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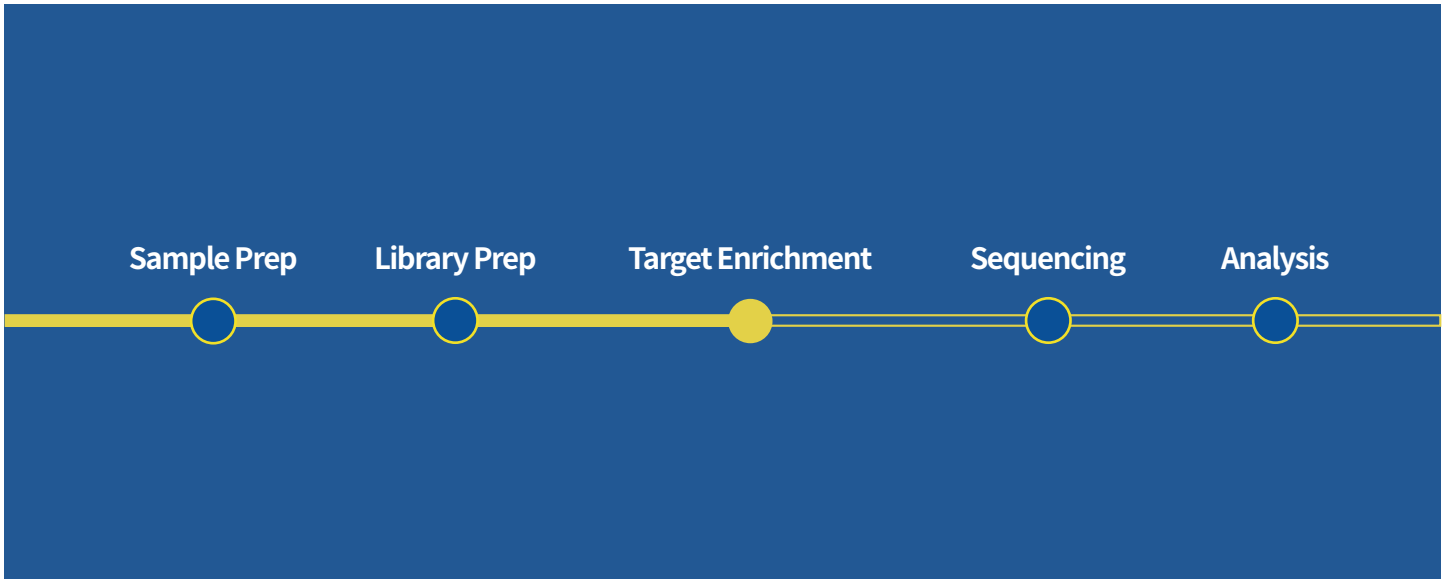
# TargetSeq One® Hyb & Wash Kit v2.0

# Protocol for AIExome® V3 Series

For AIExome® Human Exome Panel V3 series only.

English Version
Version: C.2(E), March 2024
Document Number: PROT220606



# Version Notes

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## Protocol Notes

The protocol provides an instruction for hybridization-based capture using TargetSeq One® Hyb & Wash Kit v2.0 with TargetSeq® Target Probes (AIExome® Human Exome Panel V3 series). Before you begin, please read this protocol carefully, and strictly follow the protocol for experiment.

## Product Notes

TargetSeq One® Hyb & Wash Kit v2.0 is used forhybridization-based capture on target regions and generates captured sequencing libraries of Illumina® and DNBSEQ® platform.

## History

| Version | Date | Description |
|---|---|---|
| C.2(E) | March, 2024 | Minor correction |
| C.1(E) | June, 2022 | Minor correction |
| C.0(E) | June, 2022 | First release in English |
| C.0 | June, 2022 | Optimization for new product system |
| B.0 | March, 2022 | Optimization of format |
| A.0 | November, 2021 | Initial release |

# Materials from iGeneTech

The reagents of hybridization-based capture experiment include TargetSeq One® Hyb & Wash Kit v2.0, TargetSeq® Blocking Oligo, TargetSeq® Target Probes and TargetSeq® Cap Beads.

TargetSeq One® Hyb & Wash Kit v2.0 and TargetSeq® Blocking Oligo are library type specific. Please choose the correct version according to the library type.

