NileHiFi® Long Amplicon PCR Kit

Cat. No. PC002 (50 reactions)

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I. Introduction

NileHiFi® Long Amplicon PCR Kit is capable of amplifying templates up to 18kb of human gDNA, 30kb of λ phage DNA and other long genomic DNA. This kit is also recommended for amplifying GC rich or complex targets. NileHiFi® Long Amplicon PCR Kit is the ideal choice for generating long PCR products with high sensitivity and processivity.

II. Contents and Storage

Catalog#	Component	Size	Storage
PC002-01	Long Amplicon DNA Polymerase (1 U/μl)	100 μl	-20℃
PC002-02	2× Long Amplicon PCR Mix	1.3 ml	
PC002-03	50 mM Mg^{2+}	50 μl	

Avoid repeated freezing-thawing.

DNA Polymerase Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

III. Important Notes:

1. Mix reagents thoroughly by gently inverting tubes several times while avoiding bubbles, and then briefly centrifuge before use.
2. Store the kit at –20°C. Avoid storage or leaving reagents at 4°C or room temperature. Avoid light exposure at all times.
3. Avoid repeated freezing-thawing.
4. Follow standard procedures for PCR to avoid nucleic acid contamination and non-specific amplification.
5. Read all procedures before setting up the PCR reaction.

IV. Procedure

1. Primer design

- DNA fragment ≤ 8 kb

Use professional primer design software or web design primers such as Primer Premier 5 or NCBI Primer Blast. If the DNA fragment is ≤8kb, the length of the primer is recommended to be 20-25 bp, and the Tm value is recommended to be higher than 55 °C. The Tm value of the upstream and downstream primers should avoid the

difference, and the estimation should be made. The 3' end of the primer should avoid more than 3 binary repeats. sequence.

(2) DNA fragment ≥ 8kb

The primer length is recommended to be 25-30 bp, and the Tm value is recommended to be higher than 60 °C. The Tm value of the upstream and downstream primers should not be too high, and the GC content of the primer 3' should not be too high. Due to the amplification of macromolecular DNA fragments, it is recommended to optimize the primer design and use it preferentially.

2. Sample preparation（25/40/50 μl）

	25 μl	40 μl	50 μl	Concentration
2× Long Amplicon PCR Mix (Mg^{2+}、dNTP plus)[a]	12.5 μl	20 μl	25 μl	1×
Forward Primer	0.4 μM	0.4 μM	0.4 μM	0.4 μM
Reverse Primer	0.4 μM	0.4 μM	0.4 μM	0.4 μM
Template	100 ng/100 pg	100 ng/100 pg	100 ng/100 pg	100 ng/100 pg
Long Amplicon DNA Polymerase (1U/μl)	1 μl	1.5 μl	2 μl	Various
ddH_2O	Up to 25 μl	Up to 40 μl	Up to 50 μl	——

a. The optimal Mg^{2+} concentration of Long Amplicon DNA Polymerase is 1.5~3 mM. If the specific target band is not obtained, please adjust and optimize according to the FAQ of the section V.

b. If the template is gDNA, 50 ng or more should be used. If the template is plasmid DNA, 100 pg can be used.

c. Adjust the amount of polymerase added to different volumes of PCR reactions, such as 1 μl/25 μl; 1.5 μl/40 μl; 2 μl/50 μl.

3. PCR procedure

(1) DNA fragment ≤8 kb:

You can try a 3 step PCR first. If the specificity is not high enough, you can refer to the section VI for FAQ and solutions to make appropriate adjustments.

95℃[a]	2 min	
95℃	30 sec	30 cycles
55～60℃[b]	30 sec	
72℃	40 sec/kb	
72℃	5 min	
Keep in 4-10℃		

(2) For high GC% template or fragments larger than 8 kb:

it is recommended to use a 2 step PCR.

95℃	2 min	
95℃	30 sec	30 cycles
68℃[c]	40-50 sec/kb	
72℃	5 min	
Keep in 4-10℃		

a. Pre-denaturation time should be at least 2 minutes to completely melt DNA and activate the polymerase completely.

b. Stepwise temperature should be adjusted according to the Tm value of the primers. If the Tm value of the primers exceeds 55 °C, the continuous temperature can be set to 60 °C. If the Tm value of the primers is lower than 55 °C, the continuous temperature can be set to 55 °C;

c. According to the Tm value of the long fragment primer, the primer design principle is as described above, and the extension speed is as follows:

