VAHTS Target Capture Hybridization and Wash Kit

NC103

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Instruction for Use

Version 22.1

Vazyme

Contents

01/Product Description

The VAHTS Target Capture Hybridization and Wash Kit is specifically developed for use in combination with Vazyme's other VAHTS Target Capture products. The well-designed hybridization and post-capture wash systems ensure the stability and uniformity of the capture. All the reagents provided in the kit have undergone rigorous quality control and functional testing to ensure the optimal stability and repeatability of the experiment.

02/Components

	Components	NC103-01 24 rxns	NC103-02 96 rxns
Box 1	2 × Hybridization Buffer	408 μl	2 × 816 μl
	Hybridization Enhancer	130 μl	520 μl
	Human Cot-1 DNA	120 μl	480 μl
	2 × Beads Wash Buffer	5 ml	20 ml
	10 × Wash Buffer S	1 ml	4 ml
	10 × Wash Buffer I	750 μl	3 ml
	10 × Wash Buffer II	500 μl	2 ml
	10 × Wash Buffer III	500 μl	2 ml
	VAHTS HiFi Amplification Mix 2	600 μl	4 × 600 μl
Box 2	CA-28 Streptavidin Beads	2.4 ml	9.6 ml

03/Storage

Box 1: Store at -30 ~ -15℃ and transport at ≤0℃.
Box 2: Store at 2 ~ 8℃. Adjust the shipping method according to the destination.

04/Applications

This product is intended for hybridization capture of exons or other target regions, as well as subsequent beads capture and wash. It should be used in conjunction with other VAHTS Target Capture products.

05/Self-prepared Materials

1. Compatible reagents

Components	Name	Remark
ND627	VAHTS Universal Plus DNA Library Prep Kit for Illumina V2	Pre-library preparation
NC001	VAHTS Target Capture Core Exome Panel	Exon panel
NC101	VAHTS Target Capture Universal Blockers and Post-PCR Primer Mix for Illumina-TS	Adapter blocking and amplification of Illumina TruSeq DNA libraries
NCM101	VAHTS Target Capture Universal Blockers and Post-PCR Primer Mix for MGI-SI	Adapter blocking and amplification of MGI single-indexed libraries
NCM102	VAHTS Target Capture Universal Blockers and Post-PCR Primer Mix for MGI-DI	Adapter blocking and amplification of MGI dual-indexed libraries
N411	VAHTS DNA Clean Beads	Purification of libraries obtained by post-capture PCR enrichment
EQ121	Equalbit 1 × dsDNA HS Assay Kit	Determination of the final library concentration after capture

2. Other materials:

   Absolute ethanol, ddH_2O, 0.1 × TE, elution buffer (10 mM Tris-HCl, pH 8.0 - 8.5), 200 μl low binding PCR tube, 1.5 ml low binding Nuclease-free EP tube, and magnetic stand.

