Genie Fusion Ultra High-Fidelity DNA Polymerase

Code: MORV0001 **Code: MORV0001-500** **Code: MORV0001-1000**
Pack Size: 100U **Pack Size: 500U** **Pack Size: 1000U**

1. Introduction

Genie Fusion Ultra High-Fidelity DNA Polymerase is a next generation enzyme for robust PCR with ultra high-fidelity. With extremely low error rates x53-fold lower than Taq, Genie Fusion Ultra High-Fidelity DNA Polymerase sets a new level of performance for standard and long fragment PCR amplification.

Employing a unique extension factor as well as specificity-promoting and plateau-inhibiting factors Genie Fusion greatly improves long range amplification ability, specificity, and PCR yield. Genie Fusion is capable of amplifying long fragments such as 40 kb λ DNA, 40 kb plasmid DNA, 20 kb genomic DNA and 10 kb cDNA. In addition, Genie Fusion has a excellent resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Genie Fusion contains two monoclonal antibodies inhibiting the 5´→3´ polymerase activity and 3´→5´ exonuclease activity at room temperature which enables Genie Fusion to perform super-specific Hot-Start PCRs. The amplification generates blunt-ended products, which are compatible with GenieClone DNA Assembly Cloning Kit (Assay Genie, Cat. No. # MORV0004).

Key Features

- Ultra high-fidelity DNA polymerase with error rates x53-fold lower than conventional Taq and x6-fold lower than Pfu
- Excels at amplifying long fragments such as 40 kb λ DNA, 40 kb plasmid DNA, 20 kb genomic DNA and 10 kb cDNA
- Superior performance with GC-rich templates
- Extreme resistance to PCR inhibitors
- Excellent choice for direct PCR from bacteria, fungi, plant tissues, animal tissues and whole blood samples
- Formulated with dual monoclonal antibodies for super specific Hotstart PCR
- Compatible with GenieClone DNA Assembly Cloning Kit (MORV0004)

2. Package Information

Components	MORV0001 100 U	MORV0001-500 500 U	MORV0001-1000 1000 U
Genie Fusion Ultra High-Fidelity DNA Polymerase (1 U/μl)	100 μl	MORV0001 x 5	MORV0001 x 10
2 X Genie Fusion Buffer	2 X 1.25 μl		
dNTP Mix (10 mM each)	100 μl		
10 x Loading buffer	1.25 μl		

3. Storage

Store at -20℃; avoid repeated freeze-thawing.

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4. Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of whole dNTPs into acid-insoluble products in 30 minutes at 74℃ with activated salmon sperm DNA as the template / primer.

5. Quality Control

Residual Endonulease Test: The product was tested in a reaction containing 10U of Genie Fusion Ultra high-fidelity DNA polymerase and 0.3 µg of Supercoiled pBR322 DNA. After incubation at 37℃ for 4 hours, there was no change in DNA bands determined by agarose gel electrophoresis.

Residual gDNA Test: The residual nucleic acids in 10U of the enzyme was tested with 16S rDNA-specific TaqMan qPCR. The residual gDNA is less than 10 copies.

Functional Assay: 1U of this enzyme was used in a 50 µl PCR reaction (30 sec/kb extension time) to amplify 5 fragments of various lengths and different GC content. λ DNA, 50 ng of plasmid DNA, 100 ng of human genome DNA and 1 ul of cDNA from Hela cells was used as templates. After 35 cycles, 5ul of the PCR products were analysed via 1% agarose gel electrophoresis and EB staining. Each reaction resulted in a single band of the expected size.

6. Experimental Process

6.1 Standard PCR

Recommended PCR System

Keep all components on ice during the experiment. All components need to be mixed thoroughly after thawing and put back at -20℃ immediately after using.

