sCAP Hybridization and Wash Kit V2

RK20277

www.abclonal.com

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Contents

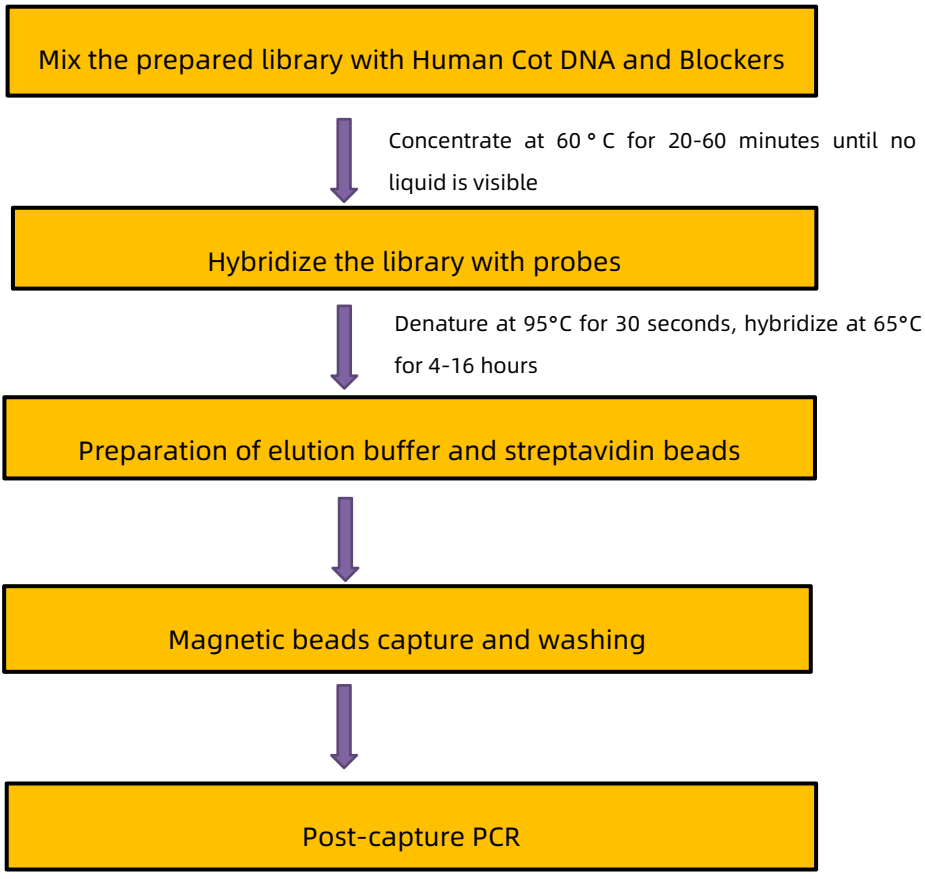
1. Product Overview

ABclonal's hybrid capture system offers reagents suitable for Illumina and MGI high-throughput sequencing platforms, including Hybridization and Wash buffer, Universal blockers, Human Cot DNA, capture beads, PCR amplification reagents, and amplification primers.

The Universal blockers, Human Cot DNA, capture beads, PCR amplification reagents, and amplification primers can all be used with the customer's own reagents and be sold separately.

The components of the reagent kit undergo strict quality control, including component contamination testing, functional assay validation, application scenario testing, and inter-batch stability testing.

