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

<table>
<thead>
<tr>
<th>Catalog#</th>
<th>Component</th>
<th>Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC002-01</td>
<td>Long Amplicon DNA Polymerase (1U/μl)</td>
<td>100 μl</td>
<td>-20°C</td>
</tr>
<tr>
<td>PC002-02</td>
<td>2× Long Amplicon PCR Mix</td>
<td>1.3 ml</td>
<td></td>
</tr>
<tr>
<td>PC002-03</td>
<td>50mM Mg²⁺</td>
<td>50 μl</td>
<td></td>
</tr>
</tbody>
</table>

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 PrimerPremier 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)

<table>
<thead>
<tr>
<th></th>
<th>25 µl</th>
<th>40 µl</th>
<th>50 µl</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2× Long Amplicon PCR Mix (Mg²⁺ + dNTP plus)</td>
<td>12.5 µl</td>
<td>20 µl</td>
<td>25 µl</td>
<td>1×</td>
</tr>
<tr>
<td>Forward Primer</td>
<td>0.4 µM</td>
<td>0.4 µM</td>
<td>0.4 µM</td>
<td>0.4 µM</td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>0.4 µM</td>
<td>0.4 µM</td>
<td>0.4 µM</td>
<td>0.4 µM</td>
</tr>
<tr>
<td>Template</td>
<td>100 ng/100 pg</td>
<td>100 ng/100 pg</td>
<td>100 ng/100 pg</td>
<td>100 ng/100 pg</td>
</tr>
<tr>
<td>Long Amplicon DNA Polymerase(1U/µl)</td>
<td>1 µl</td>
<td>1.5 µl</td>
<td>2 µl</td>
<td>Various</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>Up to 25 µl</td>
<td>Up to 40 µl</td>
<td>Up to 50 µl</td>
<td>——</td>
</tr>
</tbody>
</table>

a. The optimal Mg²⁺ 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. PCRprocedure

(1) DNA fragment ≤ 8kb:

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.
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:

<table>
<thead>
<tr>
<th>Agarose</th>
<th>0.6%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis condition:</td>
<td>90 V • 10 min</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>PCR product</th>
<th>≤12 kb</th>
<th>≥12 kb</th>
<th>λ DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extension speed</td>
<td>40 sec/kb</td>
<td>50 sec/kb</td>
<td>30 sec/kb</td>
</tr>
</tbody>
</table>
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: 140V, 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

<table>
<thead>
<tr>
<th>Questions</th>
<th>Causes</th>
<th>Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>No specific fragments</td>
<td>Primer</td>
<td>Refer to the section IV Operation Flow 1. Primer design are optimized. It is recommended to design 2 or more primers for optimal use.</td>
</tr>
<tr>
<td></td>
<td>Template</td>
<td>To increase the purity of the template, use appropriate</td>
</tr>
</tbody>
</table>
### Concentration of Mg²⁺

It is recommended to optimize the reaction system by appropriately increasing the final concentration of Mg²⁺ 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

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>95°C</td>
<td>2 min</td>
</tr>
<tr>
<td>95°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>x=70°C × x=1</td>
<td>20 sec</td>
</tr>
<tr>
<td>72°C</td>
<td>40-50 sec/kb</td>
</tr>
<tr>
<td>95°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>68°C</td>
<td>40-50 sec/kb</td>
</tr>
</tbody>
</table>

- 15 cycles
- 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**
Extend the time (1 min/kb) if the conditions are 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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