WISCEN
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# xCapSeq™ DNA Probe Ultra Target Capture

# Operating instruction

**(Cat#HC015,Version5.0)**

欣基（杭州）生物科技有限公司
WisGen BioSciences Co., Ltd

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## Overview

This protocol includes the steps necessary for target enrichment of a next generation sequencing library prepared from DNA or RNA, using xCapSeq™ DNA Panels. In brief, the steps of this protocol include:

1. Preventing unintentional hybridization between library molecules.
2. Performing hybridization using one of the many options of the xCapSeq™ Ready-to-use Panel or an xCapSeq™ Custom Panel and the xCapSeq™ Ultra Hyb&Wash buffer kit and xCapSeq™ SA Beads.
3. Perform bead capture of the hybridized mixture.
4. Perform post-capture washes.
5. Post-capture PCR to amplify the library for sequencing.

After preparing an NGS library using one of the methods described in the **Input recommendations section**, this procedure describes the process for using the xCapSeq™ Universal Blockers to prevent off-target fragments from annealing to the intended target sequence via adapter-to-adapter hybridization. The type of xCapSeq™ Ultra Blocker depends upon your chosen method of adapter ligation and/or sequencing instrument that will be used. WisGen offers a variety of different options that can be reviewed **in the Consumables and Equipment section**.

Once the adapters have been blocked, the protocol describes the conditions necessary for hybridization of one of the DNA or one of the xCapSeq™ Custom DNA Panels.These panels contain capture probes that were individually synthesized and pooled, which ensures equal representation of each probe in the panel.

The hybridization procedure presented here is specific to the xCapSeq™ Ultra Hyb&Washing Buffer Kit, which includes three products:
xCapSeq™ Ultra Hyb&Washing Buffer Reagents, xCapSeq™ Ultra Blocker and

xCapSeq™ SA Beads. Both components are compatible with any of the WisGen xCapSeq™ DNA Panels. There are instructions to perform hybridization capture either in plates or tubes depending on the number of targeted sequencing experiments that you will be performing.

After the probes are hybridized to the target fragments, the procedure for separating the desired DNA targets from the off-target fragments is presented. Since each probe has a 5' biotin modification, this procedure describes the use of streptavidin-coated magnetic beads to capture the probe and targeted DNA duplexes. Post-capture washes are performed to further remove non-specifically bound DNA. WisGen provides a selection of compatible suppliers of the necessary reagents and magnetic stands **in the Consumables and Equipment section**.

After removal of the non-specific DNA from the sample, the procedure for post-capture PCR is described using a PCR master mix with the xLIBPreP™ Universal HIFI Amplication Kit. WisGen offers suggestions for the number of cycles for amplification based on the number of probes in the panel as a starting point to create enough DNA in the final captured library.

# Description

### xCapSeq™ DNA Probes or Panels

xCapSeq™ DNA Probes (probes) are Oligo containing biotin modifications at the 5' end and are commonly used for targeted capture in second generation sequencing. Combinations of probes are called Panels, and xCapSeq™ DNA Panels can be optimised and expanded by adding additional custom capture probes or used in combination with other Panels. Probes can also be used to complement or improve areas where Ready-to-use Panel is underperforming.

WisGen xCapSeq™ DNA Panel has pre-designed and pre-synthesized Panels for different targeted capture applications, please contact us for more information. If you would like to add custom probes to a Panel or mix multiple Panels for capture experiments, please contact us (order@wisgen.cn) for expert, personalised advice.

