TA-Blunt Ligation Kit Manual（3rd edition）

Code No. 315-06541	For 5 reactions
Code No. 311-06543	For 50 reactions

- Description -

Nippon Gene has been offering the “Ligation-Convenience Kit” which can be used to perform DNA ligation reactions simply and efficiently, in contrast to the long reaction times of the conventional ligation method using T4 DNA Ligase singly. However, in general, ligation efficiency varies depending on the shape of the DNA ends.

“TA-Blunt Ligation Kit” is a ligation kit specialized for TA cloning and blunt end ligation using a buffer of Nippon Gene’s own composition. “TA-Blunt Ligation Kit” offers higher DNA ligation efficiency than the “Ligation-Convenience Kit”.

I. Characteristics

- This product is a kit for performing highly efficient TA cloning and ligation of blunt end DNA.
- The 5×Ligation Mix includes a reaction buffer required for DNA ligation, ATP, DTT, T4 DNA Ligase and the like. Highly efficient DNA ligation reactions can be performed by adding 1/5 of the volume of the 5×Ligation Mix and 1/10 of the volume of the 10×Enhancer solution to the reaction system.
- Since the 5×Ligation Mix and the 10×Enhancer solution are not frozen at -20°C, these can be used immediately after taking out of the refrigerator and do not require complicated thawing operations.
- After completing the ligation reaction, the product can be used for transformation as-is.

II. Contents of kit

Reagent	For 5 reactions*	For 50 reactions*
5×Ligation Mix	20 µl×1	100 µl×2
10×Enhancer solution	10 µl×1	50 µl×2

* Number of reactions when used in 20 µl reaction system

Vector DNA is not included in this kit.

III. Storage method

—20°C storage

- Since this product is not frozen at -20°C storage, there is no need to worry about reduction of stability due to freezing and thawing, and there is no need for complicated thawing operations.
- Handle on ice during usage.

IV. Protocol and experimental examples

1. Protocol

(1) Preparation of DNA solution

Prepare 14 µl of DNA solution by combining a proper molar ratio of vector DNA (about 0.025 pmol) and insert DNA fragment.[*1]

(2) Preparation of ligation reaction solution

Add 2 µl of 10 × Enhancer solution to 4 µl of 5 × Ligation Mix and mix.[*2]

(3) Ligation reaction

React at 16°C for 30 min.[*3]

(4) Transformation

Use the reaction solution for transformation as-is.[*4-6]

Vector DNA Insert DNA ddH_2O or TE [*1]	up to 14 µl
10 × Enhancer solution	2 µl
5 × Ligation Mix	4 µl
Total	20 µl

↓

React at 16°C for 30 min.[*3]

↓

Transformation[*4-6]

*** <u>The ratio of the amount of vector to 10 × Enhancer solution is very important.</u>**

Use about 0.025 pmol vector for the reaction in 20 µl reaction system（about 50 ng in the case of 3 kbp vector）.

*** When the ligation reaction is performed in a 10 µl reaction system, use half the amount of DNA, 10 × Enhancer solution and 5 × Ligation Mix that would be used in a 20 µl reaction system.**

2. Investigation of the vector : insert molar ratio

The vector : insert molar ratio in ligation greatly affects ligation efficiency. The tables below show the molar ratios at which the best results were obtained at Nippon Gene when insert DNAs of various lengths were ligated and used for transformation under the conditions described below.[*3]

1. Plasmid vector ligation

(1) Blunt end ligation

Insert size	200 bp	500 bp	1 kbp	3 kbp
Vector	1	1	1	1
Insert	6-10	3-6	3	0.5-1

Vector: pBluescript II SK(+) cleaved with *Eco*R V.
Stratagene Inc. (0.025 pmol)
Insert: Insert DNA cleaved with *Eco*R V
(0.0125 pmol, 0.025 pmol, 0.0375 pmol, 0.15 pmol, 0.25 pmol)
Ligation reaction: 16°C, for 30 min.

