COSMO BIO CO., LTD.
Inspiration for Life Science

TOYOBO

Ligation high

DNA Ligation Kit

Instruction Manual

(Code No.: LGK-101)

Distributor

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Contents

Caution

All the reagents in this kit are intended for research usage. Do not use them for diagnosis or in clinical practice. Please observe the general laboratory precautions and pay attention to safety while using this kit.

1. Introduction

The ligation of the DNA fragment procedure is frequently performed in gene manipulation experiments. The conventional procedure for ligation reaction is complicated involving setting the conditions depending on the substrate and adding each reaction composition separately. Furthermore, it is difficult to achieve highly efficient transformation in the case of insert insertion ligation, etc.
To solve these problems, TOYOBO developed a kit to complete the ligation reaction using a simple and highly efficient procedure. All the reagents required for the ligation reaction are contained in the reaction solutions of this kit, so the kit is applicable to various types of ligation. The standard results obtained with "Ligation high" are shown in this operating manual.

2. Protocol

[1] Usage method

- This product should be stored at -20°C.
- "Ligation High" is thawed in ice. Spontaneous thawing occurs within 5 to 10 minutes.
- The DNA solution used for ligation is mixed and prepared.
- Half or an equivalent amount of "Ligation high" is mixed with the DNA solution.
- The reaction is performed at 16°C for 30 minutes.
- The reaction solution can be used for transformation as it is after completion of the reaction.

[2] For efficient ligation reaction

- When ligation efficiency is poor, it is advisable to purify the DNA by ethanol precipitation, etc.
- To suppress the reaction fluid volume, the amount of DNA solution is decreased, and this is mixed with half of "Ligation high" (refer to p.2).
- The transformation efficiency is decreased if a large amount of reaction solution is added to the competent cell. When it is necessary to add a large amount of reaction solution, it is advisable to condense DNA by ethanol precipitation, etc.
- The ligation efficiency is influenced by the concentration of salt. To increase the efficiency, DNA is dissolved with a TE buffer *1 that does not contain salt before the ligation reaction is performed (refer to p.7).

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*1 10 mM Tris-HCI, pH 8.0/1 mM EDTA

3. Characteristics of "Ligation high" *1

[1] High ligation efficiency

The efficiency is more than 50 times higher compared to when T4 DNA ligase is used.

[2] Reaction with a smaller amount of solution

Half or an equivalent amount of "Ligation high" is mixed with the DNA solution.

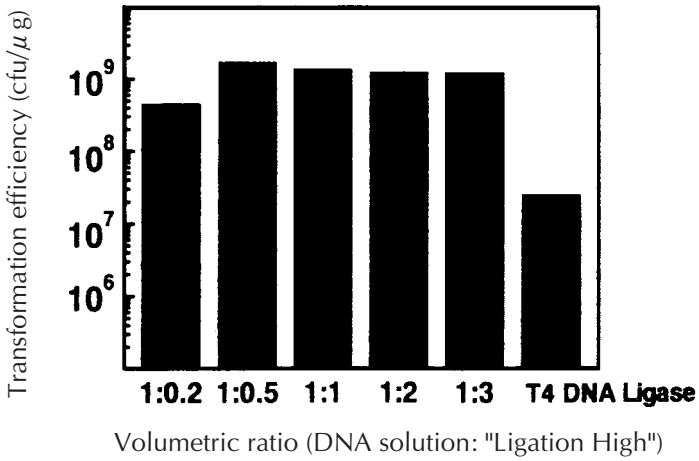


Fig. 1. Difference in transformation efficiency by volumetric ratio (DNA solution:Ligation High)

[3] Excellent stability

The ligation efficiency is not decreased even after freezing and thawing 50 times.

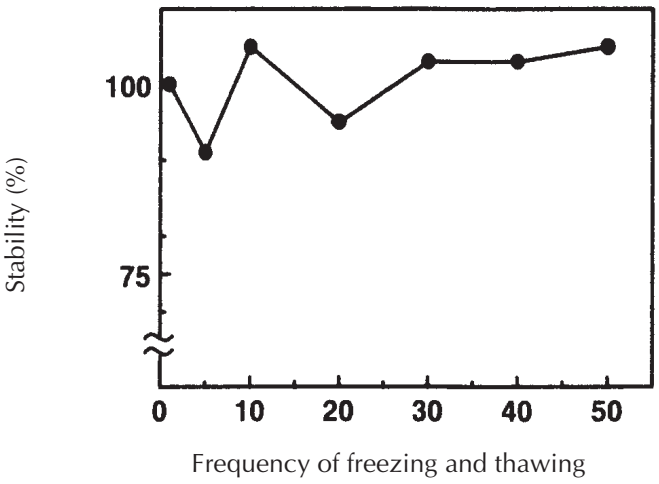


Fig. 2. Changes in transformation efficiency due to freezing and thawing

*1 All the results shown here are the results of self-ligation. Self-ligation is performed using the method described on p.7 (dissolution in TE buffer).

