For Research Use Only. Not for use in diagnostic procedures. Compatible with Illumina and MGI sequencing platforms.

If you have any questions, please contact:

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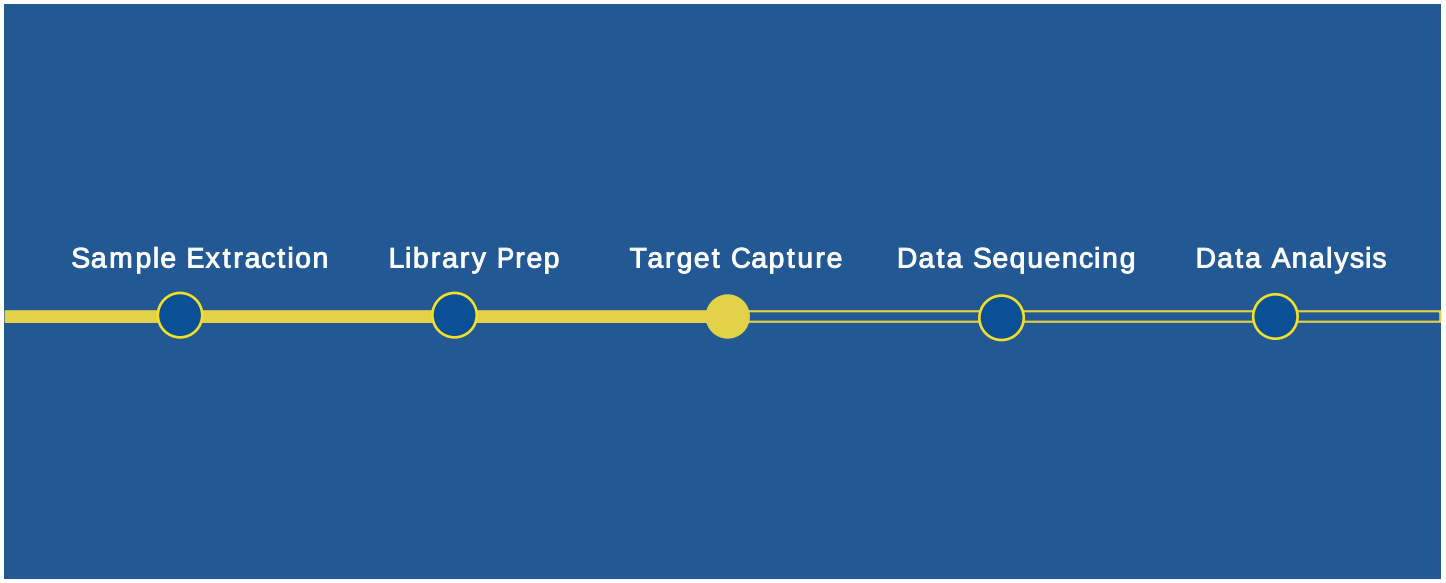
TargetSeq One® Hyb & Wash Kit v3.0

User Manual

Applies to Series products of TargetSeq® DNA Probes HT

Version NO.: A.1, June, 2025

Document NO.: PROT250302



Version Notes

Manufacturer: iGeneTech (Beijing) Bioscience Co., Ltd.

Instructions for Use

This manual is applicable to TargetSeq One® Hyb & Wash Kit v3.0, as to products of TargetSeq® DNA Probes HT, please read this manual carefully before use and strictly follow the instructions provided herein to conduct the experiment.

Overview of the Kit

TargetSeq One® Hyb & Wash Kit v3.0, a liquid-phase probe hybridization capture kit designed for genomic target region, suitable for Illumina and MGI high-throughput sequencing platforms. iGeneTech® designs probes for genomic target region based on multi-factor algorithm and synthesizes biotin-labeled specific capture probes. This kit can capture and enrich target sequences, improving capture performance while significantly reducing the sequencing data volume.