(2) TA cloning

Insert size	200 bp	500 bp	1 kbp	3 kbp
Vector	1	1	1	1
Insert	6-10	3-6	1-6	0.5-1

Vector: pGEM®-T
Promega Inc. (0.025 pmol)
Insert: PCR product
amplified by Gene *Taq* NT (Code No. 318-03231)
(0.0125 pmol, 0.025 pmol, 0.0375 pmol, 0.15 pmol, 0.25 pmol)
Ligation reaction: 16°C, 30 min

2. Linker ligation

Insert size	8 bp
Vector	1
Insert	700-1000

Vector: pUC19 (0.03 pmol) cleaved by *Hin*c II
Insert: Linker *Eco*R V
(0.09 pmol, 0.3 pmol, 1.5 pmol, 3 pmol, 6 pmol, 15 pmol, 21 pmol, 30 pmol)
Ligation reaction: 16°C, 30 min

3. Experimental example

< Experimental example 1: TA cloning >

Comparison to T4 DNA Ligase

(1) A 500 bp DNA fragment derived from lambda DNA was PCR amplified with *Taq* DNA Polymerase.

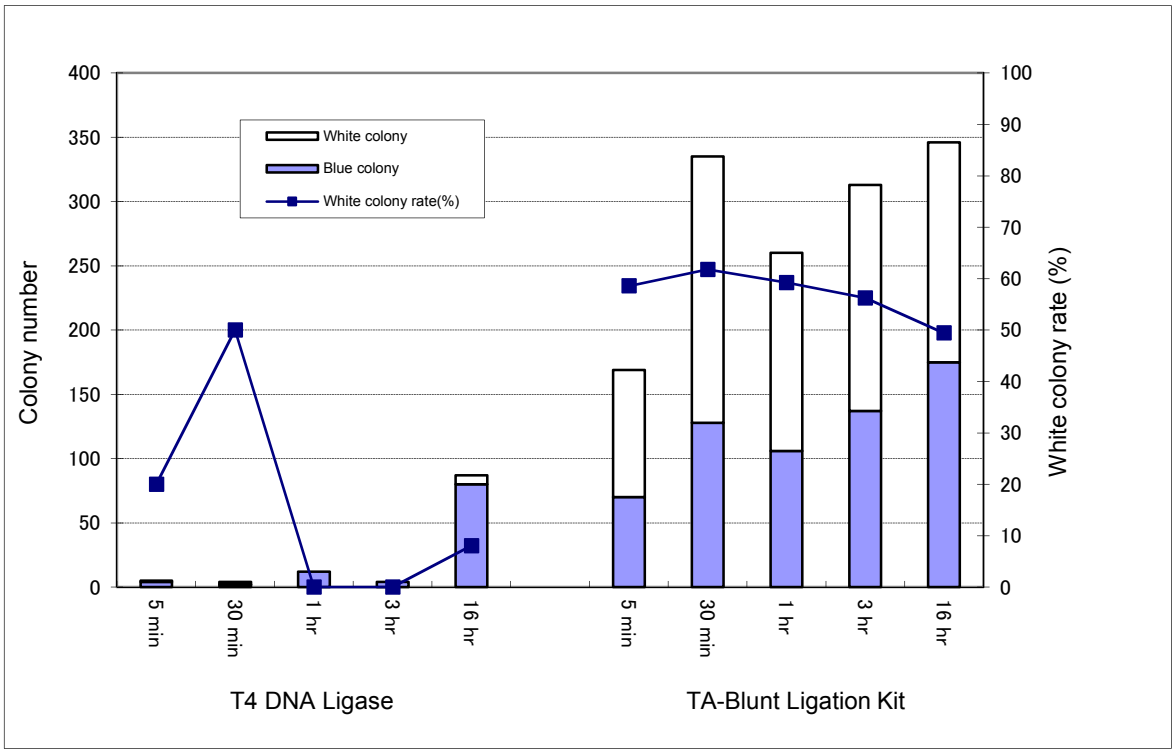
(2) Fourteen µl of DNA solution containing 50 ng of pGEM®-T vector (0.025 pmol) and 25 ng of insert DNA fragment (insert/vector molar ratio is about 3) was prepared.[*1]

(3) Two µl of 10 × Enhancer solution and 4 µl of 5 × Ligation Mix were added to 14 µl of the DNA solution and mixed.

(4) The mixture was reacted at 16°C for 5 min to 16 hrs.