4. Examples of usage

[1] Insert insertion ligation

1. Method

- Five μl of DNA solution was prepared by adding 1,444 bp fragments (5-125 ng, 5-125 fmol) of pUC18/*Taq* I to dephosphorated pBluescript II/*Ban* III (50 ng, 25 fmol).
- Half (2.5 μl) or an equivalent amount (5 μl) of "Ligation high" was mixed with DNA solution and this was reacted at 16°C for 30 minutes.
- The competent cell of *E. coli* JM109 [*1] was transformed with 2 μl of the reaction solution, and this was incubated in an LB plate that contained X-Gal, IPTG and ampicillin. The transformation efficiency was determined from the number of white colonies formed.
- The result of the 16-hour reaction using T4 DNA ligase was used as the control.

2. Results and discussion

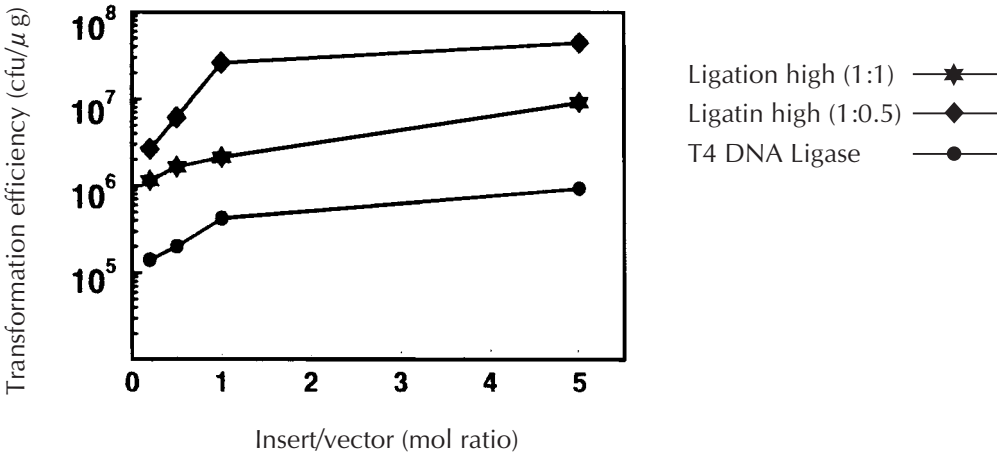


Fig. 3. Influence of insert/vector mol ratio on ligation efficiency

Table 2: Ratio of white colonies to the total number of colonies

(%)

Insert/vector (mol ratio)	Ligatin high (Volumetric ratio 1:1)	Ligatin high (Volumetric ratio 1:0.5)	T4 DNA Ligase
0.2	52	63	12
0.5	50	65	21
1.0	56	61	27
5.0	66	72	39

The mol ratio of insert/vector was 1.0 or more, indicating a favorable result.

[*1] The transformation efficiency was 1.07 x 10^9 cfu/μg pBluescript II

[2] Linker ligation

1. Method

- Five μl of DNA solution was prepared by adding phosphorated *Eco*R I linker (2.6-130 ng, 0.5-25 pmol) to dephosphorated pBluescript II/*Hin*c II (100 ng, 50 fmol).
- Half (2.5 μl) or an equivalent amount (5 μl) of "Ligation high" was mixed with the DNA solution, and this was reacted at 16°C for 30 minutes.
- The competent cell of *E. coli* JM109 [*1] was transformed with 2 μl of reaction solution, and this was incubated in an LB plate that contained X-Gal, IPTG and ampicillin. The transformation efficiency was determined from the number of white colonies formed.
- The result of the 16-hour reaction using T4 DNA ligase was used as the control.