Component	Volume
ddH_2O	up to 50 µl
2× Genie Fusion Buffer [a]	25 µl
dNTP Mix (10 mM each)	1 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Genie Fusion Ultra High-Fidelity DNA Polymerase (1 U/µl)	1 µl
Template DNA [b]	X µl

a. 2× Genie Fusion Buffer contains Mg2+. The final concentration of Mg2+ is 2 mM.

b. Optimal reaction concentration varies in different templates. In a 50 µl reaction, the recommended template usage is as follows:

Templates	Input Template DNA
Genomic DNA	50 - 400 ng
Plasmid or Virus DNA	10 pg -30 ng
cDNA	1 - 5 µl (≤ 1/10 of the total volume of PCR system)

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Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation [a]	95°C	30 sec/ 3 min	1
Denaturation	95°C	15 sec	25 - 35
Annealing [b]	56 – 72 °C	15 sec	
Extension [c]	72°C	30 - 60 sec/kb	
Final Extension	72°C	5 min	1

a. For pre-denaturation, the recommended temperature is 95°C, and the recommended time is 30 sec for plasmid / virus DNA and 3 min for genomic DNA / cDNA.
b. For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72°C, the annealing step can be removed (two-step PCR). If necessary, annealing temperature can be further optimized in a gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature is helpful to improve poor amplification specificity.
c. Longer extension time is helpful to increase the amplification yield.

6.2 Long Range PCR

Genie Fusion Ultra High-Fidelity DNA Polymerase can perform a long-range amplification with high specificity and yields. If the standard PCR protocol (Section 6.1) yields insufficient results, the following Touch-Down, two-step PCR is recommended.

Steps	Temperature	Time	Cycles
Pre-denaturation	95°C	3 min	1
Denaturation	92°C	15 sec	5
Extension	74°C	60 sec/kb	
Denaturation	95°C	15 sec	5
Extension	72°C	60 sec/kb	
Denaturation	95°C	15 sec	5
Extension	70°C	60 sec/kb	
Denaturation	95°C	15 sec	25
Extension	68°C	60 sec/kb	
Final Extension	68°C	5 min	1

It is recommended to use high-quality templates and long primers. Increasing the input of template DNA may be helpful to improve the amplification yield.

6.3 Direct PCR with Crude Samples

Genie Fusion has excellent resistance to PCR inhibitors and can be used for direct PCR amplification of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Crude materials that have been successfully amplified with Genie Fusion are as follows:

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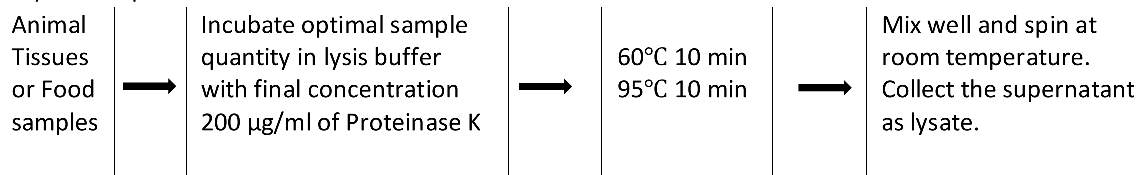
Sample Type	Amplification Method	Temperature Recommendation (for a 50 µl PCR system)
Whole Blood	Direct PCR	1 - 5 µl
Filter Paper Dry Blood	Direct PCR	1 - 2 mm^2 filter paper
Cultured Cells	Direct PCR	Low amounts of cells
Yeast	Direct PCR	Single clone or 1 µl suspension
Bacteria	Direct PCR	Single clone or 1 µl suspension
Mod	Direct PCR	Low amount of sample
Sperm	Direct PCR	Low amount of sample
Plankton	Direct PCR	Low amount of sample
Plant Tissue	Direct PCR	1 - 2 mm^2 tissue
Mouse Tail	PCR with lysate	1 - 5 µl lysate*
Food	PCR with lysate	1 - 5 µl lysate*

*Lysate Preparation:



Proteinase K and Lysis Buffer: 20 mM of Tris-HCl, 100 mM of EDTA, 0.1% SDS, pH 8.0 (not included in this kit).