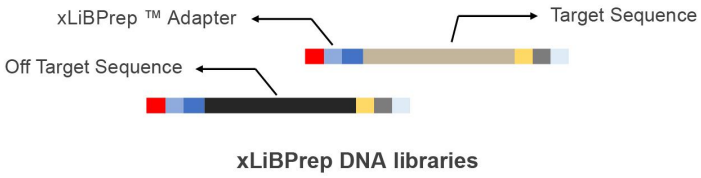
### xCapSeq™ Ultra Blocker

xCapSeq™ Ultra Blocker could be used to blocker adaptor of illumina/GeneMind platform and MGI platform, xCapSeq™ Ultra Blocker for illumina-TS(Truseq) can be used to block illumina/GeneMind library containing single or dual 6 nt, 8 nt or 10 nt index. xCapSeq™ Ultra Blocker for MGI-SI/DI can be used to block MGI library containing single or dual barcode.

### xCapSeq™ Hyb&Washing Buffer Kit Ultra

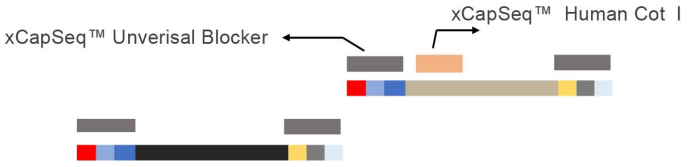
xCapSeq™ Ultra Hyb&Washing Buffer Kit can be used to xCapSeq DNA Probe or Panel rapid hybridization which could be short to 2 hr hybridization to get excellent performance. For exome capture, pervious experiment have shown that the performance for 2 ~ 4hr hybridization is same to 16hr hybridization.

# Target Capture Workflow



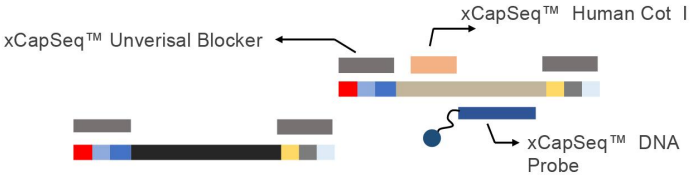

**xLiBPrep DNA libraries**

Add xCapSeq Ultra Blocker



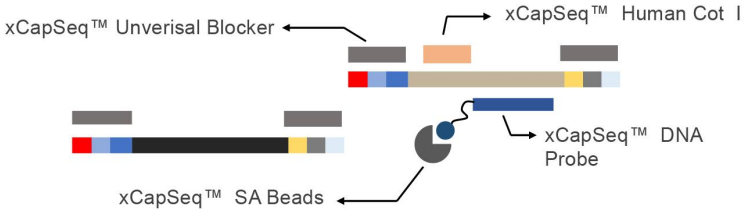

**Blocking DNA libraries**

Hybridize targets to captue probes




**Hybridized library fragments**

Inculcate with magnetic streptavidin beads




**SA-Coat Beads-Bound xCapSeq™ DNA Probe**

Wash off-target DNA fragments
Isolate targets with magnet

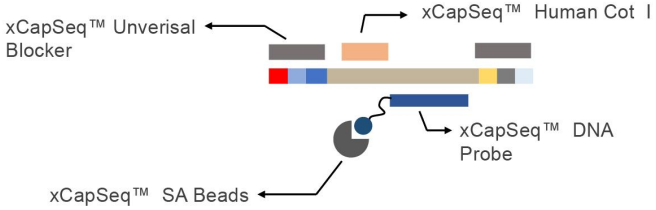



Figure 1. Desired prepared library fragments are separated from off-target fragments using hybridization capture. First, xCapSeq™ Ultra Blockers are mixed with prepared library fragments to prevent adapter-to-adapter hybridization. Blocked library fragments are then annealed to the 5' biotinylated oligonucleotide probes from an xCapSeq™ Ready-to-use DNA Panels or an xCapSeq™ Custom DNA Panels. The probe and fragment duplexes are then separated from the unbound fragments by streptavidin-coated magnetic bead purification. The resulting library is enriched for targeted sequences.

