 30 min 25℃, 5 min
(2) Cloning of PCR product into T-vector	1 : 1	16℃, 30 min
(3) Intramolecular ligation of linear DNA (self circularization)	1 : 1	16℃, 30 min
(4) Linker Ligation, Adaptor Ligation Insertion of Linker DNA into plasmid vector Linker [Adaptor] ligation to cDNA	1 : 1 1 : 2	16℃, 30 min
(5) Insertion of DNA fragments into λ-phage vectors* (*in vitro* packaging)	1 : 2	25℃, 10 min

* The examples shown in this manual represent average values obtained with DNA Ligation Kit <Mighty Mix>.

II. Components

Ligation Mix 150 μl x 5
Components support 100 reactions when 7.5 μl of Ligation Mix is used per reaction.

III. Storage

-20℃
Before using the kit, thaw on ice (for 5 - 10 min) and mix thoroughly by pipetting. The performance of the DNA Ligation Mix was confirmed to be unaffected up to at least 50 freeze-thaw cycles. (Refer to V. Stability)

IV. Protocols and Examples

(1) Ligation of DNA fragment with plasmid vector

The Standard Protocol should be used for general ligation reactions. When performing sticky-ended DNA ligations or when the highest efficiencies are not required, the Rapid Protocol offers good efficiency in a shorter period of time. Refer to the "[Example 4] Ligation with Rapid Protocol" to compare efficiencies.

[Standard Protocol]

1. Combine the linearized plasmid vector DNA and the DNA fragment to be inserted in a total volume of 5 - 10 μl. We recommend using TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) for dissolving DNA. The recommended vector : insert ratio is 25 fmol vector : 25 - 250 fmol insert. (Note : 25 fmol of pUC118 DNA (3,162 bp) corresponds to approx. 50 ng).
2. Add one volume of Ligation Mix (5 - 10 μl) to the DNA solution and mix thoroughly.
3. Incubate at 16℃ for 30 minutes＊1.
4. The ligation reaction mixture can be used directly for transformation with *E.coli* competent cells. When performing transformation immediately following ligation, add 10 μl of the ligation mixture to 100 μl of competent cells＊2.

＊1 If good results are not obtained, the reaction time can be extended overnight.
＊2 If more than 10 μl of the ligation reaction mixture must be used for transformation, then the ligated DNA should be ethanol precipitated prior to use.

NOTE : The ligation reaction mixture should not be used directly in electroporation. The ligated DNA should be ethanol precipitated and dissolved in a low salt buffer such as TE buffer prior to use.

[Rapid Protocol]

1. Combine the linearized plasmid vector and the DNA fragment to be inserted in a total volume of 5 - 10 μl. We recommend using TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) for dissolving DNA. Recommended vector : insert ratios are 25 fmol vector : 25 - 250 fmol insert (Note : 25 fmol of pUC118 DNA (3,162 bp) corresponds to approx. 50 ng).
2. Add one volume of Ligation Mix (5 - 10 μl) to the DNA solution and mix thoroughly.
3. Incubate at 25℃ for 5 minutes＊1.
4. The ligation reaction mixture can be used directly for transformation with *E.coli* competent cells. When performing transformation immediately after ligation, add 10 μl of the ligation mixture to 100 μl of competent cells＊2.

＊1 Higher temperatures (> 26℃) will inhibit the formation of circular DNA. The reaction temperature should be strictly kept at 25℃ when using the Rapid Protocol.
＊2 If more than 10 μl of the ligation reaction mixture must be used for transformation, the ligated DNA should be ethanol precipitated prior to use.

NOTE : The ligation reaction mixture should not be used directly in electroporation. The ligated DNA should be ethanol precipitated and dissolved in a low salt buffer such as TE buffer prior to use.

