Limited liability company
«Biolabmix»
TIN 5408278957 CAT 540801001
630090, Novosibirsk obl., Novosibirsk,
st. Injenernaya, building № 28
Tel/Fax: +7(383)363-51-91, Tel: +7(383)363-22-40
E-mail: sales@biolabmix.ru

Fusion DNA polymerase (Pfu-Sso7d)

Cat. Number: E-11001, E-11005

Enzyme description:

The Fusion DNA polymerase is a recombinant polypeptide consisting of fused thermostable DNA polymerase *Pyrococcus furiosus* (Pfu) and DNA-binding protein of thermophilic archaea *Saccharolobus solfataricus* (Sso7d). The Sso7d protein binds to the minor groove of double-stranded DNA and additionally stabilizes the polymerase complex with the template. As a result, the Fusion DNA polymerase has increased processivity, fidelity, and resistance to PCR inhibitors compared to native Pfu DNA polymerase [1]. The Fusion DNA polymerase has 5'→3' polymerase activity, 3'→5' exonuclease activity, and synthesizes products with blunt ends.

Applications

DNA polymerase fusion is a good choice for routine gene cloning and can be used to generate long or hard amplicons by PCR. Examples of the use of Fusion DNA polymerase are presented at the end of the description.

Source

Fusion DNA polymerase was isolated from an *E. coli* strain containing a plasmid with cloned fusion genes for *Pyrococcus furiosus* thermostable DNA polymerase (Pfu) and *Saccharolobus solfataricus* DNA-binding protein (Sso7d).

Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 74°C.

Enzyme concentration and packaging: 2 U/µl.

Cat. No.	Product Name	Quantity	Volume
E-11001	Fusion DNA polymerase	100 U	50 µl
E-11005		500 U	250 µl

Storage Buffer

20 mM Tris-HCl (pH 7.5 at 25°C), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml BSA, 0.1% Tween 20, 50% glycerol.

Quality Control

Each batch of the enzyme is tested for electrophoretic purity in SDS-PAGE, enzyme activity, and nonspecific DNase activity.

Protocol for standard PCR reaction

1. Mix individual components in a tube according to the table (for optimal results keep the components and reaction mixture on ice):

Component	Reaction mixture 25 µl	Reaction mixture 50 µl	Final concentration
5x Fusion DNA Polymerase Reaction Buffer [1]	5 µl	10 µl	1x
50x dNTP mix (10 mM each, NM10-0100)	0,5 µl	1 µl	200 µM of each dNTP
Forward primer, 2 µM	2,5 µl	5 µl	200 nM [2]
Reverse primer, 2 µM	2,5 µl	5 µl	200 nM [2]
Template DNA	variable	variable	1 pg - 250 ng [3]
Fusion DNA polymerase, 2 u/µl	0,5 µl	1 µl	2.0 u/50 µl reaction mixtures
Nuclease-free water	up to 25 µl	up to 50 µl	-

[1] 5x Reaction buffer supplied with enzyme: 1.3 ml for E-11001 and 6.5 ml for E-11005 (1x buffer contains 2 mM $MgCl_2$).
[2] primer concentration can vary within 10-500 nM.
[3] for genomic DNA, it is recommended to use from 50 to 250 ng per reaction, for plasmids and viral DNA - from 1 to 10 ng.

2. Gently mix the contents of the tube and remove droplets by short centrifugation.
3. Transfer the tubes with the reaction mixture to a preheated cycler (95-98°C).
4. Use the following program for standard PCR:

Step	Temperature and time	The number of cycles
Initial denaturation	95-98°C, 120-30 seconds	1 cycle
Denaturation	95°C, 5-10 seconds	25-35 cycles
Primer annealing [1]	50-72°C, 10-30 seconds	
Elongation	72°C, 15-30 seconds /1 kb	
Final extension	5-10 minutes	1 cycle

[1] When amplifying large fragments (≥ 5 kb), it is recommended to skip this step.

5. Analyze PCR products in agarose gel. Samples must first be mixed with gel loading buffer.

Storage and transportation conditions

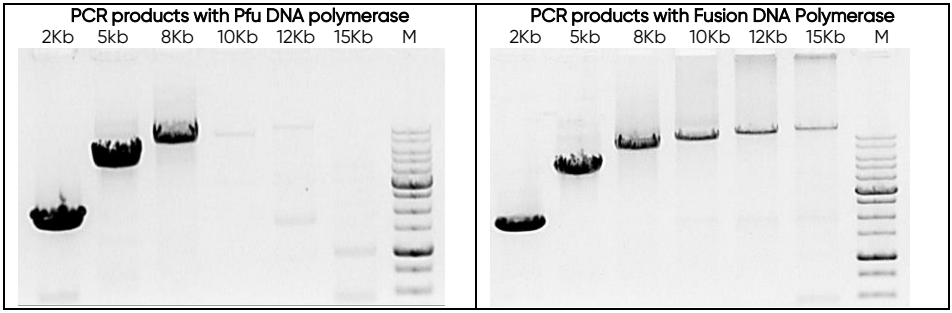
Store at -20°C.
Transportation at temperatures not exceeding +8°C is allowed for up to three days.

