Apostle MiniEnrich ES Hybrid Capture of DNA Libraries (for Illumina®) User Manual

MiniEnrich ES Hybrid Capture Reagents

MiniMax NanoBlockers (for Illumina®)

Panels

For Research Use Only.

2024 April

Statement

This instruction is intended for use with the Apostle MiniEnrich ES Hybrid Capture Reagents, Panels and Blockers on Illumina® platforms.

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

Content

Introduction

Panels

Panels within 5'-biotinylated probes are predesigned and optimized for targeted capture applications in next generation sequencing. Panels can be upgraded by either expanding with spike-in probes or combining with other panels.

If you plan to use Panels as spike-in panels, contact us (support@apostlebio.com) for professional and specific recommendations.

MiniMax NanoBlockers (for Illumina®)

MiniMax NanoBlockers (for Illumina®) are universal blockers for Illumina® platforms. The MiniMax NanoBlockers act by reducing non-specific binding of adapter sequences, which improves on-target rate and enrichment efficiency. MiniMax NanoBlockers (for Illumina®) work with Illumina libraries with single/dual indexes and 6 nt/8 nt/10nt indexes.

MiniEnrich ES Hybrid Capture Reagents

MiniEnrich ES Hybrid Capture Reagents is a kit optimized for the targeted capture with rapid hybridization elution steps of Panels/Probes developed by Apostle.

Catalog#	Item	Package/Storage
1005402	MiniEnrich ES Hybrid Capture Reagents, 16 rxn	Box 1/–25 ~ –15°C Box 2/2 ~ 8°C
1005401	MiniEnrich ES Hybrid Capture Reagents, 96 rxn	Box 1/–25 ~ –15°C Box 2/2 ~ 8°C

Package#	Component	Volume 1005402 (16 rxn)	Volume 1005401 (96 rxn)	Storage
Box1	MiniEnrich ES Hyb #1	380 µL	2×1150 µL	–25 ~ –15°C
	MiniEnrich Hyb #2	120 µL	720 µL	–25 ~ –15°C
	Human Cot DNA	110 µL	650 µL	–25 ~ –15°C
	MiniEnrich ES Wash Buffer	4×4 mL	5×19 mL	–25 ~ –15°C
	Sealing Oil	250 µL	1.5 mL	–25 ~ –15°C
Box2	Streptavidin Beads	950 µL	5.7 mL	2 ~ 8°C

Library Input Consideration

Library Preparation

This protocol was verified with libraries prepared by Apostle MiniEnrich EZ DNA Library Preparation Module v2, Apostle MiniMax DNA Universal Library Preparation Kit (for Illumina®) and Apostle MIniMax cfDNA Library Preparation Kit (for Illumina®).

Please adjust the length of fragmented DNA to match sequencing mode.

Library Input

For genome DNA, 500 ng of each library for hybrid capture is recommended. In most cases, multiplexing up to 12 samples (6 µg of total DNA) has limited impact on capture performance.

DNA Concentration

A vacuum concentrator is recommended for concentrating DNA. Although the beads-based concentration method (refer to **Appendix**) is quicker, the reproducible and minor GC bias occurs during bead-based concentration.

Equipment and Consumable

Equipment

Item	Description
Digital electrophoresis	Agilent 2100 Electrophoresis Bioanalyzer® system (Cat # G2939AA) Agilent 2200 TapeStation system (Cat # G2965AA) Bioptic Qsep100 capillary gel electrophoresis system or equivalent
Pipettor	General laboratory supplier
Thermal cycler	General laboratory supplier
Benchtop centrifuge	General laboratory supplier
Microcentrifuge	General laboratory supplier
Vortex mixer	General laboratory supplier
Magnet stand	Tube protocol: Thermo Fisher DynaMag™ -96 Side Magnet（Cat # 12331D） Thermo Fisher DynaMag™ -PCR Magnet (Cat # 492025) or equivalent Plate protocol: Thermo Fisher Magnum™ EX Universal Magnet Plate (Cat # AM10027) Alpaqua Magnetic Stand-96 (Cat # A000380) or equivalent
Fluorometer	Thermo.Fisher.Qubit™.3.0.Fluorometer.(Cat.#.Q33216) or equivalent
Timer	General laboratory supplier
Vacuum concentrator	Eppendorf.Concentrator.plus.(Cat.#.5305000193) Thermo.Fisher.Scientific.SpeedVac.system.(Cat.#.SC210A-115) or equivalent

Capture Regents from Apostle

Item	Description	Catalog#	Storage
Panels/Probes	www.apostlebio.com	Custom/ Commercial	–25 ~ –15°C
MiniEnrich ES Hybrid Capture Reagents	MiniEnrich ES Hybrid Capture Reagents, 16 rxn Box 1 Box 2 MiniEnrich ES Hybrid Capture Reagents, 96 rxn Box 1 Box 2	1005402 1005401 	 –25 ~ –15°C 2 ~ 8°C –25 ~ –15°C 2 ~ 8°C