# Consumables and Equipment

**Consumables—WisGen**

| Item | Description | Catalog# | Stroge |
|---|---|---|---|
| Probes (DNA Probes) | Custom Predesigned | Varies | -30 ～ -15 °C |
| xCapSeq™Hyb& Washing Buffer Kit Ultra | 12 rxns | HC015-012 | -30 ～ -15 °C |
| | 96 rxns | HC015-096 | -30 ～ -15 °C |
| xCapSeq™ Ultra Blocker for illumina-TS | 12 rxns | HC021-012 | -30 ～ -15 °C |
| | 96 rxns | HC021-096 | -30 ～ -15 °C |
| xCapSeq™ Ultra Blocker for MGI-SI | 12 rxns | HC022-012 | -30 ～ -15 °C |
| | 96 rxns | HC022-096 | -30 ～ -15 °C |
| xCapSeq™ Ultra Blocker for MGI-DI | 12 rxns | HC023-012 | -30 ～ -15 °C |
| | 96 rxns | HC023-096 | -30 ～ -15 °C |
| xLIBPreP™ Universal HIFI Amplication Kit (for illumina) | 24 rxns | NC004-024 | -30 ～ -15 °C |
| | 96 rxns | NC004-096 | -30 ～ -15 °C |
| xLIBPreP™ Universal HIFI Amplication Kit (for MGI) | 24 rxns | NC009-024 | -30 ～ -15 °C |
| | 96 rxns | NC009-096 | -30 ～ -15 °C |
| xCapSeq™ SA Beads | 1 mL | MB001-001 | 2~8 °C |
| | 5 mL | MB001-005 | 2~8 °C |
| | 100 mL | MB001-100 | 2~8 °C |
| xCapSeq™ SPRI Beads | 50 ml | MB002-050 | 2~8 °C |
| | 450 ml | MB002-450 | 2~8 °C |
| QuantiFast™ HS DNA Kit (for Qubit) | 500 rxns | QC001-500 | 2~8 °C |
| QuantiFast™ Tube (for Qubit) | 500 tubes/pack | C001-R-500 | Room temp |
| xMag™ Magnet (1.5 mL *16 position) | 1.5 mL *16 position | MR-B-16-3 | Room temp |
| xMag™ Magnet (0.2 mL *16 position) | 0.2 mL *16 position | MR-C-16-4 | Room temp |

**Consumables—From other suppliers**

| Item | Supplier | Catalog# |
|---|---|---|
| Nuclease-Free Water | General laboratory supplier | Varies |
| Ethanol absolute (AR) | General laboratory supplier | Varies |
| 0.1× TE buffer | General laboratory supplier | Varies |
| 0.2 ml PCR MicroTubes (Nuclease-Free) | Low adsorption，general laboratory supplier | Varies |
| 0.5 ml MicroTubes (Nuclease-Free) | Low adsorption，general laboratory supplier | Varies |
| 1.5 ml MicroTubes (Nuclease-Free) | Low adsorption，general laboratory supplier | Varies |
| 10 μL Pipet Tips (Nuclease-Free) | Low adsorption，general laboratory supplier | Varies |
| 200 μL Pipet Tips (Nuclease-Free) | Low adsorption，general laboratory supplier | Varies |
| 1 mL Pipet Tips (Nuclease-Free) | Low adsorption，general laboratory supplier | Varies |
| 0.2 mL PCR Strip Tubes&Caps (Nuclease-Free) | Low adsorption，general laboratory supplier | Varies |

**Equipment**

| Item | Description |
|---|---|
| Thermal cycler | Bio-Rad C/S1000 or T100 |
| Water bath or heating block | General laboratory supplier |
| Microcentrifuge | General laboratory supplier |
| Vortex mixer | General laboratory supplier |
| Fluorescence-based DNA quantitation system, such as Qubit® fluorometer (Thermo Fisher Scientific) for final quantitation of library | Various suppliers |
| Digital electrophoresis system | Agilent 2100 Electrophoresis Bioanalyzer® system (cat # G2939AA), Agilent 4200 TapeStation® System (cat # G2991BA), or equivalent |