Update Information

Version	Date	Description
A.1	June, 2025	Minor corrections
A.0	March, 2025	Initial release of English version

Kit components

Reagents used for hybridization capture include TargetSeq One® Hyb & Wash Kit v3.0, TargetSeq® DNA Probes HT, TargetSeq® Eco Universal Blocking Oligo, and TargetSeq® Cap Beads & Nuclease-Free Water.

TargetSeq One® Hyb & Wash Kit v3.0 and TargetSeq® Eco Universal Blocking Oligo are related to the type of the library. Please select the correct version accordingly.

TargetSeq One® Hyb & Wash Kit v3.0

Product Name	Composition	Storage
TargetSeq One® Hyb & Wash Kit v3.0	TargetSeq One® Hyb & Wash Kit v3.0 (Module A)	-20℃ ± 5℃
	TargetSeq One® Hyb & Wash Kit v3.0 (Module B)	15℃ ~ 25℃

TargetSeq One® Hyb & Wash Kit v3.0 (Module A)

Cap color	Component	Total amount		Storage
		24 rxn	96 rxn	
Purple	Hyb Human Block	120 μL	540 μL	-20℃ ± 5℃
Purple	TargetSeq One® Hyb Buffer v3	650 μL	2*1300 μL	
Orange	Post PCR Master Mix	660 μL	2*1350 μL	
Orange	Post PCR Primer (25 μM)*	48 μL	192 μL	

*TargetSeq One® Hyb & Wash Kit v3.0 (Module A) is available in two different versions due to the types of library: Module A for Illumina or Module A for MGI DI. Consistent with Module A, Post PCR Primer (25 μM) is also available in two versions: Post PCR Primer (25 μM, for Illumina) or Post PCR Primer (25 μM, for MGI DI) .

TargetSeq One® Hyb & Wash Kit v3.0 (Module B)

Cap color	Component	Total amount		Storage
		24 rxn	96 rxn	
Bottle	TargetSeq One® Wash Buffer v3	45 mL	180 mL	15℃ ~ 25℃

TargetSeq® Eco Universal Blocking Oligo

TargetSeq® Eco Universal Blocking Oligo can block library adapters during hybrid capture process, which can block libraries up to 6ug.

Cap color	Component	Total amount		Storage
		24 rxn	96 rxn	
Purple	TargetSeq® Eco Universal Blocking Oligo*	48 μL	200 μL	-20℃ ± 5℃

*TargetSeq® Eco Universal Blocking is available in two versions: for Illumina or for MGI DI. Please select the appropriate Blocking Oligo according to the type of the library.

TargetSeq® DNA Probes HT

Cap color	Component	Total amount				Storage
		4 rxn	24 rxn	96 rxn	960 rxn	
Yellow	TargetSeq® DNA Probes HT*	10 μL	56 μL	216 μL	2*1080 μL	-20℃± 5℃

*TargetSeq® DNA Probes HT provides 4/24/96/960 rxn regarding pre-defined panels. Customized panels only offer 96/960 rxn.

TargetSeq® Cap Beads & Nuclease-Free Water

Cap color	Component	Total amount			Storage
		24 rxn	5 mL each	50 mL each	
Green / bottle	TargetSeq® Cap Beads*	1320 μL	5 mL	50 mL	2 ~ 8℃
Whilte / bottle	Nuclease-Free Water	1320 μL	5 mL	50 mL	

*TargetSeq® Cap Beads are streptavidin magnetic beads for hybridization capture, which is different from IGT® Pure Beads.

Materials Supplied by User

The followings are the recommended brands by iGeneTech. You can also use alternative reagents, instruments and consumables that meet the experimental requirement.

