1. Introduction

Our product hyPerFUsion™ high-fidelity PCR Kit offers superior performance for major PCR applications with high fidelity, powerful amplification ability and faster amplification speed. The high fidelity makes hyPerFUsion DNA Polymerase a superior choice for cloning or other subsequent applications. HyPerFUsion 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.

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. Our hyPerFUsion polymerase has already been capable of amplifying fragment as long as 10 kb in assays.

2. Component

This product contains hyPerFUsion DNA Polymerase (**1 U/μL)** and 5X hyPerFUsion HF Buffer (**Mg2+ plus**), separate MgCl2 solution (50 mM) and DMSO (100%).

3. Guidelines for using hyPerFUsion DNA Polymerase

3.1. Set up the appropriate reactions on ice

We recommend setting all reaction components on ice. Experimenters should carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. HyPerFUsion Polymerase should be pipetted carefully and gently because of the storage buffer containing high glycerol content.

It is important that the hyPerFUsion DNA Polymerase should be added to the PCR mixture **in the last**, since the enzyme exhibits 3′→5′ exonuclease activity that can degrade primers in the absence of dNTPs.

Please pay attention to protocols with hyPerFUsion DNA Polymerase 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	
5×HF buffer	4 μL	10 μL	1X
2.5 mM dNTP Mixture	1.6 μL	4 μL	200 μM each
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
DMSO (optional)	(0.6 μL)	(1.5 μL)	(3%)
hyPerFUsion DNA polymerase	0.4 μL	1 μL	0.02 U/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. DMSO addition is recommended for GC-rich amplicons. DMSO is not recommended for amplicons with very low GC ratio or amplicons that are > 20 kb.

c. 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.

d. The optimal amount of polymerase depends on the amount of template and the length of the PCR product. Usually 1 U of hyPerFUsion DNA Polymerase per 50 µL reaction volume will lead to good results, but the optimal amount can range from 0.5 to 1 U per 50 µL reaction depending on the amplicon length and difficulty. It is not recommended to exceed 2 U/50 µL (0.04 U/µL), especially for amplicons that are > 5kb.

e. High quality dNTPs should be used for optimal amplification with hyPerFUsion DNA Polymerase. The polymerase cannot read dUTP-derivatives or dITP in the template strand so the use of these analogues or primers containing them is not recommended.

f. Mg2+ has been added into the buffer, you don't need to add Mg2+ repeatedly. If the chelators (e.g. EDTA) are present, or you want to improve the performance, it might be necessary to increase the Mg2+ concentration in addition. We have separate MgCl2 provided. The Mg2+ concentration in 5×HF buffer is 10 mM, which means final Mg2+ concentration in the reaction solution is 2 mM.

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

Experimenters should quickly 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.

3.3. Cycling Conditions

	Temperature	Time	Cycles
Initial denaturation	98℃	1min	1
Denaturation	98℃	15s	variable
Annealing	55-58℃	15s	
Extension	72℃	15–30s per kb	
Final extension	72℃	2min	1
Hold	4℃	+∞	1

a. We recommend an 1 minute 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. For most templates, we recommend use a temperature between 55-58℃ for 10-30 secs. If necessary, 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.

d. A 2-step protocol may be recommended when primer Tm values are at least 69℃(> 20 nt) or 72℃ (≤ 20 nt). In the 2-step protocol the combined annealing/extension step should be performed at 72 °C even when the primer Tm is > 72 °C.

	Temperature	Time	Cycles
Initial denaturation	98℃	1min	1
Denaturation	98℃	15s	variable
Extension	72℃	15–30s per kb	
Final extension	72℃	2min	1
Hold	4℃	+∞	1

3.4. Electrophoresis

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

4. Note

a. HyPerFUsion polymerase annealing temperature is different from many common DNA polymerases (such as Taq DNA polymerase). For the setting of the annealing temperature for the experiment, you might try 55-58 ℃. 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. Use hyPerFUsion DNA Polymerase at 0.5–1.0 U per 50 µL reaction volume. Do not exceed 2 U/50 µL.

d. Use 200 µM of each dNTP. Do not use dUTP.

e. HyPerFUsion DNA Polymerases produce blunt end DNA products.

f. HyPerFUsion enzyme has powerful 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.

g. The PCR products generated by using hyPerFUsion DNA Polymerase have blunt ends. If cloning is the next step, then blunt-end cloning is recommended. If T/A-cloning is preferred, then DNA should be purified prior to A-addition (Any remaining hyPerFUsion DNA Polymerase will degrade the A overhangs, creating blunt ends again.). Addition of an untemplated -dA can be done with Taq DNA Polymerase or Klenow exo–. Experimenters could incubate the purified PCR product with 1x Taq buffer, 2.5 mM MgCl2, 0.2 mM dATP and 1 U Taq DNA polymerase in 10 µL reaction mixture up to 30 min at 72 °C.