iGeneTech
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TargetSeq One® BisCap® Target Enrichment Workflow

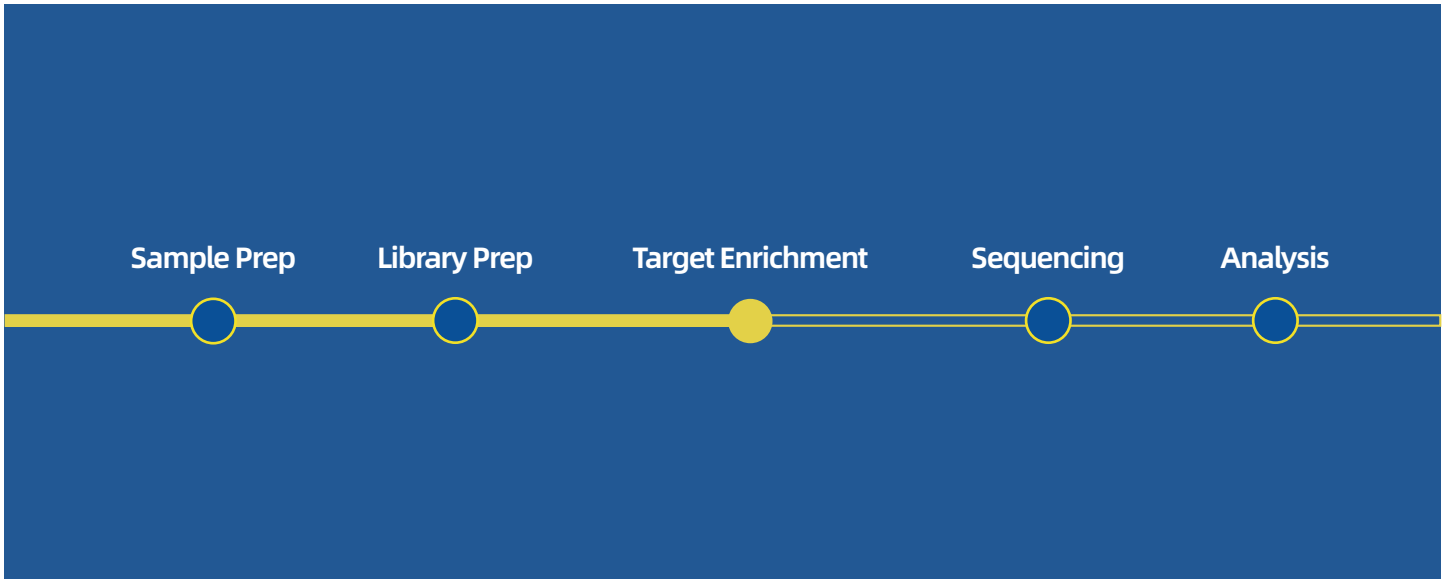
Protocol

For use with:

· TargetSeq One® BisCap® Hyb & Wash Kit

· BisCap® Target Probes

Version: A.0(E), March 2024
Document Number: PROT240302



Version Notes

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Protocol Notes

The protocol provides an instruction for hybridization-based capture using TargetSeq One® BisCap® Hyb & Wash Kit with BisCap® Target Probes (panel size < 30Mb). Before you begin, please read this protocol carefully, and strictly follow the protocol for experiment.

Product Notes

TargetSeq One® BisCap® Hyb & Wash Kit is used for hybridization-based capture on target regions for methyl-seq and generates captured sequencing libraries of Illumina and MGI platform.

History

Version	Date	Description
A.0	March, 2024	Initial release

Materials from iGeneTech

The reagents for hybridization-based capture experiment include TargetSeq One® BisCap® Hyb & Wash Kit, TargetSeq® Blocking Oligo, BisCap® Target Probes and TargetSeq® Cap Beads.

TargetSeq One® BisCap® Hyb & Wash Kit and TargetSeq® Blocking Oligo are library type specific. Please choose the correct version according to the library type.