Capture Regents from Apostle

Item	Description	Catalog#	Package/Storage
MiniMax NanoBlockers (for Illumina®)	MiniMax NanoBlockers (for Illumina®), 16 rxn	1006101	–25~–15°C
	MiniMax NanoBlockers (for Illumina®), 96 rxn	1006102	–25~–15°C

Consumable

Item	Description
Absolute ethanol	General laboratory supplier, analytical grade
TE buffer	MiniMax.TE.Buffer or equivalent (10 mM Tris, 0.1 mM EDTA)
Purification reagents	Beckman-Coulter.Agencourt®.AMPure®.XP.-.PCR.Purification.beads. (Cat.#.A63880) MiniMax.SP.Beads or equivalent
PCR reagents	**PCR Master Mix:** Kapa Biosystems HiFi HotStart ReadyMix (Cat # KK2601) MiniMax 2X HiFi PCR Master Mix **Amplification Primer Mix II:** MiniMax Amplification Primer Mix II or equivalent
1.7 mL microtubes	Axyge®.MAXYMum.Recovery.Microtubes,.1.7.mL.(Cat # MCT-175-L-C) or equivalent
0.2.mL.microtubes	Axygen®.MAXYMum.Recovery PCR.Tubes,.0.2.mL.flat.cap.(Cat.#.PCR-02-L-C) or equivalent
96-well plate	Eppendorf.96.Well.LoBind.PCR.Plates,.Semi-skirted.(Cat # 0030129504) or equivalent
PCR Plate Sealing Film	Bio-Rad.Microseal®.B.PCR.Plate.Sealing.Film,.adhesive,.optical.(Cat #.MSB1001) or equivalent

Workflow

Workflow	Duration
Perform Hybridization Concentrate libraries Perform hybridization reaction	Dependent on methods 0.5-4 hr STOP
⬇	
Perform Wash Prepare buffers Wash streptavidin beads Perform bead capture Perform washes	 5 min* 10 min* 45 min 20 min
⬇	
Perform Post-capture PCR	30.min
⬇	
Purify and Quantify Library	30.min STOP

* Perform during hybridization reaction.

STOP Safe stopping point

Hybridization Capture of DNA Librariy

This protocol is validated by the MiniEnrich Exome Core Panel (from Apostle).

Tube Protocol

This protocol is only for a maximum of 6 capture reactions by using individual tubes. Please refer to the Plate Protocol for more capture reactions.

Step 1: Perform Hybridization Reaction

1. Thaw MiniEnrich ES Hyb #1 and MiniEnrich Hyb #2 of MiniEnrich ES Hybrid Capture Reagents to room temperature.

Note: Inspect the tube of MiniEnrich ES Hyb #1 for possible crystallization of salts. If crystals exist, heat the tube up to 65°C with shaking occasionally until the solution is completely solubilized. The heating process may take minutes to hours.

2. When using a vacuum concentrator for DNA concentration, please preheat the instrument and adjust the temperature to 60°C.

Note: Considering no GC bias, we highly recommend using vacuum system for library concentration. However, if you require a quicker turnaround, please refer to the beads-based method in Appendix: MiniMax SP Beads DNA Concentration Protocol.

3. Set up each reaction in a low-bind 0.2/1.5 mL tube on ice as follows:

Component	Volume Per Capture Reaction	Quantity
Library Pool	500.ng/library	1–12
Human.Cot.DNA	5.µL	
MiniMax NanoBlockers (for Illumina®)	2.µL	

Important notes:

- To maintain the complexity of each library, it is recommended to use at least 50% yield of each library for hybridization.
- For FFPE DNA or cfDNA samples, it is recommended to mix libraries constructed from samples with same quality.

4. Mix thoroughly and centrifuge to collect the contents.
5. Place the tube in the preheated vacuum concentrator.
6. Dry down the mixture and seal the tube prior to use.

Safe Stopping Point: Make sure to seal the tube. The sample can be stored at RT overnight or –25~–15 °C for longer.