2. Results and discussion

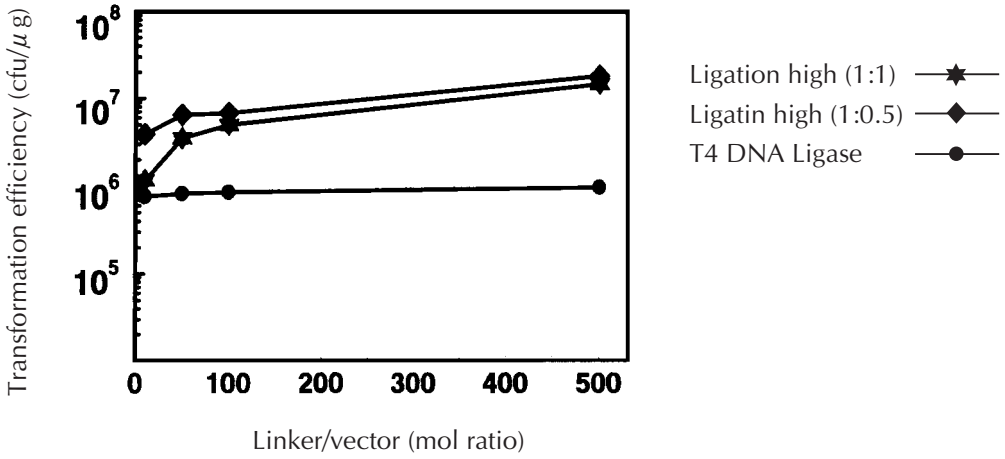


Fig. 3. Influence of mol ratio of linker/vector on ligation efficiency

(%)

Linker/vector (mol ratio)	Ligatin high Volumetric ratio 1:1	Ligatin high Volumetric ratio 1:0.5	T4 DNA Ligase
10	51	57	23
50	54	65	24
100	57	64	38
500	54	76	38

The mol ratio of linker/vector was 100 or more, indicating a favorable result.

[*1] The transformation efficiency was 1.07 x 10^9 cfu/μg pBluescript II.

[3] Phage ligation

1. Method

- Five μl of DNA solution was prepared by adding the test insert (200 ng) to the *Eco*R I arm (250 ng) of λ ZAP II.
- Half (2.5 μl) or an equivalent amount (5 μl) of "Ligation high" was mixed with the DNA solution, and this was reacted at 16°C and 26°C for 10 minutes to 1 hour.
- *In vitro* packaging was performed with 4 μl of reaction solution using GIGA-PACK III Gold. This was infected with *E. coli* XL1-Blue, and the transduction efficiency was determined.
- The result of the 16-hour reaction using T4 DNA ligase was used as the control.

2. Results and discussion

Table 3: Influence of reaction conditions in phage ligation

(pfu/μg λ ZAP II)

Reaction conditions	Ligatin high (1:1)	Ligatin high (1:0.5)	T4 DNA ligase
16°C × 10 min	2.20×10^5	7.68×10^6	ND
16°C × 30 min	5.20×10^5	8.32×10^6	ND
16°C × 60 min	3.00×10^6	1.15×10^7	ND
16°C × 16 hr	3.21×10^6	9.35×10^6	2.15×10^5
26°C × 10 min	4.80×10^5	5.12×10^5	ND
26°C × 30 min	1.20×10^6	3.20×10^6	ND
26°C × 60 min	2.00×10^6	3.84×10^6	ND
26°C × 16 hr	1.19×10^6	3.56×10^6	3.09×10^5

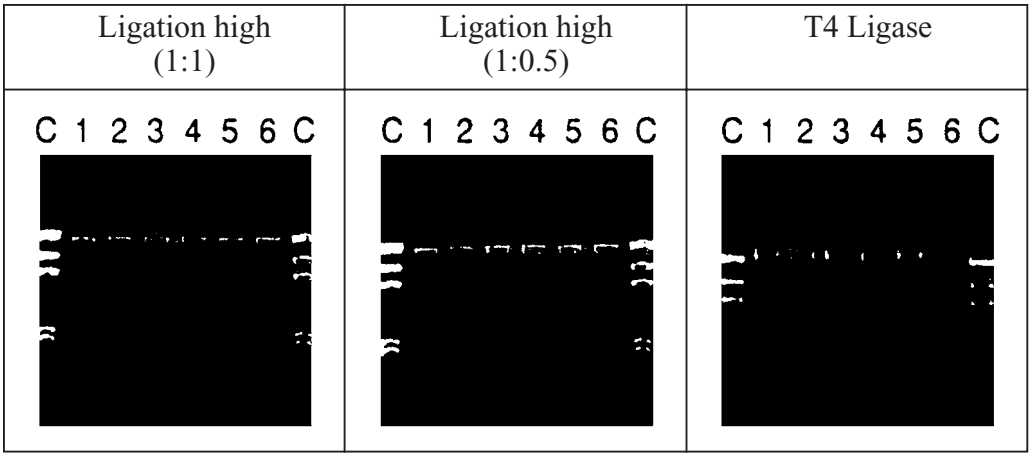
- No marked difference in ligation efficiency was observed between the reaction temperatures of 16°C and 26°C.
- Sufficient ligation efficiency was obtained after 30 minutes of reaction.