TargetSeq One® BisCap® Hyb & Wash Kit

TargetSeq One® BisCap® Hyb & Wash Kit has 3 different versions, for Illumina, MGI SI and MGI DI libraries. TargetSeq One® BisCap® Hyb & Wash Kit consists of 3 different modules as below.

Kit	Module	Storage
TargetSeq One® BisCap® Hyb & Wash Kit [1]	TargetSeq One® BisCap® Hyb & Wash Kit (Module A)	-20℃ ± 5℃
	TargetSeq One® Hyb & Wash Kit (Module B)	15℃ ~ 25℃
	TargetSeq One® Hyb & Wash Kit (Module C) [1]	-20℃ ± 5℃

[1] TargetSeq One® BisCap® Hyb & Wash Kit has 3 different versions, for Illumina, MGI SI and MGI DI libraries, which consists with different Module C, i.e., TargetSeq One® Hyb & Wash Kit (Module C, for Illumina) , TargetSeq One® Hyb & Wash Kit (Module C, for MGI SI), and TargetSeq One® Hyb & Wash Kit (Module C, for MGI DI) , respectively.

TargetSeq One® BisCap® Hyb & Wash Kit (Module A)

Screw Cap	Components	Amount		Storage
		16 rxn	96 rxn	
Violet	Hyb Human Block	88 μL	540 μL	-20℃ ± 5℃
Violet	RNase Block	88 μL	540 μL	
Violet	Hyb Buffer	360 μL	2*1080 μL	
Violet	BisCap® Enhancer	180 μL	1080 μL	

TargetSeq One® Hyb & Wash Kit (Module B)

Screw Cap	Components	Amount		Storage
		16 rxn	96 rxn	
/ [1]	Binding Buffer	14 mL	84 mL	15℃ ~ 25℃
/ [1]	Wash Buffer 1	4 mL	24 mL	
/ [1]	TargetSeq One® Wash Buffer	18 mL	108 mL	

[1] "/" means in bottle

TargetSeq One® Hyb & Wash Kit (Module C)

Screw Cap	Components	Amount		Storage
		16 rxn	96 rxn	
Orange	Post PCR Master Mix	450 μL	2*1350 μL	-20℃ ± 5℃
Orange	Post PCR Primer (25 μM) [1]	32 μL	192 μL	

[1] Post PCR Primer (25 μM) has 3 different versions, for Illumina, MGI SI and MGI DI libraries, which is in corresponding to the version of Module C.

TargetSeq® Blocking Oligo

iGeneTech provides two TargetSeq® Blocking Oligo to be chosen from, i.e., TargetSeq® Universal Blocking Oligo and TargetSeq® Eco Universal Blocking Oligo with blocking capacity up to 6 μg and 3 μg libraries, repectively. TargetSeq® Blocking Oligo also has different versions for different types of library, such as Illumina TS library, Illumina ssDNA library, MGI SI and MGI DI library. Please choose the appropriate version accoroding to the multiplexing choice and the library type.

TargetSeq® Universal Blocking Oligo

Screw Cap	Component	Amount			Storage
		4 rxn	16 rxn	96 rxn	
Violet	TargetSeq® Universal Blocking Oligo	10 μL	36 μL	200 μL	-20℃ ± 5℃

TargetSeq® Eco Universal Blocking Oligo

Screw Cap	Component	Amount		Storage
		16 rxn	96 rxn	
Violet	TargetSeq® Eco Universal Blocking Oligo	36 μL	200 μL	-20℃ ± 5℃

BisCap® DNA Probes

Screw Cap	Component	Amount		Storage
		16 rxn	96 rxn	
Red	BisCap® Target Probes	36 μL	216 μL	<-70℃

TargetSeq® Cap Beads & Nuclease-Free Water

Screw Cap	Components	Amount			Storage
		1000 μL each	5 mL each	50 mL each	
Green or / [1]	TargetSeq® Cap Beads [2]	1000 μL	5 mL	50 mL	2 ~ 8℃
White or / [1]	Nuclease-Free Water	1000 μL	5 mL	50 mL	

[1] "/" means in bottle

[2] TargetSeq® Cap Beads is Streptavidin-coated magnetic beads for hybridization-based capture and is different from IGT® Pure Beads. Dynabeads ™ MyOne ™ Streptavidin T1 from Thermo Fisher (Cat#65602) is an alternative of TargetSeq® Cap Beads.

