Protocol

Ligation Protocol

Utilizing T4 DNA Ligase

Introduction

GoldBio T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. GoldBio T4 DNA ligase joins DNA fragments with either cohesive or blunt termini and repairs single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids. In addition, GoldBio T4 DNA ligase can be used to clone restriction enzyme-generated DNA fragments and PCR products. T4 DNA ligase can also be used for Next-gen library preparation and self-circularization of linear DNA. Finally, T4 DNA ligase can also join linkers and adapters to cohesive or blunt-ended DNA and repair nicks in duplex DNA, RNA, or DNA/RNA hybrids. Here, we describe a general protocol for the use of GoldBio T4 DNA Ligase to ligate vector and insert DNA.

Materials

- T4 DNA Ligase (GoldBio Catalog # T-410 or T-411)
- 10x T4 DNA Ligase Reaction Buffer

Not supplied:

- H_2O PCR grade

Note: T4 DNA Ligase is inhibited by metal chelators, phosphate and ammonium ions, KCl and NaCl at a concentration higher than 50mM. It can be inactivated by incubating at 70°C for 15 minutes or by adding EDTA.

Note: The storage buffer contains 50mM Tris-HCl, 50mM KCl, 1mM DTT, 0.1mM EDTA, 50% glycerol, pH 7.5 at 25°C.

Note: 1x T4 DNA Ligase Reaction Buffer contains 50mM Tris-HCl, 10mM $MgCl_2$, 10mM DTT, 1mM ATP, pH 7.5 at 25°C.

Note: One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (250 ng/µl) in a total reaction volume of 20 µl in 30 minutes at 16°C in 1x T4 DNA ligase reaction buffer.

Storage and Handling

- Store GoldBio T4 DNA Ligase and the 10x T4 DNA Ligase Reaction Buffer at -20°C.

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- These products may be shipped on blue ice and should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.
- Thaw on ice and mix by gentle vortexing. After thawing, these products should be kept on ice before use. These products can be refrozen for storage.

Method

1. Set up a reaction according to Table 1, in a microcentrifuge tube, on ice. Use a molar ratio of 1:3 vector to insert DNA (see Calculations section).

Table 1. Set up of ligation reaction (400 units/µl Ligase)

Component	20 µl Reaction
Vector DNA	x µl
Insert DNA	x µl
10x T4 Ligase Buffer	2.0 µl
T4 DNA Ligase	1.0 µl
Nuclease-free water	Up to 20.0 µl

2. Gently mix the reaction and centrifuge briefly.

3. For cohesive ends, incubate at room temperature for 10 minutes or 16°C overnight.

4. For blunt ends, incubate at room temperature for 2 hours or 16°C overnight.

5. Heat-inactivate at 70°C for 15 minutes.

6. Cool on ice and transform 2 µl of the reaction into 50 µl of competent cells.

Calculations

1. To calculate ng of insert to add to ligation reaction with known amount of vector, first calculate the ratio coefficient (x).

$$\frac{bp\ size\ vector}{bp\ size\ insert} = x$$

2. Determine the quantity of vector (y) to use.

Note: Generally, 10 ng of vector should be sufficient.

3. Calculate the quantity of insert to use at 1:1 ratio (z).

$$\frac{y}{x} = z$$

4. Calculate the quantity of insert needed for a 3:1 insert:vector molar ratio ligation reaction.

$$3 \times z = Quantity\ of\ insert$$

Associated Products

- T4 UvsX DNA Recombinase (GoldBio Catalog # T-414)
- T4 UvsY Protein (GoldBio Catalog # T-415)
- T4 gp32 Protein (GoldBio Catalog # T-416)
- T4 DNA Helicase (gp41) (GoldBio Catalog # T-417)
- T4 gp46 Protein (GoldBio Catalog # T-418)
- T4 gp47 Protein (GoldBio Catalog # T-419)
- T4 gp59 Protein (GoldBio Catalog # T-420)
- T4 DNA Ligase – 2000 units/µl (GoldBio Catalog # T-411)

References

Engler, M. J. and Richardson, C. C. 1982. DNA Ligases. In: P.D. Boyer (Ed.) The Enzymes, vol. XV, Academic Publishers, New York, pp. 3-29.