Protocol

TD-P Revision 1.0

Creation Date: 5/31/2018
Revision Date: 7/17/2018

Blunting DNA ends by 3'-overhang removal or fill-in of 3' recessed-end

Utilizing T4 DNA Polymerase

Introduction

Polymerase Chain Reaction (PCR) is a powerful technique used to amplify DNA through the use of the enzyme T4 DNA Polymerase. GoldBio T4 DNA Polymerase has DNA-dependent DNA polymerase activity and potent 3'→5' exonuclease activity. GoldBio T4 DNA Polymerase is ideal for 3'-overhang removal or 5'-overhang fill-in to form blunt ends, probe labeling using replacement synthesis, DNA library preparation for Next-generation sequencing, ligation-independent cloning of PCR products and second strand synthesis in site-directed mutagenesis. Here, we describe a general protocol for the use of GoldBio T4 DNA Polymerase in the blunting of ends by 3' overhang removal or 3' recessed-end fill-in.

Materials

- T4 DNA Polymerase (GoldBio Catalog # T-412)
- 10x T4 DNA Polymerase Buffer

Not supplied:

- 100µM dNTPs (GoldBio Catalog # D-900)
- H_2O PCR Grade

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

Note: 10x T4 DNA Polymerase Buffer contains 500mM Tris-HCl, 100mM $MgCl_2$, 50mM DTT, pH 7.5 at 25°C.

Note: T4 DNA Polymerase concentration is 5 units/ul. One unit of T4 DNA Polymerase converts 10 nmol of dNTPs into acid-insoluble material in 30 minutes at 37°C under standard assay conditions.

Note: T4 DNA Polymerase is inactivated by incubating at 70°C for 15 minutes.

Storage and Handling

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

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- 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

Blunting ends by 3' overhang removal or 3' recessed-end fill-in.

1. Dissolve linear DNA or PCR product in 1x reaction buffer supplemented with 100µM dNTPs.

2. Add 1 unit T4 DNA Polymerase per µg DNA.

3. Incubate at room temperature for 5-30 minutes.

4. Stop the reaction by heating at 70°C for 20 minutes.

Associated Products

- dNTP Mix (GoldBio Catalog # D-900)
- T4 Polynucleotide Kinase (PNK) (GoldBio Catalog # T-413)
- 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 – 400 units/µl (GoldBio Catalog # T-410)
- T4 DNA Ligase – 2000 units/µl (GoldBio Catalog # T-411)

References

Tabor, S. and Struhl, K. 1989. DNA-dependent DNA polymerases. In F. M. Ausebel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl (Ed.). *Current Protocols in Molecular Biology*, 3.5.10-3.5.12. New York: John Wiley & Sons, Inc.

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. (2nd ed), 5.44-5.47. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Kunkel, T. A., Roberts, J. D. and Zakour, R. A. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol*, 154:367-82.