3. Instruments:

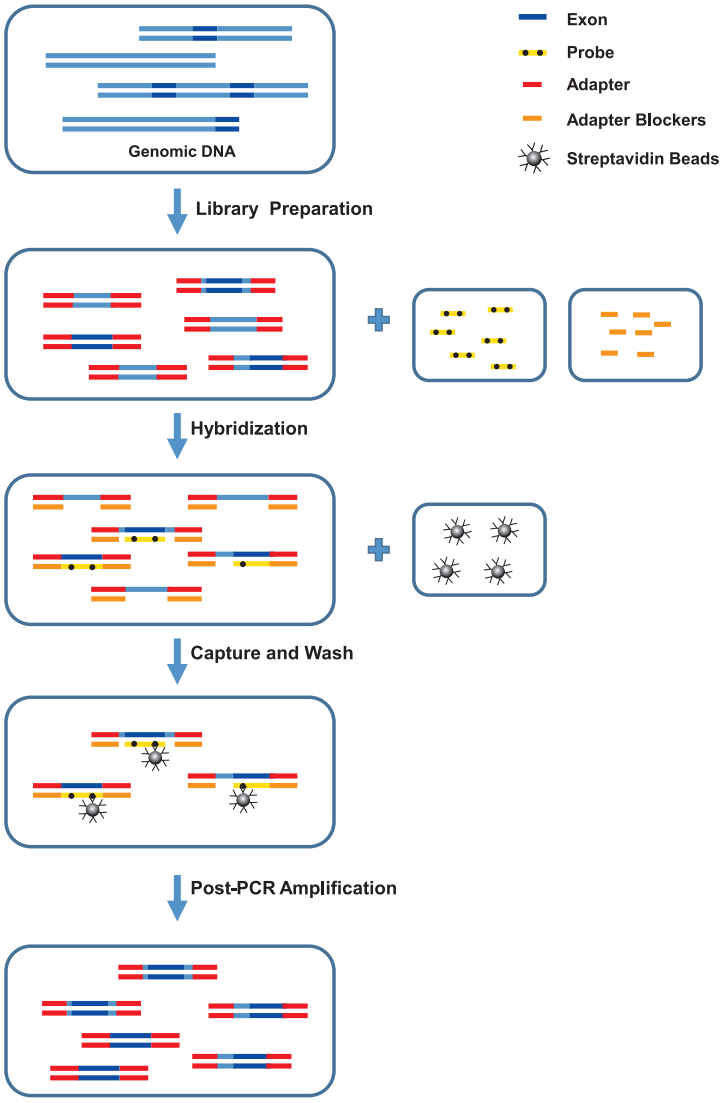
   Vacuum concentrator, PCR instrument, metal bath apparatus, vortex mixer, handheld centrifuge, Qubit fluorometer, and Agilent Technologies 2100 Bioanalyzer or other equivalents.

06/Notes

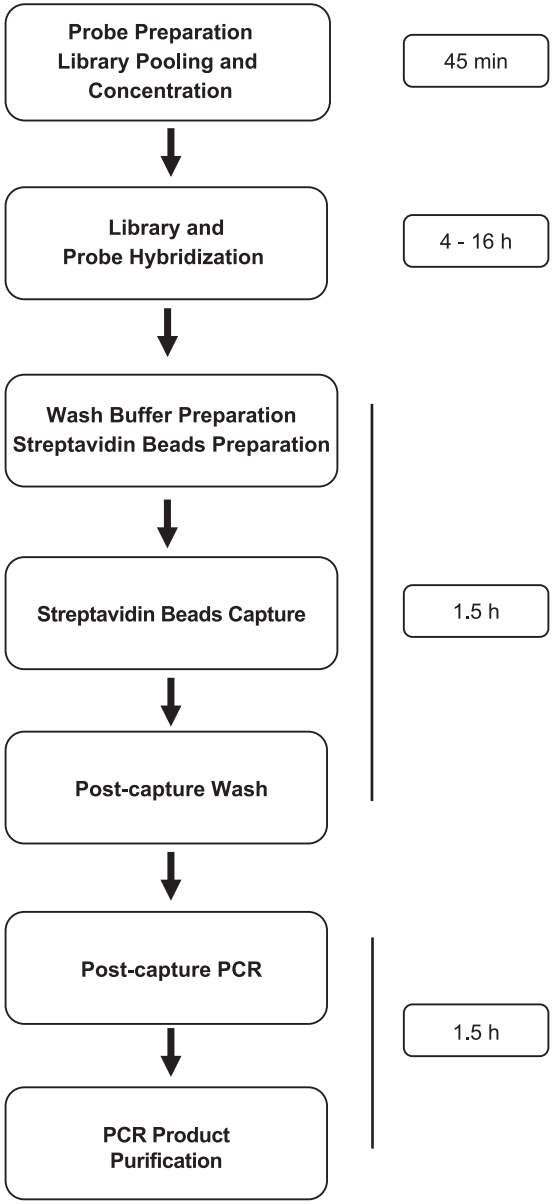
For research use only. Not for use in diagnostic procedures.