(5) After the reaction, 1 µl of the reaction solution was used for transforming 30 µl of JM109 competent cells, and the generated colonies were counted. Also, as a control, a similar ligation reaction was performed using T4 DNA Ligase as a single body. The transformation efficiency of JM109 competent cells used in this experiment was 1.41×10^8 cfu/µg (pBR322 DNA).[*4, 5]

Effect of reaction time in TA cloning



Ligation reaction time and obtained number of white colonies

Reaction time	5 min	30 min	1 hr	3 hrs	16 hrs
TA-Blunt Ligation Kit	99	207	154	176	171
T4 DNA Ligase	1	2	0	0	7

Result

When the TA-Blunt Ligation Kit was used, it was found that ligation was sufficiently completed within a reaction time of 30 min.

< Experimental example 2: blunt end ligation >

Comparison with T4 DNA Ligase

(1) pBluescript II SK(+) was cleaved with *Eco*R V, dephosphorylated, extracted with phenol/chloroform/isoamyl alcohol, precipitated with ethanol and then dissolved in TE buffer.[*1]

(2) A 1000 bp DNA fragment derived from lambda DNA was cleaved with *Eco*R V.

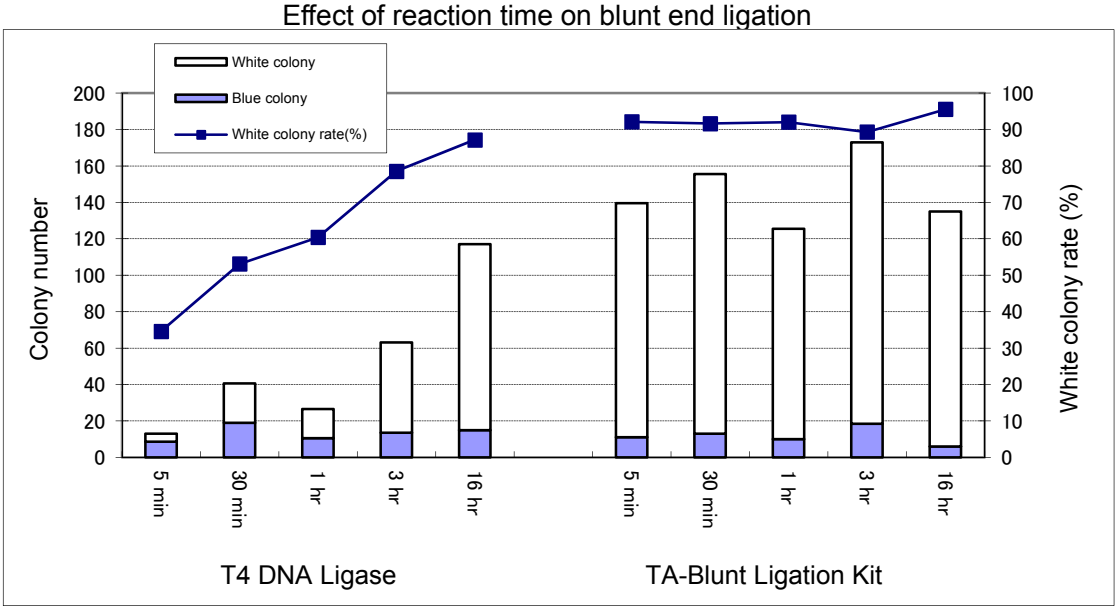
(3) Fourteen µl of DNA solution containing 50 ng of pBluescript II SK(+) and 50 ng of insert DNA fragment (insert/vector molar ratio is about 3) were prepared.[*1]

(4) Two µl of 10 × Enhancer solution and 4 µl of 5 × Ligation Mix were added to 14 µl of DNA solution and mixed.

(5) The mixture was reacted at 16°C for 5 min to 16 hrs.

(6) After the reaction, 1 µl of the reaction solution was used for transforming 30 µl of JM109 competent cells, and the generated colonies were counted. Also, as a control, a similar ligation reaction was performed using T4 DNA Ligase as a single body. The transformation efficiency of JM109 competent cells used in this experiment was 1.41×10^8 cfu/µg (pBR322 DNA).[*4, 5]

Effect of reaction time on blunt end ligation



Ligation reaction time and obtained number of white colonies

Reaction time	5 min	30 min	1 hr	3 hrs	16 hrs
TA-Blunt Ligation Kit	129	143	116	155	129
T4 DNA Ligase	5	22	16	50	102

Result

When the TA-Blunt Ligation Kit was used, it was found that ligation was sufficiently completed within a reaction time of 30 min.