Reagents

NO.	Name	Recommended Products	Supplier
1	Ethanol Absolute	General laboratory supplier	General laboratory supplier
2	Nuclease-free water	Nuclease-Free Water	Ambion (AM9930)
3	Magnetic Beads (for Purification)*	IGT® Pure Beads	iGeneTech (C80661)
		Agencourt AMPure XP Kit	Beckman Coulter (A63880)
4	Fragment analyzer	S2 Cartridge (Standard Cartridge)	BiOptic (C105101)
5	Nucleic acid quantification reagent	Qubit dsDNA HS Assay Kit	Thermo Fisher (C47257)

Equipments

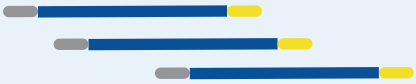
NO.	Name	Recommended Products	Supplier
1	96-well magnetic rack, 0.2 mL module	DynaMag-96 Side	Thermo Fisher (12331D)
2	Fragment analyzer	Qsep 100	BiOptic (Qsep100)
3	Nucleic acid quantification	Qubit™ 4.0 Fluorometer	Thermo Fisher (Q33238)
4	ThermoMixer，0.2 mL block	Eppendorf ThermoMixer® C	Eppendorf (5382000015)
5	Vortex Mixer	General laboratory supplier	General laboratory supplier
6	Minicentrifuge	General laboratory supplier	General laboratory supplier
7	Ice box	General laboratory supplier	General laboratory supplier
8	PCR instrument	General laboratory supplier	General laboratory supplier
9	Vertical Rotating Mixer, 0.2ml block	General laboratory supplier	General laboratory supplier
10	Water bath kettle	General laboratory supplier	General laboratory supplier
11	Vacuum concentrator and pump	General laboratory supplier	General laboratory supplier

Consumables

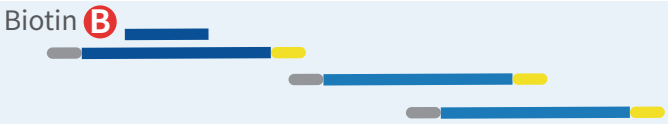
NO.	Name	Recommended Products	Supplier
1	0.5 mL Qubit tube	Qubit® Assay Tubes	Thermo Fisher (Q32856)
2	0.2 mL PCR tube	General laboratory supplier	General laboratory supplier
3	0.2 mL 8-calandria	General laboratory supplier	General laboratory supplier
4	10 μL pipette tip	General laboratory supplier	General laboratory supplier
5	200 μL pipette tip	General laboratory supplier	General laboratory supplier

Workflow

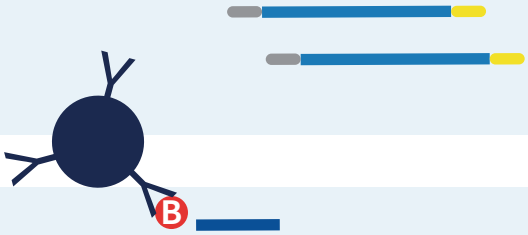
① Dry Down Library
20~30 min



② Perform Hybridization
2~18 h

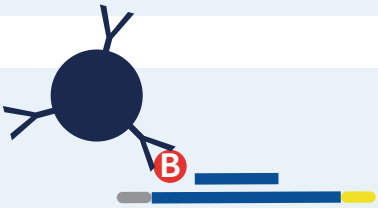


③ Wash TargetSeq® Cap Beads
10~15 min

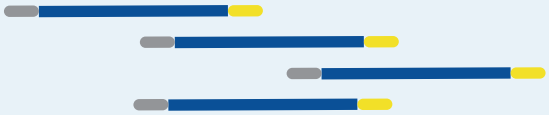


④ Bind Captured Targets to Magnetic Beads
40 min

⑤ Post-capture PCR
30 min



⑥ Purification and QC
40 min



Before you start

Welcome to use TargetSeq One® Hyb & Wash Kit v3.0. Please confirm the following points before use:

☐ Always use reagents and consumables that are certified sterile, DNase/RNase-Free.
Wipe down work area and pipette with an RNase- and DNA-cleaning product.

☐ Please ensure that the temperature of the Thermal Cycler and metal bath is accurate and stable. Accurate temperature control is crucial for effectively removing non-specific products.

☐ Control and qualify the pre-capture library.

