Protocol

1. Introduction

Our product 2×HyPerFUsion® High-Fidelity Master Mix (With dye) offers superior performance for major PCR applications with high fidelity, powerful amplification ability and faster amplification speed. HyPerFUsion® high-fidelity DNA polymerases are composed of a DNA-binding domain fused to a Pyrococcus-like proofreading polymerase. Its unique structure, a novel Pyrococcus-like enzyme fused with a processivity-enhancing domain, increases fidelity and speed. The high fidelity makes hyPerFUsion® high-fidelity DNA polymerase a superior choice for detection or other subsequent applications. HyPerFUsion® high-fidelity DNA polymerase possesses one of the most accuracy with an error rate that is 50-fold lower compared to Taq DNA Polymerase and 6-fold lower than Pyrococcus Furiosus DNA Polymerase (Pfu).

HyPerFUsion® DNA polymerase possesses 5′→ 3′ polymerase activity and 3′→ 5′ exonuclease activity. It will generate blunt-ended products in the amplification products without an A overhang which appears in the product amplified with Taq polymerase. The polymerase has been capable of amplifying fragment as long as 10 kb in our assays. In PCR reaction, elongation rate of HyPerFUsion® DNA polymerase is about 15-30 sec/kb depending on the complexity of the gene.

2×HyPerFUsion® High-Fidelity Master Mix (With dye) is a ready-to-use 2×premix solution containing polymerase, dNTPs, an already optimized buffer system and dye. Our mix is ideal for any detection and the PCR product can be directly electrophoresed after the amplification without the need to add a loading buffer.

If the subsequent experiment is cloning, product 2×HyPerFUsion® High-Fidelity Master Mix(Cat No. K1039) is your superior choice which doesn't contain a dye.

2. Guidelines

2.1. Set up the appropriate reactions on ice

We recommend setting all reaction components on ice. Experimenters should carefully unfreeze the tube before opening and ensure homogeneity.

It is intensively recommended that the 2×HyPerFUsion® High-Fidelity Master Mix (With dye) should be added to the PCR mixture **at last**, since the enzyme exhibits 3′→5′ exonuclease activity that can degrade primers in the absence of dNTPs.

Please pay attention to protocols with 2×HyPerFUsion® High-Fidelity Master Mix that may differ from protocols with other standard polymerases due to the nature of HyPerFUsion DNA Polymerase.

COMPONENT	20 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
ddH2O	add to 20 µL	add to 50 µL	
10 µM Forward Primer	0.8 µL	2 µL	0.4 µM
10 µM Reverse Primer	0.8 µL	2 µL	0.4 µM
Template	variable	variable	< 250 ng
2×HyPerFUsion® High-Fidelity Master Mix (With dye)	10 µL	25 µL	

a. The recommended final primer concentration is 0.4 µM, but it can be varied in a range of 0.2–1.0 µM, you can adjust the concentration. Oligonucleotide primers are generally recommended as a length between20–40 bp and ideally having a GC content of 40–60%.

b. Use of high quality, purified DNA templates could greatly enhance the success of PCR reactions. For low complexity DNA (e.g. **plasmid, viral, λ** or **BAC DNA**), DNA template amount can be 1 pg–10 ng per 50 µL reaction volume. For high complexity **genomic** DNA, the amount of DNA template should be 50–250 ng per 50 µL reaction volume. If the template DNA is obtained from a **cDNA** synthesis reaction, the volume of the template should not exceed 10% of the final PCR reaction volume.

2.2. Gently mix the reaction and spin down in microcentrifuge.

Mix and briefly centrifuge the reaction tube, then transfer the reactions to a thermocycler preheated to the denaturation temperature (98°C).

If the thermocycler does not have a heated lid, overlay the sample with mineral oil.

2.3. Cycling Conditions

	Temperature	**Time**	**Cycles**
Initial denaturation	98℃	1min	1
Denaturation	98℃	15sec	25-35 cycle(variable)
Annealing	55-58℃(Adjustable)	15sec	
Extension	72℃	15–30s per kb	
Final extension	72℃	2min	1
Hold	4℃	+∞	1

a. We recommend a 1min initial denaturation at 98 °C for most templates. Some templates may require longer initial denaturation time and the length of the initial denaturation time can be extended up to 3 minutes.

b. The optimal annealing temperature for HyPerFUsion DNA Polymerase might differ significantly from that of Taq-based polymerases.
The HyPerFUsion DNA Polymerase has the ability to stabilize primer-template hybridization. As generally recommended, anneal for 10–30 seconds at **55-58° C.** If you can't generate an ideal product or yield, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination.

c. Extension time depends on amplicon length and complexity. For low complexity DNA (e.g. **plasmid, viral, λ** or **BAC DNA**) use an extension time of 15 seconds per 1 kb. For high complexity genomic DNA 30 seconds per 1 kb is recommended. For some cDNA templates, the extension time can be increased up to 40 seconds per 1 kb to obtain optimal results.
For generally use, 20 second per 1 kb is recommended.

2.4. Electrophoresis

If needed to subject the PCR product to agarose gel electrophoresis, our product **SYBR Safe DNA Gel Stain (Cat: A8743)** is available.

3. Note

a. HyPerFUsion DNA Polymerase annealing temperature is different from many common DNA polymerases (such as Taq DNA polymerase). If you can't generate ideal experimental results under this temperature, you may set gradient annealing temperature to optimize the experimental conditions.

b. Use 15–30 s/kb for extension. Do not exceed 1 min/kb.

c. HyPerFUsion DNA Polymerase produces blunt end DNA products.

d. HyPerFUsion DNA Polymerase has powful polymerization ability. Experimenters should operate on ice during the whole experiment of PCR, otherwise the enzyme is active at room temperature. It is possible to polymerize primers to form primer dimers under room temperature, which cause the depletion of the primers, thus the PCR efficiency will decrease.