7. Application Examples

7.1 Amplification of gDNA

Human genomic DNA fragments of 0.6 kb, 1.0 kb, 2.6 kb, 3.0 kb, 4.0 kb, 5.1 kb, 6.2 kb, 7.1 kb, 8.5 kb, 10.6 kb, 17.8 kb, 20.3 kb, and 21.4 kb were amplified. The Tm of all primers are approximately 60°C (calculated in Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

ddH_2O	up to 50 µl
2× Genie Fusion Buffer	25 µl
dNTP Mix (10 mM each)	1 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Genie Fusion Ultra High-Fidelity DNA Polymerase (1 U/µl)	1 µl
Template DNA	X µl

Recommended PCR System

Steps	Temperature	Time	Cycles
Pre-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	35
Annealing	60°C	15 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

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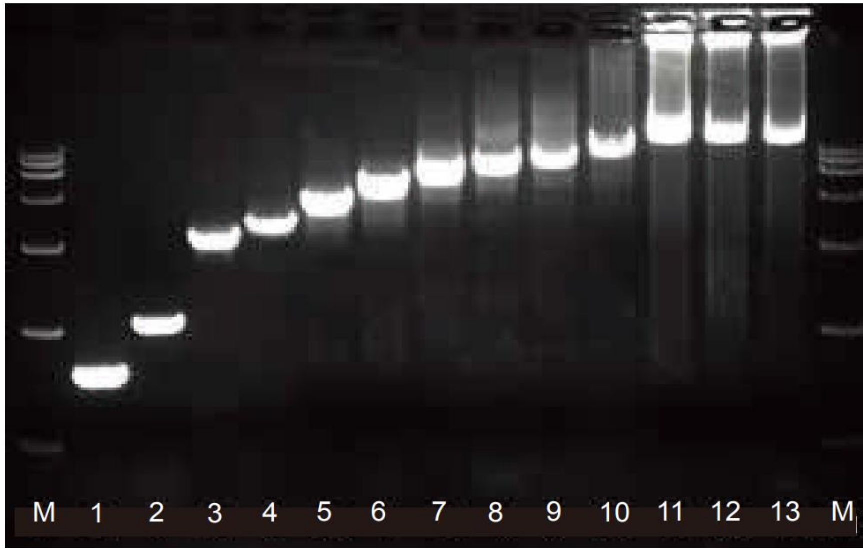

M: 15kb DNA Marker
1: 0.6 kb
2: 1.0 kb
3: 2.6 kb
4:3.0 kb
5: 4.0 kb
6: 5.1 kb
7: 6.2 kb
8: 7.1 kb
9: 8.5 kb
10: 10.6 kb
11: 17.8 kb
12: 20.3 kb
13: 21.4 kb

7.2 Amplification of Crude Samples

1. Using Human whole blood as a template, a target fragment of 1,295 bp was amplified with Genie Fusion Ultra High-Fidelity DNA Polymerase, a high-fidelity DNA polymerase from company A, and a high-fidelity DNA polymerase from company B. The Tm of all primers are around 60°C (calculated in Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

Component	Volume
ddH_2O	up to 50 µl
2× Genie Fusion Buffer	25 µl
dNTP Mix (10 mM each)	1 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Genie Fusion Ultra High-Fidelity DNA Polymerase (1 U/µl)	1 µl
Whole Blood*	X µl

* The inputs of the whole blood are 1µl, 2µl, 4µl, respectively.

