www.toyobo.co.jp/e/bio

Instruction manual Ligation high Ver.2 0810

F1016K

Ligation high Ver.2

LGK-201 750 µl (100 reactions)
Store at -20°C

Contents

[1] **Introduction**

[2] **Components**

[3] **Protocol**

[4] **Application data**

Example 1. Cohesive end ligation
Example 2. Blunt end ligation
Example 3. Linker ligation
Example 4. TA cloning using purified PCR products
Example 5. TA Cloning using unpurified PCR products
Example 6. Phage vector ligation

[5] **Troubleshooting**

CAUTION
All reagents in this kit are intended for research purposes. Do not use for diagnostic or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

[1] Introduction

Description

The ligation reaction is an essential step in genetic recombination experiments. For this reaction, T4 DNA ligase has been widely used. Ligation high Ver.2 is a highly efficient premixed T4 Ligase reagent.

Features

- Effective ligation of cohesive, blunt and A overhang DNA fragments can be achieved.

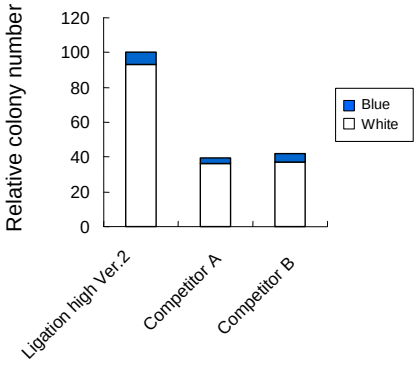

Fig. 1 Comparison of TA cloning efficiency
Colony number of Ligation high Ver.2 corresponds to 100

- Will not freeze at -20℃. No need to thaw.
- Just mix ligation high Ver.2 with an equal volume or with double the volume of the solution containing DNA fragments.

[2] Components

Ligation high Ver.2 750 µl × 1Vial (100 reactions)

Notes:

In the case of -30℃ storage or long-term storage under -20℃, the reagent might freeze or form white precipitates. The frozen reagent can be thawed by incubation at room temperature in short time. No decrease in reaction efficiency is observed following 5 freeze-thaw cycles. The white precipitates should be dissolved prior to use.The white precipitates can also be dissolved by incubation at room temperature with vortexing at low speed in short time.

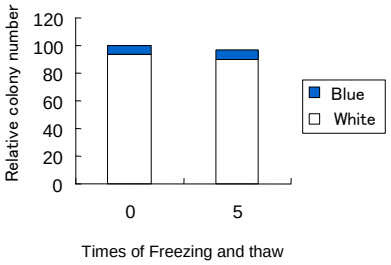

Fig. 2 Effect of freezing and thaw for activity of Ligation high Ver.2
Ligation efficiency was estimated by the TA cloning test.
Colony number at 0 times of freezing and thaw corresponds to 100.
No negative effect was detected by 5-time-freezing and thaw.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

[3] Protocol

Vector DNA＋Insert DNA[*1]	7.5 µl
Ligation high Ver.2	3.75-7.5 µl[*2]
16℃、30 min.[*3*4]	
↓	
Transform 100 µl competent cells using 1 -10 µl reaction solution [*5]	

*1 Ligation efficiency is decreased by adding excess salts. DNA fragments should be in low salt solutions (e.g. 10 mM Tris-HCl [pH 8.0], 1 mM EDTA)
*2 Adding an equal volume of Ligation high Ver.2 to the DNA solution is recommended in the case of TA cloning.
*3 The reaction can be prolonged for up to 2 hr. Standard cohesive end ligations can be completed within 5 min.
*4 The reaction can be performed at 4℃-25℃.
*5 The volume of ligation reaction added should be less than 10% of the volume of the competent cell suspension. For electro-transformation, ligation reactions should be purified, because salts inhibit the transformation.

Table 1 Recommended reaction conditions

	DNA : Ligation reagent (Volume)	Reaction condition
Cohesive end ligation	1 : 1 or 1 : 0.5	16℃、5-30 min
Blunt end ligation	1 : 1 or 1 : 0.5	16℃、30 min
Linker ligation	1 : 1 or 1 : 0.5	16℃、30 min
T vector ligation (Purified PCR procuct)	1 : 1	16℃、30 min
T vector ligation [*1*2] (Unpurified PCR product)	1 : 1	16℃、30 min
Phage vector ligation	1 : 1 or 1 : 0.5	16℃、30 min

*1 When PCR reactions contain primer dimers or unexpected PCR products, the desired products should be purified.