7. Thaw MiniEnrich Exome Core Panel on ice.
8. Prepare the Hybridization Master Mix in the tube from step 6 as follows:

MiniEnrich ES Hyb #1	8.5 µL
MiniEnrich Hyb #2	2.7 µL
Nuclease Free Water	1.8 µL
MiniEnrich Exome Core Panel	4 µL
Sealing Oil*	10 µL*
Total	**17/27 µL***

*** It is recommended to perform an evaporation test on the thermal cycler before hybridization. If the evaporation is observed, Sealing Oil can be added to prevent capture abnormalities caused by the evaporation of reaction solution.**

9. Pipet the mixture for 15-20 times. Briefly centrifuge and incubate the tube at 25°C for 5-10 min.
10. Vortex and centrifuge to collect the contents. Transfer all the solution to a new low-bind 0.2 mL PCR tube.
11. Incubate the tube in the thermal cycler programmed as follows:

Hybridization Program (heated lid set at 100°C)	
95°C	30.sec
65°C	0.5–4.hr
65°C	Hold
Wash Program (heated lid set at 100°C)	
65°C	Hold

Step 2: Perform Washing

Prepare Buffers

1. Thaw MiniEnrich ES Wash Buffer of MiniEnrich ES Hybrid Capture Reagents to room temperature, mix thoroughly and centrifuge to collect the contents.

Note: If MiniEnrich ES Wash Buffer cannot be fully thawed, it can be incubated in a water bath at 65°C until it's co-mpletely thawed. Mix well by inversion; do not vortex to avoid foaming and compromising subsequent op-erations.

2. Equilibrate the streptavidin beads to room temperature for a minimum of 30 min prior to use.
3. Aliquot the following Wash buffers into low-bind tubes.

Component	Volume Per Capture Reaction	Storage
MiniEnrich ES Wash Buffer	820 µL	Aliquot 160 µL of MiniEnrichES Wash Buffer in duplicate, heat to 65°C, and leave the remaining buffer at room temperature.

Note: The above heated MiniEnrich ES Wash Buffer need to be incubated at 65°C for a minimum of 15 min.

4. Prepare the Bead Resuspension Mix in a low-bind tube as follows:

MiniEnrich ES Hyb #1	8.5.µL
MiniEnrich Hyb #2	2.7.µL
Nuclease.Free.Water.	5.8.µL
Total	**17 µL**

Wash Streptavidin Beads

1. Vortex the streptavidin beads for 15 sec at high-speed. Add 50 µL of streptavidin beads per capture into a single 0.2/1.5 mL low-bind tube.

8. Add 100 µL of MiniEnrich ES Wash Buffer at **room temperature** to the centrifuge tube, and then mix well by gently pipetting up and down for 10 times; perform brief centrifugation and place the tube on the magnetic stand for 1-2 min until the solution is completely clear, and discard the supernatant using a pipette. Remove the centrifuge tube from the magnetic stand.

3. Repeat step 2 once.

4. Resuspend the beads by using 17 µL of **Bead Resuspension Mix** per capture. Resuspend the beads gently and transfer all solution into a new low-bind 0.2 mL PCR tube for each capture reaction.

2. Incubate the tube at 65°C for 5 min prior to use.

Perform Capture Reaction

1. After the hybridization reaction (0.5–4 hr), initiate the Wash program immediately.
2. Transfer the resuspended preheated streptavidin beads to the 0.2 mL tube containing the library. Briefly vortex until the beads are fully resuspended.

Note: Operate quickly by using barrier pipette tips.

3. Place the sample tube in the thermal cycler and incubate at 65 °C for 45 min. Keep the tube in thermal cycler and gently pipette up and down for 8-10 times every 10-12 min to ensure complete resuspension of the beads.

Heated Washes

Note: Operate quickly and be careful to minimize bubble formation during pipetting.

1. After incubation, take the sample tube out of the thermal cycler. Transfer 150 µL of 65°C MiniEnrich ES Wash Buffer into the tube, and then mix well by gently pipetting up and down for 15-20 times.
2. Place the tube on the magnet stand until the solution is clear (~30 sec). Carefully remove and discard the supernatant without disturbing the beads.
3. Remove the tube off the magnet stand, add 150 µL of 65°C MiniEnrich ES Wash Buffer to the tube. Briefly mix by pipetting up and down for 15-20 times and incubate in the thermal cycler at 65°C for 5 min.

Room Temperature Washes

1. Place the PCR tube on the magnetic stand for 30 sec, and then pipette and discard the supernatant after the solution is completely clear; perform brief centrifugation, and then use a 10-µL pipette tip to remove all residual buffer; add 150 µL of MiniEnrich ES Wash Buffer at room temperature into the tube, and then mix well by gently pipetting up and down for 10-15 times (do not vortex); transfer all reaction solutions with beads to new 0.2 mL low-bind PCR tubes. Incubate at temperature for 2 min while alternating with vortexing for 30 sec and resting for 30 sec, to ensure the mixture remains homogenous.

Note: After the MiniEnrich ES Wash Buffer at 65°C is discarded, the residual Buffer must be discarded using a small tip; after adding the MiniEnrich ES Wash Buffer at room temperature and mixing well by pipetting up and down, the reaction solution must be transferred to a new centrifuge tube, otherwise the quality of subsequent sequenc-ing data may be affected.

