Ligation-Convenience Kit Manual (4th edition)

Code No. 319-05961	For 100 ligations
Code No. 315-05963	For 10 ligations

- Description –

The first important step of gene cloning is to ligate the target gene to a vector DNA. The efficiency of the ligation reaction varies depending on the shape of the DNA terminal, and until now the ligation reaction has needed to be optimized for each reaction. Further, the ligation reaction requires 1-16 hours using a conventional T4 DNA ligase, and it has been difficult to obtain high transformation efficiency. The Ligation-Convenience Kit can solve these problems and provide highly efficient ligation in a short period of time.

I. Characteristics

- This product is a kit for performing DNA ligation rapidly and simply.
- The 2×Ligation Mix contains all the components required for DNA ligation such as the reaction buffer, ATP, DTT, T4 DNA Ligase, etc. The DNA ligation reaction can be performed by adding an equal volume of 2×Ligation Mix to the DNA solution.
- By using this kit, the ligation reaction can be performed in 5-30 min regardless of the shape of the DNA terminal. Further, the DNA solution in which the ligation reaction has been completed can be used for transformation and *in vitro* packaging as-is.

II. Contents of kit

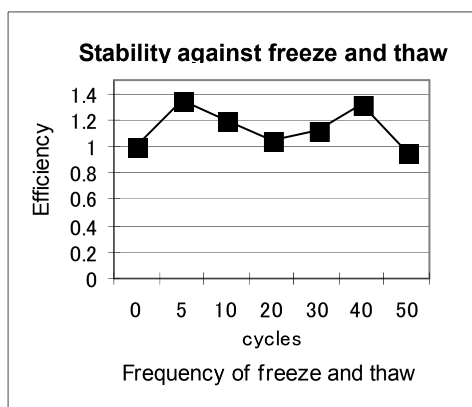
Reagent	For 100 ligations *	For 10 ligations *
2×Ligation Mix	250 μl×4	100 μl×1

* When used in 20 μl reaction system

III. Storage and melting method

Storage at −20℃

- At the time of use, thaw completely on ice and mix well by pipetting.
- No reduction in reaction efficiency was found in up to 50 cycles of freezing and thawing.



IV. Protocol and examples of experiments

1. Protocol

(1) Preparation of DNA solution

Prepare 10 µl of DNA solution by mixing a vector DNA and an insert DNA fragment at a proper molar ratio.[*2]

(2) Preparation of ligation reaction solution

Add an equal volume of 2×Ligation Mix to the DNA solution and mix.

(3) Ligation reaction

React at 16°C for 5-30 min.[*1]

(4) Transformation or *in vitro* packaging

Use the reaction solution for transformation or *in vitro* packaging as-is.[*4-9]

Vector DNA Insert DNA ddH_2O or TE [*2]	} up to 10 µl
2×Ligation Mix	10 µl
Total	20 µl

↓

React at 16°C for 5-30 min.[*1]

↓

Transformation or *in vitro* packaging [*4-9]

2. Investigation of the vector : insert molar ratio

The vector : insert molar ratio in ligation has a significant effect on the ligation efficiency. Shown below are the results obtained at Nippon Gene when ligating various length of inserts and transforming with them. The molar ratios which gave the best results under the specific conditions are summarized in the table below.[*3]

1. Plasmid vector ligation

(1) Sticky end

Insert length	200 bp	600 bp	1000 bp	3000 bp
Vector	1	1	1	1
Insert	10	5-10	2-10	0.5-2

Vector: pUC19 (0.03 pmol) cut by *Eco*R I
Insert: insert DNA (0.015 pmol, 0.03 pmol, 0.06 pmol, 0.15 pmol, 0.3 pmol) cut by *Eco*R I
Ligation reaction: 16°C, 5 min

(2) Blunt end

Insert length	200 bp	600 bp	1000 bp	3000 bp
Vector	1	1	1	1
Insert	5	5	2-10	0.5-2

Vector: pUC19 (0.03 pmol) cut by *Sma* I
Insert: insert DNA (0.015 pmol, 0.03 pmol, 0.06 pmol, 0.15 pmol, 0.3 pmol) cut by *Sma* I
Ligation reaction: 16°C, 5 min

