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Blitz DNA Polymerase

Instruction manual

Components:

Blitz DNA polymerase 2 U/ul	1x0,1 ml
-2,5X Blitz buffer	1x1,25 ml

1. Introduction

Blitz DNA Polymerase is a highly thermostable DNA polymerase from the hyperthermophilic archaeum *Pyrococcus furiosus*.

The modifications of amino acid structure of the native Pfu results in shorter extension times (30 s/kb), more robust and high yield amplification, and the ability to extend long templates in a fraction of the time, making Blitz a superior choice for cloning.

This enzyme is suitable for all PCR applications requiring greater accuracy or long amplicons.

The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction and also exhibits 3'→5' exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. It has no 5'-exonuclease activity.

- Blitz DNA polymerase is suitable for amplification of NGS DNA-library.
- The error rate of Blitz DNA Polymerase in PCR is comparable with the wild type DNA polymerase
- Single enzyme generate amplicons over 10 kb on genomic templates.
- With Blitz, dUTP can be used instead of dTTP.
- Blitz is very tolerant to PCR inhibitors (allows up to 10 % blood in the PCR reaction).
- 2.5X Reaction buffer with $MgCl_2$ and dNTPs provides an optimal enzyme performance in nearly all types of PCR.

2. Protocol

Important points before starting:

The 3'→5' exonuclease activity associated with Blitz DNA Polymerase may degrade the primers. It is therefore important that D NA Polymerase be added last to the reaction mixture.

Mg^{2+} is critical to achieve optimal activity with Blitz DNA Polymerase. The final Mg^{2+} concentration in 1X Buffer is 2 mM. The optimal Mg^{2+} concentration is affected by dNTP concentration, the template being used and supplements that are added to the reaction. This can also be affected by the presence of chelators (e.g. EDTA). Mg^{2+}can be optimized in 0.5 mM increments.

Optimal amounts of template DNA in the 50 µl reaction volume are 0.05-1 ng for both plasmid and phage DNA, and 0.05 – 0,5 µg for genomic DNA. Higher amounts of template increase the risk of generating of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

Since Blitz polymerase is not a "hot start", we recommend prepare master mix on ice.

1. Thaw all reagents, template DNA and primers.
2. Prepare a reaction mix according to Table 1.

Table 1. Reaction setup

Component	Volume/ 25 ul reaction	Final concentration
2.5X Buffer (with $MgCl_2$ and dNTPs)	10 ul	1X
Forward primer (10 uM)	0,5 - 1 ul	0,2 - 0,4 uM
Reverse primer (10 uM)	0,5 - 1 ul	0,2 - 0,4 uM
Template DNA	Variable	1 ng - 250 ng (for high complexity genomic DNA)
Blitz 2U/ul	0,25 ul	0,5 U
Nuclease Free H2O	to 25 ul	

- A final primer concentrations of 0.4 µM is optimal for most applications. However, for individual determination of optimal primer concentration, a primer titration from 0.2 µM to 1 µM can be performed.

The amount of reagents in table 1 is given for the reaction volume of 25 µl. For another final volume, change proportionally the amount of all reagents.

3. Mix the reaction thoroughly, and dispense appropriate volumes into PCR tubes or plates.
4. Add template DNA to the individual PCR tubes or wells containing the reaction mix, than add Blitz DNA polymerase
5. Program your thermocycler according to the program outlined in Table 2.
6. Place the PCR tubes or plates in the thermocycler, and start the cycling program.

Table 2. PCR conditions

Cycle Step	Tempera-ture	Time	Num-ber of cycles
Initial Denaturation	98 ºC	30 s	1
Denaturation	98 ºC	5 s	25-35
Annealing	55ºC – 72 ºC	15 s	
Elongation	72ºC	30s/kb	

3. Shipping and Storage

Blitz DNA polymerase is shipped at ambient temperature (below 25°C) up to 7 days, at +4 ºC up to 30 days, at a temperature of less than –16 ºC for a long time.

Blitz DNA polymerase upon arrival should be stored at –20°C, and has a shelflife of 24 months when stored properly under these conditions.

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