Recommended PCR System

Steps	Temperature	Time	Cycles
Pre-denaturation [a]	95°C	3 min	
Denaturation	95°C	15 sec	} 35
Annealing*	60/63/70°C	15 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

* The annealing temperatures for 1.3 kb, 3.6 kb and 8.5 kb of target fragments are 60°C, 63°C, and 70°C, respectively

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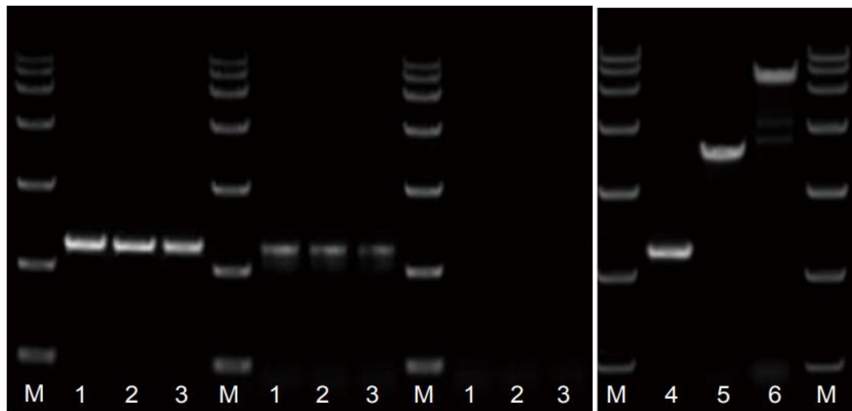
Genie Fusion Ultra High-Fidelity DNA Polymerase | High-Fidelity DNA Polymerase Company A | High - Fidelity DNA Polymerase Company B | Genie Fusion Super-Fidelity DNA Polymerase



M: 15 kb DNA Marker
1: 1.3 kb (1 µl of blood)
2: 1.3 kb (2 µl of blood)
3: 1.3 kb (4 µl of blood)
4: 1.3 kb (2 µl of blood)
5: 3.6 kb (2 µl of blood)
6: 8.5 kb (2 µl of blood)

*The high-fidelity polymerases from company A and B were used according to their own protocols.

2. Using tomato leaf, rice leaf and rice as templates and the purified genomic DNA from rice leaf as a positive control, target fragments of 1.3 kb were amplified with Genie Fusion Ultra High-Fidelity DNA Polymerase, a high fidelity DNA polymerase from company A and a high fidelity DNA polymerase from company B. The Tm of all primers are approximately 60°C (calculated in Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

ddH_2O	up to 50 µl
2× Genie Fusion Buffer	25 µl
dNTP Mix (10 mM each)	1 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Genie Fusion Ultra High-Fidelity DNA Polymerase (1 U/µl)	1 µl
Plant Tissues*	X µl

*The recommended diameter of the plant tissues is 0.3 - 3 mm.

Recommended PCR System

Steps	Temperature	Time	Cycles
Pre-denaturation [a]	95°C	3 min	
Denaturation	95°C	15 sec	35
Annealing	60°C	15 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

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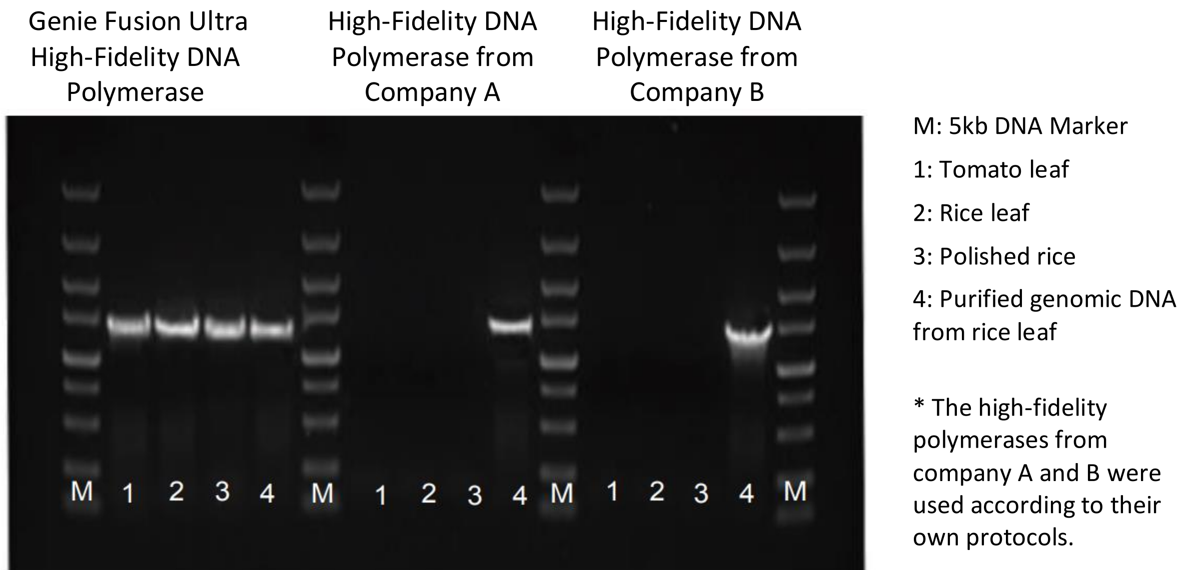