2. After brief centrifugation of the PCR tube, place it on a magnetic stand for 30 sec, and then pipette and discard the supernatant after the solution is completely clear; perform brief centrifugation, and then use a 10-µL pipette tip to remove the residual buffer; add additional 150 µL of MiniEnrich ES Wash Buffer at room temperature into the tube and then mix well by ge-ntly pipetting up and down for 10-15 times. Incubate the mixture at room temperature for 2 min while alternating with vortexing for 30 sec and resting for 30 sec, to ensure the mixture remains homogenous.

Note: If using a desktop vortex mixer, please control the rotation speed at ~1500 rpm to ensure thorough mixing, otherwise the quality of subsequent sequencing data may be affected.

3. After brief centrifugation of the PCR tube, place it on a magnetic stand for 30 sec, and then pipette and discard the supernatant after the solution is completely clear; perform brief centrifugation, and then use a 10-µL pipette tip to remove the residual buffer.
4. Remove the PCR tube off the magnet stand and add 22.5 µL Nuclease Free Water into the tube with magnetic beads for

Step 3: Perform Post-capture PCR.

Note: The beads shall be included and not be discarded.

Step 3: Perform Post-capture PCR

1. Thaw MiniEnrich 2X HiFi PCR Master Mix and MiniEnrich Amplification Primer Mix II on ice. Mix thoroughly and centri-fuge to collect the contents.

Note: When choosing the PCR Master Mix from other vendors, the conditions of on-bead PCR should be optimized.

2. Set up each amplification reaction in a 0.2 mL PCR tube on ice as follows:

MiniMax 2X.HiFi.PCR.Master.Mix	25.µL
MiniEnrich Amplification Primer Mix II	2.5.µL
Beads with captured DNA	22.5.µL
Total	**50 µL**

3. Incubate the tube in a thermal cycler programmed as follows, with the heated lid set at 105°C.

98°C	45.sec	1 cycle
98°C 60°C 72°C	15.sec 30.sec 30.sec	Varies (refer to the following table)
72°C 4°C	1.min Hold	1 cycle –

Panel Size	Hybridization Time	Recommended Post-PCR Cycles			
		1-plex (0.5 µg)	4-plex (2 µg)	8-plex (4 µg)	12-plex (6 µg)
> 10 Mb (MiniEnrich Exome Core Panel)	0.5-4 hr	13-10	11-8	10-7	9-6
1 Mb-10 Mb	0.5-4 hr	16-13	14-11	13-10	12-9
50 Kb-1 Mb	0.5-4 hr	17-14	15-12	14-11	14-11
1 Kb-50 Kb	0.5-4 hr	18-15	16-13	14-12	14-12

Note: The number of PCR cycles should be optimized by sample types, panel sizes, hybridization times and the number of pooled libraries per capture.

Step 4: Purify and Quantify Library

Note: Equilibrate the MiniMax SP Beads to room temperature for a minimum of 30 min prior to use. The 80% ethanol must be freshly prepared.

1. After amplification, place the tube on the magnet stand until the solution is clear (~2 min). Transfer all supernatant into a new 0.2 mL PCR tube.
2. Add 75 µL of MiniMax SP Beads into each tube. Mix thoroughly and then incubate the tube at 25°C for 5-10 min. Briefly centrifuge and then place the tube on the magnet stand until the solution is clear (~5 min). Carefully remove and discard the supernatant without disturbing the beads.
3. Keeping the tube on the magnet stand, add 150 µL of freshly prepared 80% ethanol without disturbing the beads.
4. Incubate the tube on the magnet stand at room temperature for 30 sec. Carefully remove and discard the ethanol.
5. Repeat step 4 once.
6. Briefly centrifuge and then place the tube on the magnet stand. Carefully remove all residual ethanol without disturbing the beads by using a 10-µL pipette tip.
7. Dry the beads on the magnet stand at room temperature for 2-5 min.

Note: Over-dried beads may be more difficult to suspend.

5. Remove the tube off the magnet stand. Thoroughly resuspend the beads in 20-22 µL of TE Buffer. Incubate the tube at 25°C for 2-5 min.
9. Briefly centrifuge and place the tube on the magnet stand. Incubate for 1-2 min until the solution is clear. Transfer the clear supernatant to a new 0.2/1.5 mL tube. Avoid transfer of MiniMax SP Beads.
10. Quantify the purified captured library by using a fluorescence-based method (e.g., Qubit dsDNA HS Assay kit) or equivalent assay.
11. Analyze the average size of the captured library by using a digital electrophoresis system (e.g., Agilent 2100 Bioanalyzer, Bioptic Qsep100) or other similar system.

