

Genie Fusion Ultra High-Fidelity 2X Master Mix

Code: MORV0002

Code: MORV0002-5

Code: MORV0002-15

Pack Size: 1ml / 40 rxns

Pack Size: 5x1ml / 200 rxns

Pack Size: 15x1ml / 600 rxns



1. Introduction

Genie Fusion Ultra High-Fidelity 2x Master Mix contains 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 PCR amplification, 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 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.

Genie Fusion Ultra High-Fidelity 2X Master Mix contains Genie Fusion Ultra High-Fidelity DNA Polymerase, dNTPs and an optimized buffer system. Protective agents in the Genie Fusion Ultra High-Fidelity 2X Master Mix allow for repeat freeze-thaw cycles while maintaining excellent performance. Amplification generates blunt-end products which are compatible with which are compatible with GenieClone DNA Assembly Cloning Kit (Assay Genie, Cat. No. #MORV0004).

Key Features

- Optimized 2x mastermix containing Ultra high-fidelity DNA polymerase, dNTPs and next-generation buffer system
- 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. Product

Components	MORV0002	MORV0002-5	MORV0002-15
Genie Fusion Ultra High-Fidelity 2X Master Mix	1 ml	5 x 1ml	15 x 1ml
	40 x 50ul reactions	200 x 50ul reactions	800 x 50ul reactions

3. Storage

Store at -20°C: 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°C with activated salmon sperm DNA as the template / primer.

5. Quality Control

Residual Endonuclease Test: The product was tested in a reaction containing 25 µl Genie Fusion Ultra High-Fidelity 2X Master Mix and 0.3 µg of Supercoiled pBR322 DNA. After incubation at 37°C for 4 hours, there was no change in DNA bands determined by agarose gel electrophoresis.

Residual gDNA Test: The residual nucleotides in 25 µl of Genie Fusion Ultra High-Fidelity 2X Master Mix was tested by SYBR® Green qPCR using specific primers of gDNA. The residual gDNA is less than 10 copies.

Functional Assay 1: In a 50µl PCR system with 1U of Genie Fusion Ultra High-Fidelity 2X Master Mix, 100 ng of human genomic DNA was used as a template. After 35 cycles, 1/10 of PCR products were detected by 1% agarose gel electrophoresis. A single DNA band of 8.2 kb was detected after EB staining.

Functional Assay 2: In a 50µl PCR system with 1U of Genie Fusion Ultra High-Fidelity 2X Master Mix, 10 ng of λ DNA was used as template. After 30 cycles, 1/10 of PCR products were detected by 1% agarose gel electrophoresis. A single DNA band of 15 kb was detected after EB staining.

6. Experimental Process

6.1 Standard PCR

Recommended PCR System

Keep all components on ice during the experiment. Mix components thoroughly after thawing and replace at -20°C immediately use.

ddH ₂ O	Up to 50 µl
Genie Fusion Ultra High-Fidelity 2X Master Mix	25 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Template DNA ^a	X µl

The Assay Genie PCR Enhancer is recommended for unsuccessful amplification of fragments with GC content > 60%.

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

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

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation ^a	95°C	30 sec/ 3 min	1

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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 T_m of the primers. If the T_m 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 For Long-fragment 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:

Sample Type	Amplification Method	Temperature Recommendation (for a 50 µl PCR system)
Whole Blood	Direct PCR	1 - 5 µl

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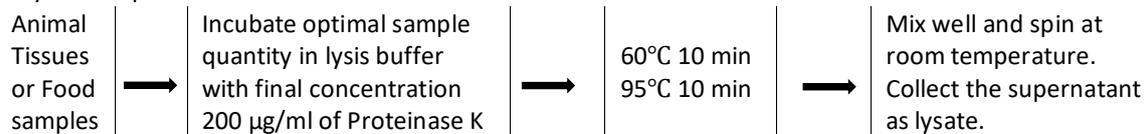
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Filter Paper Dry Blood	Direct PCR	1 - 2 mm ² filter paper
Cultured Cells	Direct PCR	Low amounts of cells
Yeast	Direct PCR	Single clone or 1 µl suspension
Bacteria	Direct PCR	Single clone or 1 µl suspension
Mod	Direct PCR	Low amount of sample
Sperm	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

Taking human genomic DNA as templates, the target 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, respectively. The T_m of all primers are approximately 60°C (calculated in Primer Premier 5). The reaction system and program are as follows:

ddH ₂ O	Up to 50 µl
Genie Fusion Ultra High-Fidelity 2X Master Mix	25 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Human Genomic DNA (100ng/µl)	1 µl