Materials Supplied by User

The materials below are recommended by iGeneTech. Please select the appropriate materials to start the experiment.

Reagents

Item	Recommended Reagents	Supplier (Cat #)
Ethanol Absolute	Varies	Varies
Nuclease-Free Water	Nuclease-Free Water	Ambion (AM9930)
Magnetic Beads (for Purification)*	Agencourt AMPure XP Kit	Beckman Coulter (A63880)
	IGT® Pure Beads	iGeneTech (C80661)
Fragment Analyzer	Agilent DNA 1000 Kit	Agilent (5067-1504)
Nucleic Acid Quantification Assay	Qubit dsDNA HS Assay Kit	Thermo Fisher (C47257)

* Not to be confused with TargetSeq® Cap Beads.

Equipment

Item	Recommended Equipment	Supplier (Cat #)
96-well Magnetic Stand, 0.2 mL block	DynaMag-96 Side	Thermo Fisher (12331D)
Fragment Analyzer	Agilent 2100 Bioanalyzer system	Agilent (G2939AA)
Nucleic Acid Quantification Instrument	Qubit® 4.0 Fluorometer	Thermo Fisher (Q33238)
Dry Bath Incubator	Varies	Varies
Vortex Mixer	Varies	Varies
Mini Centrifuge	Varies	Varies
Ice Block	Varies	Varies
Thermal Cycler	Varies	Varies
Vertical Rotating Mixer, 0.2mL block	Varies	Varies
Vacuum Concentrators & Pumps	SPD2010 Integrated SpeedVac	Thermo Fisher (SPD2010-220)

Consumables

Item	Recommended Eonsumables	Supplier (Cat #)
Qubit tubes, 0.5 mL	Qubit® assay tubes	Thermo Fisher (Q32856)
PCR tubes, 0.2 mL	Varies	Varies
8-tubes strip, 0.2 mL	Varies	Varies
Pipette tips, 10 µL	Varies	Varies
Pipette tips, 200 µL	Varies	Varies

