VAHTS™ HiFi Amplification Mix

N616-01/02

Version 9.1

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Introduction

VAHTS™ HiFi Amplification Mix is a new High-fidelity PCR amplification master mix, which is applicable to high-throughput sequencing library PCR amplification. The master mix is based on VAHTS™ HiFi DNA Polymerase, which is the new generation of DNA polymerase with high yield and fidelity engineered from Pfu DNA Polymerase. The sensitivity of this enzyme has been greatly improved, with extremely high amplification efficiency and extensive adaptability of the template. It greatly improves the amplification output and the platform period. At the same time, the amplification mismatch rate is 1/52 of Taq DNA Polymerase and 1/6 of Pfu DNA Polymerase, ensuring the authenticity of library amplification. VAHTS™ HiFi Amplification Mix, with an optimized buffer system, enables low preference of high-throughput sequencing library, wide template input range, high yield, and high stability amplification. This product contains all the components required for PCR amplification, and contains library amplification primers for Illumina sequencing platforms. It can be used easily by only adding a library template. It contains special protective regents, which enables long-term storage and can maintain a stable activity after repeated freezing and thawing. The amplified product is blunt and can be used directly for blunt-end cloning.

Components

Components	N616-01 (24 rxn)	N616-02 (96 rxn)
VAHTS HiFi Amplification Mix	600 µl	4 × 600 µl
PCR Primer Mix 3 for Illumina	120 µl	480 µl

Storage

The Kit should be stored at -30 ~ -15℃ and transported at -20 ~ 0℃.

Quality control

Residual endonuclease detection: 25 µl of this product and 0.3 µg of pBR322 DNA are incubated at 37℃ for 4 h. After agarose gel electrophoresis, the electrophoresis bands of the plasmid should not change.

Residual E. coli DNA detection: 25 µl of the remaining nucleic acid in this product is detected by TaqMan qPCR specific for E.coli gDNA, and the E.coli genome residue is less than 10 copies.

Functional detection:

Add 25 µl of this product to the 50 µl of PCR reaction system, take 0.4 ng and 200 ng DNA library as template, respectively. Perform 12 and 3 cycles of library amplification and purify the product by Qubit and the total yield should be more than 1 µg.

Add 25 µl of this product to the 50 µl of PCR reaction system, take 10 ng of human genome DNA and 0.5 µM of amplification primers to amplify 1 kb of fragment. After 30 cycles, perform agarose gel electrophoresis, a specific 1 kb product band should be observed.

Protocol

Reaction system

All operations should be on the ice, invert VAHTS HiFi Amplification Mix up and down after thawing, immediately put it back to -20℃ after use.

Purified ligation products	x µl
PCR Primer Mix 3 for Illumina*	5 µl
VAHTS HiFi Amplification Mix	25 µl
Sterilized distilled water	To 50 µl

*For non-illumina sequencing platforms, the corresponding library amplification primers must be changed.

2. Perform qPCR reaction at the following cycling conditions:

Steps	Temperature	Time	Cycles
Hot lid On	105℃		
Pre-denaturation	95℃	3 min	1
denaturation	98℃	20 sec	
Annealing	60℃*a	15 sec	2 - 19*c
Extension	72℃	30 sec*b	
Complete extension	72℃	5 min	1
Hold	4℃		

*a. The annealing temperature should be adjusted according to the Tm value of the primer. For the common Illumina sequencing platform library, it is recommended to set the temperature to 60℃.

*b. For libraries with special-length, the extension time can be increased as appropriate.

*c. Select proper cycles according to the table below:

Library type	Initial amount of template	1μg of yields required number of cycles
DNA	100 pg	16 - 19
	1 ng	12 - 15
	10 ng	8 - 12
	100 ng	5 - 8
	500 ng	2 - 5
	1 μg	2 - 5
mRNA	10 ng	16 - 19
	100 ng	12 - 16
	1 μg	11 - 14

▲ For special library or library constructed by low quality template, such as library constructed by FREE samples, ChIP DNA library and so on, 2 - 4 cycles can be increased.

Trouble shooting

◇ Sequencing platform compatibility

The PCR Primer Mix 3 for Illumina included in the kit is suitable for the Illumina sequencing platform; for other sequencing platforms, replace the primers with the corresponding libraries.

◇ The concentration of primers for library amplification

For library amplification primers that are not Illumina sequencing platforms, it is recommended that the primer concentration be adjusted within the range of 10 - 20 μM.

◇ Other Notes

① It is recommended to use a purified high quality library template and follow the instructions.

② For special libraries, such as low-quality libraries and long-fragment libraries, the annealing temperature, extension time, and amplification cycle numbers can be adjusted according to the reaction procedure to obtain the best amplification results.

③ When adjusting the extension time, it should not exceed 1 min/kb.