< Experimental example 3: ligation reaction at a room temperature of 25ºC >

Comparison of 16ºC and 25ºC

（1） pBluescript II SK(+) was cleaved with *Eco*R V, dephosphorylated, extracted with phenol/chloroform/isoamyl alcohol, precipitated with ethanol and then dissolved in TE buffer.[*1]

（2） A 1000 bp DNA fragment derived from lambda DNA was cleaved with *Eco*R V.

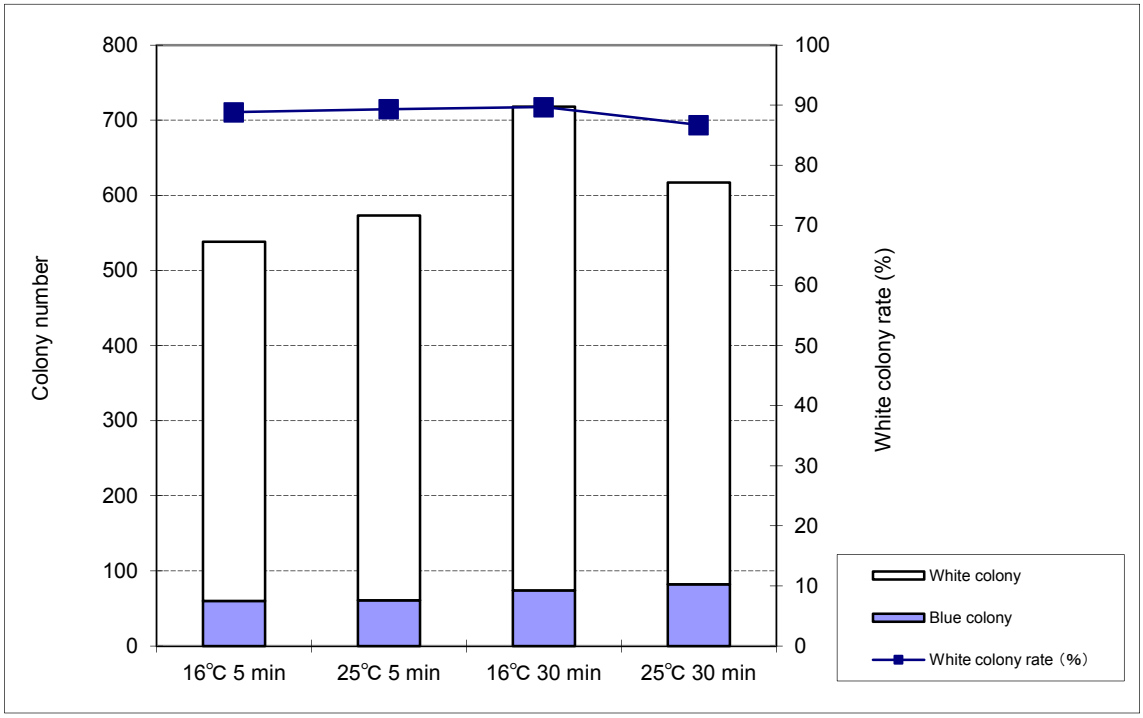
（3） Fourteen μl of DNA solution containing 50 ng of pBluescript II SK(+) and 50 ng of insert DNA fragment (insert/vector molar ratio is about 3) were prepared.[*1]

（4） Two μl of 10 × Enhancer solution and 4 μl of 5 × Ligation Mix were added to 14 μl of DNA solution and mixed.

（5） The mixture was reacted at 25ºC or 16°C for 5 min to 30 min.

（6） After the reaction, 1 μl of the reaction solution was used for transforming 30 μl of JM109 competent cells, and the generated colonies were counted. Also, as a control, a similar ligation reaction was performed using T4 DNA Ligase as a single body. The transformation efficiency of JM109 competent cells used in this experiment was 1.41×10^8 cfu/μg（pBR322 DNA）.[*4, 5]

Comparison of ligation efficiency at 16°C and 25°C



Result

Ligation could be performed at 25°C with a ligation efficiency equivalent to 16°C.