Workflow

① Dry Down the Library
20 ~ 30 min

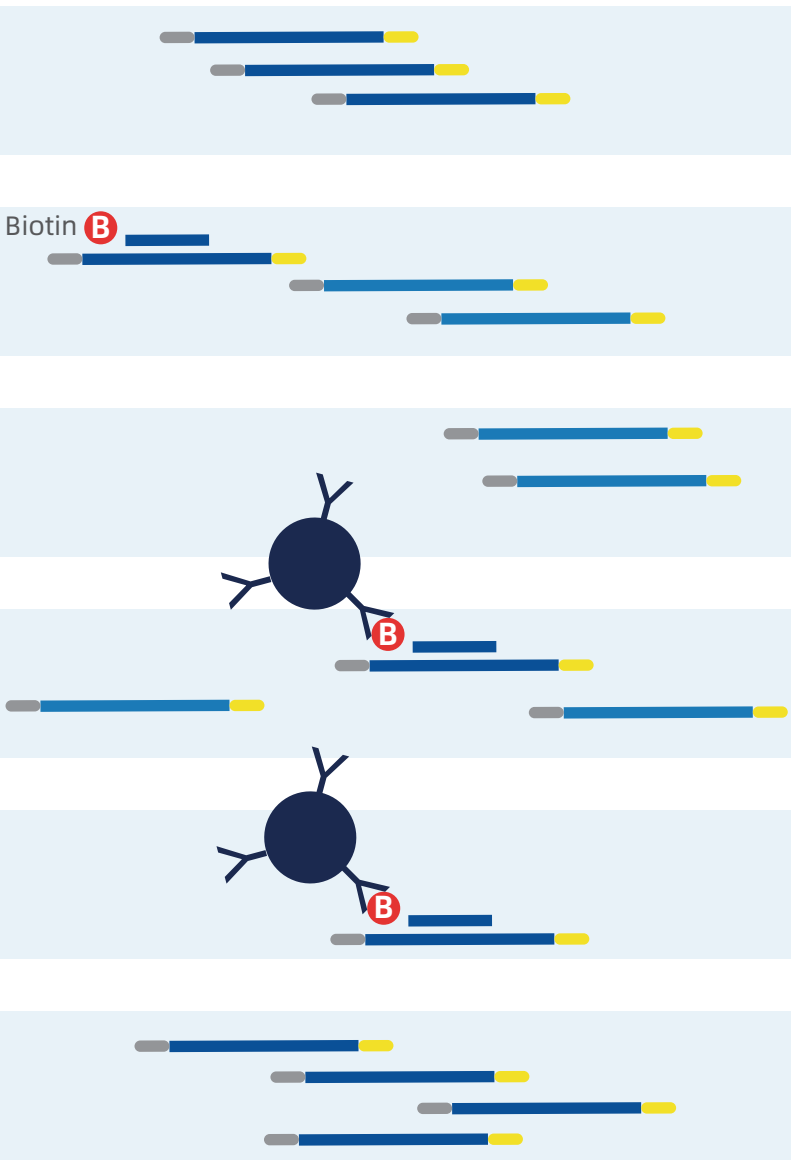
② Hybridization-based Capture
12 ~ 18 h

③ Prepare the Beads
10 ~ 15 min

④ Bind Captured Targets to Magnetic Beads
90 min

⑤ Post-Capture Amplification
1 h

⑥ Purification and QC
40 min



Before You Begin

To start, please confirm the following requirements are met.

- ☐ Always use reagents and consumables that are certified sterile, DNase/RNase-Free. Wipe down work area and pipettes with an RNase- and DNA-cleaning product.
- ☐ Pre-captured library should be quantified and pass the Quality Control test.
- ☐ The recommended time for hybridization is 12 ~ 18 h. Please arrange experiment appropriately.
- ☐ Please select the correct version of Blocking Oligo and Post PCR primer which are matched to the pre-captured library.
- ☐ The protocol is intended to be used for BisCap® Target probes.

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

STEP 1 Preparation before Hybridization

The experiment needs about 24 h, please arrange experiment appropriately.

1.1 Thaw Hyb Human Block, RNase Block, BisCap® Enhancer and TargetSeq® Blocking Oligo on ice. Once reagents are thawed, mix each of them thoroughly by quick vortexing to avoid any localized concentrations. Centrifuge briefly before use.

1.2 Thaw BisCap® DNA Probes on ice. Vortex and centrifuge briefly before use. Place back on ice.

1.3 Thaw pre-captured library on ice. Mix thoroughly and centrifuge briefly.

1.4 Thaw Hyb Buffer at room temperature. Pre-heat at 65℃ to dissolve any precipitate if presents. Vortex and centrifuge briefly. Aliquot according to the requirements and incubate the aliquots at 65℃.

Experiment Notes

STEP 2 Drying and Hybridization

Experiment Notes

This section outlines the vacuum evaporation method for the library concentration process which is the recommended method. However, if the magnetic bead concentration method (optional) is preferred, please refer to **Appendix 1**.

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. Vortex and spin down the sample before use.

The total library amount for hybridization capture should not exceed 2 μg.

Please select the correct TargetSeq® Blocking Oligo to proceed the hybridization based on the total library input of each reaction as well as the library type.

- TargetSeq® Universal Blocking Oligo Kit can block up to 6 μg libraries (not appplicable for this experiment).
- TargetSeq® Eco Universal Blocking Oligo Kit can block up to 3 μg libraries with versions for Illumina TS library, Illumina ssDNA library, MGI SI and MGI DI library.