# Reagents Details

### Part I: Hyb&Wash buffer Reagents

| Component | 12 rxns Cat#(HC015-012) | 96 rxns Cat#(HC015-096) | Stroage |
|---|---|---|---|
| xCap **Ultra** Hyb Buffer | 180 μL | 1440 μL | -30 ～ -15°C |
| xCap **Ultra** Enhancer | 60 μL | 480 μL | -30 ～ -15°C |
| xCap 10× WB | 576 μL | 4608 μL | -30 ～ -15°C |
| xCap 2× EB | 960 μL | 7680 μL | -30 ～ -15°C |

### Product II. Blocking Reagents (Configuration according to platform)

| Component | 12 rxns Cat#(HC021-012) | 96 rxns Cat#(HC021 -096) | Storage |
|---|---|---|---|
| xCapSeq™ **Ultra** Blocker for illumina-TS | 24 μL | 192 μL | -20° C |

| Component | 12 rxns Cat#(HC022 -012) | 96 rxns Cat#(HC022-096) | Storage |
|---|---|---|---|
| xCapSeq™ **Ultra** Blocker for MGI-SI | 24 μL | 192 μL | -20° C |

| Component | 12 rxns Cat#(HC023 -012) | 96 rxns Cat#(HC023-096) | Storage |
|---|---|---|---|
| xCapSeq™ **Ultra** Blocker for MGI-DI | 24 μL | 192 μL | -20° C |

### Product III: PCR Reaction Reagents (Configuration according to platform)

| Component | 24 rxns Cat#(HC011 -024) | 96 rxns Cat#(HC011 -096) | Storage |
|---|---|---|---|
| 2× HiFi Master Mix | 600 μL | 1.2 mL*2 tube | -20° C |
| PCR Primer Mix for illumina(20 μM ) | 120 μL | 480 μL | -20° C |

| Component | 24 rxns Cat#(HC017 -024) | 96 rxns Cat#(HC017 -096) | Storage |
|---|---|---|---|
| 2× HiFi Master Mix | 600 μL | 1.2 mL*2 tube | -20° C |
| PCR Primer Mix for MGI SI(20 μM ) | 120 μL | 480 μL | -20° C |

| Component | 24 rxns Cat#(HC018 -024) | 96 rxns Cat#(HC018 -096) | Storage |
|---|---|---|---|
| 2× HiFi Master Mix | 600 μL | 1.2 mL*2 tube | -20° C |
| PCR Primer Mix for MGI DI (20 μM ) | 120 μL | 480 μL | -20° C |

**Product IV: Beads Reagents**

| Component | 1 mL Cat#(MB001 -001) | 5 mL Cat#(MB001 -005) | 100 mL Cat#(MB001 -100) | Storage |
|---|---|---|---|---|
| xCapSeq™ SA Beads | 1 mL | 5 mL | 100 mL | 2~8° C |

| Component | 50 mL Cat#(MB002 -050) | 450mL Cat#(MB002 -450) | Storage |
|---|---|---|---|
| xCapSeq™ SPRI Beads | 50 mL | 450 mL | 2 ~ 8° C |

# Input Recommendations

### Input for library preparation

This protocol was developed with libraries prepared from multiple library preparation kits, including the xLIBPreP™ Enyzmetics DNA Library Kit, xLIBPreP™ Fast DNA Library Kit, and third-party kits. For optimal results, we recommend using fragmented DNA between 150~350 bp of illumina、 GeneMind platform , 200~400 bp of MGI platform.

### Input for capture

We recommend using 500 ng of each prepared library for hybridization capture. For exome captures, multiplexing has been investigated on up to 8 samples (125 ng of each library ,1 μg total DNA) and showed limited impact on data quality. Using less input for capture can result in higher duplicate rates, lower mean coverage, and poor coverage uniformity.

# Tube Protocol

This protocol has been developed for a maximum of 6~8 capture reactions using individual tubes.