2. Experimental Procedure



After completion, the library can be stored at -20℃

3. Product Components

Classification	Catalog	Size	Components	Storage
sCAP Hybridization and Wash Kit V2	RK20277	4 / 16 RXN	2X Bead Washing Buffer	-20℃
			2X Hybridization Buffer III	-20℃
			10X High Stringency Buffer	-20℃
			10X Low Stringency Buffer	-20℃
			10X Washing Buffer I	-20℃
			10X Washing Buffer II	-20℃
			Hybridization Buffer Enhancer	-20℃
Streptavidin Beads	RK20270	4 / 16 RXN	Streptavidin beads	4℃
Human Cot DNA	RK20268	4 / 16 RXN	Human Cot DNA	-20℃
Blocking Oligos for Illumina	RK20269	4 / 16 RXN	ILM Universal Blockers-X1	-20℃
Blocking Oligos for MGI	RK20278	4 / 16 RXN	MGI Universal Blockers-S	-20℃
	RK20279	4 / 16 RXN	MGI Universal Blockers-D	-20℃
Amplification Module for Illumina	RK20274	40 / 120 μL	10X ILM PCR Primers	-20℃
	RK20726	200 / 600 μL	2X PCR Mix	-20℃
Amplification Module for MGI	RK20272	40 / 120 μL	MGI PCR Primer Mix	-20℃
	RK20273	40 / 120 μL	10X MGI UDI Primers	-20℃
	RK20726	200 / 600 μL	2X PCR Mix	-20℃

Note: Streptavidin magnetic beads, Human Cot DNA, Blockers and Amplification Module need to be purchased separately.

4. Storage

Transportation and Storage: Except for Streptavidin beads, which should be stored and transported at 4°C, ABclonal's hybrid capture kit and other matching reagents must be stored at -25°C to -15°C. These kits are sensitive to temperature, so long-distance transportation should preferably use dry ice or a combination of dry ice and ice packs.

5. Product Applications

Targeted sequencing can enrich and sequence only the interested genomic regions, effectively reducing sequencing costs. It also allows for deep sequencing of target regions, increasing the sensitivity and accuracy of detecting genetic variations within these regions. This makes it particularly suitable for genetic typing and detecting rare mutations. Since hybridization probe capture does not require PCR primer design, the likelihood of missing mutations is lower, and it performs better in terms of sequence complexity. ABclonal's independently developed NGS library preparation hybrid capture system has good sensitivity and specificity, making it widely applicable in research on complex disease-related pathogenic genes and health screening, among many other fields.

6. Other Materials Required

1. Clean Beads

Recommend high-quality and stable DNA purification magnetic beads, such as AFTMag NGS DNA Clean Beads (ABclonal, Cat.RK20257).

2. DNA Quality Control

Agilent's 2100 Bioanalyzer and related consumables are used for DNA fragment size analysis, while Life's Qubit nucleic acid quantification instrument and related reagents are used for DNA concentration measurement.

3. Other materials

ABQubit dsDNA quantification assay kit (ABclonal, Cat.NO. RK30140 ; RK30141), 80% ethanol (ethanol needs to be prepared at the time of use), Nuclease-Free Water, among other materials.

4. Other Consumables and Instruments

Low-binding EP tubes, pipette tips, low-binding thin-walled PCR tubes (200 μL), a magnetic rack, single- or multi-channel pipettors, a PCR machine, a vortex mixer, a desktop microcentrifuge, and a rotary evaporator.

7. Precautions

1. Reagent Preparation

1.1 Streptavidin Beads and DNA purification magnetic beads should be equilibrated to room temperature before use. It is recommended to take them out from 4°C and let them equilibrate at room temperature for half an hour in advance to avoid reduced yield. Streptavidin Beads should be thoroughly mixed before use.

1.2 Before using the reagents, ensure complete dissolution without precipitation by briefly centrifuging them to the bottom of the tube. The 10X High Stringency Buffer and 10X Low Stringency Buffer may become slightly cloudy when dissolved at room temperature. In this case, they can be clarified by incubating at 65°C and then diluting to 1X for use. Additionally, if crystals are observed in the 2X Hybridization Buffer before use, it should be incubated at 65°C until completely dissolved before use. After using the kit components, please store them promptly at -25°C to -15°C.

1.3 Before the experiment, familiarize yourself with the entire SOP. Pre-set the vacuum concentrator, PCR machine, and temperature-controlled devices such as a metal bath. Pay attention to accurately set the reaction temperature and the lid temperature.

2. Library Concentration

2.1 User recommends using a vacuum concentrator to concentrate the mixed DNA library. This method is simple to operate and results in low DNA library loss, allowing for the acquisition of high-quality library samples.