3. Using mouse tail lysate as a template, a target fragment of 2.5 kb was amplified with Genie Fusion Ultra High-Fidelity DNA Polymerase, a high-fidelity DNA polymerase from company A, and a high-fidelity DNA polymerase from company B. The Tm of all primers are approximately 60℃ (calculated in Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

ddH_2O	up to 50 µl
2× Genie Fusion Buffer	25 µl
dNTP Mix (10 mM each)	1 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Genie Fusion Ultra High-Fidelity DNA Polymerase (1 U/µl)	1 µl
Mouse Tail Lysate	2 µl

Recommended PCR System

Steps	Temperature	Time	Cycles
Pre-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	35
Annealing	60°C	15 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	5 min	

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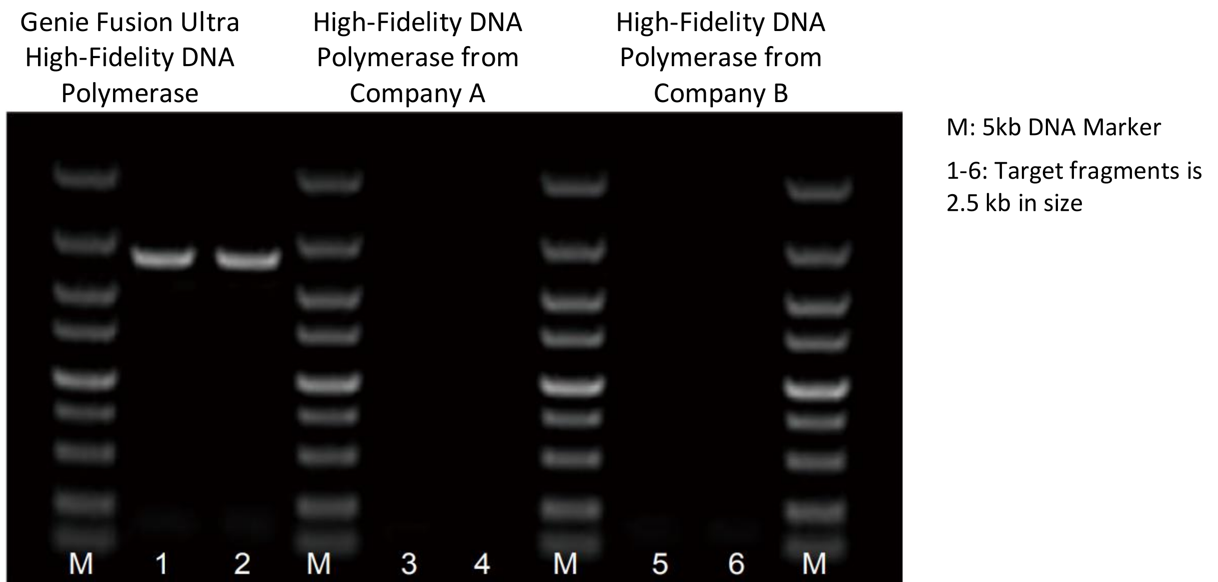