1. Check the adapter type of the pre-library before use and select the correct Blockers. If the Blockers used do not match the pre-library adapter type, the on-target rate will be reduced, and the hybridization capture may fail.
2. Strict temperature control is required during hybridization and washing. Inaccurate temperature control may result in a lower on-target rate and uniformity of the capture library.
3. The concentration of pre-libraries with beads may lead to GC bias, and it is recommended that pre-libraries be concentrated using a vacuum concentrator.
4. After washing the streptavidin beads obtained in 08-6/Post-capture Wash, resuspend the beads in Nuclease-free ddH_2O; do not discard the beads.

07/Mechanism & Workflow



Mechanism of Hybridization Capture



Workflow of Hybridization Capture

08/Experiment Process

08-1/Library Pooling and Concentration

1. Prepare the following reaction system in a 1.5 ml Nuclease-free EP tube:

Components	Volume
Pre-library	X μl (500 ng of each library, If multiplexing samples, Do not exceed 5 μg int total)
Cot-1 DNA	5 μl
Universal Blockers	2 μl

▲When the probes are hybridized to multiple libraries, the total volume of libraries should not exceed 5 μg.

▲When the probes are hybridized to multiple libraries, the volume of each library is recommended to be 500 - 1,000 ng and must be no less than 125 ng.

The Blockers are suitable for the libraries with different adapter types are listed in the table below:

Cat. No.	Universal Blockers Type	Scope of Application
NC101	VAHTS Target Capture Universal Blockers and Post-PCR Primer Mix for Illumina-TS	Illumina TruSeq libraries
NCM101	VAHTS Target Capture Universal Blockers and Post-PCR Primer Mix for MGI-SI	MGI single-indexed libraries
NCM102	VAHTS Target Capture Universal Blockers and Post-PCR Primer Mix for MGI-DI	MGI dual-indexed libraries

2. Dry down the mixture of 08-1/Library Pooling and Concentration/Step 1 by a vacuum concentrator (temperature ≤50℃).

▲ If subsequent hybridization capture will not be performed immediately, store the concentrated library at -30 ~ -15℃ for no more than 24 h.

08-2/Library and Probe Hybridization

1. Take out the kit from -20℃. Thaw the reagents and mix them well. According to the following table, add the reagents to the EP tube of 08-1/Library Pooling and Concentration/Step 2.

Components	Volume
2 × Hybridization Buffer	8.5 μl
Hybridization Enhancer	2.7 μl
Nuclease-free ddH_2O	1.8 μl
Probe	4 μl
Total	17 μl

2. Keep the tube at room temperature for 10 min. Mix the contents well by pipetting up and down. Transfer the solution to a new 200 μl low binding PCR tube.

▲ Be sure to use PCR tubes with good airtightness and resistance to high temperature for this step to avoid evaporation of the reaction solution during hybridization.

3. Place the PCR tube into the PCR instrument and run the following program:

Temperature	Time
Heating lid, 100℃	On
95℃	30 sec
65℃	4 - 16 h

08-3/Reagent Preparation

1. Wash Buffer preparation

Prepare the reagents according to the following table. Dilute the Buffer to 1 ×. The Buffer volumes specified in the table are for one capture reaction only. If N capture reactions are planned, multiply the volumes by a factor of 1.1 × N.

▲Be sure to incubate the 10 × Wash Buffer I at 65℃ for at least 10 min before use to ensure complete dissolution.

Buffer Name	Buffer Volume	Nuclease-free ddH_2O Volume	1 × Working Solution Volume (per rxn)	Temperature
2 × Beads Wash Buffer	200 µl	200 µl	400 µl	Room temperature
10 × Wash Buffer I	30 µl	270 µl	300 µl	110 µl at 65℃, the rest at room temperature
10 × Wash Buffer II	20 µl	180 µl	200 µl	Room temperature
10 × Wash Buffer III	20 µl	180 µl	200 µl	Room temperature
10 × Wash Buffer S	40 µl	360 µl	400 µl	65℃

▲The 1 × Wash Buffer I and 1 × Wash Buffer S used for heated wash must be placed at 65℃ for about 20 min before use. The buffer should be avoiding excessive pre-warming to protect from evaporating.