(3) TA cloning

Insert length	200 bp	600 bp	1000 bp	3000 bp
Vector	1	1	1	1
Insert	10	5	5	1

Vector: pGEM®-T Easy; Promega Inc. (0.03 pmol)
Insert: PCR product amplified by Gene *Taq* NT (Code No. 318-03231)
(0.015 pmol, 0.03 pmol, 0.06 pmol, 0.15 pmol, 0.3 pmol)
Ligation reaction: 16°C, 5 min

2. Linker ligation

Insert length	8 bp
Vector	1
Insert	Not less than 50

Vector: pUC19 (0.03 pmol) cut by *Hin*c II
Insert: Linker *Eco*RV (Code No. 318-02251)
(0.3 pmol , 1.5 pmol , 3 pmol , 15 pmol)
Ligation reaction: 16°C, 5 min

3. Examples of experiments

< Example (1) Sticky end ligation >

Comparison with T4 DNA Ligase

(1) pUC19 DNA was cut with *Eco*R I, dephosphorylated and extracted with phenol/chloroform/isoamyl alcohol, and then dissolved in TE buffer.*2

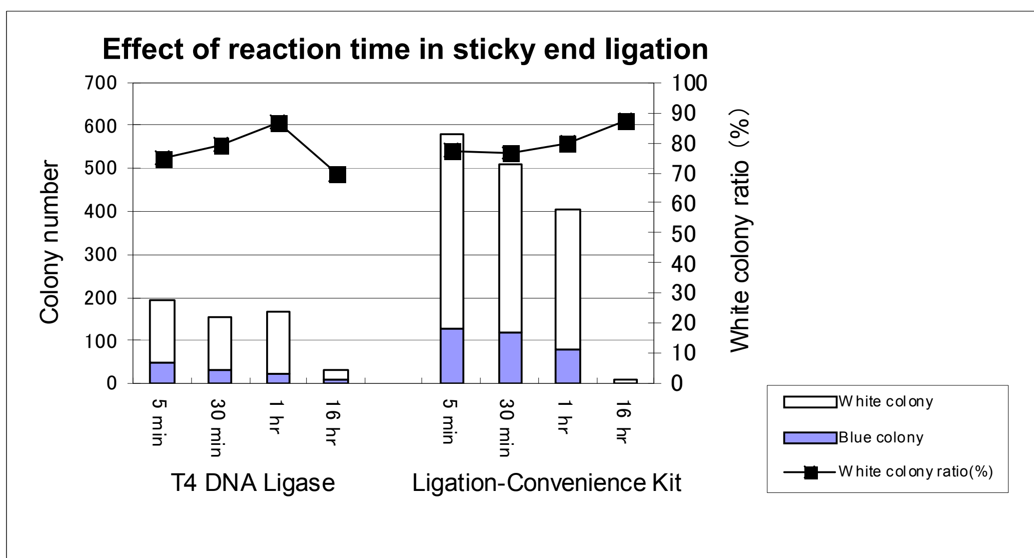
(2) A 500 bp DNA fragment derived from lambda DNA was cut with *EcoR* I.

(3) 10 μl of DNA solution containing 50 ng of pUC19 DNA and 20 ng of the insert DNA fragment (insert/vector molar ratio≒2) was prepared.*2

(4) 10 μl of 2× Ligation Mix was added to 10 μl of DNA solution and mixed.

(5) The reaction was carried out at 16°C for 5 min to 16 hr.