## TargetSeq One® Hyb & Wash Kit v2.0

TargetSeq One® Hyb & Wash Kit v2.0 has 3 different versions, for Illumina, MGI SI and MGI DI libraries. TargetSeq One® Hyb & Wash Kit v2.0 consists of 3 different modules as below.

| Kit | Module | Storage |
|---|---|---|
| TargetSeq One® Hyb & Wash Kit v2.0* | TargetSeq One® Hyb & Wash Kit v2.0 (Module A) | -20°C ± 5°C |
| | TargetSeq One® Hyb & Wash Kit v2.0 (Module B) | 15°C ~ 25°C |
| | TargetSeq One® Hyb & Wash Kit (Module C)* | -20°C ± 5°C |

*TargetSeq One® Hyb & Wash Kit v2.0 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® Hyb & Wash Kit v2.0 (Module A)

| Screw Cap | Components | Amount | | Storage |
|---|---|---|---|---|
| | | 16 rxn | 96 rxn | |
| Violet | Hyb Human Block | 88 µL | 540 µL | -20°C ± 5°C |
| Violet | RNase Block | 88 µL | 540 µL | |
| Violet | TargetSeq One® Hyb Buffer v2 | 360 µL | 2*1080 µL | |

### TargetSeq One® Hyb & Wash Kit v2.0 (Module B)

| Screw Cap | Components | Amount | | Storage |
|---|---|---|---|---|
| | | 16 rxn | 96 rxn | |
| /* | Binding Buffer | 14 mL | 84 mL | 15°C ~ 25°C |
| /* | TargetSeq One® Wash Buffer 2 v2 | 18 mL | 108 mL | |
| /* | Wash Buffer 1 | 4 mL | 24 mL | |

*"/" 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°C ± 5°C |
| Orange | Post PCR Primer (25 µM)* | 32 µL | 192 µL | |

*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 3 different versions, for Illumina, MGI SI and MGI DI libraries.

Please choose 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°C ± 5°C |

### 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°C ± 5°C |

## TargetSeq® Target Probes

| Screw Cap | Component | Amount | | Storage |
|---|---|---|---|---|
| | | 16 rxn | 96 rxn | |
| Red | TargetSeq® Target Probes* | 36 µL | 216 µL | <-70°C |

*Please note that this protocol is for AIExome® Human Exome Panel v3 series only.

## TargetSeq® Cap Beads & Nuclease-Free Water

| Screw Cap | Components | Amount | | | Storage |
|---|---|---|---|---|---|
| | | 1000 µL each | 5 mL each | 50 mL each | |
| Green or /* | TargetSeq® Cap Beads** | 1000 µL | 5 mL | 50 mL | 2 ~ 8°C |
| White or /* | Nuclease-Free Water | 1000 µL | 5 mL | 50 mL | |

*"/" means in bottle
**TargetSeq® Cap Beads is Streptavidin-coated magnetic beads for hybridization-based capture and different from IGT® Pure Beads. Dynabeads ™ MyOne ™ Streptavidin T1 by 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.

## Equipments

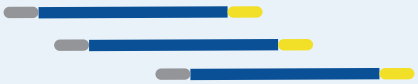
| Item | Recommended Equipments | Supplier (Cat #) |
|---|---|---|
| 96-well Magnetic Stand, 0.2 mL block | DynaMag-96 Side | Thermo Fisher (12331D) |
| Fragment Analyzer | Agilent 2100 Bioanalyzer system | Agilent (G2939AA) |
| Nucleic Acid Quantification Instrument | Qubit® 4.0 Fluorometer | Thermo Fisher (Q33238) |
| Thermal Mixer, 0.2 mL block | Eppendorf ThermoMixer® C | Eppendorf (5382000015) |
| 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

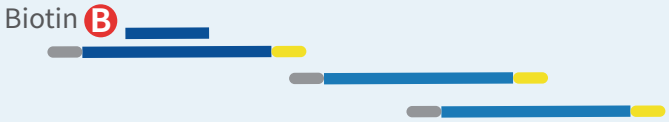
| Item | Recommended Eonsumables | Supplier (Cat #) |
|---|---|---|
| Qubit tubes, 0.5 mL | Qubit® assay tubes | Thermo Fisher (Q32856) |
| PCR tubes, 0.2 mL | Varies | Varies |
| 8-tubes strip, 0.2 mL | Varies | Varies |
| Pipette tips, 10 µL | Varies | Varies |
| Pipette tips, 200 µL | Varies | Varies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

