NZYLong 2× Green Master Mix

Catalogue number: MB13902, 500 U
MB13903, 1000 U
MB13904, 5000 U

Description

NZYLong 2× Green Master Mix is a premixed ready-to-use solution containing NZYLong DNA polymerase (MB003), an engineered DNA polymerase designed to amplify long target DNA sequences, generally of 20 kb and beyond. A wide range of long PCR products can be generated using lambda DNA or human genomic DNA as starting template. The master mix contains dNTPs, reaction buffer and additives at optimal concentrations for the efficient amplification of an extensive range of DNA templates. $MgCl_2$ final concentration is 2.5 mM, allowing the implementation of a variety of PCR protocols. In addition, reactions assembled with NZYLong 2× Green Master Mix may be directly loaded onto agarose gels. There are two dyes (blue and yellow) in the mix that allow monitoring the progress of electrophoresis. NZYLong 2× Green Master Mix is not suitable when direct fluorescent or absorbance readings are required without prior purification of the amplified DNA from PCR. We recommend using the master mix version without dyes – NZYLong 2× Colourless Master Mix (MB332) – or purifying the PCR product using NZYGelpure (MB011) before performing any other protocol. NZYLong DNA polymerase generates a mixture of A-overhang-ended (predominantly) and blunt-ended PCR products, being suitable for cloning with NZYTech´s TA PCR cloning kits (MB053 or MB137).

Storage temperature

NZYLong 2× Green Master mix should be stored at -20 °C, in a constant temperature freezer. Minimize the number of freeze-thaw cycles by storing in pre-prepared working aliquots of the master mix. The Mix maybe stored at 4 °C for up to 7 days.

Unit definition

One unit is defined as the amount of enzyme required to catalyse the incorporation of 10 nmoles of dNTPs into acid insoluble material in 30 minutes at 72 °C.

Enzyme concentration: 0.2 U/µL

Standard Protocol

The following standard protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of primers and template DNA) may vary, although PCR optimization is usually not required. In case you need to fine-tune primer concentrations, test the recommended variations provided in brackets in the table below.

1. Gently mix and briefly centrifuge the master mix after thawing. Set up the PCR reaction on ice and add water first and the remaining components in the order specified in the table below. A single reaction mixture of 50 µL should combine the following components:

Primers	0.35 (0.25-0.5) µM
Template DNA	50 ng-0.5 µg
NZYLong 2× Green Master Mix	25 µL
Nuclease-free water	up to 50 µL

2. Mix and quickly pulse the reactions.

3. Perform PCR using the following cycling parameters.

Cycle step	Temp.	Time	Cycles
Initial denaturation	94 °C	2 min	1
Denaturation	94 °C	20 s	25-35
Annealing	*	30 s	
Extension	68 °C	1 min/kb	
Final Extension	68 °C	1.5 min/kb	1

*Annealing temperature should be optimised for each primer set based on the primer T_m; typically it should be T_m-5 °C.

4. Analyse the PCR products by agarose gel electrophoresis (0.6-0.8%, w/v) and visualise with GreenSafe Premium (MB132) or any other mean.

Primer Design

Optimal primer design is critical for long-range amplifications. PCR primers should be designed to have 18–35 bases in length and a GC content of 45-60%. Pay special attention to avoid sequences that might produce internal secondary structures. The 3´-ends of the primers should not be complementary to avoid the production of primer-dimers, and it is recommended to have at least 2 Cs or Gs. Ideally, both primers should have nearly identical melting temperatures (T_m) allowing their annealing with the denatured template DNA at roughly the same temperature. For long PCRs avoid using primers that have been previously subjected to multiple freezing-thawing cycles. Note that primer annealing and DNA extension can be combined into one step if primers are designed to have a Tm ≥ 70 ºC.

DNA template

The amplification of long PCR products requires high quality gDNA retaining long DNA fragments (it is impossible to amplify a 20 kb product from damaged gDNA with an average fragment size of 5 kb, for example). The optimal amount of starting material for long PCR may vary from 50-500 ng of genomic DNA template, depending on the size and quality of the template. Lower amounts of DNA template (typically 10-100 ng) can be used for the amplification of lambda or plasmid DNA. Try to add the DNA as the latest component to the PCR reaction and avoid pipetting after

this. To retain DNA integrity, avoid multiple freeze-thawing cycles stock DNA solutions and keep working DNA at 4 ºC.

Cycling conditions

It is highly recommended to use incubation and extension temperatures as high as required by the experiment. An extension performed at 68 ºC favours the accumulation of long PCR products without compromising enzyme performance.

Quality control assays

Purity

NZYLong DNA polymerase purity is >90% as judged by SDS-PAGE followed by Coomassie Blue staining.

Genomic DNA contamination

NZYLong 2× Green Master Mix must be free of any detectable genomic DNA contamination as evaluated through PCR.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with NZYLong 2× Green Master Mix for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a GreenSafe Premium-stained agarose gel. There must be no visible nicking or cutting of the nucleic acid.

Functional assay

NZYLong 2× Green Master Mix is tested for performance in a polymerase chain reaction (PCR) of different-sized DNA fragments (10, 15 and 20 kb) from human genomic DNA. The resulting PCR products are visualized as single bands in a GreenSafe Premium-stained agarose gel.

Troubleshooting

No product amplification or low yield
• Inadequate annealing temperature
The reaction mix composition may affect the melting properties of primers and DNA. Adjust the annealing temperature to accommodate the primer with the lowest melting temperature (5 ° to 10 °C lower than T_m).
• Presence of PCR inhibitors
Some DNA isolation procedures, particularly genomic DNA isolation, can result in the co-purification of PCR inhibitors. Reduce the volume of template DNA in reaction or dilute template DNA prior to adding to the reaction. Diluting samples even 1:10,000 has been shown to be effective in improving results, depending on initial DNA concentration.
• Template DNA damaged or degraded
An intact, high-quality template is essential to achieve a reliable amplification of large DNA fragments. Extreme care must be taken in the preparation and handling of DNA. Always use purified high-quality DNA as template.
• Concentration of Mg^{2+} is too low
Mg^{2+} is included in the Master Mix at a final concentration of 2.5 mM, which is sufficient for most targets. For some targets, higher Mg^{2+} concentration may be required. Titrate from 2.5 mM to 4 mM (final concentration) in 0.5 mM increments. (Note: $MgCl_2$ is not provided in separate tubes).
Presence of non-specific bands
• Non-specific annealing of primers
Adjust annealing conditions and/or design another set of primers, by increasing the length and avoiding complementary sequences.
• Primer degradation
Check the quality and concentration of primer solutions. We recommend to prepare small-volume working aliquots from the stock solution. Avoid using primers subjected to multiple freezing-thawing cycles.

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Certificate of Analysis

Test	Result
Enzyme purity*	Pass
Genomic DNA contamination*	Pass
DNase contamination	Pass
Functional assay	Pass

*These assays were performed exclusively with the enzyme

Approved by:

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