Recommended PCR Program

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

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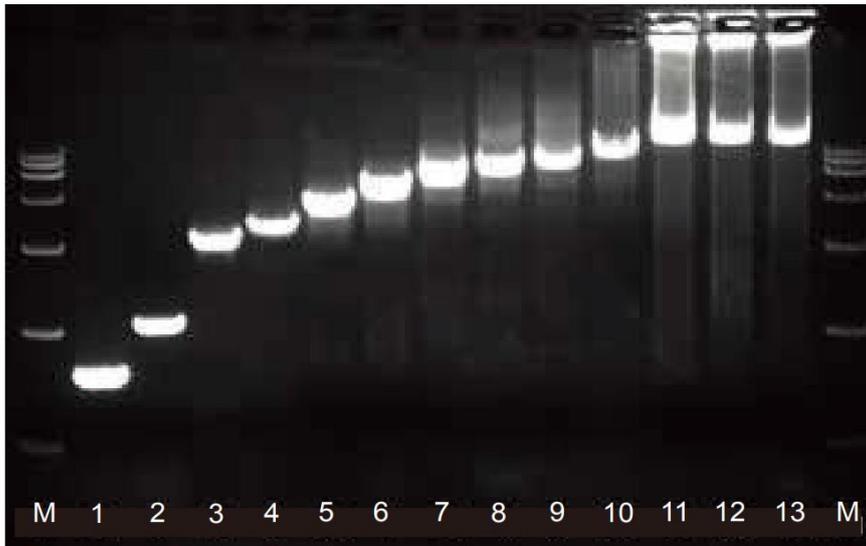
Pack Size: 1ml / 40 rxns

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PCR Analysis | Agarose Gel Electrophoresis



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, a high-fidelity DNA polymerase from company A, and a high-fidelity DNA polymerase from company B. The T_m of all primers are around 60°C (calculated in Primer Premier 5). The reaction system and program are as follows:

ddH ₂ O	Up to 50 µl
Genie Fusion Ultra High-Fidelity 2X Master Mix	25 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Whole Blood*	x µl

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

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation	95°C	3 min	
Denaturation	95°C	15 sec	
Annealing*	60/63/70°C	15 sec	} 35
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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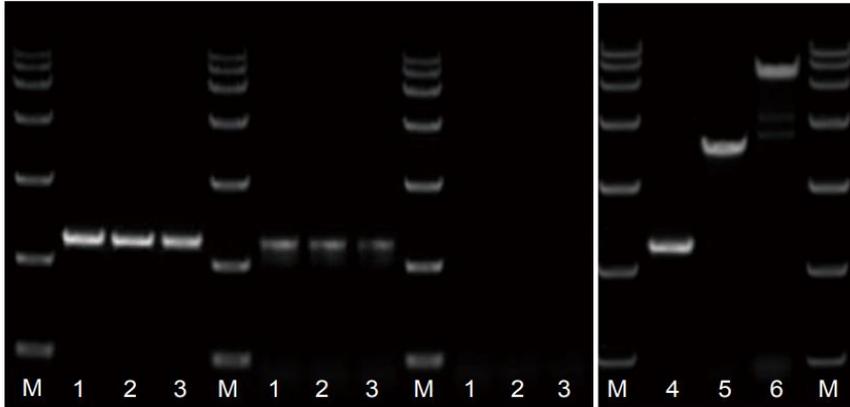
Pack Size: 5x1ml / 200 rxns

Pack Size: 15x1ml / 600 rxns



PCR Analysis | Agarose Gel Electrophoresis

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



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

*Use the high-fidelity polymerase of company A and B according to their own protocols.

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

ddH ₂ O	Up to 50 μ l
Genie Fusion Ultra High-Fidelity 2X Master Mix	25 μ l
Primer 1 (10 μ M)	2 μ l
Primer 2 (10 μ M)	2 μ l
Plant Tissues*	x μ l