☐ The recommended time for hybridization is 2 ~ 18 h, please arrange experiment appropriately.

☐ Please select the Blocking Oligo and Post PCR primer which are matched to Illumina® or MGI pre-capture library.

The experiment can be started if all the conditions above are met.

STEP 1 Dry down library

Experimental Notes

Reagents required:

- Hyb Human Block
- TargetSeq® Eco Universal Blocking Oligo
- TargetSeq® DNA Probes HT
- TargetSeq One® Hyb Buffer v3

Instruments required:

- Vortex Mixer
- Minicentrifuge
- Ice box
- Thermal Mixer

1.1. Take Hyb Human Block from -20℃ refrigerator, place it on ice to thaw. After thawing, briefly vortex and centrifuge. Keep it on ice.

1.2. Take the TargetSeq® Eco Universal Blocking Oligo from -20℃ refrigerator and place them on ice to to thaw. Briefly vortex and centrifuge. Keep it on ice.

1.3. Take the probes from -20℃ refrigerator, place them on ice to thaw. Briefly vortex and centrifuge. Keep it on ice.

1.4. Take the libraries for hybridization capture from -20℃ refrigerator and place them on ice to thaw. Briefly vortex and centrifuge. Keep it on ice.

1.5. Take TargetSeqOne® Hyb Buffer v3 and thaw at room temperature. Briefly vortex and centrifuge. If precipitate is present, please heat the TargetSeqOne® Hyb Buffer v3 on Thermal Mixer at 37℃. Vortex and make sure all precipitate is dissolved.

STEP 2 Perform Hybridization

Reagents required:

- TargetSeq One® Hyb Buffer v3
- Hyb Human Block
- TargetSeq® Eco Universal Blocking Oligo
- Nuclease-Free Water
- TargetSeq® DNA Probes HT

Instruments required:

- Vacuum concentrator
- PCR Instrument
- Vortex Mixer
- Minicentrifuge

The following steps adopt the vacuum concentration scheme. If the laboratory does not have the conditions for vacuum concentration, magnetic bead concentration can be selected as alternatives. The detailed steps are provided in Appendix I.

2.1. Add 750 ng library to the PCR tube. If multiplexing libraries in a single hybridization-based capture reaction, add 500 ng library each.

2.2. Place the PCR tube in a vacuum concentrator, open the cap of the tube and concentrate the libraries to dry.

Before concentrating the library, it is suggested to estimate the concentration time using same volume of water. Over drying will cause loss of the library.

2.3. After the library drying down completed, prepare the Hybridization Master Mix according to following table:

Reagents	Volume
TargetSeq One® Hyb Buffer v3	18 μL
Hyb Human Block	5 μL
TargetSeq® Eco Universal Blocking Oligo*	2 μL
Nuclease-Free Water	3 μL
TargetSeq® DNA Probes HT	2 μL
Total Volume	30 μL

*TargetSeq® Eco Universal Blocking Oligo is available in two versions: for Illumina or for MGI DI. Please select the appropriate Blocking Oligo according to the type of the library.

2.4. Add the Hybridization Master Mix to the dried library, gently vortex for 30 s to ensure the library at the bottom of the tube is dissolved, and spin briefly.

2.5. Place the PCR tube on the thermal cycler, and start the program as indicated below.

Temperature	Time
Heat lid temperature 95℃	
85℃	5 min
57℃	Hold

2.6. Incubate for 2 ~ 18 h. Start next step (STEP 3) 30 min before the incubation finishes.

Experimental Notes

STEP 3 Wash TargetSeq® Cap Beads

Reagents required:

- Cap Beads
- 80% Ethanol (freshly prepared)
- TargetSeq One® Wash Buffer v3 (room temperature)

Instruments required:

- Magnetic rack
- Vortex Mixer
- Minicentrifuge

3.1. Take the Cap Beads from -4 ℃ refrigerator, gently vortex to mix well, place at room temperature to equilibrate for 30 min.

