

Product Data Sheet

Product Name: T4 DNA Ligase(with 10X Ligation Buffer)

Cat. No.: GF03018

Protocol

Instructions:

1. Connection of PCR products or enzyme-digested fragments with a regular vector:
 - a. Take 1-2 µg of the vector, digest with the enzyme overnight, or for at least 3-5 hours. Ensure thorough digestion to avoid excessive self-ligation in subsequent steps.
 - b. After digesting the vector, you can use a purification kit, such as a PCR purification kit/DNA purification kit, for purification. You can also use the conventional phenol-chloroform extraction and ethanol precipitation method for vector purification. For cases where the enzyme digestion produces larger fragments (greater than 50-60 bp), gel purification is recommended.
 - c. For PCR products: After gel electrophoresis of PCR products, recover DNA fragments of the expected size. Gel purification of DNA fragments can be done using a kit, such as a DNA gel extraction kit. Alternatively, you can use methods like repeated freeze-thaw cycles for DNA fragment recovery.
 - d. For recovered PCR products or other plasmids or DNA fragments that need enzyme digestion, digest with an appropriate restriction enzyme and then purify the digested product.
Note: Enzyme digestion in this step does not need to be overly thorough. Typically, an enzyme digestion efficiency of over 80-90% is sufficient. Enzyme digestion in this step usually takes 1-2 hours. Enzyme-digested products can be purified using a kit, such as a PCR purification kit/DNA purification kit, or by conventional phenol-chloroform extraction and ethanol precipitation.
 - e. Take approximately 25-100 ng of the enzyme-digested and purified vector and add 3 times the molar amount of the insert fragment. Refer to the table below for setting up the ligation reaction system.

Note 1: Often, it is challenging to quantify the amounts of vector and insert fragment due to their low quantities after recovery. In such cases, you can estimate roughly based on the brightness of the gel bands before recovery. Typically, you can use one of the bands from a DNA molecular weight marker as a reference and estimate the ratio of the brightness of your target band to that reference band. Then, calculate the ratio of the final amounts of vector and insert fragment based on the expected yield during purification or gel recovery.

Note 2: Usually, using 0.2-0.5 µl of ligase per reaction is sufficient. If you want to further increase ligation efficiency, you can increase the amount of ligase to 1 µl.

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Vector	About 50-100ng
Insert Fragment	Approximately 3 times the molar amount of the vector
10X Ligation Buffer	2 µl
Double distilled water or Milli-Q water	to 20 µl
T4 DNA ligase	0.2-0.5 µl
Total volume	20 µl

f. Gently mix by pipetting or mild vortexing, centrifuge briefly at room temperature to collect the liquid at the bottom of the tube.

g. Incubate the ligation reaction at 20-25°C for 1-2 hours or at 16°C overnight.

Note 1: For blunt-end ligation, overnight incubation is necessary. For blunt-end ligation, using T4 DNA ligase alone results in lower efficiency; it is recommended to use Biyuntian's fast DNA ligation kit (D7002 or D7003).

Note 2: For rapid screening of expected clones, you can follow this approach: After a 1-2 hour incubation of a 20 µl blunt-end ligation reaction, take 10 µl for direct transformation into E. coli, and incubate the remaining 10 µl at 16°C overnight. If you obtain the desired clones the next day, you can proceed to the next experiment. If the transformed E. coli the next day does not yield the expected clones, you can use the remaining ligation products from the overnight incubation for a second round of E. coli transformation.

h. Subsequently, you can directly use the ligation products for transformation into competent bacteria.

2. Connection of PCR products and T vector:

a. After gel electrophoresis of PCR products, recover DNA fragments of the expected size. Gel purification of DNA fragments can be done using a kit, such as a DNA gel extraction kit. Alternatively, you can use methods like repeated freeze-thaw cycles for DNA fragment recovery.

b. Take an appropriate amount of the T vector according to the T vector's manual and add 3 times the molar amount of the insert fragment. Refer to the table below for setting up the ligation reaction system.

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Note 2: Usually, using 0.2-0.5 μ l of ligase per reaction is sufficient. If you want to further increase ligation efficiency, you can increase the amount of ligase to 1 μ l.

T Vector	Appropriate amount
Insert Fragment	Approximately 3 times the molar amount of the vector
10X Ligation Buffer	2 μ l
Double distilled water or Milli-Q water	to 20 μ l
T4 DNA ligase	0.2-0.5 μ l
Total volume	Approximately 20 μ l

c. Gently mix by pipetting or mild vortexing, centrifuge briefly at room temperature to collect the liquid at the bottom of the tube.

d. Incubate the ligation reaction at 20-25°C for 1-2 hours or at 16°C overnight. Note: For rapid screening of expected clones, you can follow this approach: After a 1-2 hour incubation of a 20 μ l blunt-end ligation reaction, take 10 μ l for direct transformation into E. coli, and incubate the remaining 10 μ l at 16°C overnight. If you obtain the desired clones the next day, you can proceed to the next experiment. If the transformed E. coli the next day does not yield the expected clones, you can use the remaining ligation products from the overnight incubation for a second round of E. coli transformation.

e. Subsequently, you can directly use the ligation products for transformation into competent bacteria.

3. Connection of Linker or RNAi fragments with a vector:

a. Perform vector digestion and purification steps as in Step 1a and 1b.

b. Annealing of Linker or RNAi fragments can be done using an appropriate DNA annealing buffer, such as Annealing Buffer for DNA Oligos (5X).

c. For Linker or RNAi fragments longer than 8 bp, perform the ligation reaction with the vector in a

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ratio of 5:1 to 10:1. For example, if the vector is 0.03 pmol, the insert fragment can be 0.15 to 0.3 pmol. For Linker fragments shorter than 8 bp, adjust the ratio to be greater than 10:1.

d. Follow steps 1e-1h, in addition to the insert fragment amount.

4. Connection of DNA self-circularization:

Refer to Step 1e, replacing the insert fragment with an appropriate amount of water. Follow the remaining steps as in steps 1f-1h.

5. For other types of DNA fragment connections, refer to the methods mentioned above.

Common Issues:

1. Low transformation efficiency or very few positive clones after ligation reaction:

a. The competent bacteria may have low transformation efficiency; use plasmid as a positive control and simultaneously test the transformation efficiency of competent cells.

b. Try to improve the purity of the vector or insert fragments. For blunt-end ligation, consider extending the ligation time appropriately.

c. The vector may not have been digested thoroughly; use undigested vector as a negative control.

d. Use the storage solution for DNA as a negative control to check if there are issues with the competent bacteria.

Storage:-20°C

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