Long Flash PCR Master Mix (TBS4044)

Description

The Long Flash PCR Master Mix is a convenient, high-performance solution that includes Super DNA Polymerase, dNTPs, unique elongation factors, and an optimized buffer system, allowing users to perform PCR by simply adding primers and template DNA. The Super DNA Polymerase features both 5′→3′ polymerase and 3′→5′ exonuclease (proofreading) activity, with a fidelity approximately 80 times higher than that of Taq polymerase and generates blunt-end products. It's hot-start capability ensures the enzyme remains inactive at temperatures ≤55°C and becomes fully active after a 30-second incubation at 98°C. This mix is particularly suitable for amplifying DNA fragments from 100 bp up to 40 kb, with excellent performance for fragments ≥10 kb. With a fast extension rate of 10 seconds per kilobase, it significantly shortens PCR reaction time. Offering high fidelity, strong amplification efficiency, and resistance to inhibitors, the Long Flash PCR Master Mix is well-suited for downstream applications such as molecular cloning and first-, second-, and third-generation sequencing.

Components

Component	
2 × Long Flash PCR Master Mix	1 mL
ddH_2O	1 mL

Features

- Long fragments: The human genomic DNA template can amplify a 34kb target fragment.
- Ultra-fast: The extension time can be as fast as 1 sec/kb (target fragment within 2 kb)
- High GC: The human genomic DNA template can amplify 40% to 80% GC content.
- High resistance to adversity: Effectively amplify samples such as blood and mouse tail crude extract.

Shipping and Storage:

-20°C.

Protocol

The following are examples of conventional PCR reaction systems and conditions. In practical operation, corresponding improvements and optimizations should be fragment size.

PCR Reaction System

All operations should be performed on ice. After the components are decomposed and frozen, please mix thoroughly. After use, please store them back at -20℃ in a timely manner.

Reagent	20μLReaction system	Final Conc.
2×Long Flash PCR Master Mix	10μL	1×
Forward Primer,10μM	0.5-1μL	0.2-0.4μM
Reverse Primer,10μM	0.5-1μL	0.2-0.4μM
Template DNA	appropriate amount	< 200ng
ddH_2O	up to 10μL	/

PCR Reaction Program

Step	Temperature	Time	
Pre denaturation	98°C	30s-3 min[1)]	
Denaturation	98°C	10 s	30-35cycles
Annealing	Determine based on primer Tm[2)]	10 s	
Extension	72°C	1-10 s/kb[3)]	
Final extension	72°C	5 min	

Note:

1) Pre denaturation: The pre denaturation time for templates such as plasmid DNA, λ DNA, and simple genomic DNA can be set to 30 s-1 min. For complex templates such as crude samples, high GC, and human genomes, the pre denaturation time can be extended to 3 minutes.

2) Annealing: The 2×Long Flash PCR Master Mix contains a high ion concentration, and the reaction annealing temperature can be set 2-3°C higher than the theoretical primer Tm value. If ideal amplification efficiency cannot be obtained, the annealing temperature can be gradient changed for optimization; When non-specific reactions occur, increase the annealing temperature appropriately.

3) Extension: Set the extension time according to the length of the target fragment. For fragments≥10kb, set the extension time to 10s/kb. If the fragment is too long and the amplification band is weak, increase the extension time appropriately. For amplified fragments<10kb, set the extension time to 1-5s/kb.

4) Cycle number: The cycle number can be set according to the downstream application of the amplified product. If the cycle number is too few, the amplification amount is insufficient, and the cycle number is too many, the probability of mismatch will increase. Therefore, while

365 San Aleso Ave, Sunnyvale, CA 94085, USA Website: www.tribioscience.com
Tel: 408-498-0197/833-697-8998 Email: order@tribioscience.com

ensuring product yield, the cycle number should be minimized as much as possible

Amplification Tips

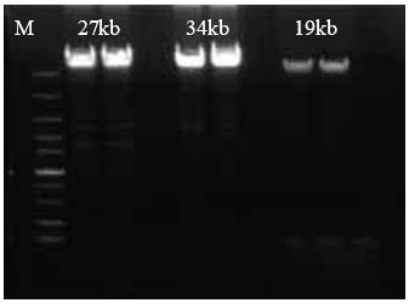
Target fragment length	Suggest extending the time
<2kb	1-2 sec/kb
2-5kb	2-5sec/kb
5-10kb	5-10sec/kb
>10kb	10-20sec/kb

Experimental Case

1. Long fragments

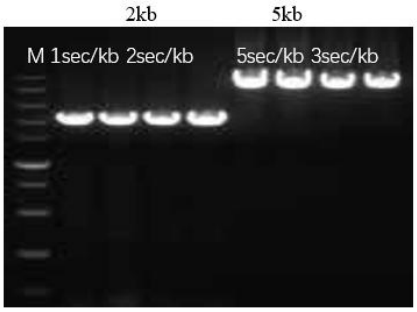
Experimental Design 1: Using human genomic DNA as a template, Long Flash PCR Master Mix was used to amplify long target fragments of 19kb, 27kb, and 34kb, respectively. The experimental data are as follows:

The experimental results indicate that Long Flash PCR Master Mix can effectively amplify a 34kb target fragment.



2. Ultra-fast

Experimental Design 2: Using human genomic DNA as a template, Long Flash PCR Master Mix was used to amplify target fragments of 2kb and 5kb, respectively (with extension times set to 1sec/kb, 2 sec/kb, 3 sec/kb, and 5 sec/kb). The experimental data are as follows:



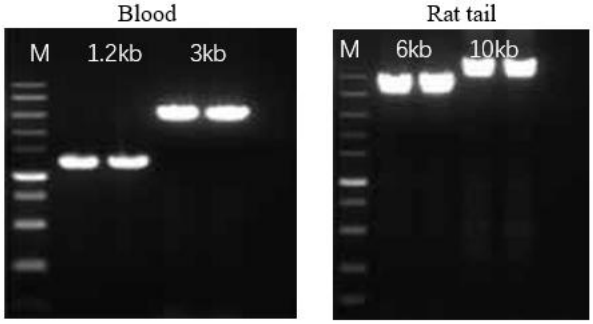
3. High GC

Experimental Design 3: Using human genome DNA as a template, Long Flash PCR Master Mix was used to amplify target fragments with 40% and 80% GC content, respectively. The experimental data are as follows:

4. High resistance to adversity

Experimental Design 4: Using blood and mouse tail lysate as templates, Long Flash PCR Master Mix was used to amplify target fragments of different lengths. The experimental data are as follows:

The experimental results indicate that Long Flash PCR Master Mix can effectively amplify blood and mouse tail crude extract samples.



Relative Products

TBS4001: Tribo™ 2x Sybr qPCR Super Mix
TBS4002: Tribo™ 2x TaqMan qPCR Super Mix
TBS4003: Tribo™ 2x Genotyping PCR Ready Mix
TBS4006: Tribo™ Reverse Transcription Reaction
TBS4007: One-Step Sybr qRT-PCR
TBS4008: One-Step TaqProbe qRT-PCR Kit
TBS4032: Gene Assembly Kit
TBS4033: Mouse Fast Genotyping System

This product is for research only.