[Example 1] Vector ligation of stick-ended DNA

50 ng of pUC118 *Hind* III/BAP (Cat. #3324), (25 fmol) was mixed with 2.5 - 150 fmol of 564 bp *Hin*d III-digested lambda DNA fragment at insert : vector ratios ranging from 0.1 to 6, in a total volume of 7.5 μl. One volume (7.5 μl) of Ligation Mix was added to the DNA solution. The combined solution was then incubated at 16℃ for 30 minutes. A part of the solution was used directly to transform *E.coli* JM109 competent cells and colonies were formed on LB-Amp plates containing X-Gal and IPTG. The transformation efficiency of *E.coli* JM109 competent cells was 7.3×10^8 cfu/μg pUC118 DNA.

Transformation efficiencies, obtained by counting the number of white colonies, are shown in Figure1.

Insert/vector (molor ratio)	(colonies/μg vector)		white colonies/ total colonies (%)
	white	blue	
—	3.8×10^4	2.5×10^5	13.0
1/10	5.6×10^5	2.4×10^5	70.2
1/4	1.1×10^6	2.4×10^5	82.9
1	4.3×10^6	2.4×10^5	92.6
3	8.2×10^6	3.3×10^5	96.2
6	9.1×10^6	2.4×10^5	97.4

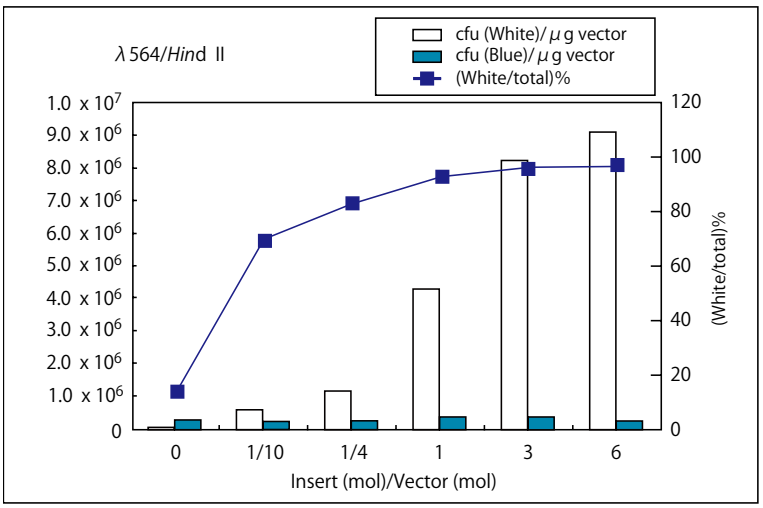


Figure 1. Vector ligation using *Hin*d III-digested λ DNA fragment (564 bp)

[Example 2] Vector ligation of blunt-end DNA

50 ng of pUC118 *Hin*c II/BAP (Cat. #3322), (25 fmol) was mixed with 2.5 - 150 fmol of 500 bp *Hin*c II-digested lambda DNA fragment at insert : vector ratios ranging from 0.1 to 6, in a total volume of 7.5 μl. One volume (7.5 μl) of Ligation Mix was added to the DNA solution. The combined solution was then incubated at 16℃ for 30 minutes. A part of the solution was used directly to transform *E.coli* JM109 competent cells and colonies were formed on LB-Amp plates containing X-Gal and IPTG. The transformation efficiency of *E.coli* JM109 competent cells was 7.3 x 10^8 cfu/μg pUC118 DNA.

Transformation efficiencies, obtained counting the number of white colonies, are shown in Figure 2.

Insert/vector (molor ratio)	(colonies/μg vector)		white colonies/ total colonies (%)
	white	blue	
—	1.6 x 10^4	2.1 x 10^5	6.7
1/10	5.6 x 10^5	2.3 x 10^5	70.6
1/4	1.1 x 10^6	2.2 x 10^5	83.0
1	4.2 x 10^6	3.0 x 10^5	93.4
3	7.5 x 10^6	2.6 x 10^5	96.7
6	6.7 x 10^6	2.7 x 10^5	94.8