3.2. Prepare 80% ethanol in advance using ethanol absolute and Nuclease-Free Water, and store at room temperature.

Please use TargetSeq® Cap Beads, other magnetic beads like C1, M270, M280 or Purification Magnetic Bead are not suitable for capture.

3.3. Gently votex the Cap Beads for 30 s before use. Add 50 µL of Cap Beads to a new PCR tube and place on magnetic stand for 1 min until the solution becomes clear. Then remove and discard the supernatant.

The TargetSeq One® Wash Buffer v3 used in STEP 3 should be stored and used at room temperature. Please do not preheat it in advance.

3.4. Remove the PCR tubes from the magnetic stand, add 180 µL of TargetSeq One® Wash Buffer v3, mix by pipetting to resuspend the magnetic beads.

3.5. Spin briefly on minicentrifuge, place the PCR tube on magnetic stand for 1 min until the solution becomes clear, then remove the supernatant.

3.6. Repeat steps 3.4 ~ 3.5 one time (for a total of 2 washes).

3.7. Remove the PCR tube from the magnetic stand, add 180 µL of TargetSeq One® Wash Buffer v3, mix by pipetting or vortexing, and proceed immediately to STEP 4.

STEP 4 Target Capture of Libraries

Reagents required:

- Cap Beads Resuspension
- TargetSeq One® Wash Buffer v3 (preheat at 63℃)
- 80% Ethanol(freshly prepared)
- Nuclease-Free Water

Instruments required:

- Vertical Rotating Mixer
- Thermal Mixer
- Magnetic rack
- Vortex Mixer
- Minicentrifuge

4.1. Preheat TargetSeq One® Wash Buffer v3 on thermal cycler at 63℃.

4.2. Keep the hybridization products from STEP 2 on PCR instrument. Add 180 µL Cap Beads resuspension prepared in STEP 3.7. to the hybridization products and mix well by pipetting up and down.

4.3. Close the tube caps, remove the PCR tubes from the PCR instrument and place on a vertical rotating mixer at a speed not exceeding 10 rpm for 15 min at room temperature (If vertical rotating mixer is not available, then tubes can be inverted and mixed several times every 5 min at room temperature for 15 min).

4.4. The PCR tubes spin briefly, and place them on a magnetic stand for 2 min. After the solution becomes clear, discard the supernatant.

4.5. Remove the PCR tubes from the magnetic stand, add 150 µL of preheated TargetSeq One Wash Buffer v3 (63℃) gently mix by pipetting, spin briefly and incubate at 63℃ for 5 min on Thermal Mixer.

4.6. Remove the PCR tubes from Thermal Mixer, spin briefly, and place them on a magnetic stand for 2 min. Discard the supernatant after the solution becomes clear.

Please ensure that the temperature of the Thermal Mixer is accurate and stable. Precise temperature control is essential for effective removal of non-specific products.

4.7. Repeat steps 4.5 to 4.6 three times. For panels coverage region ＜ 200 kb, repeat steps 4.5 to 4.6 four times.

Step 4.6 : After incubating at 63℃ for 5 min, **transfer the total solution into a NEW PCR tube** in the second and final round, which is conductive to improve the stability of the capture efficiency.

4.8. Keep the PCR tubes on the magnetic stand, add 200 µL of 80% ethanol to PCR tube, let it stand for 30 s, then completely remove and discard the ethanol solution (remove all residual ethanol using a 10 µL pipette), and air dry the magnetic beads at room temperature to ensure the residual ethanol to evaporate completely.

4.9. Add 24 µL Nuclease-Free Water to the PCR tube, remove the PCR tube from the magnetic stand, briefly vortexing or pipette to mix well. Proceed immediately to the next step.

Don't discard the TargetSeq® Cap Beads!
Captured libraries are on the TargetSeq® Cap Beads.

STEP 5 Post-capture PCR

Experimental Notes

Reagents required:

- Post PCR Master Mix
- Post PCR Primer

Instruments required:

- PCR Instrument
- Vortex Mixer
- Minicentrifuge

5.1. Take out the Post PCR Master Mix and Post PCR Primer from -20°C refrigerator, place them on ice box to thaw. Store on ice box after they were dissolved.