2.2 Users can also use magnetic beads to concentrate the mixed DNA library, which does not require the purchase of a vacuum concentrator. This

method can be better compatible with automation work, but there is a certain loss of DNA library, an increase in reagent demand, and a slight GC bias. The detailed operation method can be found in the appendix.

2.3 After concentration, the library mixture must be completely dried to avoid inaccuracies in the hybridization system, which can affect the efficiency of library capture.

3. The Number of Hybridization Samples

3.1 ABclonal's hybrid capture system can meet various needs from 1 to 12 samples. In general, a library mixing input ratio of >50% is recommended (library mixing input ratio = library amount entering the hybridization reaction / total output of actual library construction for all samples * 100%).

3.2 For libraries with uniform size distribution and requiring the same amount of sequencing data, it is recommended to mix at an input ratio of >50% of 500 ng/library to increase library richness and reduce sequencing data duplication.

3.3 For libraries with uneven size distribution, large differences in sample quality, and different requirements for sequencing data, it is recommended to mix in proportion to the molar amount under the premise of a library mixing ratio of >50%. Generally, it is recommended to mix libraries with similar sample qualities, which helps to balance the output of data between different sub-libraries.

4. Hybridization Time

4.1 ABclonal's hybrid capture system can be applied to hybridization times of 2-16 hours. However, compared to a 16-hour hybridization, different panel sizes show a slight decrease in total library capture, capture efficiency, and coverage uniformity with shorter hybridization times.

4.2 Generally, compared to medium to large panels (>0.4 Mb), small panels (<0.4 Mb) are more affected by shorter hybridization times. For those with time constraints, it is possible to try shortening the hybridization time for large panels, but this approach is not recommended for small panels.

5. The Use of Capture Reagents for Illumina and MGI Platforms

5.1 Due to the different library structures (single-end index library and double-end index library) for Illumina and MGI platforms, different blocker closure reagents and amplification primers are required during hybridization and PCR amplification. However, other matching reagents can be used interchangeably.

Library Types	Blockers		PCR Primer	
	Catalog	Name	Catalog	Name
Illumina single index library Illumina dual Index library	RK20269	ILM Universal Blockers-X1	RK20274	10X PCR Primers
MGI single Index library	RK20278	MGI Universal Blockers-S	RK20272	MGI PCR Primer Mix
MGI Dual Index library	RK20279	MGI Universal Blockers-D	RK20273	10X MGI UDI Primers

6. PCR Cycles

Library yield after capture is related to many factors. Apart from the influence of experimental operations, there are several theoretical

influencing factors: sample type, initial mixed library total amount, panel size, hybridization time, PCR cycle number, etc. To obtain good experimental data, the PCR cycle number after capture should be controlled as much as possible, while meeting the requirements of sequencing volume.

6.1 Illumina platforms perform cluster amplification of libraries exponentially before sequencing, so the total amount of libraries required for sequencing is relatively low. Depending on the panel size, refer to the recommended cycle number in the table below at the beginning of the experiment, and adjust it later based on specific results if needed:

Probe Panel size	1-plex	4-plex	8-plex	12-plex
>100,000 probes	10 cycles	8 cycles	7 cycles	6 cycles
10,000-100,000 probes	12 cycles	10 cycles	9 cycles	8 cycles
500-10,000 probes	13 cycles	11 cycles	10 cycles	10 cycles
1-500 probes	14 cycles	12 cycles	11 cycles	11 cycles

6.2 MGI sequencing platforms require circularization of the library before sequencing, followed by rolling circle amplification to form DNA nanoballs. Therefore, the total amount of libraries required for sequencing is higher than that for Illumina platforms. For a single captured library, it is recommended to have a total library amount of >300 ng before circularization; when multiple captured libraries are mixed, the recommended total library amount after mixing is >300 ng. Depending on the panel size, refer to the recommended cycle number in the table below at the beginning of the experiment, and adjust it later based on specific results if needed:

Probe Panel size	1-plex	12-plex
>100,000 probes	8	6
10,000-100,000 probes	10	7
500-10,000 probes	14	11
1-500 probes	15	12

7. Temperature Control

Temperature control is crucial during the hybridization, capture, and elution steps of the hybrid capture process, as it directly affects the success rate of the experiment and the performance of sequencing data. Special attention should be paid to temperature control, especially when operating at 65°C, ensuring that the temperature deviation of the temperature control instrument does not exceed 0.5°C. Here are some key points:

7.1 During hybridization, the temperature needs to be maintained at 65°C using a PCR machine with a lid temperature set to 100°C.