(6) After the reaction, 50 μl of competent cells of JM109 was transformed with 5 μl of the reaction solution, and the number of developed colonies was counted. Also, as a control, a similar ligation reaction was performed using T4 DNA Ligase by itself. Further, the transformation efficiency of the competent cells of JM109 is 1.3 × 10^8 cfu/μg (pBR322 DNA).*4, 5



Relation between ligation reaction time and transformation efficiency (white colony count)

Reaction time	5 min	30 min	1 hr	16 hr
Ligation-Convenience Kit	**450**	390	323	7
T4 DNA Ligase	145	123	146	23

[Results]

The above results indicate that the ligation reaction is sufficiently completed with a reaction time of 5 min when the Ligation-Convenience Kit is used. When the ligation time is extended to 16 hr, however, the transformation efficiency tends to decrease.*3

< Example (2) Blunt end ligation >

Comparison with T4 DNA Ligase

(1) pBluescript II SK(+) was cut with *Eco*RV, dephosphorylated and extracted with phenol/chloroform/isoamylalcohol, and then dissolved in TE buffer.*2

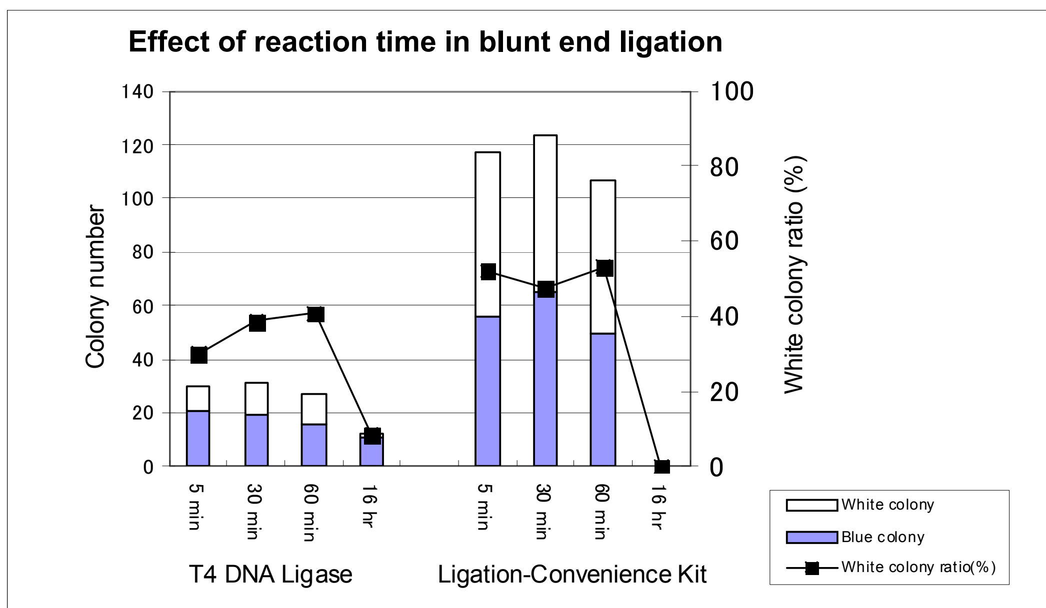
(2) A 500 bp DNA fragment derived from lambda DNA was cut with *Eco*RV.

(3) 10 μl of DNA solution containing 50 ng of pBluescriptⅡSK(+) and 20 ng of the insert DNA fragment (insert/vector molar ratio≒2.4) was prepared.*2

(4) 10 μl of 2×Ligation Mix was added to 10 μl of the DNA solution and mixed.

(5) The reaction was carried out at 16°C for 5 min to 16 hr.

(6) After the reaction, 50 μl of competent cells of JM109 was transformed with 5 μl of the reaction solution, and the number of developed colonies was counted. Also, as a control, a similar ligation reaction was performed using T4 DNA Ligase by itself. Further, the transformation efficiency of the competent cells of JM109 is 1.3×10^8 cfu/μg (pBR322 DNA).*4, 5



Ligation reaction time and obtained white colony count (number of white colonies)

Reaction time	5 min	30 min	1 hr	16 hr
Ligation-Convenience Kit	**61**	59	57	0
T4 DNA Ligase	9	12	11	1

[Results]

It is found that the ligation reaction is sufficiently completed with a reaction time of 5 min when the Ligation-Convenience Kit is used. When the ligation time is extended to 16 hr, however, the transformation efficiency tends to decrease.*3

< Example (3) Linker ligation >

Comparison with T4 DNA Ligase (investigation of linker : vector molar ratio)