STOP **Safe Stopping Point:** The purified library can be stored at –25~–15°C for up to one week.

Plate Protocol

This protocol is only for a maximum of 32 capture reactions by using 96-well plate in a single operation.

Step 1: Perform Hybridization Reaction

1. Thaw MiniEnrich ES Hyb #1 and MiniEnrich Hyb #2 of MiniEnrich ES Hybrid Capture Reagents to room temperature.

Note: Inspect the tube of MiniEnrich ES Hyb #1 for possible crystallization of salts. If crystals exist, heat the tube up to 65°C with shaking occasionally until the solution is completely solubilized. The heating process may take minutes to hours.

2. The plate must be sealed properly during hybridization. Otherwise, the evaporation may lead to capture failure. Please refer to **Attention: Hybridization** for details.

3. When using a vacuum concentrator for DNA concentration, please preheat the instrument and adjust the temperature to 60°C.

Note: Considering no GC bias, we highly recommend using vacuum system for library concentration. However, if you require a quick turnaround, please refer to the beads-based method in Appendix: MiniMax SP Beads DNA Concentration Protocol.

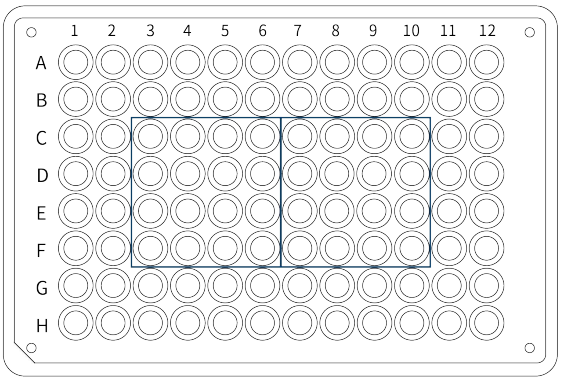
4. Set up each reaction in a low-bind 96-well plate as follows:

Component	Volume Per Capture Reaction	Quantity
Library Pool	500.ng/library	1–12
Human.Cot.DNA	5.μL	
MiniMax NanoBlockers (for Illumina®)	2.μL	

Important notes:

- To maintain the complexity of each library, it is recommended to use at least 50% yield of each library for hybridization.
- For FFPE DNA or cfDNA samples, it is recommended to mix libraries constructed from samples with same quality.
- To avoid evaporation, it is recommended to use wells in the middle of the plate. The wells which contain DNA will be distinguishable after drying down the plate. Please mark the wells in advance.

5. Mix thoroughly and centrifuge to collect the contents.
6. Place the plate in the preheated vacuum concentrator.
7. Dry down the mixture and seal the sample plate prior use.



Safe Stopping Point: Make sure to seal the sample plate. The sample can be stored at RT overnight or –25~–15°C for longer.

8. Thaw MiniEnrich Exome Core Panel on ice.
9. Prepare the Hybridization Master Mix in a low-bind tube. Multiply by the number of reactions and add 10% overflow.

MiniEnrich ES Hyb #1	8.5 µL
MiniEnrich Hyb #2	2.7 µL
Nuclease Free Water	1.8 µL
MiniEnrich Exome Core Panel	4 µL
Sealing Oil*	10 µL*
Total	**17/27 µL***

*** It is recommended to perform an evaporation test on the thermal cycler before hybridization. If the eva-poration is observed, Sealing Oil can be added to prevent capture abnormalities caused by the evaporation of reaction solution.**

10. Vortex and centrifuge to collect the contents.
11. Add 17 µL of the Hybridization Master Mix to each well of the plate from the tube above. Securely seal the plate with a Microseal B seal and incubate at 25°C for 5-10 min. After the incubation, pipette up and down repeatedly to ensure that the DNA is completely eluted from the tube wall.
12. Vortex and briefly centrifuge the plate.
13. Incubate the plate in the thermal cycler programmed as follows:

Hybridization Program (heated lid set at 100°C)	
95°C	30.sec
65°C	0.5–4 hr
65°C	Hold
Wash Program (heated lid set at 100°C)	
65°C	Hold

Step 2: Perform Washing

Prepare Buffers

1. Thaw MiniEnrich ES Wash Buffer of MiniEnrich ES Hybrid Capture Reagents to room temperature, mix thoroughly and centrifuge to collect the contents.

Note: If MiniEnrich ES Wash Buffer cannot be fully thawed, it can be incubated in a water bath at 65°C until it's com-pletely thawed. Mix well by inversion; do not vortex to avoid foaming and compromising subsequent opera-tions.