References

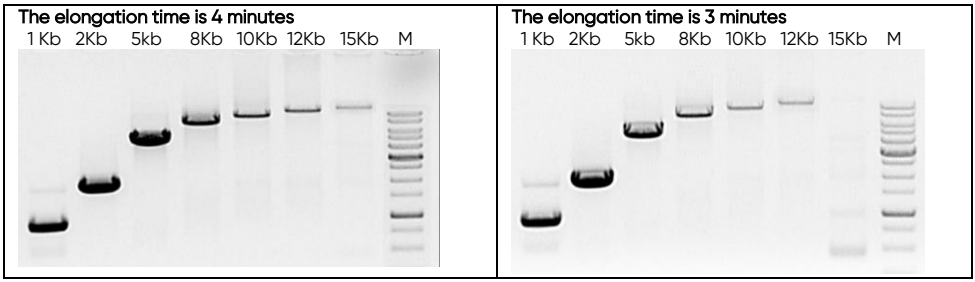
1. Wang, Y., Prosen, D. E., Mei, L., Sullivan, J. C., Finney, M., & Vander Horn, P. B. (2004). A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance in vitro. Nucleic acids research, 32(3), 1197-1207. DOI: 10.1093/nar/gkh271

Examples of using Fusion DNA polymerase compared to Pfu DNA polymerase, which was used as a precursor in the construction of Fusion DNA polymerase. The reactions were based on the conditions described by Wang Y. et al. [DOI: 10.1093/nar/gkh271]. The DNA of lambda phage (0.5 ng/µl) was used as a template, and the primer structure was indicated in the mentioned work. The reaction program was two-stage, and the total number of cycles was 25.

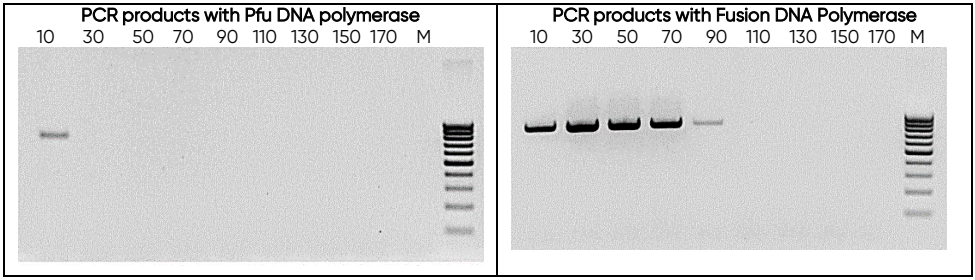
1. Comparison of Fusion DNA polymerase and Pfu DNA polymerase to synthesize long fragments. The elongation time was 7.5 minutes in all cases. M - DNA ladder "Sky-High" (S-8000).



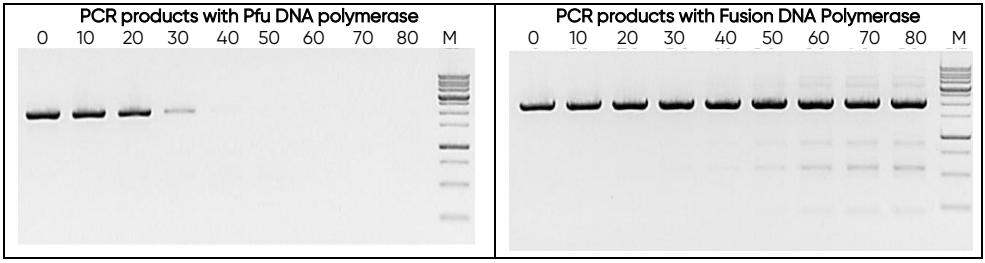
2. Analysis of the speed of DNA fragment synthesis using Fusion DNA polymerase. Only the elongation time was changed (4 and 3 minutes, respectively).



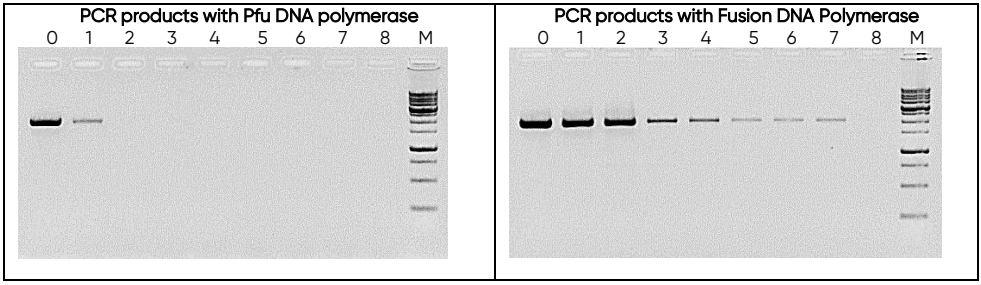
3. Analysis of the resistance of Pfu DNA polymerase and Fusion DNA polymerase to salt using the example of amplification of a 0.9 Kb fragment in the presence of potassium chloride. Elongation time was 1 minute. Lanes: 0-170, samples with potassium chloride added to the reaction mixtures up to 170 mM, respectively; M - DNA ladder "Step 100" (S-8100).



4. Analysis of the resistance of Pfu DNA polymerase and Fusion DNA polymerase to guanidine hydrochloride using the example of amplification of a 2 Kb fragment. Elongation time was 2 minutes. Lanes: 0-80, samples with guanidine hydrochloride added to the reaction mixtures up to 80 mM, respectively; M - DNA ladder "Sky-High" (S-8000).



5. Analysis of the resistance of Pfu DNA polymerase and Fusion DNA polymerase to sodium citrate using the example of amplification of a 2 Kb fragment. Elongation time was 2 minutes. Lanes: 0-8, samples with sodium citrate added to the reaction mixtures up to 8 mM, respectively; M - DNA ladder "Sky-High" (S-8000).



6. Analysis of the resistance of Pfu DNA polymerase and Fusion DNA polymerase to sodium dodecyl sulfate (SDS) using the example of amplification of a 2 Kb fragment. Elongation time was 2 minutes. Lanes: 0-0.04, samples with SDS added to the reaction mixtures up to 0.04% (w/v), respectively; M - DNA ladder "Sky-High" (S-8000).