# Workflow

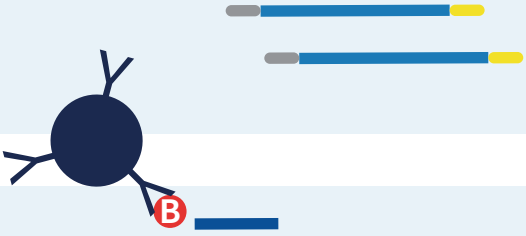
① Dry Down the Library
20 to 30 min



② Hybridization-based Capture
12 to 18 h



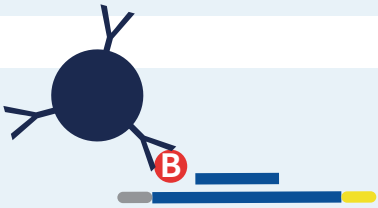

③ Prepare the Beads
10 to 15 min



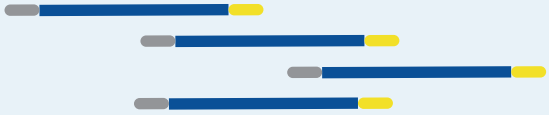
④ Bind Captured Targets to Magnetic Beads
90 min




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.
- ☐ Control and quantify the pre-capture library.
- ☐ The recommended time for hybridization is 12 to 18 h. Please arrange experiment appropriately.
- ☐ Please select the Blocking Oligo and Post PCR primer which are matched to the Illumina® or DNBSEQ® platform pre-capture library.
- ☐ The protocol is intended to be used for TargetSeq® Target Probes of AIExome® Human Exome Panel v3 series only.

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, and TargetSeq® Blocking Oligo on ice. Once reagents are thawed, mix each of them thoroughly by quick vortexing to avoid any localized concentrations. Briefly spin down vortexed reagents before use.

1.2 Thaw TargetSeq® Target Probes on ice. Vortex and spin down the reagent before use. Place back on ice.

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

1.4 Vortex TargetSeq One® Hyb Buffer v2 to mix well. If precipitate is present, please heat the TargetSeq One® Hyb Buffer v2 at 37℃. Vortex and make sure all precipitate is dissolved.

## 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, refer to Appendix 1.

2.1 Add 750 ng library to a 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.

Please select the correct TargetSeq® Blocking Oligo to proceed the hybridization based on the total library input of each reaction.
- TargetSeq® Universal Blocking Oligo Kit can block up to 6 µg libraries.
- TargetSeq® Eco Universal Blocking Oligo Kit can block up to 3 µg libraries.

2.2 Dry down the pre-captured library in a SpeedVac system (or a similar evaporator device). Open the cap 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-capture library.

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

| Component | Volume per reaction |
|---|---|
| TargetSeq One® Hyb Buffer v2 | 13 µL |
| Hyb Human Block | 5 µL |
| TargetSeq® Blocking Oligo | 2 µL |
| RNase Block | 5 µL |
| Nuclease-Free Water | 3 µL |
| TargetSeq® Target Probes | 2 µL |
| **Total Volume** | **30 µL** |

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

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

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

| Temperature | Time |
|---|---|
| Heat lid temperature 85°C | |
| 80°C | 5 min |
| **50**°C | **Hold** |

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

# STEP 3 Preparation before Capture

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

3.2 Prepare the 80% ethanol for 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°C, until the precipitate is dissolved.

3.4 Preheat the TargetSeq One® Wash Buffer 2 v2 to 50°C.

3.5 Add 100 µL of TargetSeq® Cap Beads to a new PCR tube, and place the PCR tube containing TargetSeq® Cap Beads 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 vortex for 10 sec or pipet to mix well.

3.7 Spin briefly on a mini centrifuge, and place the PCR tube containing TargetSeq® Cap Beads 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 and 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 vortex for 10 sec or pipet to mix well.

## Experiment Notes

# STEP 4 Target Capture of Libraries

Experiment Notes

Regents required:

- Wash Buffer 1
- TargetSeq One® Wash Buffer 2 v2
- 80% Ethanol (freshly prepared)
- Nuclease-Free Water
- TargetSeq® Cap Beads

Equipments required:

- Thermal Mixer
- Magnetic Stand
- Vertical Rotating Mixer

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

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

4.3 Remove the PCR tube from the vertical rotating mixer. Spin briefly on a mini centrifuge, place the PCR tube containing TargetSeq® Cap Beads 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 containing TargetSeq® Cap Beads from the magnetic stand. Add 150 µL of Wash Buffer 1, pipet to mix well. Then incubate the PCR tube on the vertical rotating mixer, mix gently (< 10 rpm) for 15 min at room temperature.

4.5 Remove the PCR tube from the vertical rotating mixer. Spin briefly on a mini centrifuge, place the PCR tube containing TargetSeq® Cap Beads 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 containing TargetSeq® Cap Beads from the magnetic stand. Add 150 µL of pre-heated TargetSeq One® Wash Buffer 2 v2, pipet to mix well. Spin briefly on a mini centrifuge. Incubate the PCR tube(s) on the thermal mixer at 50°C for 10 min.