7.2 During Streptavidin Beads capture, the reaction system should be kept at 65°C in a PCR machine with a lid temperature set to 70°C. The PCR tubes should be gently shaken every 10-12 minutes to prevent bead settling. When shaking, do it quickly to keep the reaction system at 65°C and avoid temperature drop.

7.3 The temperature requirements are strict during the thermal elution step. Each operation should be kept at 65°C to avoid temperature drop. Gentle inversion can be used to mix without generating bubbles.

7.4 The ambient temperature in the laboratory must be stable at 20-25 ° C. Low temperatures can affect the stability of the elution experiment.

8. Instrument and Consumable Selection

8.1 When performing overnight hybridization in a PCR instrument, it is recommended to test the loss of the hybridization system in advance: use 17 μL of distilled water instead of the hybridization system for testing, react at 65°C for 12 hours, and the volume loss should be less than 0.5 μL to ensure the good sealing of PCR tubes and 96-well plates.

8.2 For experimental consumables used in the capture experiment, such as centrifuge tubes and pipette tips, please use low-binding series to avoid sample loss.

8. Protocol

This operation mainly includes: Mix the prepared library with Human Cot DNA and Blockers; Hybridize the Library with probes; Preparation of elution buffer and streptavidin beads; Magnetic beads capture and washing; and post-capture PCR.

1. Mix the prepared library with Human Cot DNA and Blockers

1.1 Place Blockers and Human Cot DNA at room temperature to dissolve, vortex mix, centrifuge, and place on ice for later use.

1.2 Take a new 1.5 mL tube and prepare the following reaction system:

Components	Volume
DNA library*	500 ng DNA Library each
Human Cot DNA	5 μL
Blockers（Differentiate between Illumina/MGI platforms, single/dual index）	2 μL

1.3 Vortex mix thoroughly, briefly centrifuge to bring down any liquid, then concentrate using a vacuum concentrator at 60°C until no liquid is visible.

Safety stop point: The concentrated mixture can be temporarily stored at 4°C overnight or at -20°C for approximately 1-2 weeks.

2. Hybridize the Library with probes

2.1 Place the ABclonal Hybridization and Wash Kit V2 at room temperature to thaw.

Note: The 2X Hybridization Buffer must be completely dissolved without any crystals. If it does not dissolve at room temperature, incubate it in a 65° C water bath for about 10-30 minutes, vortexing occasionally until all crystals are completely dissolved.

2.2 For the concentrated mixture, add other components according to the table below:

Components	Volume
2X Hybridization Buffer	8.5 μL
Hybridization Buffer Enhancer	2.7 μL
Capture Probes	4 μL
Nuclease-Free Water	1.8 μL
Total Volume	17 μL

2.3 Vortex the mixture several times with a pipette and incubate at room temperature for 5-10 minutes.

2.4 Gently vortex the mixture again and briefly centrifuge.

2.5 Transfer the liquid from the 1.5 mL tube to a 0.2 mL PCR tube and briefly centrifuge to collect the liquid at the bottom of the tube.

2.6 Close the tube lid tightly and immediately place it into a PCR machine preheated to 95°C, with the lid temperature set to 100°C. Proceed with the reaction according to the program in the table below:

Temperature	Time
95℃	30 sec
65℃	4-16 hr
65℃	Hold

3. Preparation of elution buffer and streptavidin beads

3.1 Prepare 1X working buffer according to the number of hybridization capture reactions N, as shown in the table below. The prepared 1X Working Buffer can be stored at room temperature (15-25℃) for one month.