2.2 Add 10 μL BisCap® Enhancer to each reaction.

2.3 Dry down the pre-captured library in a SpeedVac system (or a similar evaporator device). Open the lid of PCR tube, and concentrate the libraries to dry.

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

2.4 Prepare Hybridization Master Mix as indicated below. Mix well and centrifuge briefly.

Component	Volume per reaction
Hyb Buffer (pre-heated at 65℃)	13 μL
Hyb Human Block	5 μL
TargetSeq® Blocking Oligo	2 μL
RNase Block	5 μL
Nuclease-Free Water	3 μL
BisCap® Target Probes	2 μL
Total Volume	**30 μL**

Add BisCap® Target Probes lastly for the Hybridization Master Mix, or add BisCap® Target Probes after RNase Block.

2.5 Add 30 μL of Hybridization Master Mix to the dried pre-captured library. Vortex for 30 sec to completely dissolve the dried DNA at the bottom of the tube, and spin briefly on a mini centrifuge.

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

Temperature	Time
Heat lid temperature 105℃	
95℃	5 min
65℃	**Hold**

2.7 Incubate for 12 to 18 h. Start next step (STEP 3) 30 min before the incubation finished.

STEP 3 Preparation before Capture

3.1 Vortex the TargetSeq® Cap Beads for 30 sec to mix well. Equilibrate the TargetSeq® Cap Beads to room temperature for 30 min.

3.2 Prepare the 80% ethanol for post-PCR purification. Place the 80% ethanol at room temperature.

3.3 Gently turn the Wash Buffer 1 upside down for 3-5 times to mix well. If precipitate is present, heat the Wash Buffer 1 at 37℃, until the precipitate is dissolved.

3.4 Pre-heat the TargetSeq One® Wash Buffer at 50℃.

3.5 Vortex the TargetSeq® Cap Beads again before use. Add 50 μL of TargetSeq® Cap Beads to a new PCR tube, and place the PCR tube on a magnetic stand and allow the TargetSeq® Cap Beads to fully separate from the supernatant (approximately 1 min). Remove and discard the clear supernatant.

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

3.6 Remove the PCR tube containing TargetSeq® Cap Beads from the magnetic stand. Add 180 μL of Binding Buffer, and pipet or vortex to mix well.

3.7 Short spin on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the TargetSeq® Cap Beads to fully separate from the supernatant (approximately 1 min). Remove and discard the clear supernatant.

3.8 Repeat Steps 3.6 ~ 3.7 two more times (three times in total).

3.9 Remove the PCR tube containing TargetSeq® Cap Beads from the magnetic stand. Add 180 μL of Binding Buffer, and pipet or vortex to mix well. Proceed to STEP 4 immediately.

Experiment Notes

STEP 4 Target Capture of Libraries

Experiment Notes

Reagents Required:

- Wash Buffer 1
- TargetSeq One® Wash Buffer
- 80% Ethanol (freshly prepared)
- Nuclease-Free Water
- Cap Beads Suspension Solution

Equipment Required:

- Dry Bath Incubator
- Magnetic Stand
- Vertical Rotating Mixer

4.1 Keep the PCR tube containing hybridization reaction mixture (from STEP 2) on thermal cycler at 50℃, and rapidly transfer the 180 µL of TargetSeq® Cap Beads to hybridization reaction mixture, and pipet to mix well.

4.2 Close the lid and remove the PCR tube from thermal cycler. Then incubate the PCR tube on a vertical rotating mixer, mixing gently (< 10 rpm) for 30 min at room temperature. If there is no vertical rotating mixer, manually mix the PCR tube for 30 min at room temperature by gently turning upside down for 5 sec every 5 min.

4.3 Remove the PCR tube from the vertical rotating mixer and spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand, and allow the TargetSeq® Cap Beads to fully separate from the supernatant (approximately 2 min). Remove and discard the clear supernatant.