2. Preparation of magnetic beads resuspension

Prepare the Beads Resuspension Mix in low binding tube as the following table. The reagent volumes given in the table are for one capture reaction only. If N capture reactions are planned, multiply the volumes by a factor of 1.1 × N.

Components	Volume
2 × Hybridization Buffer	8.5 µl
Hybridization Enhancer	2.7 µl
Nuclease-free ddH_2O	5.8 µl
Total	17 µl

08-4/CA-28 Streptavidin Beads Wash

The amount of CA-28 Streptavidin Beads required for a single capture reaction is 100 µl. Prepare the required amount of beads for N capture reactions as described below.

▲CA-28 Streptavidin Beads must be prepared to ready to use. Avoid drying out the beads during use.

1. Take the CA-28 Streptavidin Beads out from the 4℃ and equilirate to room temprature.
2. Mix the beads thoroughly by vortexing for 15 sec.
3. Add 100 µl of CA-28 Streptavidin Beads to a new 200 µl low binding PCR tube.
4. Place the tube on the magnetic stand until the solution becomes clear.
5. Remove and discard the clear supernatant, ensuring that the beads remain in the well.
6. Remove the tube from the magnetic stand, add 200 µl of 1 × Beads Wash Buffer, and vortex-shake the tube for 10 sec.
7. After instantaneous centrifugation, let the PCR tube stand on the magnetic stand until the solution becomes clear. Remove and discard the clear supernatant, ensuring that the beads remain in the well.
8. Repeat Step 6 - 7 once again, and the beads are washed twice in total.
9. Remove the tube from the magnetic stand and add 17 µl of magnetic beads resuspension prepared in 08-3/Reagent Preparation/Step 2.

08-5/CA-28 Streptavidin Beads Capture

1. Ensure the tube of 08-2/Library and Probe Hybridizationn/Step 3 is kept in the PCR instrument at all times. Place the beads resuspended in 08-4/CA-28 Streptavidin Beads Wash/Step 9 at the PCR instrument (heating lid, 100℃) for 1 min. Quickly transfer the pre-warmed beads into the reaction solution obtained in 08-2/Library and Probe Hybridization/Step 3. Pipet the solution up and down 15 times to mix the contents well.

   ▲Do not take the reaction solution tube out from the PCR instrument when adding beads to the hybridization product; be sure to keep the reaction solution in the PCR instrument at all times.

2. Incubate the mixture at 65℃ for 45 min, every 15 min, remove the tube from the PCR instrument and gently vortex to ensure the sample is fully resuspended.

   ▲This step should be completed as fast as possible to avoid great changes in the temperature of the hybridization product in the PCR tube. After vortexing, gently shake the tube until the reaction solution sinks to the bottom, without the need for instantaneous centrifugation.

08-6/Post-capture Wash

1. Heated wash at 65℃ (heating lid, 65℃)

▲During heated wash, keep the PCR tube in the PCR instrument at 65℃.

a. Set both the heating lid temperature and incubation temperature of the PCR instrument to 65℃ in advance.

b. After beads capture, transfer the PCR tube in 08-5/CA-28 Streptavidin Beads Capture/Step 2 into the PCR instrument of step a.

c. Add 100 µl of 1 × Wash Buffer I (pre-warmed to 65℃) to the PCR tube. Pipet the mixture up and down 10 times to mix it well.

d. Place the tube on the magnetic stand for 30 sec. Remove the supernatant after the solution becomes clear.

e. Quickly put the PCR tube back in the PCR instrument. Immediately add 180 µl of 1 × Wash Buffer S (pre-warmed to 65℃) to the PCR tube and pipet the solution up and down 10 times for thoroughly mixing. Cap the tube and incubate it in the PCR instrument at 65℃ for 5 min.

   ▲This step should be completed as fast as possible so that the temperature of the hybridization product in the PCR tube can return to 65℃ quickly.

f. Mix the solution thoroughly by gently pipetting up and down 10 times every 2.5 min during incubation.

g. After the reaction, take out the PCR tube and perform instantaneous centrifugation. Place the PCR tube on the magnetic stand and remove the supernatant after the solution becomes clear.

h. Repeat Step e - g once again, and the capture product undergoes heated wash with pre-warmed Wash Buffer S twice in total.