Components	Concentrated Buffer	Nuclease-Free Water	1X Working Buffer
2X Bead Washing Buffer	N * 160 μL	N * 160 μL	N * 320 μL
10X Low stringency Buffer	N * 28 μL	N * 252 μL	N * 280 μL
10X High stringency Buffer	N * 32 μL	N * 288 μL	N * 320 μL
10X Washing Buffer I	N * 16 μL	N * 144 μL	N * 160 μL
10X Washing Buffer II	N * 16 μL	N * 144 μL	N * 160 μL

Note: If the 10X Low Stringency Buffer is cloudy, place the vial in a 65°C water bath until it is completely dissolved.

3.2 According to the number of hybridization capture reactions N, preheat the diluted 1X Low Stringency Buffer and 1X High Stringency Buffer volumes as shown in the table below for at least 30 minutes before use.

Components	Volume	Temperature
1X Low stringency Buffer	N * 120 μL	65 ℃
1X High stringency Buffer	N * 320 μL	65 ℃

3.3 Keep the remaining N * 160 μL of 1X Low Stringency Buffer at room temperature for subsequent room temperature elution.

Note: During the preparation of the biotinylated beads, the beads do not need to be dried after each removal of the supernatant on the magnetic rack. Proceed immediately to the next step.

3.4 Take the streptavidin beads out of the 4°C refrigerator in advance and place at room temperature for at least 30 minutes.

3.5 Vortex the beads thoroughly to ensure complete suspension.

3.6 For each hybridization reaction, add 50 μL of Streptavidin Beads to a low-binding PCR tube.

3.7 Add 100 μL of 1X Bead Washing Buffer and pipette up and down 10 times.

3.8 Place the PCR tube on the magnetic rack until the solution clears, approximately 1 minute, then remove the supernatant.

3.9 Repeat steps 3.8 and 3.9 twice (a small amount of buffer residue will not affect the binding of the library to the beads).

3.10 Add the bead resuspension buffer prepared according to the components in the table below to the beads in each hybridization reaction:

Components	Volume
2X Hybridization Buffer	8.5 μL
Hybridization Buffer Enhancer	2.7 μL
Nuclease-Free Water	5.8 μL
Total Volume	17 μL

3.11 Vortex the beads thoroughly to mix, then gently centrifuge for a moment to collect the beads at the bottom of the tube.

4. Magnetic beads capture and washing

4.1 Keep the hybridization mixture from step 2.6 at 65°C, add the bead mixture (product from step 3.2.9) to the hybridization mixture, gently vortex to mix, and briefly centrifuge.

4.2 Place the PCR tubes in a preheated PCR machine at 65°C for 45 minutes (lid at 75°C). Remove the PCR tubes every 10-12 minutes and gently shake to prevent bead settling.

4.3 Avoid liquid splashing onto the tube lid as much as possible during mixing. When removing and shaking, do it quickly to maintain the reaction system at 65°C and minimize temperature drop.

Note 1: The thermal elution step requires strict temperature control. Try to maintain it at 65°C to avoid temperature drop.

Note 2: When operating on multiple samples simultaneously, each time you add preheated 1X Low Stringency Buffer and 1X High Stringency Buffer, change the tip for each sample to avoid insufficient buffer.

4.4 Immediately after removing from the PCR machine, add 100 μL preheated 1X Low Stringency Buffer, gently pipette up and down about 10 times, taking care to prevent bubble formation.

4.5 Place the PCR tubes on the magnetic rack until the solution clears, approximately 1 minute.

4.6 Remove the tubes from the magnetic rack, add 150 μL preheated 1X High Stringency Buffer, gently pipette up and down about 10 times, taking care to prevent bubble formation.

4.7 Place the PCR tubes back into the PCR machine at 65°C for 5 minutes.

4.8 Place the PCR tubes on the magnetic rack until the solution clears, approximately 1 minute.

4.9 Repeat steps 4.6 - 4.8 once.

Note: During the elution step, after each removal of the supernatant, the beads do not need to dry; proceed immediately to the next step.

