DNA Ligation Kit, Mighty Mix (Cat.# 6023)

Application: Use of DNA Ligation Kit, Mighty Mix for cohesive- and blunt- end ligation

The DNA Ligation Kit, Mighty Mix (Cat. # 6023) kit contains a single, pre-mixed ligation solution that is suitable for all types of ligations. This experiment demonstrates the use this kit for cohesive end ligation (Example 1) and blunt end ligation (Example 2).

Example 1: Cohesive (sticky) end ligation

In this example, a λ DNA fragment digested with *Hin*dIII is inserted into pUC118 that was also digested with *Hin*dIII.

Methods

A total of 50 ng (25 fmol) of *Hin*dIII-digested, BAP treated pUC118 vector (Cat. #3324) was mixed with 2.5 - 150 fmol of a 564 bp *Hin*dIII-digested λ DNA fragment at insert:vector ratios ranging from 0.1 to 6. The total volume of DNA was 7.5 µl. One volume (7.5 µl) of Ligation Mix was added to the DNA solution. The reaction mixture was then incubated at 16°C for 30 minutes.
An aliquot of the ligation reaction was used directly to transform *E. coli* JM109 competent cells. Colonies were selected on LB-Amp plates containing X-Gal and IPTG. (The transformation efficiency of *E. coli* JM109 competent cells was 7.3×10^8 cfu per µg pUC118 DNA.)

Results

The transformation efficiencies, obtained by counting the number of white colonies, are listed in Table 1 and graphically depicted in Figure 1.

Table 1. Transformation efficiency using different molar ratios of *Hin*dIII-digested vector and insert.

Insert/vector (molar 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

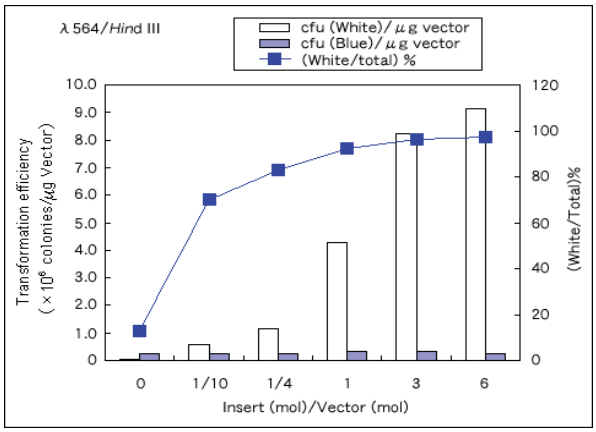

Figure 1. Cohesive-end ligation of a *Hin*dIII-digested λ DNA fragment (564 bp) with *Hin*dIII-digested, BAP treated pUC118 vector.

- Continued -

Example 2: Blunt End Ligation

Methods

A total of 50 ng (25 fmol) of *Hin*cII-digested, BAP-treated pUC118 vector (Cat. #3322) was mixed with 2.5 - 150 fmol of a 500 bp *Hin*cII-digested DNA fragment at insert:vector ratios ranging from 0.1 to 6. The total volume of DNA was 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°C for 30 minutes.

A portion of the solution was used directly to transform *E. coli* JM109 competent cells. Colonies were selected 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.)

Results

The transformation efficiencies, obtained by counting the number of white colonies, are listed in Table 2 and graphically depicted in Figure 2.

Table 2. Transformation efficiency using different molar ratios of *Hin*cII-digested vector and insert.

Insert/vector (molar ratio)	(colonies/µg vector)		White colonies/total colonies (%)
	White	Blue	
-	1.6×10^4	2.1×10^5	6.7
1/10	5.6×10^5	2.3×10^5	70.6
1/4	1.1×10^6	2.2×10^5	83.0
1	4.2×10^6	3.0×10^5	93.4
3	7.5×10^6	2.6×10^5	96.7
6	6.7×10^6	2.7×10^5	94.8

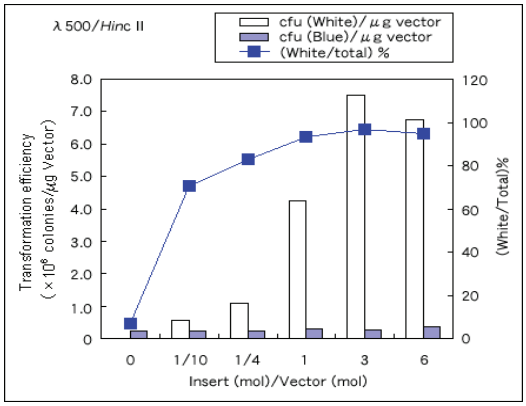

Figure 2. Blunt-end ligation of a *Hin*cII-digested DNA fragment (500 bp) with *Hin*cII-digested, BAP-treated pUC118 vector.

- Continued -

For both ligation experiments, the PerfectShot™ Insert Check PCR Mix (Cat. #RR020A, discontinued)* was used to determine whether the white colonies contained the expected DNA inserts (Table 3).

Table 3. The ratio of colonies containing correct insert to white colonies.

Insert	Insert:Vector	
	0.1 : 1	**3: 1**
λ564/*Hin*dIII	21/24	24/24
λ500/*Hin*cII	24/24	22/24

Conclusions

An insert:vector ratio of approximately 3 is recommend when performing ligations with this product. In general, a greater number of white vs. blue colonies were obtained when the insert:vector ratio was greater than 1. No further improvement in ligation efficiency was observed when the ratio was greater than 6.

Moreover, for both cohesive end and blunt end reactions, the majority of transformants contained the desired ligation product.