## Guidelines

1. The duration of hybridization should be kept consistent for all samples within a project. For GC-rich or small panels (<1000 probes), longer hybridization times (up to 16 hr) may improve performance.
2. This protocol does not require library evaporation before hybridization, and can be performed directly after pooling the library for hybridization, which requires attention to the volume of hybridisation and the amount of library input in advance.

## Before you start

1.Create the following PCR programs:

| HYB program (lid set at 105°C) | |
|---|---|
| 95°C | 5 min |
| 68°C | 2~16 h |
| 68°C | Hold |
| **WASH program (lid set at 75°C*)** | |
| 68°C | Hold |

* It is critical to reduce the lid temperature to 75°C for the WASH program.

3. Thaw xCapSeq™ DNA Panels at room temperature (RT, 15~25°C). Mix thoroughly and centrifuge briefly.

## Perform hybridization reaction

**1.** Reagent

xCapSeq™ Ultra Blocker:

Remove from -20°C refrigerator and melt at 4°C~room temperature.

xCapSeq™ Ultra Hyb_Buffer:

Remove from -20°C refrigerator and melt at 4°C~room temperature.

xCapSeq™ Enhancer :

Remove from -20°C refrigerator and melt at 4°C~room temperature.

**Note : Ultra Hyb_buffer will appear to crystallize, Heating at 65 ° C is required to thoroughly mix the solution and maintain it in a turbid state, while using it in a 65 ℃ hot bath.**

**2.** Adding the following component in 0.2 mL PCR tube.

| Component | Amount or Volume |
|---|---|
| Single or pooled barcoded library | x μL * |
| xCap Ultra Blocker | 2 μL |
| **xCap Ultra Hyb Buffer** | 15 μL |
| **xCap Ultra** Enhancer | 5 μL |
| Probe* | 2 μL |
| Nuclease-Free Water | 11-x μL |
| Total Volume | 35 μL |

① The input of each DNA library is recommended for 500 ng/library, Previous experiments have shown that library input could be as low as 125 ng satisfy hybridization requirements, but duplication rate will increase and uniformity will slight drop down;

② To maintain library complexity, ≥50% of the total number of per library is recommended for hybridization;

③ For special samples, such as FFPE-derived DNA, cfDNA, etc., libraries of the same quality as the original sample are recommended for hybridization.

**3.** Vortex to mix well.

* For more hybridization reactions, transfer to an 8-plex PCR tube.

**4.** Incubate the sample tubes in the thermal cycler at the HYB program.

| HYB program (lid set at 105°C) | | |
|---|---|---|
| Step | Temp (°C) | Time |
| Step 1 | 95 | 5 min |
| Step 2 | 68 | 2~16 h |

* For WES Capture , Recommended for overnight at 16h.

## Prepare buffers

**Note: Before preparing the buffers, take out the xCapSeq™ SA Beads box, which contains the xCapSeq™ SA beads from storage at 4°C. The beads need to be at room temperature for a minimum of 30 min before performing the wash.**

**1.** Dilute the following xCapSeq™ Wash buffers to create 1X working solutions per reaction*

| Buffer Name | Buffer (μl) | + dd H20 (μl) | Total (μl) | Storage |
|---|---|---|---|---|
| 10X WB* | 48 | 432 | 480 | Aliquot into two tubes (160 μL each). Heat tubes to 68°C in a water bath or heating block. The remaining solution should be kept at room temperature. |
| 2X EB | 80 | 80 | 160 | Keep at room temperature. |

1) 10X WB will have a precipitate, shake well to dissolve the aspirate can have a precipitate, can 68°C to help dissolve .

2) 1X WB requires two temperature placements, 68°C and room temperature, please note the distinction. 1X working solutions can be stored at room temperature for 30 days.

## Perform bead capture

1) Place the 1X WB (150 µL aliquot) in a per-warm 68°C thermal cycle or water bath for at least 15 min.