4.10 Add 150 µL room temperature 1X Low Stringency Buffer and vortex until homogenous.

4.11 Incubate at room temperature for 2 minutes, vortexing every 30 seconds to ensure homogeneity.

4.12 After incubation, briefly centrifuge and place on the magnetic rack for 1 minute until the solution clears, then remove the supernatant.

4.13 Remove the tubes from the magnetic rack, add 150 µL room temperature 1X Washing Buffer I, vortex until homogenous.

4.14 Incubate at room temperature for 2 minutes, vortexing every 30 seconds to ensure homogeneity.

4.15 After incubation, briefly centrifuge and place on the magnetic rack for 1 minute until the solution clears, then remove the supernatant.

4.16 Remove the tubes from the magnetic rack, add 150 µL room temperature 1X Washing Buffer II, vortex until homogenous.

4.17 Incubate at room temperature for 2 minutes, vortexing every 30 seconds to ensure homogeneity.

4.18 After incubation, briefly centrifuge and place on the magnetic rack for 1 minute until the solution clears, then remove the supernatant. Remove the magnetic rack after completely removing residual buffer with a 10 µL tip.

4.19 Add 22.5 µL Nuclease-Free Water, pipette up and down at least 10

times to completely resuspend the beads. Proceed to PCR amplification without discarding the beads.

Note: The captured product and beads are to be used together for PCR amplification.

5. Post-Capture PCR

5.1 Prepare the amplification system according to the table below:

Illumina Library Amplification System:

Components	Volume
Library-bounded beads（Product from Step 4.19）	22.5 μL
10X ILM PCR Primers	2.5 μL
Gloria Nova HS 2X PCR Mix for NGS	25 μL
Total Volume	50 μL

MGI Single-Index Library Amplification System：

Components	Volume
Library-bounded beads（Product from Step 4.19）	22.5 μL
MGI PCR Primer Mix	2.5 μL
2X PCR Mix	25 μL
Total Volume	50 μL

MGI Dual Index Library Amplification System：

Components	Volume
Library-bounded beads（Product from Step 4.19）	22.5 μL
10X MGI UDI Primers	2.5 μL
2X PCR Mix	25 μL
Total Volume	50 μL

5.2 Vortex, briefly centrifuge the tubes to collect all liquid at the bottom. Proceed immediately with the PCR reaction, following the program in the table below:

Temperature	Time	Cycles
98 ℃	45 s	1
98 ℃	15 s	
60 ℃	30 s	Variable*
72 ℃	30 s	
72 ℃	1 min	1
4 ℃	Hold	1

5.3 Recommended PCR cycles for overnight hybridization on the Illumina platform:

Probe Panel size	1-plex	4-plex	8-plex	12-plex
>100,000 probes	10 cycles	8 cycles	7 cycles	6 cycles
10,000-100,000 probes	12 cycles	10 cycles	9 cycles	8 cycles
500-10,000 probes	13 cycles	11 cycles	10 cycles	10 cycles
1-500 probes	14 cycles	12 cycles	11 cycles	11 cycles

5.4 Recommended PCR cycles for overnight hybridization on the MGI platform:

Probe Panel size	1-plex	12-plex
>100,000 probes	8 cycles	6 cycles
10,000-100,000 probes	10 cycles	7 cycles
500-10,000 probes	14 cycles	11 cycles
1-500 probes	15 cycles	12 cycles

Note: For 4-hour rapid hybridization, add one additional cycle compared to overnight hybridization.

5.5 Add 75 μL (1.5X) of AFTMag NGS DNA Clean Beads to each sample tube, and mix thoroughly by pipetting or shaking.

5.6 Incubate at room temperature for 5 minutes, then transfer to a magnetic rack until the solution clears, approximately 2 minutes.

5.7 After the solution clears, carefully aspirate and discard the supernatant, being careful not to aspirate or touch the beads.

5.8 Add 125 μL of 80% ethanol, incubate at room temperature for 1 minute, then carefully aspirate and discard the ethanol, being careful not to aspirate or touch the beads.

5.9 Repeat step 5.8.

5.10 Air-dry the beads at room temperature until the bead surface is no longer reflective, approximately 1-3 minutes. Be careful not to overdry the beads.