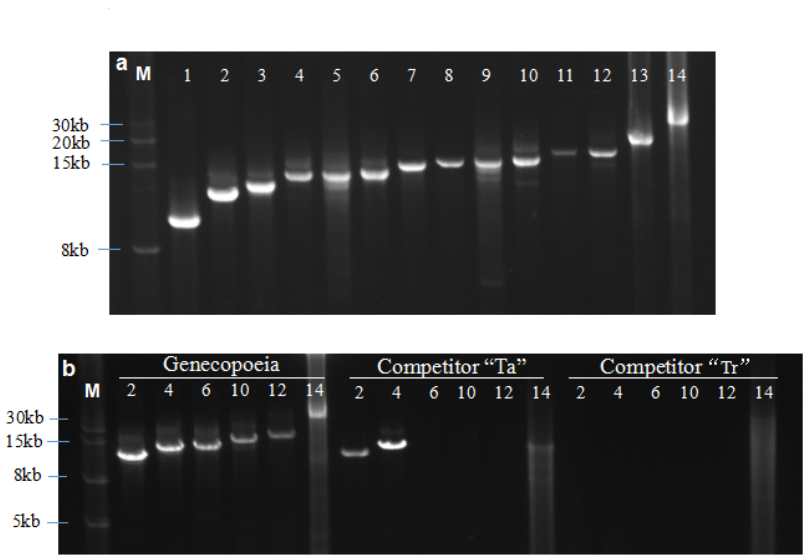
PCR product	≤12 kb	≥12kb	λ DNA
Extension speed	40 sec/kb	50 sec/kb	30 sec/kb

4. Agarose gel electrophoresis

（1） PCR products should be detected by electrophoresis with 1×TAE buffer. Long-length PCR products are recommended to use low-concentration agarose gels and use low-voltage extension electrophoresis time to distinguish long fragments, for example:

Agarose：0.6%

Electrophoresis condition: 90 V，10 min ➡ 20 V，6~16 h：



track	long PCR	
1	EGFR	9.9 kb
2	EGFR	12.0 kb
3	BRCA I	12.1 kb
4	EGFR	13.9 kb
5	BRCA I	14.0 kb
6	β-Globin	14.2 kb
7	BRAF	15.5 kb
8	PTEN	16.0 kb
9	BRCA I	16.0 kb
10	EGFR	16.3 kb
11	PTEN	18.0 kb
12	EGFR	18.0 kb
13	λ DNA	20 kb
14	λ DNA	30 kb

Figure 1. The NileHiFi® long amplicon PCR kit was used to amplify different long fragments of DNA. a. The length of the fragments is randomly selected and contains corresponding mutation targets (such as V600E, L858R, dE746-A750, etc.). b. Performance of GeneCopoeia's long amplicon PCR kit compared with equivalent kits from competitor "Ta" and competitor "Tr".

(2) Short-segment PCR products are recommended for using a 1% agarose gel, for example:

Agarose：1%

Electrophoresis condition:：140 V，20 min

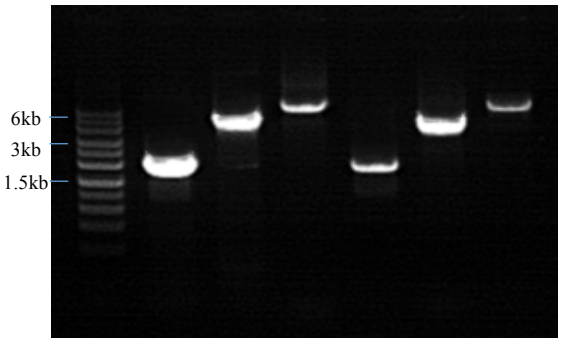


Figure 2. Genecopoeia's Long Amplicon PCR Kit short fragment amplification. Fragments are randomly selected and contain corresponding mutation targets (eg, V600E, dE746-A750, etc.).

V. FAQ

Questions	Causes	Solutions
No specific fragments	Primer	Refer to the section IV Operation Flow 1. Primer design are optimized. It is recommended to design 2 or more primers for optimal use.
	Template	To increase the purity of the template, use appropriate

		amount of template DNA, the genomic DNA should not be less than 50 ng, and the plasmid DNA should be 100 pg.
	Concentration of Mg^{2+}	It is recommended to optimize the reaction system by appropriately increasing the final concentration of Mg^{2+} by a gradient of 0.5 mM.
	PCR Program	1. If the 3-step PCR result is not satisfactory, try 2-step PCR; Slowdown PCR increases specificity 95℃ 2 min 95℃ 30 sec x=70℃，x=x-1 20 sec } 15 cycles 72℃ 40-50 sec/kb 95℃ 30 sec 68℃ 40-50 sec/kb } 20 cycles
Smear	Primer Tm value	Refer to the section IV Operation Flow 1. Primer design principles are optimized. It is recommended to design 2 or more primers for optimal use.
	Polymerase	Adjust the amount of polymerase, such as 0.6 μl / 25 μl or 1.5 μl/25 μl。
	Primer concentration	Adjust from 0.2 μM to 0.4 μM.
	Extension time	Properly reduce the extension time.
	PCR program	Reduce the number of cycles appropriately, for example 25~30.
Low specific product yield	Template	Increase the amount of template appropriately, for example 200 ng
	Extension time	Extent the time (1 min/kb) if the conditions are has good specificity and no serious smear phenomenon.
	PCR program	The number of cycles can be set to 32~35, which can increase the yield of PCR products.

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