*2 When sufficient amplification is confirmed, 0.5-1 µl of PCR product can be used directly. The reaction conditions are as follows;

H_20	X
PCR product	0.5-1
Ligation high Ver.2	7.5
	15 (µl)

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

[4] Application data

Example 1. Cohesive end ligation

Dephosphorylated pUC19/*Hin*dIII, (50 ng, 25 fmol,) was mixed, at different ratios, with the 546 bp DNA fragment of *Hin*dIII digested phage lambda DNA. An equal volume of Ligation high Ver.2, 7.5 µl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 µl of reaction mixture, and cultured on LB/Amp (X-Gal) plates O/N.

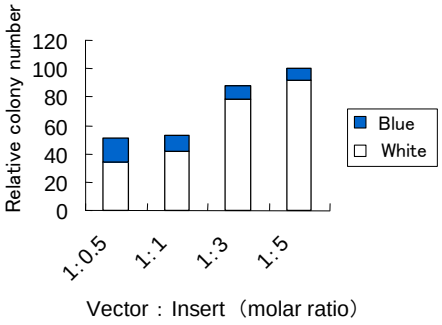

Fig. 3 Cohesive end ligation
Colony number at 1:5 corresponds to 100

Example 2. Blunt end ligation

Dephosphorylated pUC19/*Hin*cII, (50 ng, 25 fmol) was mixed, at different ratios, with purified PCR product (500 bp: from high-fidelity PCR enzyme[*1]). Half or an equal volume of Ligation high Ver.2, 3.75 or 7.5 µl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 µl of reaction mixture, and cultured on LB/Amp (X-Gal) plates O/N.

[*1]High-fidelity PCR enzyme generates blunt ends.

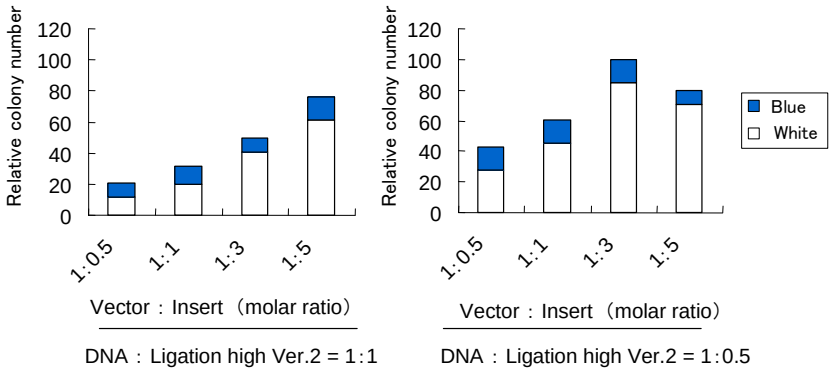

Fig. 4 Blunt end ligation
Colony number on the condition of DNA : Ligation high Ver.2 = 1: 0.5 and Vector : Insert = 1 : 3 corresponds to 100.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

Example 3. Linker ligation

Dephosphorylated pUC19/*Hinc*II（50 ng, 25 fmol）was mixed at different ratios with phosphorylated *Hin*dIII linker (10mer). Half or an equal volume of Ligation high Ver.2, 3.75 or 7.5 µl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 µl of reaction mixture, and cultured on LB/Amp (X-Gal) plates O/N.

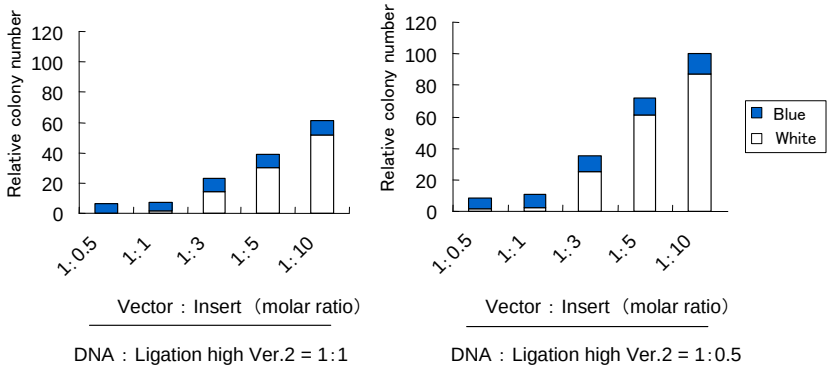

Fig. 5 Linker ligation

Colony number on the condition of DNA ： Ligation high Ver.2 = 1 : 0.5 and Vector ：Insert = 1 : 10 corresponds to 100.

