AmpliPLUS HiFi PCR Master Mix (2X)

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BIO-5189-200
BIO-5189-1000

Applications: High-fidelity PCR amplification of DNA fragments up to 20kb, PCR for cloning, DNA labeling, genotyping, colony PCR, site-directed mutagenesis and Sanger sequencing.

Nuclease Assay: No detectable contaminating nucleases activities.

Functional Assay: AmpliPLUS HiFi PCR Master Mix (2X) was tested for amplification of 20kb of human genomic material, and its fidelity was assessed through next-generation sequencing.

1.0 DESCRIPTION

AmpliPLUS HiFi PCR Master Mix (2X), a convenient 2X high-fidelity PCR Master Mix, contains DNA polymerase, Mg^{2+}, dNTPs and PCR stabilizers as well as enhancers in an optimized reaction buffer for robust and versatile high-fidelity PCR of long fragments. The unique polymerase, possesses a 5′ → 3′ DNA polymerase activity and a 3′ → 5′ exonuclease activity, is an improved enzyme for an enhanced amplification ability (including regions with GC content over 64%) and speed (20secs/kb). Importantly, the augmented performance of AmpliPLUS HiFi PCR Master Mix (2X) also results in fidelity that is approximately 100 times higher than Taq DNA polymerase, making it an ideal alternative for applications where higher accuracy is needed, such as cloning, site-directed mutagenesis and sequencing.

AmpliPLUS HiFi PCR Master Mix (2X) is free of contaminating nucleases and capable of amplifying long fragments of up to 20kb and DNA templates extracted from varying sample types (i.e., human cell, yeast, leaf, and stool). The PCR product is free of A overhang at the 3′ end and is suitable for blunt-end cloning. For TA cloning, an additional step of A-tailing is necessary.

2.0 PROTOCOL

The following recommendation serves as a starting point and general guideline for any PCR. Reaction conditions such as incubation time and annealing temperature may vary and need to be optimized. PCR reactions should be prepared in a DNA-free environment with dedicated pipette and aerosol-resistant tips. Controls and template DNA shall be stored separately.

1) Thaw the AmpliPLUS HiFi PCR Master Mix (2X) on ice.
2) Once thawed, mix the AmpliPLUS HiFi PCR Master Mix (2x) by inverting the tube 10 times, and briefly spin down the tube content.
3) Prepare the PCR reaction, as suggested in Table 1 below, in a sterile nuclease-free PCR tube on ice.

Table 1. PCR Master Mix Setup

Component	Volume (µl) in 20µl reaction	Final concentration
AmpliPLUS HiFi PCR Master Mix (2X)	10.0	1X
Forward primer, 10µM	1.0	0.5µM
Reverse primer, 10µM	1.0	0.5µM
DNA template[1]	Variable	Human genomic DNA: 50 – 100ng Plasmid DNA: 1 – 5ng Phage DNA: 1 – 5ng Bacterial genomic DNA: 10 – 100ng
Nuclease-free water	Add to 20µl	

1) Cap the tube(s), invert/flick the tube(s) 10 times to mix and briefly spin down the content to remove bubbles, if any.
2) Place the reaction tube(s) in thermal cycler and set the cycling profile, as suggested in Table 2.

Table 2. PCR Cycling Profile

Step	Temperature	Duration	Cycle(s)
Initial Denaturation[1]	95°C	3 mins	1
Denaturation	98°C	30 secs	25-35[4]
Annealing[2]	42-65°C	10 – 30 secs	
Extension[3]	72°C	20secs/kb	
Final Extension	72°C	2-7 mins	1
Hold	4-12°C	∞	1

1 95°C for 3 minutes is generally sufficient for all template types, including templates of high GC content (64-76%).

2 The annealing temperature, in general, should be 3-5°C lower than the melting temperature (Tm) of the primers. Since the results from primer Tm calculations can vary depending on the method used, it is advisable to perform a gradient PCR to identify the optimum annealing temperature.

3 Extension time should be set, in accordance with the size of the target fragment. The amplification efficiency of this product is 20secs/kb.

4 The number of amplification cycles should be kept within 25-35 cycles. Whenever possible, a lower number of cycles is preferred as this will minimize non-specific amplification and shorter amplification time, while maintaining its high-fidelity amplification. Gradually increase or decrease the number of cycles, if deemed necessary.

3.0 IMPORTANT CONSIDERATIONS

3.1 AmpliPLUS HiFi PCR Master Mix (2X).

AmpliPLUS HiFi PCR Master Mix (2X) contains all the necessary components for the intended PCR except for the template DNA and the PCR oligonucleotides. The composition of this master mix is designed to give optimal PCR results.

3.2 Cloning of PCR product

When cloning fragments amplified with AmpliPLUS HiFi PCR Master Mix (2X), blunt end cloning is highly recommended. If TA cloning is required, it can be accomplished by A-tailing to the blunt PCR product. However, before adding the overhangs it is crucial to remove polymerase by purifying the PCR product. Any polymerase residual may degrade the A overhangs, reverting the product to blunt ends.

3.3 Oligonucleotides/Primers

The recommended final primer concentration is 0.5µM. If required, the primer final concentration may be optimized between 0.2µM and 1.0µM.

4.0 TROUBLESHOOTING

Observations	Action plans
Absence or low yield of PCR product	1. Ensure that the AmpliPLUS HiFi PCR Master Mix (2X) is fully thawed on ice, and invert to mix at least 10 times before use. 2. Check the design of the primers, their purity, melting temperatures and ensure that optimized annealing temperature is used for amplification. 3. Titrate template amount to ensure appropriate amount of template is added for amplification. 4. Check the integrity of the template DNA. Repeat DNA extraction, if deemed necessary. 5. Gradually increase (more than 35 cycles) the number of amplification cycles to increase the abundance of PCR products. 6. Make sure that the cycling protocol was performed as optimized. 7. Perform PCR with a 50µl reaction volume.
Non-specific products with high molecular weight smears	1. Ensure that the extension time used was not too long. 2. Increase annealing temperature or perform a temperature gradient PCR to determine the optimum annealing temperature. 3. Titrate template amount to ensure appropriate amount of template is added for amplification. 4. Reduce the total number of amplification cycles (fewer than 25 cycles) 5. Decrease the primer concentration. 6. Design new primers.
Non-specific products with low molecular weight smears	1. Increase annealing temperature or perform a temperature gradient PCR to determine the optimum annealing temperature. 2. Titrate template amount to ensure appropriate amount of template is added for amplification. 3. Shorten the extension time, if deemed necessary. 4. Design new primers.