

## Product components

| Components                 | Size-1      | Size-2     |
|----------------------------|-------------|------------|
|                            | 24 RXN      | 96 RXN     |
| TruePol 2X PCR Mix for NGS | 600 $\mu$ L | 1.2 mL X 2 |

## Product Description

TruePol 2X PCR Mix is a premix solution derived from a B family polymerase, which originates from a unique mutation that enhances its elongation ability. The yield and specificity were improved, and there was no obvious GC preference. 2X PCR Mix has 5' -3' continuous synthesis activity and 3' -5' exonuclease activity, and its amplification product is flat-end.

TruePol 2x PCR Mix contains DNA polymerase, buffer, Mg<sup>2+</sup> and dNTPs, etc. It is easy-to-operate, and just add DNA template, primers and water in the amplification system.

All batches of TruePol 2x PCR Mix for NGS have undergone strict quality control to ensure the stable performance of each batch of reagents.

This product applies to DNA library products, such as RK20208 Rapid Plus DNA Lib Prep Kit for Illumina, RK20255 Rapid Plus DNA Lib Prep Kit for Illumina v 2, RK20256 Rapid Plus DNA Lib Prep Kit for MGI v 2, etc. .

## Storage

Upon receipt, store all components at -20°C.

## Standard Protocol

### Notice:

- All components should be mixed well and centrifuged instantaneously before use;
- It is recommended to prepare all reaction components on ice. This is because the 3'-5' exonuclease activity of Gloria Nova HS DNA polymerase at room temperature can cause degradation of the primers. Keeping the reaction components on ice can help to minimize this effect;
- For library preparation steps or systems, please refer to the ABclonal library preparation instructions;
- This polymerase is specially developed for the NGS library amplification system. For gene cloning, please select other molecular amplification enzymes.

## Recommended Reaction

Take 50  $\mu$ L reaction systems as examples.

| Components                     | 50 $\mu$ L    |
|--------------------------------|---------------|
| TruePol 2X PCR Mix for NGS     | 25 $\mu$ L    |
| DNA Template*                  | Variable      |
| Forward Primer (20 $\mu$ M) ** | 2.5 $\mu$ L   |
| Reverse Primer (20 $\mu$ M) ** | 2.5 $\mu$ L   |
| Nuclease-free Water            | to 50 $\mu$ L |

\*The DNA template refers to the purified product of the linker connected to the magnetic beads during the library construction process.

\*\*The primers are the library primers used in the process of NGS library construction.

## Recommended PCR Program

| Step             | Temp    | Time  | Cycles                                           |
|------------------|---------|-------|--------------------------------------------------|
| Pre-denaturation | 98 °C   | 45 s  | 1                                                |
| Denaturation     | 98 °C   | 15 s  |                                                  |
| Annealing        | 60 °C   | 30 s  | Recommended number of cycles are based on input* |
| Extension        | 72 °C   | 30 s  |                                                  |
| Post-extension   | 72 °C   | 1 min | 1                                                |
| Hold             | 4-12 °C | ∞     | 1                                                |

\* Different cycle numbers are selected according to different sample types, different inputs, and different sequencing platforms, for example, short connectors using the Illumina platform/MGI platform, different starting amount templates, the recommended cycle number of 1 µg product is as follows

## Choose different cycles depending on the amount of input

| Input DNA (fragmented DNA) | Number of PCR cycles (1 µg) |
|----------------------------|-----------------------------|
| 1 ng                       | 13-15                       |
| 10 ng                      | 9-11                        |
| 25 ng                      | 7-9                         |
| 50 ng                      | 6-7                         |
| 100 ng                     | 5-6                         |
| 250 ng                     | 4-5                         |
| 500 ng                     | 3-4                         |
| 1000 ng                    | 2-3                         |

## Notes:

1. For full adapters, the maximum number of cycles can be used for amplification;
2. For MGI platform, the medium cycle number or the maximum cycle number can be used for amplification;
3. For FFPE samples, especially those with poor quality, it is necessary to increase the number of cycles by 1 -2.