7.3 Excellent Amplification of Fragments with High GC Content

Genie Fusion Ultra High-Fidelity DNA Polymerase is capable of amplifying GC-rich fragments unlike conventional polymerases. Using human genomic DNA as a template, target fragments of 654 bp, 900 bp, 800 bp, 1200 bp, 1400 bp, and 426 bp were amplified. The GC content of these amplicons was higher than 68%. High amplification efficiency is shown in the following figure. The Tm of all primers are approximately 60°C (calculated in Primer Premier 5). The PCR reaction system refers to Section 6.1, and the PCR program is as follows:

Recommended PCR System

Steps	Temperature	Time	Cycles
Pre-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	} 35
Extension	72°C	45 sec/kb	
Final Extension	72°C	5 min	

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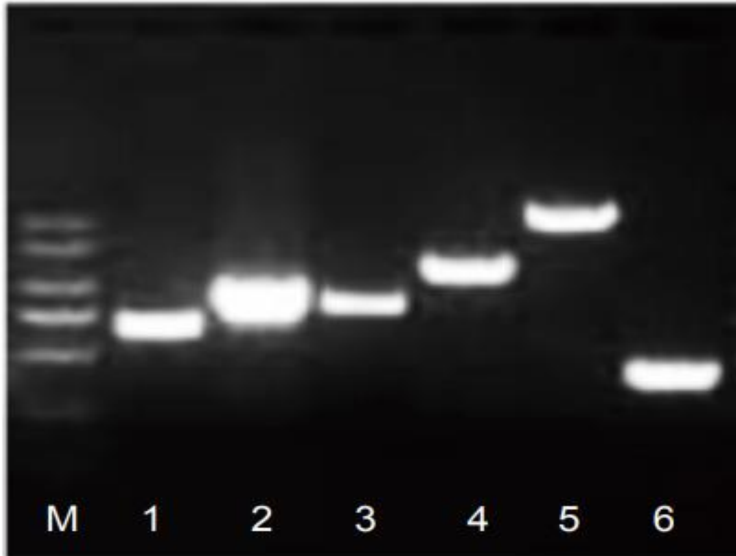
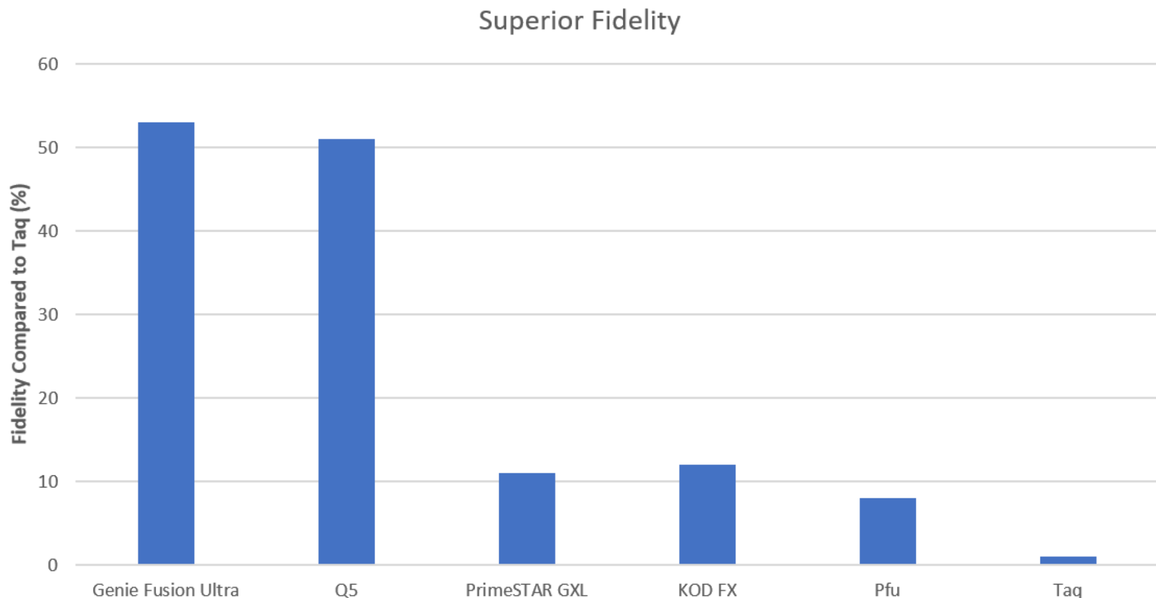