Example 4. TA cloning using purified PCR products

T vector,（50 ng, 25 fmol）was mixed, at different ratios, with purified PCR products (500 bp from Taq DNA polymerase). An equal volume of Ligation high Ver.2, 7.5 µl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 µl of reaction mixture, and cultured on LB/Amp (X-Gal) plates O/N.

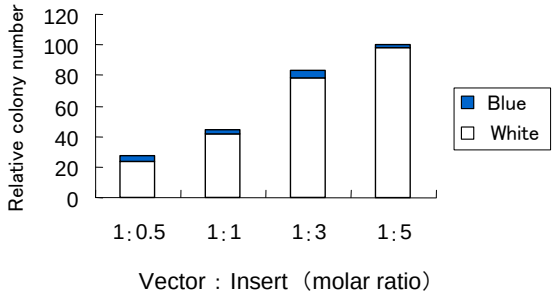

Fig. 6 TA cloning using purified products

Colony number at 1:5 corresponds to 100

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

Example 5. TA Cloning using unpurified PCR products

T vector,（50 ng, 25 fmol）was mixed, at different ratios, with unpurified PCR products (500 bp from Taq DNA polymerase). An equal volume of Ligation high Ver.2, 7.5 µl, was added and incubated at 16℃ for 30 min. *E. coli* DH5α competent cells were transformed using 10 µl of reaction mixture, and cultured on LB/Amp (X-Gal) plates O/N.

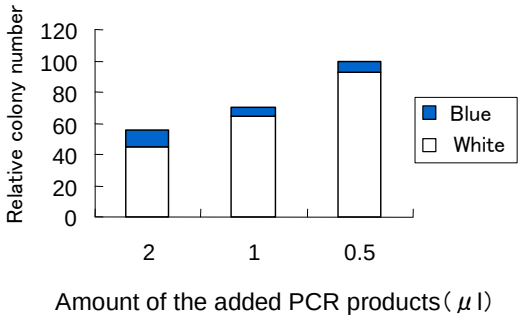

Fig. 7 TA cloning using unpurified products
Colony number on the condition of adding 0.5 µl of the PCR product corresponds to 100.

Example 6. Phage vector ligation

λ ZAP®II (500 ng), *Eco*RI digested, and pRheo/*Eco*RI test insert（225 ng, 2.8 kb）, were mixed with half or with an equal volume of Ligation high Ver.2, 3.75 or 7.5 µl, and incubated at 16℃ for 30 min. in vitro packaging was performed with 1.5 µl ligation reaction using GIGAPACK®III（Stratagene） followed by infection of *E. coli* XL-1 Blue MRF'. The *E. coli* cells were cultured O/N and plaques counted.

Table 2. Reaction conditions and efficiency

DNA soln： Ligation reagent	1 ： 0.5	1 ： 1
Efficiency (pfu/µg ZAP®II)	6.3×10^6	5.5×10^6

* λ ZAP® and GIGAPACK® are registered trademarks of STRATAGENE.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

[5] Troubleshooting

Symptom	Cause	Solution
No or low colony number	Excess amount of ligation mixture for transformation	Unpurified ligation mixtures should be added at less than 10%(V/V) of competent cell suspensions.
	Vector concentration is low	Increase the vector concentration. (See [4] Application data)
	DNA insert fragment concentration is too low.	Increase the DNA insert fragment concentration. (See [4] Application data)
	Excess salts	・DNA fragments should be dissolved in a low salt solution. ・Unpurified PCR products should be added at 0.5-1 µl per ligation reaction.
	Ends of both vector and insert are dephosphorylated.	Dephosphorylated vectors cannot ligate with unphosphorylated PCR products.
Cloning efficiency was low	Primer dimer	When the PCR reaction contains primer dimers or unexpected PCR products, the desired PCR product should be purified.
	Insufficient time for ligation reaction .	The reaction can be prolonged for up to 2hr.
	Insert fragment concentration is too low.	Increase the insert fragment concentration. (See [4] Application data)
	Self-ligation of vector	Use dephosphorylated vector.
Excess number of colonies	Self-ligation of vector	Use dephosphorylated vector.
Freezing of the reagent	Storage conditions	In the case of -30℃ storage or long-term storage under -20℃, the reagent might freeze. The frozen reagent can be thawed by incubation at room temperature in short time. No decrease in reaction efficiency is observed following 5 freeze-thaw cycles.
White precipitates are formed	Storage condition	In the case of -30℃ storage or long-term storage under -20℃, the reagent might form white precipitates. The precipitates should be dissolved prior to use. The precipitates can be dissolved by incubation at room temperature with vortexing at low speed in short time.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140