5.11 Remove the magnetic rack, add 22 μL of Nuclease-Free Water to each sample tube to resuspend the beads, and mix thoroughly by pipetting or shaking. Incubate at room temperature for 5 minutes.

5.12 Place the sample tubes on the magnetic rack until the solution clears, approximately 1-2 minutes.

5.13 Carefully aspirate 20-21 μL of the supernatant and transfer it to a new PCR tube.

Safety Stop Point: Purified PCR products can be stored at 4°C for up to 1 week, or at -20°C for long-term storage to avoid repeated freeze-thaw cycles.

6. Library Capture Quality Check

6.1 Quantify the captured library, record the concentration, and calculate the total amount. It is recommended to use a Qubit fluorescence quantifier or qPCR.。

6.2 Use the Agilent 2100 analyzer to perform peak quality check and calculate the average size of the captured library.

6.3 After quality checking, the library can be used for sequencing or stored at -20°C.

9. Appendix

Magnetic Bead-Based Concentration of Mixed Libraries

Note 1: The magnetic bead concentration method increases the reaction system compared to vacuum concentration, leading to more reagent loss, requiring additional purchase of reagents such as beads and Human Cot DNA.

Note 2: The magnetic bead concentration method inevitably results in some loss of DNA libraries. It is recommended to increase the input amount of each mixed DNA library to 700 ng.

1.1 AFTMag NGS DNA Clean Beads (ABclonal, Cat.NO.RK20257) should be taken out from 4°C and equilibrated to room temperature 30 minutes in advance.

1.2 Dissolve Blockers and Human Cot DNA at room temperature, vortex and centrifuge, then place on ice for later use.

1.3 Take a new 1.5 mL centrifuge tube and prepare the elution buffer after purification with magnetic beads, as shown in the table:

Components	Volume
Hybridization Buffer Enhancer	3.5 μL
Blockers（Differentiate between Illumina/MGI platforms, single/dual index）	2.6 μL
Nuclease-Free Water	2.4 μL
Total Volume	8.5 μL

1.4 Prepare a multi-library mixed system in a new 1.5 mL centrifuge tube as follows:

Components	Volume
DNA library	700 ng DNA Library each (X μL)
Human Cot DNA	7.5 μL
AFTMag NGS DNA Clean Beads	1.8 × (X+7.5) μL

1.5 Mix the multi-library hybridization system with the bead mixture in the centrifuge tube, vigorously vortex, and incubate at room temperature for 5 minutes.

1.6 Place the 1.5 mL centrifuge tube on the magnetic rack and let it stand for 2 minutes. After the solution clears, remove the supernatant, being careful not to touch the beads.

1.7 Add 200 μL of 80% ethanol to wash the beads, incubate for 30 seconds, and then remove the supernatant.

1.8 Repeat step 1.7.

1.9 Keep the centrifuge tube on the magnetic rack, use a 10 μL pipette to remove any residual ethanol at the bottom of the tube, and open the tube cap to air dry until no ethanol residue remains on the surface of the beads. Do not overdry.

1.10 Remove the centrifuge tube from the magnetic rack and add 8.5 μL of the bead purification elution buffer prepared in step 3. Resuspend the beads, and incubate at room temperature for 2 minutes.

1.11 Place the centrifuge tube on the magnetic rack and let it stand for 2 minutes. Transfer 6.5 μL of the supernatant to a new PCR tube.

1.12 Add the other components to the PCR tube according to the table below:

Components	Volume
Product from Step 1.11	6.5 µL
2X Hybridization Buffer	8.5 µL
Capture Probes	2 µL
Total Volume	17 µL

1.13 Gently vortex mix and briefly centrifuge.

1.14 Place the PCR tubes in a PCR machine preheated to 95 ℃, with a heated lid at 100 ℃, and perform the hybridization reaction according to the program in the table below:

Temperature	Time
95℃	30 s
65℃	4-16 hr
65℃	Hold

1.15 DNA library concentration, closure, and probe hybridization have been completed at this point.

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