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

Recommended PCR Program

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

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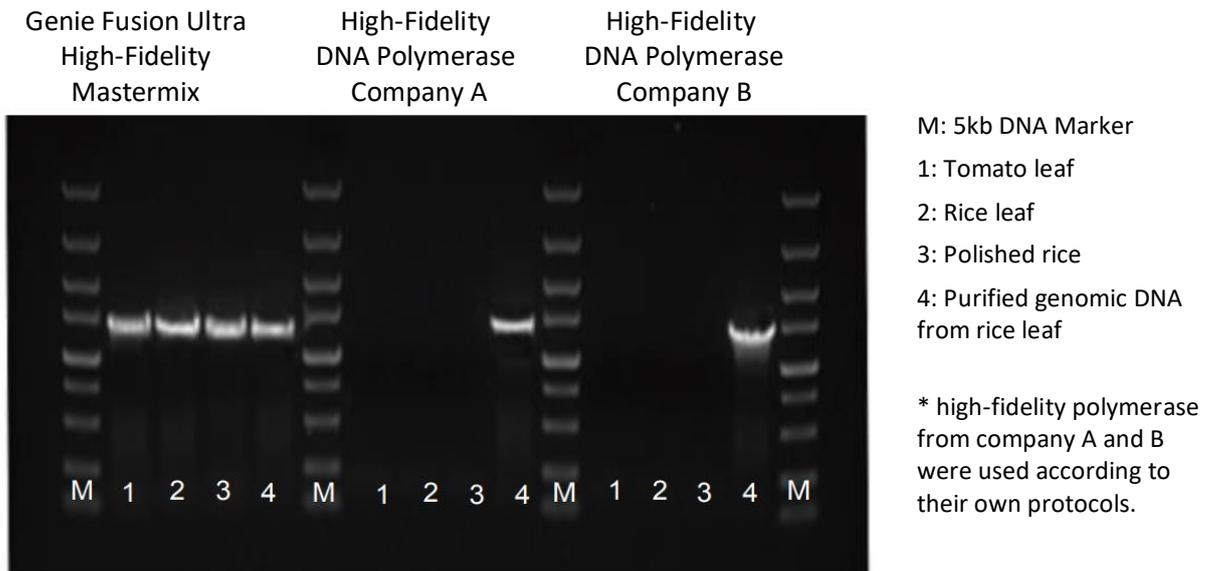
Pack Size: 1ml / 40 rxns

Pack Size: 5x1ml / 200 rxns

Pack Size: 15x1ml / 600 rxns



PCR Analysis | Agarose Gel Electrophoresis



3. Using mouse tail lysate as a template, a target fragment of 2.5 kb was amplified with Genie Fusion Ultra High-Fidelity 2X Master Mix, a high-fidelity DNA polymerase from company A, and a high fidelity DNA polymerase from company B. The T_m of all primers are approximately 60°C (calculated in Primer Premier 5). The reaction system and program are as follows:

Recommended PCR System

ddH ₂ O	up to 50 µl
Genie Fusion Ultra High-Fidelity 2X Master Mix	25 µl
Primer 1 (10µM)	2 µl
Primer 2 (10µM)	2 µl
Lysate of Mouse Tails	2 µl

Recommended PCR System

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

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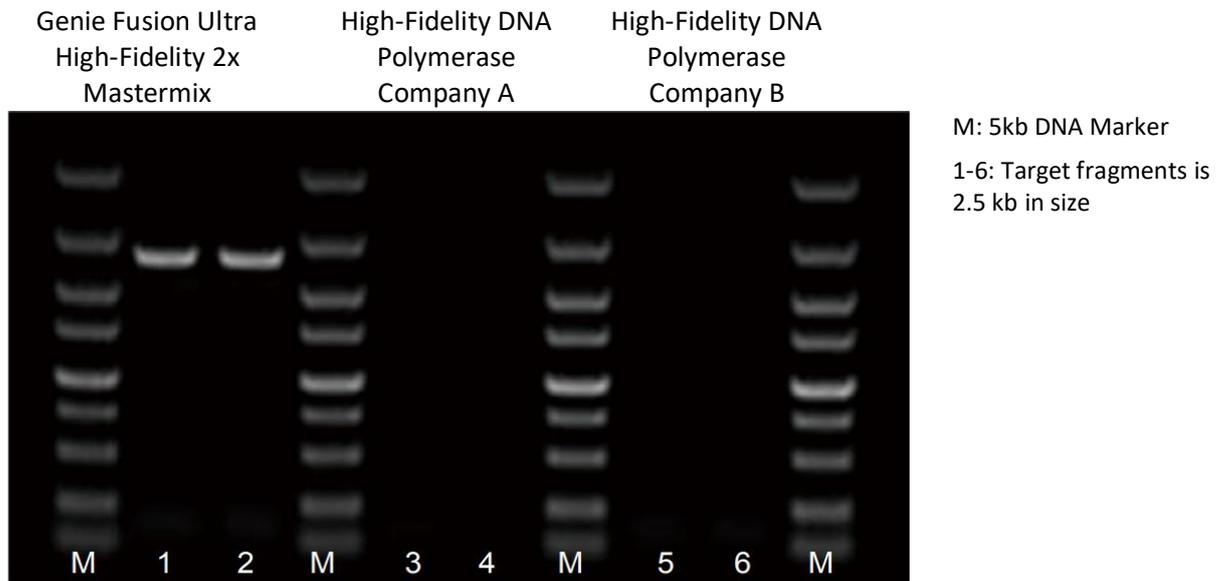
Pack Size: 1ml / 40 rxns

Pack Size: 5x1ml / 200 rxns

Pack Size: 15x1ml / 600 rxns



PCR Analysis | Agarose Gel Electrophoresis



7.3 Excellent Amplification of Fragments with High GC Content

Genie Fusion Ultra High-Fidelity 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 T_m 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 Program