2. Wash at room temperature

a. Add 180 µl of 1 × Wash Buffer **I** to the PCR tube. Vortex the tube and incubate it at room temperature for 2 min. During the incubation, vortex the tube every 30 sec to ensure thoroughly mixing.

b. After instantaneous centrifugation, place the tube on the magnetic stand for 1 min. After the solution becomes clear, carefully remove the supernatant. Add 180 µl of 1 × Wash Buffer **II** to the PCR tube. Vortex the tube and incubate it at room temperature for 2 min. During the incubation, shake the tube every 30 sec to ensure thoroughly mixing.

c. After instantaneous centrifugation, place the tube on the magnetic stand for 1 min. After the solution becomes clear, carefully remove the supernatant. Add 180 µl of 1 × Wash Buffer **III** to the PCR tube. Vortex the tube and incubate it at room temperature for 2 min. During the incubation, shake the tube every 30 sec to ensure thoroughly mixing.

d. After instantaneous centrifugation, place the tube on the magnetic stand for 1 min. After the solution becomes clear, carefully remove the supernatant. Add 20 µl of Nuclease-free ddH_2O to the PCR tube to resuspend the beads.

▲Do not discard the beads in this step.

08-7/Post-capture PCR

1. Prepare the reaction system according to the following table:

Buffer Name	Volume
Resuspended Beads of 08-6/2. Wash at room temperature step d	20 µl
2 × VAHTS HiFi Amplification Mix 2	25 µl
Post-PCR Primer Mix	5 µl

▲Select the Primer Mix corresponding to the platform and adapter type of the library.

2. Mix the solution thoroughly by pipetting up and down or vortexing. Place the PCR tube in the PCR instrument and run the following program:

Temperature	Time	Cycles
98℃	45 sec	1
98℃	15 sec	N
60℃	30 sec	
72℃	30 sec	
72℃	1 min	1
4℃	Hold	

The recommended amplification cycles, please refering to the following table:

Panel Size	1 Library	4 Libraries	8 Libraries	12 Libraries
Exome(≥30 Mb)	10	8	7	6
3 Mb	14	13	13	11
0.3 Mb	15	14	14	12

▲ Panel, blockers, and pre-library insert size can all affect the library yield, and the number of amplification cycles can be adjusted.

3. Purify the PCR product:

a. Pipet 60 µl of VAHTS DNA Clean Beads into 50 µl of the PCR product and mix thoroughly by vortexing.

b. Incubate the mixture for 5 min at room temperature. Centrifuge the PCR tube briefly and place it on a magnetic stand to separate the beads from the solution. When the solution becomes clear (it takes about 5 min), carefully remove the supernatant.

c. Place the PCR tube on the magnetic stand. Add 200 µl of freshly prepared 80% ethanol to wash the beads, incubate it at room temperature for 30 sec, and carefully remove the supernatant.

d. Repeat Step c once again.

e. Place the tube on the magnetic stand. Allow the beads to air-dry for 1 - 3 min until there is no residual ethanol.

f. Take the PCR tube off from the magnetic stand. Add 25 µl of Nuclease-free ddH_2O for elution. Centrifuge the PCR tube briefly and place it on a magnetic stand to separate the beads the solution. When the solution becomes clear (it takes about 5 min), carefully transfer 20 µl of the supernatant to a new 1.5 ml Nuclease-free EP tube.

08-8/Library Quality Control

1. Library concentration determination:

Use dsDNA-binding fluorochrome, such as the Equalbit 1 × dsDNA HS Assay Kit (Vazyme #EQ121) or other kits based on qPCR absolute quantification, such as the VAHTS Library Quantification Kit for Illumina (Vazyme #NQ101-NQ106).

2. Library size distribution determination:

Determine the size distribution of libraries using devices based on electrophoretic separation such as LabChip GX, GXII, and GX Touch (PerkinElmer); Bioanalyzer and Tapestation (Agilent Technologies); and Fragment Analyzer (Advanced Analytical).