| Product Information | |
|---|---|
| **2X HiFi PCR Master Mix** | |
| **Part Number** | P7670L |
| **Concentration** | 2X |
| **Unit Size** | 24 reactions |
| **Storage Temperature** | -25°C to -15°C |
| **Lot Number** | |
| **Reference Number** | |

**Product Description:**
2X HiFi PCR Master Mix is a high efficiency, high fidelity, and low bias PCR master mix for NGS DNA library amplification.

| Product Specifications | |
|---|---|
| **Assay** | **HiFi PCR Master Mix Functional Assay** |
| **Specification** | Functional |

**Quality Control Analysis:**
Quality of the 2X HiFi PCR Master Mix is tested functionally by amplification of a DNA library prepared from mixed bacterial genomic DNA with GC-content of 10-80%. The differences in library yield and profile among different lots must not exceed 15%. Sequencing of the amplified library must yield mapped reads >90% and normalized coverage between 0.7 and 1.3 across the full GC spectrum.

**Supplied with:**
**HiFi Enhancer (M0313L)**
Note: The HiFi enhancer can be used for optimizing workflows requiring amplification of libraries immobilized on magnetic beads.

**Usage Instructions:** two protocols are provided below, one for routine NGS library amplification and one for post-capture PCR enrichment with magnetic beads.

**Routine NGS Library Amplification**
The 2X HiFi PCR Master Mix can be used for high-fidelity amplification of the DNA library or other NGS related applications. Library amplification is generally recommended if the DNA input amount is below 100 ng. It is important that a post-ligation cleanup is performed prior to library amplification to remove unligated adapters and adapter dimers from the reaction. Please note that primers are not included with this product but can be sourced from any reputable oligonucleotide vendor. Primers should be used at final concentrations of 0.5-2 µM each.

1. Program a thermal cycler with the parameters listed in the table below. Set the instrument's heated lid to 105°C. When the thermal cycler block reaches 98°C, pause the program.

| Step | Temperature | Incubation Time | Cycle number |
|---|---|---|---|
| Initial Denaturation | 98°C | 2 min | 1 |
| Denaturation | 98°C | 20 sec | As required† |
| Annealing | 60°C* | 30 sec | |
| Extension | 72°C | 30 sec‡ | |
| Final Extension | 72°C | 1 min | 1 |
| Hold | 4°C | ∞ | N/A |

* Annealing temperature optimization may be necessary.

**Limitations of Use**
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.

† Amplification cycle number needs to be adjusted based on DNA template concentration, primer concentrations, and DNA library yield sufficient for downstream applications.
‡ 30-60 sec/kb is recommended when deciding extension time.

2. Prepare the PCR reaction in a new tube on ice by combining the DNA template, 2X HiFi PCR Master Mix, and customer-supplied primers per the table below. Mix well by pipetting up and down 8-10 times. Volumes can be scaled as needed.

| Components | Volume for 1 reaction (µL) |
|---|---|
| DNA Template | X |
| 2X HiFi PCR Master Mix | 25 |
| Primer Mix | Y |
| Nuclease-free Water | 25-X-Y |
| **Total Volume =** | **50** |

3. Pulse-spin the reaction tube and immediately transfer to the pre-heated thermal cycler (98°C). Resume the cycling program.
4. When the thermal cycler program is complete and sample block has returned to 4°C, remove the sample from block and proceed to immediately proceed to Post-Amplification Cleanup using AMPure® XP beads or other desired purification method.
5. Validate and quantify the library using gel electrophoresis, qPCR and/or Bioanalyzer.

**Post-capture PCR enrichment**

The 2X HiFi PCR Master Mix is compatible with target enrichment workflows such as those utilizing bead-based hybridization capture probes and panels. The following instructions are validated for PCR amplification of targets enriched using xGen® Lockdown® Probes and reagents from IDT. In this workflow, the targets to be amplified are immobilized on Dynabeads® M-270 Streptavidin beads. If other types of capture beads are used, the reaction may need to be optimized accordingly.

1. Program a thermal cycler with the parameters listed in the table below. Set the instrument's heated lid to 105°C. When the thermal cycler block reaches 98°C, pause the program.

| Step | Temperature | Incubation Time | Cycle number |
|---|---|---|---|
| Initial Denaturation | 98°C | 45 sec | 1 |
| Denaturation | 98°C | 15 sec | As required* |
| Annealing | 60°C | 30 sec | |
| Extension | 72°C | 30 sec | |
| Final Extension | 72°C | 1 min | 1 |
| Hold | 4°C | ∞ | N/A |

* The number of PCR cycles should be optimized according to capture probe supplier's guideline.

2. Prepare the post-capture amplification reaction mix in a separate tube per the table below.

| Components | Volume for 1 reaction (µL) |
|---|---|
| Beads with Captured DNA Template | X |
| 2X HiFi PCR Master Mix | 25 |
| Primer Mix** | Y |
| HiFi Enhancer*** | 2 |
| Nuclease-free Water | 23-X-Y |
| **Total Volume =** | **50** |

** A final concentration of 0.5 µM each primer for on-bead amplification. Please follow manufacturer/supplier's instructions.
*** 2-2.5 µL of the HiFi Enhancer should be added to each reaction to ensure high efficiency on-bead amplification. When using a higher volume of beads, scale the amount of HiFi Enhancer accordingly.

3. Pipette up and down to ensure mixing of components. Spin down gently, but ensure the beads remain in solution.
4. Transfer the reaction tube to the pre-heated thermal cycler (98°C). Resume the cycling program.
5. When the thermal cycler program is complete and sample block has returned to 4°C, remove the sample from block and proceed to immediately proceed to Post-Amplification Cleanup using AMPure® XP beads or other desired purification method.
6. Validate and quantify the library using gel electrophoresis, qPCR and/or Bioanalyzer.

| Related NGS DNA Library Preparation Products | |
|---|---|
| **Part Number** | **Description** |
| Y9410L | 5X WGS Fragmentation Mix |
| Y9420L | ER/A-Tailing Enzyme Mix |
| L6030-W-L | WGS Ligase |

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