M : 2Kb DNA Marker
1 : 654 bp | 68.1% GC Content
2 : 900 bp | 69.4% GC Content
3 : 800 bp | 71.3% GC Content
4 : 1.2 kb | 73.5% GC Content
5 : 1.4 kb | 74.7% GC Content
6 : 426 bp | 76.8% GC Content

7.4 Reliable High Fidelity

The amplification fidelity of Genie Fusion Ultra High-Fidelity DNA Polymerase is 53-fold superior to Taq DNA Polymerase and 6-fold higher than that of Pfu, which is significantly superior to other products of the same kind. The following figure shows a comparison of amplification fidelity among various polymerases detected by a Lacl Assay (Cline et al. ,Nucleic Acids Research,24: 3546-3551(1996).



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8. Important Notes

1. Use high-quality templates.
2. DO NOT use dUTP or any primers or templates that contain uracil.
3. Optimize Genie Fusion Ultra High-Fidelity DNA Polymerase according to experimental requirements but use no more than 2 U in a 50 µl reaction system.
4. Genie Fusion Ultra High-Fidelity DNA Polymerase has strong proofreading activity. Therefore, the PCR products must be purified before adding A-Tailing when TA cloning.
5. To prevent the strong proofreading activity of the Genie Fusion Ultra High-Fidelity DNA Polymerase degrading primers, the polymerase should be loaded last when making up the reaction system.
6. Primers design notes:
    - Choose C or G as the last base of the 3'-end of the primer.
    - Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer.
    - Avoid hairpin structures at the 3'-end of the primer.
    - Tm of the primers should be within the range of 55°C - 65°C (calculate using Primer Premier 5 or similar), and the Tm difference between F and R primers should be less than 1°C.
    - Additional sequence should not be included when calculating Tm of the primers.
    - GC content of the primers should be within the range of 40% - 60%.
    - The general distribution of A, G, T, C in the primers should be uniform, and avoid using regions with rich GC and rich AT.
    - Keep complementary sequence less than 5 bases within the primers or between two primers, and complementary sequence less than 3 bases at the 3'-end of the primers.
    - Please search the specificity of the designed primers by NCBI BLAST to avoid non-specific amplification.

9. Troubleshooting

No or Low Yield of PCR Products

Primers	Optimize primer design
Annealing Temperature	Set gradient annealing temperature to optimize
Concentration of Primers	Optimize the concentration of primers
Extension Time	Optimize the extension time to 30 sec/kb-1 min/kb
Cycle Numbers	Increase cycle numbers to 35 - 40
Purity of Templates	Use high-purity templates
Template Input	Refer to the recommended reaction system and increase the input
Enzymes Input	Appropriately adjust the input of high-fidelity polymerase

Unspecific or Smear Bands in Electrophoresis

Primers	Optimize primer design
Annealing Temperature	Try to improve annealing temperature and set gradient annealing temperature to optimize
Primer Concentration	Decrease the concentration of primers to final concentration as 0.2 µM

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Extension Time	Appropriately decrease the extension time when bands longer than target bands appear
Cycle Numbers	Decrease cycle number to 25 - 30
PCR Programs	Use Two-Step PCR or Touch-down PCR
Purity of Templates	Use high purity templates
Template Input	Modify or decrease templates input referring to the recommended reaction system
Enzyme Input	Appropriately adjust or decrease the input of high-fidelity polymerase

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