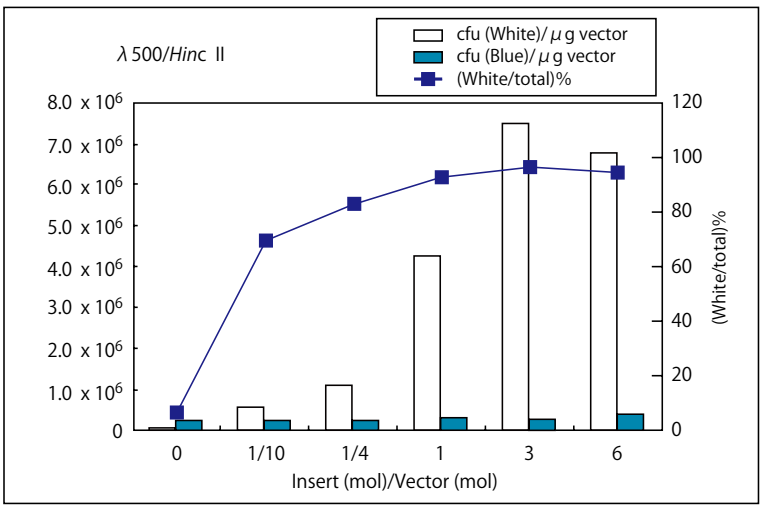


Figure 2. Vector ligation using *Hin*c II-digested λ DNA fragment (500 bp)

And whether the inserts in the white colonies are correct were examined.ed
<Proper insert colonies/White colonies>

Insert	Insert : Vector	
	0.1 : 1	3 : 1
λ 564/*Hin*d III	21/24	24/24
λ 500/*Hin*c II	24/24	22/24

Insert / Vector ratio of about 3 is recommended when performing ligations. Generally, a greater number of white vs. blue colonies are obtained when the insert : vector ratio is greater than 1. When the ratio is greater than 6, a decrease in transformation efficiency is observed. In addition, ligation of short DNA fragments frequently results in multi-ligations, that is, the insertion of multiple linked DNA fragments into a vector. As the insert : vector ratio increases, a greater number of multi-ligations are observed. Lower the insert : vector ratio when many multi-ligations are obtained.

[Example 3-1] Insertion of DNA fragments dephosphorylated at 5′ end into a 5′ -phosphorylated vector

50 ng of *Hinc* II-digested pUC 118 vector phosphorylated at 5′-terminus (25 fmol) was mixed with 25 - 750 fmol of 500 bp *Hinc* II-digested lambda DNA fragment dephosphorylated at 5′-terminus, in a total volume of 7.5 μl. One volume (7.5 μl) of Ligation Mix was added to the DNA solution. The combined solution was then incubated at 16℃ for 30 minutes. A part of the solution was used directly to transform *E.coli* JM109 competent cells and colonies were formed on L-Amp plate containing X-Gal and IPTG.
The transformation efficiency of *E.coli* JM109 competent cells was 6.2 x 10^8 cfu/μg pUC118 DNA.
Transformation efficiencies obtained counting the number of white colonies are shown in Figure 3-1.

Insert/vector ratio	Transformation efficiency (colonies/μg vector)		white colonies/ total colonies (%)
	white	blue	
-	1.1 x 10^6	8.0 x 10^7	1.3
1	3.6 x 10^6	6.4 x 10^7	5.3
5	6.7 x 10^6	5.0 x 10^7	11.9
15	5.0 x 10^6	2.6 x 10^7	16.3
30	3.6 x 10^6	1.5 x 10^7	19.1

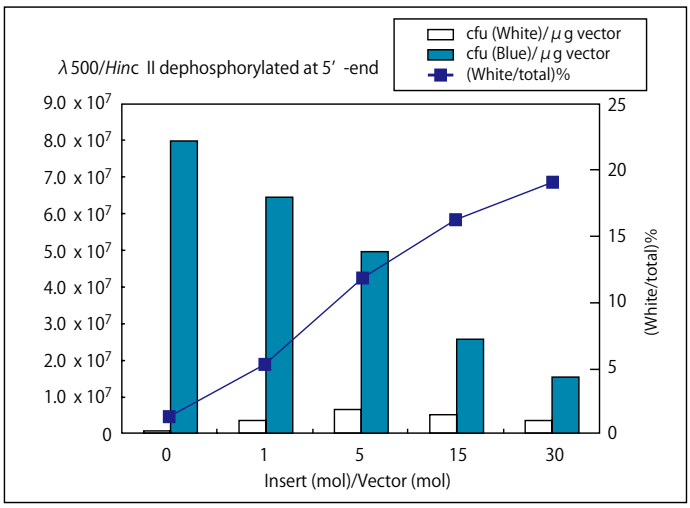


Figure 3-1.