2. Equilibrate the streptavidin beads to room temperature for a minimum of 30 min prior to use.

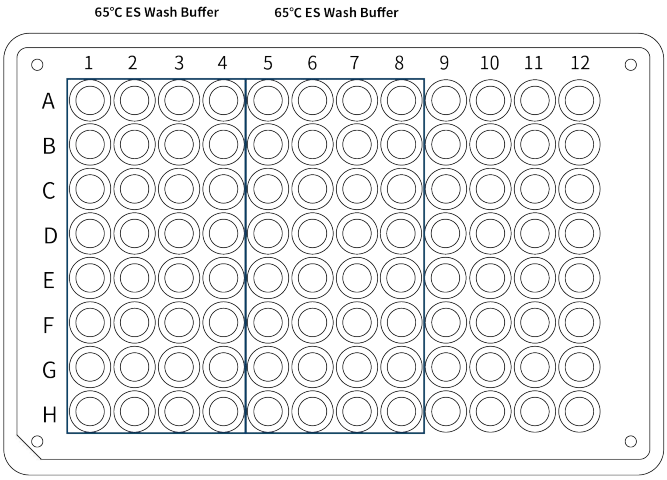
3. Prepare the MiniEnrich ES Wash Buffer and aliquot them into a new 96-well plate (taken 32 reactions as an example).

Component	Volume Per Capture Reaction	Storage
MiniEnrich ES Wash Buffer	820 µL	Aliquot 160 µL of MiniEnrich ES Wash Buffer in duplicate, heat to 65°C, and leave the remaining buffer at room temperature.

Note: Aliquot the heated MiniEnrich ES Wash Buffer into one single 96-well plate. The heated MiniEnrich ES Wash Buffer need to be incubated at 65°C for a minimum of 15 min.

Column 1–8: 160 µL of MiniEnrich ES Wash Buffer

Note: Do not discard the remaining MiniEnrich ES Wash Buffer, it will be used for subsequent elution experiments at room temperature.



4. Prepare the **Bead Resuspension Mix** in a low-bind tube. Multiply by the number of reactions and add 10% overflow.

MiniEnrich ES Hyb #1	8.5 µL
MiniEnrich Hyb #2	2.7 µL
Nuclease Free Water	5.8 µL
Total	**17 µL**

Wash Streptavidin Beads

1. Vortex the streptavidin beads for 15 sec at high-speed. Add 50 µL of streptavidin beads per capture into a new 96-well plate.

8. Add 100 µL of MiniEnrich ES Wash Buffer at **room temperature** per capture, and then mix well by gently pipetting up and down for 10 times; perform brief centrifugation and place the plate on the magnetic stand for 1-2 min until the solution is com-pletely clear, and discard the supernatant using a pipette. Remove the plate from the magnetic stand.

3. Repeat step 2 once.

4. Resuspend the beads by using 17 µL of **Bead Resuspension Mix** per capture, and then mix well by gently pipetting up and down .

2. Seal the plate and incubate the beads at 65°C for 5 min prior to use.

Perform Capture Reaction

1. After the hybridization reaction (0.5–4 hr), initiate the Wash program immediately.
2. Transfer the resuspended preheated streptavidin beads to the plate containing the library. Briefly vortex until the beads are fully resuspended.

Note: Operate quickly by using the multichannel pipettes and barrier pipette tips.

3. Place the sample plate in the thermal cycler and incubate at 65 °C for 45 min. Keep the plate in thermal cycler and gently pipette up and down for 8-10 times every 10-12 min to ensure complete resuspension of the beads.

Heated Washes

Note: Operate quickly and be careful to minimize bubble formation during pipetting.

1. After incubation, keep the sample plate in thermal cycler and then peel off the sealing film of 96-well plate. Transfer 150 µL of 65°C MiniEnrich ES Wash Buffer into each well, and then mix well by gently pipetting up and down for 15-20 times.
2. Reseal the buffer plate and closed the heated lid.
3. Take the plate out of the thermal cycler and then place it on the magnet stand until the solution is clear (~30 sec). Carefully remove and discard the supernatant without disturbing the beads.
9. Remove the plate off the magnet stand, add 150 µL of 65°C MiniEnrich ES Wash Buffer to each well. Briefly mix by gently pipetting up and down for 15-20 times and incubate in the thermal cycler at 65°C for 5 min.

Room Temperature Washes

1. Place the plate on the magnetic stand for 30 sec, and then pipette and discard the supernatant after the solution is completely clear; perform brief centrifugation, and then use a 10-µL pipette tip to remove all residual buffer; add 150 µL of MiniEnrich ES Wash Buffer at **room temperature** into each well, and then mix well by gently pipetting up and down for 10-15 times (do not vortex); transfer all reaction solutions with beads to a new 96-well plate and reseal with a new sealing film. Incubate at room temperature for 2 min while alternating with vortexing for 30 sec and resting for 30 sec, to ensure the mixture remains homogenous.