V. Caution

*1 Use about 0.025 pmol of the vector in the ligation reaction.
Also, when DNA is dissolved in a buffer containing a high concentration of salts and a buffer containing a high concentration of EDTA, ligation efficiency is markedly reduced. Prepare DNA solution in ddH_2O or TE buffer (pH 8.0).

*2 Measure the amount of 10 × Enhancer solution to be added precisely. If more than or less than a designated amount is added, ligation efficiency may be markedly reduced.

*3 Ligation efficiency may vary depending on the purity of DNA used for the ligation reaction or the restriction enzyme employed.

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

*5 If the volume of the reaction solution is 1/10 of the volume of the competent cells or more, treat the reaction solution with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1) after the ligation, recover DNA by ethanol precipitation, dissolve DNA in ddH_2O or TE buffer (pH 8.0) so that the volume is not more than 1/10 of the volume of the competent cells, and then perform transformation.

*6 When the transformation is performed by the electroporation method, treat the ligation product with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1), recover DNA by ethanol precipitation, dissolve DNA in ddH_2O or TE buffer (pH 8.0), and then perform transformation.

Q&A

Q: How should the ligation product be stored?

A: Store the product at -20°C. The product can be used for transformation experiments as-is after thawing.

Q: We have many samples. Would it be possible to prepare a pre-mix by mixing 10 × Enhancer solution and 5 × Ligation Mix before use?

A: You may prepare the pre-mix before use. However, prepare the pre-mix immediately before use and use it up immediately after preparing.

Q: Can the ligation reaction be performed at 4°C overnight?

A: Yes, the ligation reaction can be performed at 4°C overnight (16 hrs).

Q: Can the ligation product be heat-treated?

A: Yes, the ligation product can be treated at 70°C for 10 min.

Q: Can the ligation product be ethanol precipitated as-is?

A: When the ligation product is ethanol precipitated as-is, the number of colonies are markedly reduced. Perform ethanol precipitation after purifying with phenol/chloroform according to the method described in the manual(*5). Also, DNA can be recovered efficiently by ethanol precipitation using Ethachinmate.

Q: Can the ligation reaction be performed using DNA prepared by ethanol precipitation using Ethachinmate?

A: Yes.

VI. Troubleshooting

Trouble	Probable cause	Countermeasure
No ligation (no colony formation)	Mismatch of ends	Check ends.
	The ligation product is not purified when transformation is performed by electroporation.	Treat the ligation product with phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1), recover DNA by ethanol precipitation, dissolve DNA in ddH_2O or TE buffer (pH 8.0), and then perform transformation.
	The ligation product is ethanol precipitated as-is.	
Low ligation efficiency	There is too little or too much vector.	Adjust the amount of the vector to about 0.025 pmol in the 20 µl reaction system.
	There is too little or too much Enhancer solution.	Adjust Enhancer solution to be added to 1/10 of the volume of the reaction system.
	Enhancer solution is not added.	Add Enhancer solution at 1/10 of the volume of the reaction system.
	Concentration of the chelating agent such as EDTA is too high.	Dissolve DNA in TE buffer or water not containing the chelating agent and perform the ligation.
	Salt concentration of the DNA solution is too high.	Dissolve DNA in TE buffer or water not containing salts and perform the ligation.
	Ligation time is too short.	Extend the ligation time.
	The insert:vector ratio is not suitable.	Perform the ligation at an optimum molar ratio by consulting the insert:vector ratio in the manual.
	The vector has sticky ends.	This product cannot be used for sticky end ligation. We recommend to use Ligation-Convenience Kit.
Low transformation efficiency	Too much reaction solution is used.	Adjust the reaction solution to be used for the transformation to 1/10 of the volume of the competent cells. When *ECOS*™ Competent *E.coli* is used, the volume of the reaction solution to be added should be kept at 5% or lower to the competent cells.
	Low transformation efficiency of the competent cells.	Use competent cells having the transformation efficiency of not less than 1×10^7 cfu/µg.

High ratio of blue colonies	Dephosphorylation of the blunt end vector is insufficient.	Confirm the presence or absence of self-ligation beforehand.
	The T protrusion of the T vector is missing.	Use the T vector in which the T protrusion is preserved.

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