[Example 3-2] Insertion of DNA-fragment phosphorylated at 5′-end into a 5′-phosphorylated vector

50 ng of pUC118/*Hin*c II phosphorylated at 5′-terminus (25 fmol) was mixed with 25 - 750 fmol of 500 bp *Hin*c II-digested lambda DNA fragment, in a total volume of 7.5 μl. One volume (7.5 μl) of Ligation Mix was added to the DNA solution. The combined solution was then incubated at 16℃ for 30 minutes. A part of the solution was used directly to transform *E.coli* JM109 competent cells and colonies were formed on L-Amp plate containing X-Gal and IPTG. The transformation efficiency of *E.coli* JM109 competent cells was 4.6 x 10^8 cfu/μg pUC118 DNA. Transformation efficiencies obtained counting the number of white colonies are shown in Figure 3-2.

Insert/vector ratio	Transformation efficiency (colonies/μg vector)		white colonies/ total colonies (%)
	white	blue	
0	2.7 x 10^6	5.6 x 10^7	0.5
1	3.0 x 10^6	5.6 x 10^7	5.1
5	8.1 x 10^6	4.4 x 10^7	15.5
15	6.0 x 10^6	1.9 x 10^7	24.3
30	3.5 x 10^6	8.9 x 10^7	28.4

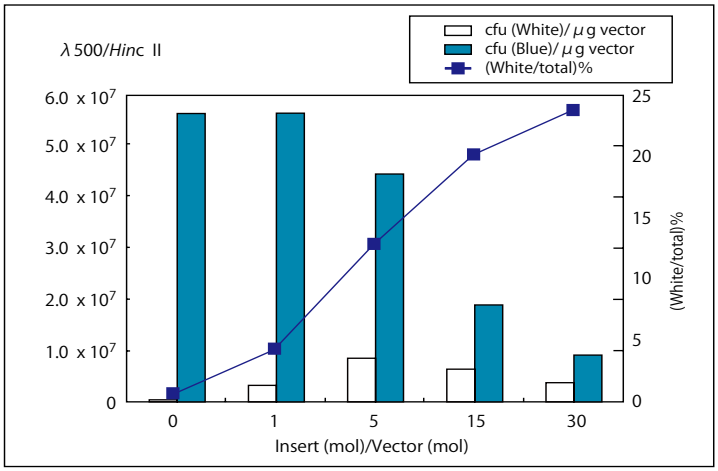


Figure 3-2.

The 5′-end dephosphorylation of vector is unnecessary for ligation reaction. Higher ratio of Insert/Vector resulted in more positive colonies. Accordingly phosphorylated vector at 5′-end can also be used for insertion of 5′-dephosphorylated DNA fragment.

[Example 4] Ligation with Rapid Protocol (at 25℃ for 5 minutes)

Ligation reactions using sticky-ended and blunt-ended DNA were performed respectively using two protocols; (1) at 25℃ for 5 minutes, and (2) at 16℃ for 30 minutes. Transformation efficiencies of the two protocols were compared. In addition, these efficiencies were compared against the ligation efficiency obtained using a kit from Company D which recommends a 25℃, 5 minute ligation.