4.7 Remove the PCR tube from the thermal mixer. Spin briefly on a mini centrifuge, place the PCR tube containing TargetSeq® Cap Beads 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 the Step 4.6 and 4.7 two more times (three times in total).

4.9 Keep the PCR tube on a magnetic stand.

4.10 Add 200 µL of 80% ethanol. Incubate at room temperature for 30 sec. Remove and discard the clear supernatant. After the final wash, remove all traces of supernatant using a 10 µL pipette.

4.11 Add 48 µL of Nuclease-Free Water. Remove the PCR tube containing TargetSeq® Cap Beads from the magnetic stand. Vortex for 10 sec or pipet to mix well. Proceed immediately to the next step.

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

# STEP 5 Post-PCR Amplification

Regents required:

- Post PCR Master Mix
- Post PCR Primer

Equipments required:

- Thermal Cycler

## Experiment Notes

5.1 Thaw Post PCR Master Mix and Post PCR Primer on ice, then vortex for 3 sec to mix well. Spin briefly on a mini centrifuge and place on ice.

5.2 Post PCR Primer is library type specific, ensure you using the correct Post PCR Primer.

5.3 Prepare PCR reaction mixture as indicated below, and vortex for 3 sec or pipet to mix well.

| Component | Volume per reaction |
|---|---|
| TargetSeq® Cap Beads Suspension from STEP 4.11 | 24 µL |
| Post PCR Primer | 1 µL |
| Post PCR Master Mix | 25 µL |
| **Total Volume** | **50 µL** |

Add only 24 µL out of 48 µL TargetSeq® Cap Beads Suspension from STEP 4.11

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

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

| Pre-capture library input | Post-PCR cycle X |
|---|---|
| 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® Target Probes. The number of post-PCR cycles is related to total pre-capture library input. As DNBSEQ® sequencing platform needs higher library inputs, it is suggested to add 2 PCR cycles to post-PCR for DNBSEQ® libraries.

5.5 Please proceed to the STEP 6 when the program finishes.

This is a safe stop point, and libraries can be stored at -20°C for up to one month.

# STEP 6 Library Purification

Regents required:

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

Equipments required:

- Magnetic Stand

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.

IGT® Pure Beads or Agencourt AMPure XP is 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 one.

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 containing magnetic beads 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 containing magnetic beads 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 to 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 containing beads from the magnetic stand. Vortex for 10 sec or pipet to mix well. Incubate at room temperature for 2 min.

6.8 Spin briefly on a mini centrifuge. Place the PCR tube containing beads on a magnetic stand and allow the beads to fully separate from the supernatant.

6.9 Transfer 23 µL of clear supernatant containing the captured library to a clean PCR tube. Captured library can be stored at -20°C 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.

Experiment Notes

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

Regents required:

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

Equipments required:

- Magnetic Stand

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. Vortex and spin down the sample before use.
2. Add 1.8× volume of magnetic beads for purification. Pipet or vortex to mix well. Incubate at room temperature for 5 min.
3. Spin briefly on a mini centrifuge. Place the PCR tube containing magnetic beads for purification 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 containing magnetic beads for purification 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 containing magnetic beads for purification 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. Spin briefly on a mini centrifuge. Place the PCR tube containing magnetic beads for purification on a magnetic stand and allow the beads to fully separate from the supernatant. Remove all traces of supernatant using a 10 µL pipette.
6. Keep the PCR tube containing magnetic beads for purification on the magnetic stand at room temperature for 3 to 5 min to dry the bead pellet.
7. Prepare Hybridization Master Mix as indicated below:

| Component | Volume per reaction |
|---|---|
| TargetSeq One® Hyb Buffer v2 | 13 µL |
| Hyb Human Block | 5 µL |
| TargetSeq® Blocking Oligo | 2 µL |
| RNase Block | 5 µL |
| Nuclease-Free Water | 3 µL |
| TargetSeq® Target Probes | 2 µL |
| **Total Volume** | **30 µL** |

8. Add 30 µL of Hybridization Master Mix to the PCR tube containing magnetic beads for purification. Vortex for 10 sec or pipet to mix well. Incubate at room temperature for 3 min.
9. Spin briefly on a mini centrifuge. Place the PCR tube containing magnetic beads for purification 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.
11. Place the PCR tube on the thermal cycler, and start the program as indicated below:

| Temperature | Time |
|---|---|
| Heat lid temperature 85°C | |
| 80°C | 5 min |
| **50**°C | **Hold** |

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

# Note

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