5.2. Post PCR Primer is library type specific, ensure using the correct Post PCR Primer. Briefly vortex Post PCR Master Mix and Post PCR Primer , spin briefly.

5.3. Prepare the PCR reaction mixture according to following table:

Reagents	Volume
TargetSeq® Cap Beads Suspension from STEP 4	24 μL
Post PCR Primer	1 μL
Post PCR Master Mix	25 μL
Total Volume	50 μL

Post PCR Primer is available in two different versions: for Illumina or for MGI DI.

5.4. After the preparation completed, mix well by pipetting up and down, and transfer it quickly to the PCR instrument. Do not use the method of vortexing followed by centrifugation to mix.

5.5. Set the PCR program as follows. Place the PCR solution on PCR instrument, and run the program:

Temperature	Time
Heat lid temperature: 105℃	
95℃	1 min
98℃	10 s
60℃	20 s
72℃	20 s
72℃	1 min
4℃	hold

Pre-capture library input	Post-PCR Cycle NO.
750 ng	N
1.5 μg	N-1
3 μg	N-2
6 μg	N-3

For post-PCR cycles, please refer to the PCR cycle number N from the tube label of TargetSeq® DNA Probes HT. The number of post-PCR cycles is related to total input of pre-capture library. As MGI sequencing platform needs higher Library inputs, it is suggsted to add 2 PCR cycles to post-PCR for MGI libraries.

5.6. After the program completed, proceed to PCR products purification in Step 6.

STEP 6 Library Purification

Reagents required:

- Purification beads (IGT® Pure Beads)
- 80% ethanol (freshly prepared)
- Nuclease-Free Water

Instruments required:

- Magnetic stand
- Vortex oscillator
- Minicentrifuge

6.1. Vortex the magnetic beads (for purification) for 30 sec to mix well. Equilibrate the magnetic beads (for purifcation) to room temperature for 30 min.

The purification step uses IGT® Pure Beads. If other brands of purification magnetic beads are selected, it is necessary to consult the corresponding supplier and determine the appropriate ratio of magnetic beads through pre-experiments to avoid purification failure.

6.2. Add 55 μL (1.1×) of magnetic beads (for purifcation) to each amplified sample. Vortex or pipette to mix well. Incubate at room temperature for 5 min.

6.3. Spin briefly on a minicentrifuge. Place the PCR tube containing both TargetSeq® Cap Beads and magnetic bead for purifcation) on a magnetic stand and allow the magnetic beads to fully seperate from the supernatant (approximately 3 min).

6.4. Keep the PCR tube containing magnetic beads on a magnetic stand. Remove and discard the clear supernatant. Add 200 μL of 80% ethanol to PCR tube. Incubate at room temperature for 30 s.

6.5. Keep the PCR tube containing magnetic beads on a magnetic stand. Remove and discard the clear supernatant. Add 200 μL of 80% ethanol to PCR tube. Incubate at room temperature for 30 s. Remove and discard the clear supernatant. (May spin briefly on a minicentrifuge and remove all residual ethanol using a 10 μL pipette).

6.6. Keep the PCR tube containing beads on the magnetic stand at room temperature for 3-5 min to dry the bead pellet.

6.7. Add 25 μL of Nuclease-Free Water, remove the PCR tube from the magnetic rack, mix by pipetting or vortexing, and let it stand at room temperature for 2 min.

6.8. Spin briefly and place the PCR tube on the magnetic rack for 2 min until the solution become clear.

6.9. Use a pipette to draw 23 μL of the supernatant and transfer it to a new PCR tube, the captured library can be stored in -20°C refrigerator for at least one month.

6.10. Take 1 μL of the library and use the Qubit dsDNA HS Assay Kit reagent to measure the library concentration on Qubit 4.0 Fluorometer, record the library concentration.