Note: After the MiniEnrich ES Wash Buffer at 65°C is discarded, the residual Buffer must be discarded using a small tip; after adding the MiniEnrich ES Wash Buffer at room temperature and mixing well by pipetting up and down, the reaction solution must be transferred to a new 96-well plate, otherwise the quality of subsequent sequencing data may be affected.

2. After brief centrifugation of the plate, place it on a magnetic stand for 30 sec, and then pipette and discard the supernatant after the solution is completely clear; perform brief centrifugation, and then use a 10-µL pipette tip to remove the residual buffer; add additional 150 µL of MiniEnrich ES Wash Buffer at **room temperature** into each well and eseal with a new sealing film. Inc-ubate the mixture at room temperature for 2 min while alternating with vortexing for 30 sec and resting for 30 sec, to ensure the mixture remains homogenous.

Note: If using a desktop vortex mixer, please control the rotation speed at ~1500 rpm to ensure thorough mixing, otherwise the quality of subsequent sequencing data may be affected.

3. After brief centrifugation of the plate, place it on a magnetic stand for 30 sec, and then pipette and discard the supernatant after the solution is completely clear; perform brief centrifugation, and then use a 10-µL pipette tip to remove the residual buffer.

4 Remove the plate off the magnet stand and add 22.5 µL Nuclease Free Water to each capture with magnetic beads for **Step 3: Performance Post-capture PCR**.

Note: The beads shall be included and not be discarded.

Step 3: Perform Post-capture PCR

1. Thaw MiniMax 2X HiFi PCR Master Mix and MiniMax Amplification Primer Mix II on ice. Mix thoroughly and centrifuge to collect the contents.

Note: When choosing the PCR Master Mix from other vendors, the conditions of on-bead PCR should be optimized.

2. Prepare the Amplification Reaction Mix, multiplied by the number of reactions on the plate and adding 10% overflow.

MiniMax 2X HiFi PCR Master Mix	25 µL
MiniMax Amplification Primer Mix II	2.5 µL
Total	**27.5 µL**

3. Add 27.5 µL of Amplification Reaction Mix into each well for a final reaction volume of 50 µL.
4. Securely seal the sample plate. Mix thoroughly and centrifuge to collect the contents.
5. Incubate the plate in a thermal cycler programmed as follows, with the heated lid set at 105°C.

98°C	45.sec	1 cycle
98°C 60°C 72°C	15.sec 30.sec 30.sec	Varies (refer to the following table)
72°C 4°C	1.min Hold	1 cycle –

Panel Size	Hybridization Time	Recommended Post-PCR Cycles			
		1-plex (0.5 µg)	4-plex (2 µg)	8-plex (4 µg)	12-plex (6 µg)
> 10 Mb (MiniEnrich Exome Core Panel)	0.5-4 hr	13-10	11-8	10-7	9-6
1 Mb-10 Mb	0.5-4 hr	16-13	14-11	13-10	12-9
50 Kb-1 Mb	0.5-4 hr	17-14	15-12	14-11	14-11
1 Kb-50 Kb	0.5-4 hr	18-15	16-13	14-12	14-12

Note: The number of PCR cycles should be optimized by sample types, panel sizes, hybridization times and the number of pooled libraries per capture.

Step 4: Purify and Quantify Library

Note: Equilibrate the MiniMax SP Beads to room temperature for a minimum of 30 min prior to use. The 80% ethanol must be freshly prepared.

1. After amplification, place the plate on the magnet stand until the solution is clear (~2 min). Transfer all supernatant into a new 96-well plate.
2. Add 75 µL of MiniMax SP Beads into each amplified capture. Mix thoroughly and then incubate at 25°C for 5-10 min.
3. Briefly centrifuge and then place the plate on the magnet stand until the solution is clear (~5 min). Carefully remove and discard the supernatant without disturbing the beads.
4. Keeping the plate on the magnet stand, add 150 µL of freshly prepared 80% ethanol without disturbing the beads. Incubate the plate on the magnet stand at room temperature for 30 sec. Carefully remove and discard the ethanol.
5. Repeat step 4 once.
6. Briefly centrifuge the plate and place the plate on the magnet stand until the solution is clear (~1 min). Remove and discard the supernatant. Moreover, remove all residual buffer by using a 10-µL pipette tip.
7. Remove the sealing film and dry the beads on the magnet stand at room temperature for 2-5 min.

Note: Over-dried beads may be more difficult to suspend.