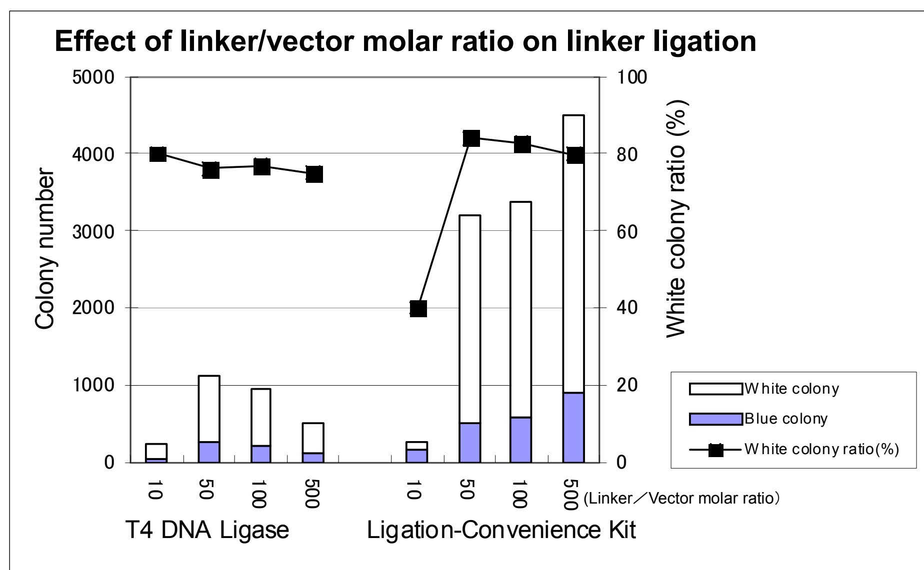
(1) pUC19 DNA was cut with *Hinc* II, dephosphorylated and extracted with phenol/chloroform/isoamyl alcohol, and then dissolved in TE buffer.*2

(2) Each 10 μl DNA solution was prepared by mixing 50 ng of pUC19 DNA vector (about 0.03 pmol) with linker DNA so that the molar ratio (linker : vector) was 10, 50, 100 or 500.*2

(3) 10 μl of 2 × Ligation Mix was added to each 10 μl of DNA solution and mixed.

(4) The reaction was carried out at 16°C for 5 min.*1

(5) After the reaction, 50 μl of competent cells of JM109 was transformed with 5 μl of the reaction solution, and the number of developed colonies was counted. Also, as a control, a similar ligation reaction was performed using T4 DNA Ligase by itself. The transformation efficiency of the competent cells of JM109 was 1.3×10^8 cfu/μg (pBR322 DNA).*4, 5



[Results]

The results above indicate that linker ligation efficiency is higher when the linker : vector molar ratio is 50-100.*3

< Example (4) Ligation to cosmid vector and *in vitro* packaging >

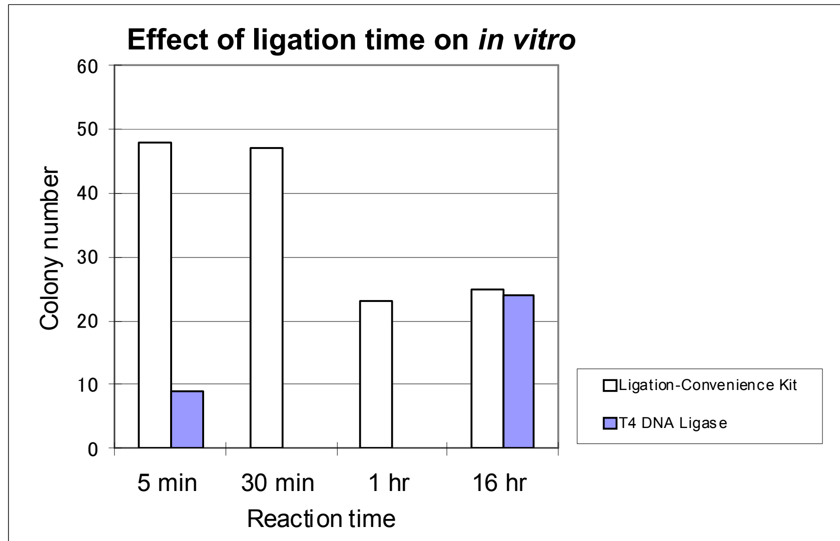
(1) 10 μl mixture containing 1 μg of Charomid 9-36 (Code No. 311-01401) linearized with *Eco*R I and 0.5 μg of a 4 kbp DNA fragment derived from ColE1 (insert : vector molar ratio≒4.5) was prepared.*2

(2) 10 μl of 2 × Ligation Mix was added to 10 μl of the DNA solution and mixed.