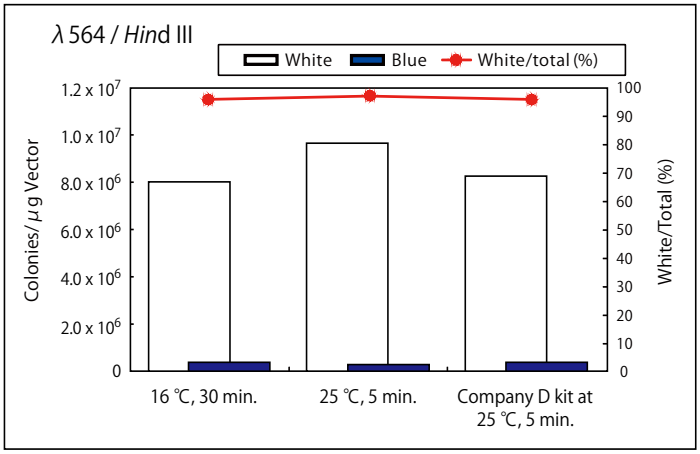


Figure 4-1. Sticky-ended DNA Ligation into Vector

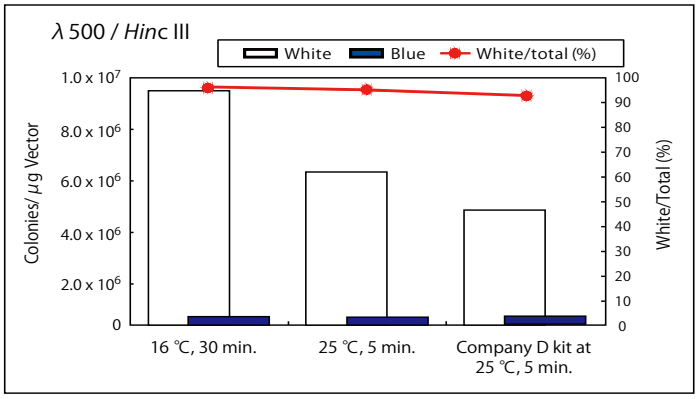


Figure 4-2. Blunt-ended DNA Ligation into Vector

The transformation efficiency of the sticky-ended DNA ligation was the same for both protocols. For the blunt-ended DNA ligation, higher efficiency was obtained using a 16℃, 30 minutes ligation reaction rather than the 25℃, 5 minute reaction. However, even when the rapid protocol (25℃, 5 minutes) was used, the transformation efficiency was higher with Takara's kit as compared to Company D's kit. Accordingly, time can be saved for performing sticky-ended DNA ligations with this kit.

(2) Ligation of PCR product with T vector

1) Prepare a DNA solution＊1 containing 13 - 25 fmol of T-vector (e.g. 25 - 50 ng of pT7 Blue T-Vector (Merck.)) and 5 - 250 fmol insert PCR fragment in a total volume of 5 - 10 μl.
2) Add one volume (5 - 10 μl) of Ligation Mix into the DNA solution and mix well with a pipette.
3) Incubate at 16℃ for 30 minutes.
4) If immediately performing a transformation following the ligation reaction, then 10 μl of the ligation solution to 100 μl of competent cells.

＊1 It is recommended dissolving the DNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or an appropriate buffer.
＊2 When ligating PCR products into T-vector, the reaction should be completed within 1 hour. Longer reaction times may result in higher background.
＊3 If more than 10 μl the ligation reaction mixture needs to be used for transformation, then the ligated DNA should be ethanol precipitated prior to use.

[Example]

25 ng of pT7Blue T-Vector (Merck.) (13 fmol) was mixed with 2,080 bp of PCR fragment (39 fmol) in the total volume of 10 μl. One volume of Ligation Mix (10 μl) was added to the DNA solution and the reaction mixture was incubated at 16℃ for 30 min. 10 μl of the ligation reaction mixture was used to transform *E.coli* JM109 competent cells (100 μl) and colonies were formed on LB-Amp plate containing X-Gal and IPTG. (The transformation efficiency of JM109 competent cells were 7.8 x 10^8 cfu/μg pUC118 DNA).
Transformation efficiency, obtained by counting the number of white colonies, were compared with the results using 4 kits from other companies.

The results demonstrate that this kit provides higher ligation efficiencies for obtaining positive (white) colonies in T-vector ligations than other companies' kits.