5. Remove the plate off the magnet stand. Add 20-22 µL of TE Buffer to each well. Mix thoroughly and incubate the plate at 25°C for 5 min.
9. Briefly centrifuge and place the plate on the magnet stand until the supernatant is clear (1-2 min). Transfer all the eluate to a new 96-well plate. Avoid transfer of MiniMax SP Beads.
10. Quantify the purified captured library by using a fluorescence-based method (e.g., Qubit dsDNA HS Assay kit) or equivalent assay.
11. Measure the average size of the captured library by using a digital electrophoresis system (e.g., Agilent 2100 Bioanalyzer, Bioptic Qsep100) or other similar system.

STOP **Safe Stopping Point:** The purified library can be stored at –25~–15°C for up to one week.

Attention

Temperature Control

- The reaction temperatures during hybridization wash steps are different. Please follow the instructions strictly.
- Please follow the instructions strictly to set the hot lid temperature during hybridization.
- The ambient temperature in the laboratory must be stable between 20-25°C. Low temperature may affect the stability and performance of the capture and wash steps.

Perform Hybridization Capture

- In most cases, it is recommended to use Plate protocol, which is repeatable and timesaving through high-throughput operation.
- For a small amount of hybridization reactions (⩽ 6) or important samples, it is recommended to use the Tube protocol, the experimental operation is relatively easier.

Library Concentration Method

- The vacuum concentration method is recommended to obtain the best quality of the hybridization library.
- The magnetic bead concentration method is fast and high-through put. However, this method produces a slight G C preference (see **Appendix** for details).

Hybridization

- The DNA library should avoid containing free linkers or linker dimers. Otherwise, it will affect the mixing accuracy of the library and the balance of data output.
- Multiple samples are mixed to construct a library of genomic DNA samples in the experiment. It is recommended to use double-sided size selection to balance the output of data.
- The hybridization buffer must be fully melted without crystals, and the library mixture must be completely evaporated to dryness. Otherwise, the inaccuracy of the hybridization system will affect the library capture efficiency.
- It is recommended to test the hybridization loss in ad vance: use distilled water instead of the hybridization system for testing. The loss should be <0.5 µL under 65°C for 12 hr, ensuring the tightness of the PCR tube and 96-well plate.

Consumable

- For experimental consumables used in capture experiments, such as centrifuge tubes, pipette tips, etc., make sure to use the low adsorption series to avoid sample loss.

Appendix

MiniMax SP Beads DNA Concentration Protocol (Optional)

Note: This optional protocol requires more beads and Human Cot DNA than vacuum concentration protocol. Equilibrate the MiniMax SP Beads to room temperature for a minimum of 30 min prior to use. The 80% ethanol must be freshly prepared.

1. Prepare the components in 1.5 mL low-bind tubes or 96-well plates according to the table below. The 96-well plates need extra sealing films for sealing.

Component	Volume Per Capture Reaction	Quantity
Library Pool	500 ng/library	1–12
Human Cot DNA	10 µL	
Nuclease Free Water	Up to 50 µL	
MiniMax SP Beads	90 µL/1.8X volume of the top two items*	

Note: * If the total volume of the Library Pool and Human Cot DNA is < 50 µL, use Nuclease Free Water to make up the volume to 50 µL, and add 90 µL MiniMax SP Beads at the same time; if the total volume of the Library Pool and Human Cot DNA is ⩾ 50 µL, then directly add the MiniMax SP Beads with 1.8X volume of the top two items.

2. Mix thoroughly and then incubate at 25°C for 10 min.
3. Place the plate or tube on the magnet stand until the supernatant is clear (2-5 min). Carefully remove and discard the supernatant without disturbing the beads.
4. Keeping the plate or tube on the magnet stand and add freshly prepared 80% fresh ethanol enough to cover the surface of the beads. Incubate at room temperature for 30 sec. Carefully remove and discard the ethanol.
5. Repeat step 4 once.
6. Place the plate or tube on the magnet stand. Carefully remove all residual ethanol without disturbing the beads by using a 10-µL pipette tip.
7. Dry the beads on the magnet stand at room temperature for 2-5 min.

Note: Dry the magnetic beads to a matte finish. Over-dried beads may be more difficult to suspend.

8. Prepare the Hybridization Reaction Mix in the well/tube as follows:

MiniEnrich ES Hyb #1	9.5.µL
MiniEnrich Hyb #2	3.µL
MiniMax NanoBlockers.(for.Illumina®)	2.µL
MiniEnrich Exome Core Panel	4.5.µL
Total	**19 µL**

9. Mix thoroughly and then incubate at room temperature for 5 min.

10. After incubation, place plate or tube on the magnet stand until the solution is clear (5-10 min). Transfer 17 µL of the supernatant to a new low-bind 0.2 mL PCR tube or 96-well plate.

11. Proceed to **Tube Protocol: Perform hybridization reaction, step 11** (Page 10), or **Plate Protocol: Perform hybridization reaction, step 13** (Page 16).