6.11. Take 1 μL of the library and use fragment analyzer to conduct fragment quality control. The fragment size should be basically consistent with the size of the pre-library.

The experiment ends here!

Appendix I: Procedure of Library Concentration using Magnetic Bead for Purification (Optional)

Reagents required:

- Magnetic Beads (IGT® Pure Beads)
- 80% ethanol (freshly prepared)
- Nuclease-Free Water

Instruments required:

- Magnetic stand
- Vortex Mixer
- Minicentrifuge

1. Add 750 ng of library to PCR tube and properly labeled, when multiplex libraries are mixed for hybridization, add 500 ng of each library.
2. Add 1.8× volume of IGT® Pure Beads to the library, gently mix by pipetting, and incubate at room temperature for 5 min.
3. Place the PCR tube on a magnetic stand for 3 min until the solution becomes clear.
4. Keep the PCR tube on magnetic stand, discard the supernatant, add 200 µL of 80% ethanol solution to the PCR tube, and let it stand for 30 s.
5. Keep the PCR tube on magnetic rack, discard the supernatant, and add 200 µL of 80% ethanol solution to the PCR tube again. Let it stand for 30 s and discard the supernatant thoroughly (you can spin briefly to bring the adhered liquid to the bottom of the tube, then use a 10 µL pipette to discard the residual ethanol at the bottom).
6. Ensure the PCR tube are placed on the magnetic rack and left at room temperature for 3-5 min to dry the magnetic beads and allow the residual ethanol to evaporate completely.
7. Prepare the Hybridization Master Mix as indicated below:

Reagents	Volume
TargetSeq One® Hyb Buffer v3	18 µL
Hyb Human Block	5 µL
TargetSeq® Eco Universal Blocking Oligo	2 µL
Nuclease-Free Water	3 µL
TargetSeq® DNA Probes HT	2 µL
Total Volume	30 µL

8. Add 30 µL of Hybridization Master Mix to the PCR tube containing magnetic beads for purifcation. Pipet to mix well and let stand at room temperature for 3 min.
9. Spin briefly and place the PCR tube on a magnetic rack for 3 min until the solution becomes clear.
10. Use a pipette to transfer 28 µL of the supernatant to a new PCR tube. Mix gently by pipetting up and down, then spin briefly.
11. Set the PCR instrument as follows, place the hybridization solution on the PCR instrument, and run the program.

Temperature	Time
Heat lid temperature 95℃	
85℃	5 min
57℃	Hold

12. It is recommended that the hybridization time be 2 ~ 18 hours, and STEP 3 be performed 30 min before end of the incubation.

Appendix II: Protocol of Small-Volume Fast Hybridization

Reagents required:

- TargetSeq One® Hyb Buffer v3
- Hyb Human Block
- TargetSeq® Eco Universal Blocking Oligo
- Nuclease-Free Water
- TargetSeq® DNA Probes HT

Instruments required:

- Vacuum concentrator
- PCR Instrument
- Vortex Mixer
- Minicentrifuge

This protocol provides hybridization of small volume which can achieve higher uniformity. If higher uniformity or fast hybridization is required, this protocol can be chosen.

1. Add 750 ng of library to PCR tube and properly labeled, when multiple libraries are mixed for hybridization, add 500 ng of each library. Add reagent follow the table and pipet to mix well.

Reagent	Volume
Library	750 ng or N*500 ng
Hyb Human Block	5 µL
TargetSeq® Eco Universal Blocking Oligo*	2 µL

*TargetSeq Eco Universal Blocking is available in two versions: for Illumina or for MGI DI. Please select the appropriate blocking Oligo according to the type of the library.

2. Dry down the libraries in a Vacuum system (or a similar evaporator device). Open the cap of PCR tube, and concentrate the libraries to dry.

Before drying down the library, it is suggested to estimate the time of concentration using same volume of water. Overdrying will cause loss of the pre-capture library.