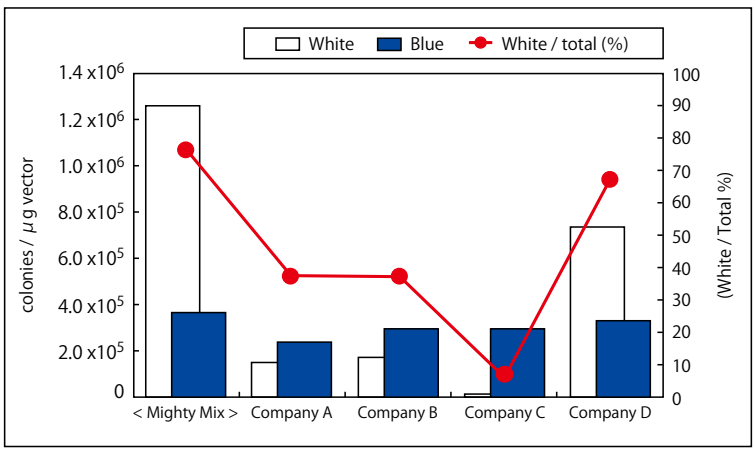


Figure 5. Ligation into T-Vector

(3) Self-circularization of linear DNA

The procedure for self-circularization of linear DNA is essentially the same as for (1) Insertion of DNA fragments into plasmid vectors. However, it is important to perform at 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 transformation efficiency.

[Example]

*Hin*c II-digested pUC118 phosphorylated at 5'-end (250 ng, 10 μl) was prepared. Ligation Mix (10 μl) was added and incubated at 16℃ for 30 minutes by following the protocol. A portion of the ligation reaction mixture solution was used to transform *E.coli* JM109 competent cells (100 μl). *E.coli* JM109 competent cells had an efficiency of 1.1×10^9 cfu/μg pUC118.

Transformation efficiencies (colonies per μg of DNA added).

DNA added	Transformation efficiencies (cfu/μg vector)
17 ng	1.2×10^7

(4) Linker, Adaptor ligation

1. Insertion of linker into a plasmid vector

Conditions for linker ligation are essentially the same as for (1) Insertion of DNA fragments into plasmid vectors.
Recommended vector/linker molar ratios are :

- phosphorylated linker : dephosphorylated vector = 10 - 1,000 : 1
- phosphorylated linker : phosphorylated vector = >100 : 1

2. Linker [Adaptor] ligation to both termini of a DNA fragment (ex. Linker ligation of cDNA)

1) Prepare 5 - 10 μl of DNA solution containing DNA fragment (10 - 100 fmol) and linker (or adaptor). Recommended DNA fragment : linker[adaptor] molar ratio is :
   - DNA fragment : linker [adaptor] = 1 : >100
2) Add twice the volume of Ligation Mix (10 - 20 μl) into the DNA solution, and incubate at 16℃ for 30 min. However, if the linker is shorter than 8 bases or if the linker has a low GC-content, then the ligation reaction should be performed at a lower temperature (<10℃) for 1 to 2 hours.
3) If the ligated DNA is to be subjected to restriction enzyme digestion, then ethanol precipitate and resuspend the DNA in an appropriate buffer prior to digestion.

[Example] Insertion of linker into a plasmid vector

50 ng of dephosphorylated vector, pUC 118 *Hin*c II/BAP (Cat. #3322) (25 fmol) and 130 ng (25 pmol) of phosphorylated *Bgl* II linkers (5'-CAGATCTG-3') were combined in a total volume of 10 μl. Ligation Mix (10 μl) was added to the DNA solution and this mixture was incubated at 16℃ for 30 minutes. A part of the solution was used to transform *E.coli* JM109 competent cells, and colonies were formed on LB-Amp plates containing X-Gal and IPTG. (The transformation efficiency of *E.coli* JM109 competent cells were 8.4 x 10^8 cfu/μg pUC118 DNA). Transformation efficiencies, obtained by counting the number of white colonies, are shown in the table below.

linker/vector (molar ratio)	cfu (White)/μg vector
0	5.3 x 10^5
1000	4.5 x 10^7

(5) Insertion of DNA fragment into λ phage vector

1. Prepare a DNA solution containing 250 ng of λ phage vector (10 fmol) and DNA fragment (30 - 100 fmol) in a total volume of 5 - 10 μl.
2. Add twice the volume of Ligation Mix (5 - 10 μl) into the DNA solution and mix well.
3. Incubate at 26℃ for 5 - 10 minutes. A higher efficiency is obtained at 26℃ rather than at 16℃. Longer reaction times may lower the efficiency. Therefore, limit the reaction time to 5 - 10 minutes.
4. Perform *in vitro* packaging.

