Long Taq DNA Polymerase

User's Instruction

Description

The conventional PCR method has some limitations, especially when amplifying DNA fragments which are more than 5kb. By changing the polymerase, buffer and reaction conditions, the amplification of long DNA fragment is possible. Long Taq DNA Polymerase is a mixed enzyme which can be used for efficient PCR reaction when using chromosome and other organelles as templates. When using human chromosome as template, more than 27kb DNA fragment can be amplified. When using λDNA as template, more than 40kb DNA fragment can be amplified.

Kit Contents

	500U
1. Long Taq DNA Polymerase (5U/μl)	100 μl
2. 10 × Long Taq Buffer (with 2.5mM Mg^{2+})	1 ml

Note

- **Long Taq DNA Polymerase:** 20mM Tris-HCl (pH8.0), 0.1 mM EDTA, 1mM DTT, 100 mM KCl, Stabilizers, 50% glycerol.
- **10×Long Taq Buffer (with 2.5mM Mg^{2+}):** 100 mM Tris-HCl (pH 8.4), 100 mM KCl, 50 mM $(NH_4)_2SO_4$, 7.5 mM $MgCl_2$, etc.

Unit Definition

One active unit is defined as the amount of enzyme required for the incorporation of 10 nmol deoxynucleotides into acid insoluble substances within 30 minutes at 74°C.

Protocol

1. Set up PCR amplification reaction as the following table (take 50 μl per well as an example):

Component	Volume
Long Taq DNA Polymerase (5U/μl)	0.5-1 μl
10×Long Taq Buffer (with 2.5mM Mg^{2+})	5 μl
dNTP Mixture (2.5 mM each)	4 μl
Template	<0.5 μg
Forward Primer (10 μM)	1 μl
Reverse Primer (10 μM)	1 μl
ddH_2O	Up to 50 μl

2. Thermocycling Conditions

	Temperature	Time
Initial Denaturation	94°C	2-5 min
30 Cycles	94°C	30 sec
	Ta	30 sec
	72°C	1 min/1-2kb
Final Extension	72°C	10 min

- **Note:** For the amplification of large fragments, especially for the fragments over 20kb, we recommend that the extension time of each cycle should be increased by 10-15 seconds at 15^{th}-30^{th} cycles. If the PCR instrument does not have the function of "automatic extension", it is recommended to increase the extension time by 1-4 minutes when setting at the beginning.

- **Recommended Setting:**

Fragment Length (kb)	Extension Time (min)	Extension/Cycle (sec)
3	2	1

6	4	2
10	7	5
15	10	5
20	14	10
25	17	10
30	20	15
35	24	15
40	27	20
45	30	20

Note

- 10×Long Taq Buffer (with 2.5mM Mg^{2+}) has high pH, and thus may form $Mg(OH)_2$ precipitation. Before use, the buffer should be incubated at 37°C for 5 mins, and then fully dissolved by votex.
- We strongly recommend to use 0.2μl thin wall PCR tube when amplifying long fragment. The thicker tube may not be able to denature the template effectively at 92°C. During denaturation, the denaturation time should be shortened and temperature should be decreased as much as possible. The initial denaturation should be carried out at 92-94°C for 2 mins (with high GC content, the denaturation time could be extended up to 5 mins). During the cycles, the denaturation time should be shortened as much as possible (10-15 secs at 92-94°C). Only if the template is rich in GC, can the denaturation time be 30 secs at 95°C. The time required for denaturation varies slightly with different PCR instruments.
- If the GC content of the template is high or the amplification template is relatively long, DMSO can be added to the reaction system to the final concentration of 1% - 8%. Generally, 2% (<30KB) or 4% (>30KB) will improve the amplification effect.
- When amplifying long fragment, the final concentration of primer should be 0.3-1μM with the length between 27-36bp. The annealing temperature is generally between 65°C and 70°C. The annealing temperature and extension temperature are basically the same in this case. Thus, the annealing extension can be carried out at the same temperature, and the two-stage amplification method can be used. Of course, if the designed primer is about 20bp, it is better to use the traditional three-stage amplification method.

- The template generally uses 0.01-2.5ng (λDNA) or 0.1-1μg (Human).
- If the reaction system contains high EDTA or chelating agent, the concentration of Mg^{2+} should be increased. If the dNTP concentration is to be increased, the Mg^{2+} concentration should be increased accordingly.

Storage

Store at -20°C for 1 year. Avoid multiple freeze-thaw cycles.