3. Prepare hybridization master mix as below after library drying down:

Reagent	Volume
TargetSeq One® Hyb Buffer v3	9 µL
Nuclease-Free Water	4 µL
TargetSeq® DNA Probes HT	2 µL
Total Volume	**15 µL**

4. Add the hybridization master mix to the dried libraries. Vortex for 30 s to completely dissolve the dried libraries at the bottom of the tube, and spin briefly.

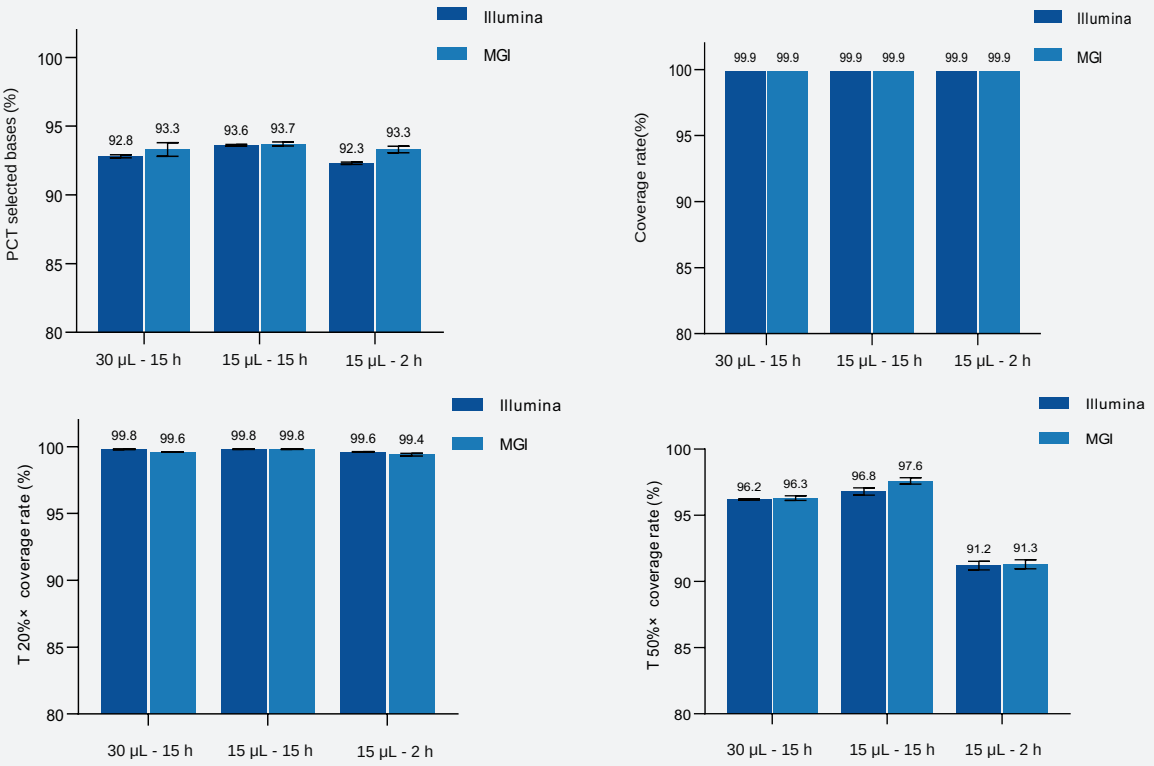
5. Set the PCR instrument as below and place the PCR tube on the thermal cycler, run the program:

Temperature	Time
Heat lid temperature: 95℃	
85℃	5 min
57℃	Hold

6. It is recommended that the hybridization time be 2 ~ 18 hours, and STEP 3 be performed 30 min before end of the program.

The following data are the test results of AIExome V5 with different hybridization volumes, for your reference.

The sample used was gDNA (Promega, G3041). Each library was captured with an input of 750 ng. Hybridization was performed according to the two volume schemes provided in this protocol, namely 30 μL or 15 μL, with hybridization time of 15 hours and 2 hours respectively.



Lab memo

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