The ligation reaction mixture can be used directly for packaging. The composition of Ligation Mix will not inhibit the λ packaging reaction as long as the ratio of ligation reaction mixture to the total packaging reaction mixture is kept to <10 % volume of the packaging lysate, even when using a commercially available packaging kit (e.g. MaxPlax Lambda Packaging Extracts (EPICENTRE Technologies), GigaPack (Agilent Technologies)). Generally 4 μl of ligation reaction mixture can be added per packaging reaction. If the total amount of DNA (i.e. total volume) of the ligation reaction mixture must be added to a packaging reaction, then the DNA should be ethanol precipitated and redissolved in TE buffer, such that a volume ratio of <10 % of the packaging lysate can be maintained.

For *in vitro* packaging using the DNA Ligation Kit <Mighty Mix>

Packaging efficiency obtained with DNA Ligation Kit <Mighty Mix> is almost the same as with the DNA Ligation Kit Ver.1 When packaging with this kit, the volume of ligation reaction mixture is one third larger than the volume of the DNA Ligation Kit Ver.1 ligation reaction mixture. As stated in the above step4, there is a limitation for the volume of the ligation reaction mixture that can be used for packaging. If the total amount of DNA of the ligation reaction mixture (i.e. total volume) must be used for packaging, then it is recommended using the Ver.1 Kit, in which a greater volume of DNA solution can be added.

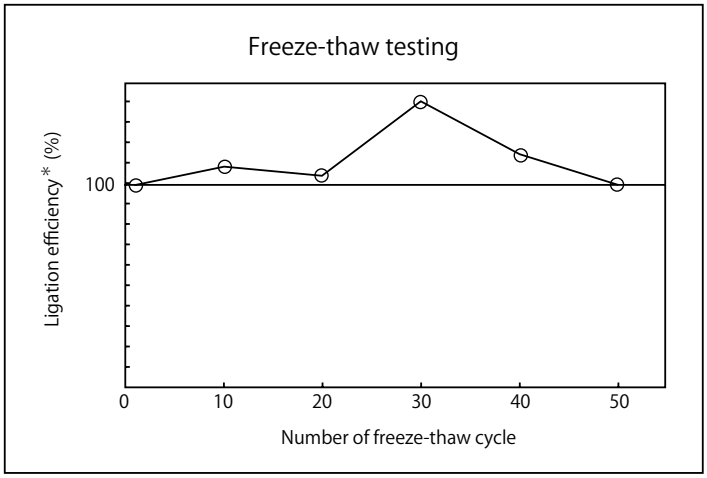
[Example]

The DNA solution was prepared by adding 400 ng of *Eco*R I/*Sal* I-digested pBR322 DNA (3.3 μl) into 1 μg *Eco*R I/*Sal* I-digested Uni-ZAP XR vector (1 μl) (Agilent Technologies). A 10 μl total volume Mighty Mix ligation reaction was performed at 26℃ for 10 minutes. 2.5 μl of the ligation reaction mixture was added for λ packaging Extracts (GigaPack III Gold or Maxplax), resulting in the formation of plaques.

	Packaging reagent	plaques/μg Uni-ZAP Vector
DNA Ligation Kit <Mighty Mix>	GigaPack III	2.6×10^6
	MaxPlax	5.5×10^6
DNA Ligation Kit Ver.1	GigaPack III	6.0×10^6
	MaxPlax	9.2×10^6

V. Stability

The influence of freeze-thaw cycles on the ligation efficiency was examined by ligation of pUC118 *Hin*d III/BAP and 564 bp of *Hin*d III digested DNA fragment. Testing verified that ligation efficiency is not lowered even after repeated 50 freeze-thaw cycles.



＊Stability was calculated assuming 100% efficiency without freeze-thaws.