(3) The reaction was carried out at 16°C for 5 min to 16 hr.

(4) Using 3 μl of the reaction-completed solution (1/10 volume of the packaging Extract) and LAMBDA INN (Code No. 317-01741), *in vitro* packaging operation was carried out.*6~8

(5) Also, as a control, the ligation reaction using T4 DNA Ligase was carried out and a similar *in vitro* packaging was carried out.



[Results]

When the Ligation-Convenience Kit is used, it is observed that a ligation reaction time of 5-30 min is sufficient. Conversely, the packaging efficiency became worse by prolonging reaction time. When T4 DNA Ligase was used by itself, the packaging efficiency did not improve much by reacting for 16 hr.[*3]

V. Notes

*1 When ligation is carried out over a long duration, e.g., 16 hr, overnight or the like, transformation efficiency may be markedly reduced.

*2 When DNA is dissolved in a high salt concentration buffer, ligation efficiency is markedly reduced. Prepare the DNA solution in ddH_2O or TE buffer (pH8.0).

*3 Ligation efficiency may be different depending on the purity of DNA used for the ligation reaction and on the employed restriction enzyme.

*4 The volume of the reaction solution to be used for transformation should be not more than 1/10 of the competent cells by volume. If too much of the reaction solution is used, the transformation efficiency may be reduced.

*5 If the volume of the reaction solution is 1/10 of the competent cells by volume or more, recover the DNA after the ligation reaction by ethanol precipitation, dissolve the DNA in ddH_2O or TE buffer (pH8.0) so that the volume of the DNA is not more than 1/10 of the competent cells by volume, and then carry out the transformation.

*6 The volume of the reaction solution to be used for packaging should be not more than 1/10 of the packaging Extract by volume. If too much of the reaction solution is used, the packaging efficiency may be reduced.

*7 If the volume of the reaction solution is 1/10 of the packaging Extract by volume or more, recover the DNA after the ligation reaction by ethanol precipitation, dissolve the DNA in ddH_2O or TE buffer (pH8.0) so that the volume of the DNA is not more than 1/10 of the packaging Extract by volume, and then carry out the transformation.

*8 Packaging is not inhibited by the use of Gigapack (Stratagene Inc.).

*9 Heat treatment of the ligation reaction completed solution causes a marked reduction of transformation efficiency. When the heat treatment is performed, heat the ligation reaction completed solution at 70°C for 10 min after diluting the ligation reaction completed solution two fold with ddH_2O (DNase-free).

VI. Troubleshooting

Problem	Presumed cause	Countermeasure
No ligation	Mismatch of the ends	Identify the ends
Low ligation efficiency	Concentration of 2 × Ligation Mix after thawing is not homogeneous	Mix well by pipetting after thawing on ice.
	Contamination of short DNA fragments	Remove the short DNA fragments generated by restriction enzyme reaction by electrophoresis or the like
	High salt concentration	Dissolve DNA in water or TE buffer containing no salts, and perform ligation.
	Long ligation time	Perform ligation within 1 hr.
Low transformation efficiency	Use of too much reaction solution	Limit the reaction solution used for transformation within 1/10 volume of competent cells.
	Insufficient vector dephosphorylation	Determine beforehand whether there is self-ligation or not.
	Heat treatment without diluting the ligation reaction solution after completing the reaction	After completing the reaction, dilute the ligation reaction solution twofold with ddH_2O (DNase-free) and then perform heat treatment (70°C for 10 min).
	Low transformation efficiency of the competent cells	Use competent cells having a transformation efficiency of 5 x 10^7 cfu/μg (pBR322 DNA) or higher.

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