4.4 Remove the PCR tube from the magnetic stand. Add 150 µL of Wash Buffer 1 and gently pipet to mix well. Replace the lid of PCR tube with a new one. Then place the PCR tube on the vertical rotating mixer, mixing gently (< 10 rpm) for 15 min at room temperature.

4.5 Remove the PCR tube from the vertical rotating mixer and spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the TargetSeq® Cap Beads to fully separate from the supernatant (approximately 2 min). Remove and discard the clear supernatant.

4.6 Remove the PCR tube from the magnetic stand. Add 150 µL of pre-heated (50℃) TargetSeq One® Wash Buffer, and pipet to mix well. Spin briefly on a mini centrifuge. Incubate the PCR tube at 50℃ for 10 min.

4.7 Remove the PCR tube from the dry bath incubator and spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand, and allow the TargetSeq® Cap Beads to fully separate from the supernatant (approximately 2 min). Remove and discard the clear supernatant.

4.8 Repeat Step 4.6 ~ 4.7 one more time. For panels with region sizes less than 200 kb, repeat Step 4.6 ~ 4.7 two more times for better performance (three times in total).

4.9 Remove the PCR tube from the magnetic stand. Add 150 µL of pre-heated (50℃) TargetSeq One® Wash Buffer, and pipet to mix well. Spin briefly on a mini centrifuge. Incubate the PCR tube at 50℃ for 10 min.

4.10 Remove the PCR tube from the dry bath incubator. Spin briefly on a mini centrifuge, and pipet to mix well. Transfer all the solution inlcuding TargetSeq® Cap Beads to a new PCR tube, and place the new PCR tube on a magnetic stand, and allow the TargetSeq® Cap Beads to fully separate from the supernatant (approximately 2 min). Remove and discard the clear supernatant.

4.11 Keep the PCR tube on a magnetic stand. Add 200 µL of 80% ethanol. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant. Remove all traces of supernatant using a 10 µL pipette. Keep the PCR tube on the magnetic stand at room temperature to dry the bead pellet. Do not overdry the bead pellet.

4.12 Add 24 µL of Nuclease-Free Water. Remove the PCR tube containing TargetSeq® Cap Beads from the magnetic stand. Vortex or pipet to mix well and spin down. Proceed to STEP 5 immediately.

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

STEP 5 Post-PCR Amplification

Experiment Notes

Reagents Required:

- Post PCR Master Mix
- Post PCR Primer

Equipment Required:

- Thermal Cycler
- Vortex Mixer
- Mini Centrifuge

5.1 Thaw Post PCR Master Mix on ice. Mix well by rotating and centrifuge briefly. Place back on ice.

5.2 Thaw Post PCR Primer on ice. Votex to mix well and centrifuge briefly. Place back on ice. Post PCR Primer is library type specific. Please ensure using the correct Post PCR Primer.

5.3 Prepare PCR reaction mixture as indicated below.

Component	Volume per reaction
TargetSeq® Cap Beads Suspension from STEP 4.12	24 µL
Post PCR Primer	1 µL
Post PCR Master Mix	25 µL
Total Volume	**50 µL**

5.4 Mix well by gently pipetting (do not vortex to mix) and centrifuge briefly.

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

Temperature	Time	PCR Cycle
Heat lid temperature 105℃		
95℃	1 min	1
98℃	20 s	X cycles
60℃	30 s	
72℃	30 s	
72℃	5 min	1
4℃	**Hold**	**1**

Pre-capture library input	Post-PCR cycle X
750 ng	N
1.5 µg	N-1

For post-PCR cycle, please refer to the PCR cycle number N from the tube label of Bis-Cap® Target Probes. The number of post-PCR cycles is related to total pre-capture library input. As MGI platform needs higher library input for sequencing, it is suggested to add 2 more PCR cycles to post-PCR for MGI libraries.

5.6 Please proceed to STEP 6 when the program finished.

STEP 6 Library Purification

Experiment Notes

Reagents Required:

- Magnetic Beads (for Purification)
- 80% Ethanol (freshly prepared)
- Nuclease-Free Water

Equipment Required:

- Thermal Cycler
- Vortex Mixer
- Mini Centrifuge

IGT® Pure Beads or Agencourt AMPure XP is the recommended magnetic beads for purification in this protocol. Please assess the volume of beads used for purification via pre-experiments if not using the recommended ones.