VI. Q&A

Q1 : What ligation conditions can be modified to improve ligation efficiency when observed efficiency is low, evidenced by low numbers of transformants ?

A1 :
- Extend the reaction time to overnight.
- For ligation of blunt-end DNA, high salt concentration of DNA solution lower the ligation efficiency. DNA solution should be desalted prior to ligation reaction.
- For ligation of sticky-ended DNA, heat the DNA solution (vector + insert DNA) at 60 - 65℃ for 2 - 3 min., cool rapidly, and then perform the ligation after adding Ligation Mix. This step will result in a higher ligation efficiency.
  If ligation efficiency is not improved after performing all above , then repurification of the vector and insert DNA is recommended.

Q2 : Can the ligation reaction mixture be directly used in electroporation ?

A2 : When performing transformation by electroporation, the DNA in the ligation reaction mixture should be ethanol precipitated and dissolved in an appropriate buffer before electroporation.

Q3 : How to ligate with cosmid vector.

A3 : Follow the protocol (1) Insertion of DNA fragments into plasmids vectors. When performing *in vitro* packaging, follow the protocol (5).

Q4 : Is it possible to use a restriction enzyme digest directly as DNA Solution in ligation with the DNA Ligation Kit ?

A4 : It is recommended that digested DNA is precipitated with ethanol and then dissolved in TE buffer before ligation reaction 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 : Can Salt (e.g. NaCl) be added to the ligation reaction mixture before ethanol precipitation ?

A5 : Yes, Salt can be added directly to the ligation reaction mixture (a final concentration of 150 mM NaCl, 2 M ammonium acetate or 300 mM sodium acetate) and then the ligated DNA is precipitated with ethanol.

Q6 : Can Ligation Solution A and B in DNA Blunting Kit (Cat. #6025) be substituted with DNA Ligation Kit <Mighty Mix> ?

A6 : They cannot be directly substituted. DNA Ligation Kit <Mighty Mix> is designed to allow a smaller reaction scale, by mixing the same volume of Ligation Mix and DNA solution. Accordingly the reaction can be influenced by the composition of DNA solution, and ligation reaction may not be carried out by using DNA Ligation Kit <Mighty Mix> instead of Ligation Solution A and B in DNA Blunting Kit (Cat. #6025). When using DNA Ligation Kit <Mighty Mix> during the reaction of DNA Blunting Kit, the sample DNA solution should be extracted with phenol and precipitated with ethanol prior to the ligation.

Q7 : Can DNA fragments that have been recovered from agarose gels be used with the DNA Ligation Kit ?

A7 : When DNA fragments recovered using a commercial DNA extraction product/ reagent (e.g. columns or silica gel) is used with the DNA Ligation Kit , To ensure high ligation efficiencies, recovered DNA fragments should be ethanol precipitated and dissolved in an appropriate buffer (such as TE) prior to use with the Ligation Kit.

VII. Notes

(1) It is recommended storing the Ligation Mix in this kit frozen at -20℃. The Ligation Mix is not inactivated by freeze-thaw cycles. Thaw the Ligation Mix on ice and mix well, e.g. by pipetting prior to use.

(2) The ligation reaction mixture can be used directly for agarose gel electrophoresis. For polyacrylamide gel electrophoresis, the ligated DNA should be ethanol precipitated prior to use.

(3) The ligation reaction mixture may turn white in color if it is subjected to phenol extraction.

(4) When performing ethanol precipitate after ligation, add one-tenth volume 3M sodium acetate (pH 5.2) or one-twentieth volume of 5 M NaCl in the ligation reaction mixture or one-twentieth volume of 5 M NaCl and add 2 - 2.5 volume of ethanol, and then cool at -20℃ for 20 minutes or -80℃ for 10 minutes. Then centrifuge at 4℃ to recover DNA. The use of precipitation carrier is also effective for recovering very small amounts of DNA.

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.1 (Cat. #6021)
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. #5801A)
E. coli HST08 Premium Competent Cells (Cat. #9128)
E. coli HB101 Competent Cells (Cat. #9051)
E. coli JM109 Competent Cells (Cat. #9052)