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

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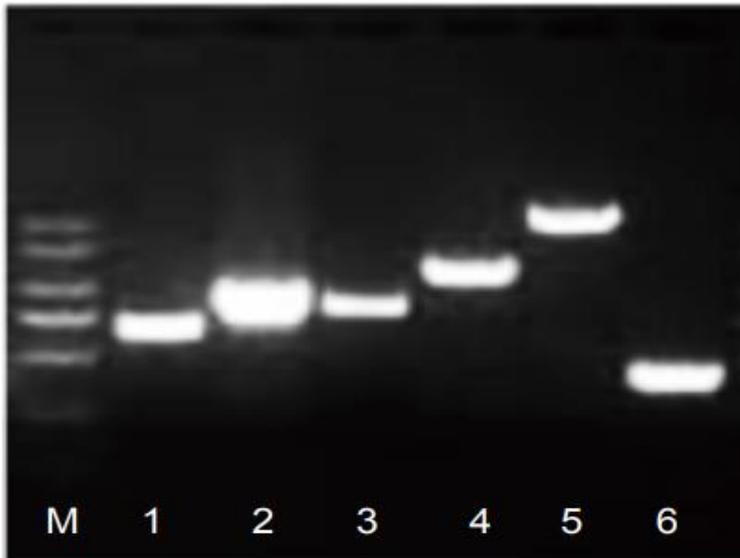
Pack Size: 1ml / 40 rxns

Pack Size: 5x1ml / 200 rxns

Pack Size: 15x1ml / 600 rxns



PCR Analysis | Agarose Gel Electrophoresis



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

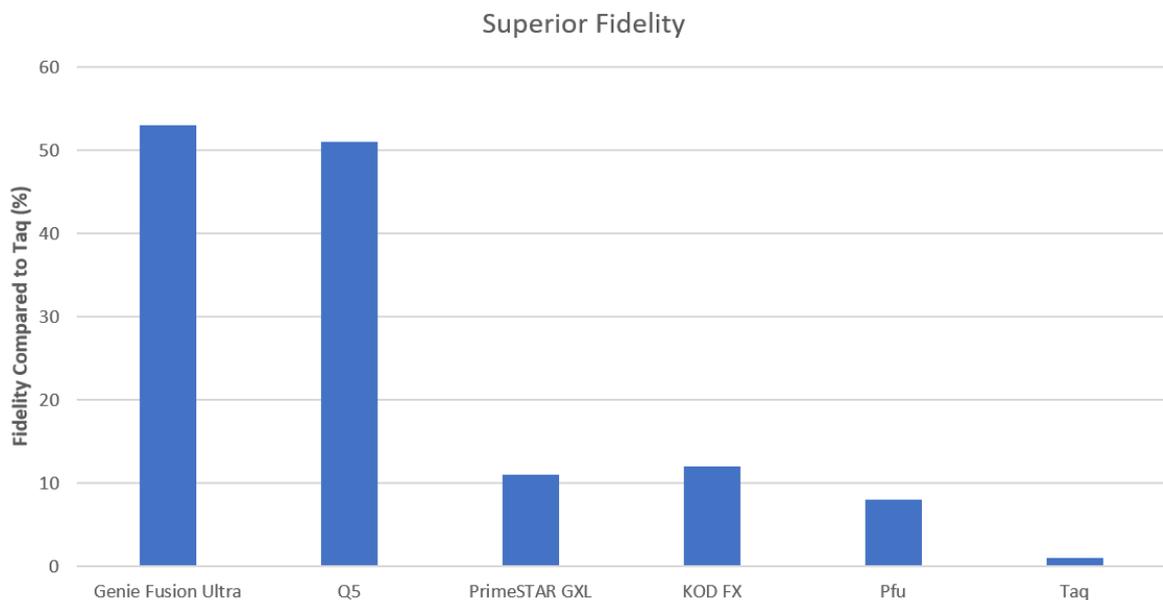
4 : 1,200 bp 73.5% GC Content

5 : 1,400 bp 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. The following figure shows a comparison of amplification fidelity between various polymerases detected by a LacI Assay (Cline et al., *Nucleic Acids Research*, 24: 3546-3551(1996)).



8. Notes

1. Use high-quality templates.
2. Do not use dUTP or any primers or templates that contain uracil.
3. Genie Fusion Super-Fidelity DNA Polymerase has strong proofreading activity. Therefore, the PCR products must be purified before adding A-Tailing when TA cloning.
4. 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 (recommend to calculate in Primer Premier 5), 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 find out the optimal one
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

Unspecific or Smear Bands in Electrophoresis

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

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Enzymes Input

Appropriately adjust or decrease the input of high-fidelity polymerase

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