**Tip: The buffers will be used during the Heated washes, but we recommend starting this incubation at the same time as the beads capture, so that the buffers will be at the correct temperature when needed.**

2) After the 2~4 hr incubation, take the the Hybridization mix tube out of the thermal cycler.
3) Once removed, stop the HYB program.
4) Immediately after the HYB program completes, start the WASH program.
5) Before use, Ensure that the SA beads have equilibrated to room temperature.
6) Mix the beads thoroughly by vortexing for 15 sec.
7) Aliquot 50 µL of SA beads per capture into 0.2 mL PCR tube, Place the tube on a magnetic rack for approximately 1 min, discard the clear supernatant.

**Note：immediately go to the next step to avoid over exposure of the beads to the air.**

8) Transfer 35 µL of the Hybridization mix to the SA beads tube.
9) Vortex to ensure that sample is fully resuspended. Gently and briefly centrifuge, if needed (10 sec at 25 x g).

**Note: This step need to use the low bind tips and rapid operation.**

10) Place the sample tube back to the thermal cycler and set a timer for 45 min.
11) Every 10–12 min(10min stop, 20min stop, 30 min stop), remove the tube from the thermal cycler and gently vortex 5 sec to ensure the sample is fully resuspended.
12) At the end of the 45 min, take the sample off the thermal cycler.

Proceed immediately to Heated wash.

## Perform Wash

### 1. Heated wash

1) Wash the beads with 1X WB.

① Add 150 μL of 1X WB pre-warmed at WASH program to a 0.2 ml sample tube (8-link row) and slowly aspirate 10 times to fully suspend the beads.

**Note: Do not shake vigorously to avoid air bubbles.**

② Place 0.2 ml tubes (8 rows) in the thermal cycler or in a warm bath at 68°C, 2 min.

③ Place 0.2 ml tube (8 rows) on a magnetic rack, separate the beads from the solution completely, and quickly remove the supernatant with a pipette.

**Note: The supernatant contains a large amount of unbound DNA, to prevent aerosol contamination, please fill the supernatant into the waste tank with 1% sodium hypochlorite solution.**

2) Repeat step 1) once again.

### 2. Room temperature wash

1) Add 150 μL of 1X WB at room temperature, add it to a 0.2 ml tube (8-link row), mix well by pipetting with a pipette.
2) Place the 0.2 ml tube (8 rows) on a magnetic rack and separate the beads from the solution completely. Remove the supernatant.
3) Add 150 μL of 1X EB at room temperature to the 0.2 ml tube (8 rows) and mix well by pipetting with a pipette.
4) Pipette the mixed suspension into a new 0.2 ml tube (8 rows) and place on a magnetic rack to completely separate the beads from the solution. Remove the supernatant.

### 3. Resuspension of Magnetic Beads

1) Remove the 0.2 ml tube (8 rows) from the magnetic rack.

2) Add 23.75 µL of Nuclease-Free Water.

3) Vortex and mix for 10 sec or more or repeatedly pipette 10 times to ensure all beads are resuspended.

**Do not discard the beads. 23.75 µL of bead resuspension containing captured DNA was used for PCR amplification.**

### Perform post-capture PCR

**1.** If a 1.5 mL tube was used for the wash, transfer the sample to a low-bind 0.2 mL PCR tube.

**2.** Add the following components to create the Amplification Reaction Mix:

| Component | Volume |
|---|---|
| Beads Resuspension | 23.75 µL |
| Amplication Primer Mix (illumina/GeneMind or MGI) | 1.25 µL |
| xLibPreP 2X HIFI Mix | 25 µL |
| Total Volume | 50 µL |

**3.** Briefly vortex shake, lightly shake or transient centrifuge.

**4.** Incubate the tubes in the thermal cycler (lid set at 105 °C) and perform PCR amplification according to the following procedure.

| Step | T(°C) | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 98 | 45 sec | 1 |
| Denaturation | 98 | 15 sec | 7* |
| Annealing | 60 | 30 sec | |
| Extension | 72 | 30 sec | |
| Final extension | 72 | 1 min | 1 |
| Hold | 4 | ∞ | 1 |

The number of PCR cycles should be optimized per panel size and the number of pooled libraries per capture, to ensure there is enough yield for sequencing.