[4] Confirmation by electrophoresis

1. Method

- Five μl of λ/*Hin*d III fragment (500 ng) was mixed with an equivalent amount (5 μl) or half (2.5 μl) of "Ligation high," and this was reacted at 16°C.
- After completion of the reaction, the DNA was collected by ethanol precipitation. This was applied to 1% agarose gel for electrophoresis.
- The result of the reaction using T4 DNA ligase was used as the control.

2. Results and discussion



C: Control (untreated)
1: 16°C × 5 min
2: 16°C × 10 min
3: 16°C × 20 min
4: 16°C × 30 min
5: 16°C × 1 hr
6: 16°C × 16 hr

Fig. 4. Electrophoresis pattern of the ligation reaction solution

- Ligation of λ/*Hin*d III was observed after only 5 minutes of reaction.

Cautions

- Depending on the shape of fragment terminal, ligation does not readily occur. If ligation efficiency is low, it is advisable to prolong the reaction time.
- The reaction solution can be used as the sample for electrophoresis without processing. However, if a clear electrophoresis pattern is required, buffer replacement by ethanol precipitation is recommended.

[5] Influence of salt concentration

1. Method

- pBluescript II/*Sca* I (5 ng) was dissolved in 5 μl of TE buffer to which salt (NaCl) was added.
- The DNA solution was mixed with an equivalent amount (5 μl) or half (2.5 μl) of "Ligation high," and this was reacted at 16°C for 30 minutes.
- The competent cell of *E. coli* JM109 [*1] was transformed with 2 μl of reaction solution, and this was incubated in an LB plate that contained ampicillin. The transformation efficiency was determined from the number of colonies formed.

2. Results and discussion

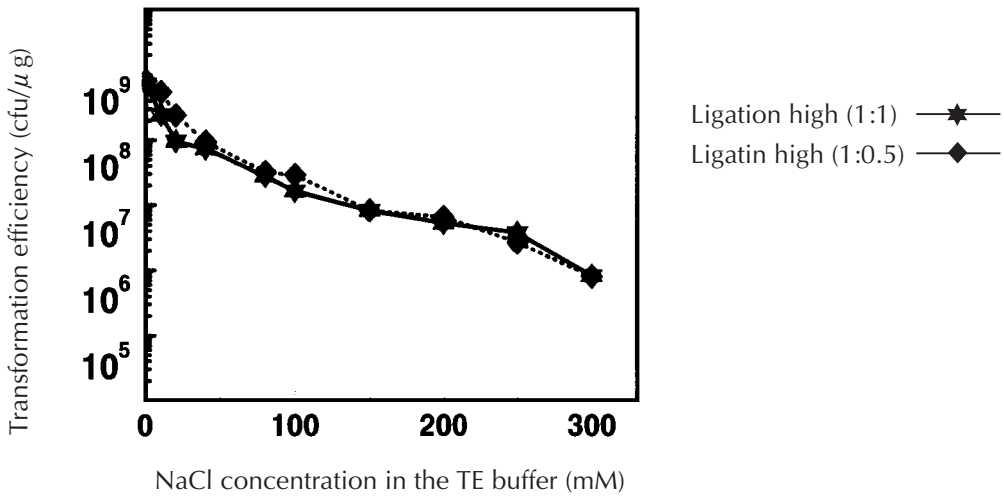


Fig. 5. Transformation efficiency by self-ligation

- When salt is contained in the buffer that dissolves the DNA, transformation efficiency tends to decrease. If high transformation efficiency is required, the DNA should be dissolved in a TE buffer that does not contain salt.

[*1] The transformation efficiency was 1.17 x 10^9 cfu/μg pBluescript II

5. List of related products

Product name	Code No.
T4 DNA Ligase	LGA-101
λ/*Hin*d III digest	DNA-010
Competent high *E. coli* JM 109	DNA-900
*Eco*R I Linker: d(pGGAATTCC)	ECO-801
pUC18 DNA	PUC-018

Manufacture

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