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

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

6.3 Spin briefly on a mini centrifuge. Place the PCR tube containing both TargetSeq® Cap Beads and magnetic beads (for purification) on a magnetic stand and allow the magnetic beads to fully separate from the supernatant (approximately 3 min).

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

6.5 Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant. (May spin briefly on a mini centrifuge and remove all traces of supernatant 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.

Do not dry the bead pellet to the point that the pellet appears cracked during any of the bead drying steps in the protocol. Elution efficiency is significantly decreased when the bead pellet is excessively dried.

6.7 Add 25 µL of Nuclease-Free Water. Remove the PCR tube from the magnetic stand. Vortex or pipet to mix well. Incubate at room temperature for 2 min.

6.8 Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the beads to fully separate from the supernatant (approximately 2 min).

6.9 Transfer 23 µL of clear supernatant containing the captured library to a clean PCR tube. The captured library can be stored at -20℃ for up to 1 month.

6.10 Quantify each captured library using Qubit dsDNA High Sensitivity Assay Kit.

6.11 Analyze the fragment size of captured library using Agilent 2100 Bioanalyzer.

The experiment ends here.

Appendix 1: Operational Procedure of Library Concentration using Magnetic Bead for Purification (Optional)

Reagents required:

- Magnetic Beads
- 80% Ethanol (freshly prepared)
- Nuclease-Free Water

Equipment Required:

- Thermal Cycler
- Vortex Mixer
- Mini Centrifuge

1. Add 750 ng of library to the PCR tube. If multiplexing libraries in a single hybridization-capture reaction, add 500 ng of library each. Add 10 µL of BisCap® Enhancer to each reaction. Vortex and spin down the sample before use.
2. Add 3× volume of magnetic beads for purification. Pipet to mix well. Incubate at room temperature for 5 min.
3. Place the PCR tube on a magnetic stand and allow the magnetic beads for purification to fully separate from the supernatant (approximately 3 min).
4. Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec.
5. Keep the PCR tube on a magnetic stand. Remove and discard the clear supernatant. Add 200 µL of 80% ethanol to the PCR tube. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant. May spin briefly on a mini centrifuge and place the PCR tube back on a magnetic stand. Remove all traces of supernatant using a 10 µL pipette.
6. Keep the PCR tube on the magnetic stand at room temperature for 3 ~ 5 min to dry the bead pellet. Do not overdry the bead pellet.
7. Prepare Hybridization Master Mix as indicated below. Mix well and centrifuge briefly.

Component	Volume per reaction
Hyb Buffer (pre-heated at 65℃)	13 µL
Hyb Human Block	5 µL
TargetSeq® Blocking Oligo	2 µL
RNase Block	5 µL
Nuclease-Free Water	3 µL
BisCap® Target Probes	2 µL
Total Volume	**30 µL**

8. Add 30 µL of Hybridization Master Mix to the PCR tube. Pipet to mix well. Incubate at room temperature for 3 min.
9. Spin briefly on a mini centrifuge. Place the PCR tube on a magnetic stand and allow the magnetic beads for purification to fully separate from the supernatant (approximately 3 min).
10. Transfer 28 µL of clear supernatant containing the pre-capture library and Hybridization Master Mix to a clean PCR tube. Spin briefly on a mini centrifuge.

Hyb Buffer is easy to crystallize and thicken at low temperature. If crystallization presents, heat the Hyb Buffer at 37°C and then transfer 28 µL of the supernatant.

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

Temperature	Time
Heat lid temperature 105℃	
95℃	5 min
65℃	**Hold**

12. Incubate for 12 to 18 h. Start next step (STEP 3 in Page 9) 30 min before the incubation finished.

Note

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Version: A.0(E), March 2024
Document Number: PROT240302