We recommend starting optimization with the following:

| Panel size | 1-plex | 4-plex | 8-plex | 12-plex |
|---|---|---|---|---|
| >100,000 probes (xCapSeq™ Exome Panel v1) | 11 cycles | 9 cycles | 8 cycles | 7 cycles |
| 10,000–100,000 probes (xCapSeq™ TMB 680 Panel ) | 12 cycles | 10 cycles | 9 cycles | 8 cycles |
| 500–10,000 probes (xCapSeq™ 160 PanCancer Panel) (xCapSeq™ xCapSeq™ 120 Hotspot Panel) | 13 cycles | 11 cycles | 10 cycles | 10 cycles |
| 1–500 probes (xCapSeq™ MRD Custom-design Panel) | 14 cycles | 12 cycles | 11 cycles | 11 cycles |

**Post-capture PCR clean up**

**Prepare 80% ethanol in advance, using 100 µL per reaction; freshly prepared 80% ethanol is recommended.**

1. Prepare 250 µL of fresh 80% ethanol per sample, multiplied by the number of samples with a 10% overfill.
2. Add 75 µL (1.5X volume) of xCapSeq™ SPRI beads to each amplified capture (transfer to a larger 1.7 mL tube, if needed).
3. After adding the beads, mix thoroughly and incubate for 5 ~10 min.
4. Place the sample tube on a magnet until the supernatant is clear (2 ~ 5 min).
5. Remove supernatant without disturbing the beads.
6. While keeping the tube on the magnet, add 125 µL of 80% ethanol, then incubate for 30s.
7. Remove the ethanol, then repeat another ethanol wash.
8. Allow the beads to air dry for 1 ~ 3 min. Do not over-dry the beads.
9. Remove the sample tube from the magnet and elute in 32 µL of Buffer EB, or equivalent (10 mM Tris- Cl, pH 8.5). Mix thoroughly. Alternatively, TE can be used.
10. Incubate for 5 min at room temperature.
11. Place the tube on a magnet until the supernatant is clear (1 ~ 2 min).
12. Transfer 30 µL of eluate to a fresh tube making sure that no beads are carried over.

**Optional stopping point: Store purified PCR fragments per your established internal laboratory procedures.**

**Quantify the library**

1. The final captured library is quantified using Qubit fluorescence quantification instrument, the concentration is recorded, and the total amount is calculated; it is recommended to use Agilent 2100 Bioanalyzer or QSEP to quality check the library peak type, quantify the captured library by fluorescence quantitative PCR, and calculate the molar concentration.

2. Reference standards for library quality control:

① Library Qubit quantification concentration not less than 1ng/μL

② Library fragment size between 300~500 bp

③ Fluorescence PCR quantification concentration not less than 5 nM, melting curve peak type single, no Dimer contamination.

3. After passing the quality control, the libraries can be sequenced or stored at -20°C.

# APPENDIX A

xCapSeq DNA Probe INPUT volume recommendation per reaction :

| Panel Name | CatLog | Probe type | Input Volume / reaction |
|---|---|---|---|
| xCapSeq™ Exome Panel,V1 | HC004 | dsDNA | 4 μL |
| xCapSeq™ 160 PanCancer Panel | HC001 | dsDNA | 2 μL |
| xCapSeq™ TMB 680 Panel | HC003 | dsDNA | 2 μL |
| xCapSeq™ 120 Hotspot Panel | HC005 | ssDNA | 2 μL |
| xCapSeq™ MRD Custom-design Panel | Various | ssDNA | 2 μL |

欣基（杭州）生物科技有限公司
WisGen BioSciences Co., Ltd
Add：浙江省杭州市钱塘区福城路 400 号 6 幢 8 层
Service： order@wisgen.cn Web : www.wisgen.cn