WISCEN
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xCapSeq™ DNA Probe Target Capture

Operating instruction Pro

(Cat#HC013,Version5.0)

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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™ 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™ Universal 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™ Hyb&Washing Buffer Kit, which includes three products:

xCapSeq™ Hyb&Washing Buffer Reagents, xCapSeq™ Universal 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.

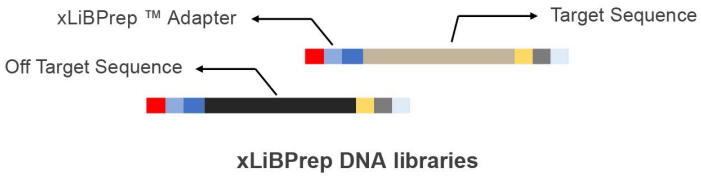
xCapSeq™ Universal Blocker

xCapSeq™ Universal Blocker could be used to blocker adaptor of illumina/GeneMind platform and MGI platform, xCapSeq™ Unverisal 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™ Universal Blocker for MGI-SI/DI can be used to block MGI library containing single or dual barcode.

xCapSeq™ Hyb&Washing Buffer Kit

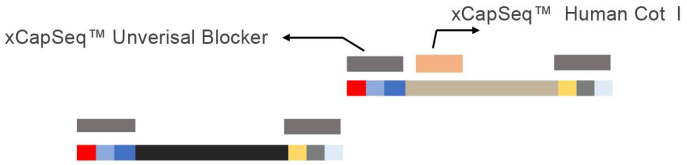
xCapSeq™ Hyb&Washing Buffer Kit can be used to xCapSeq DNA Probe or Panel rapid hybridization which could be short to 2 hr hybridization to get excellent performance. For exome capture, pervious experiment have shown that the performance for 2 ~ 4hr hybridization is same to 16hr hybridization.

Target Capture Workflow



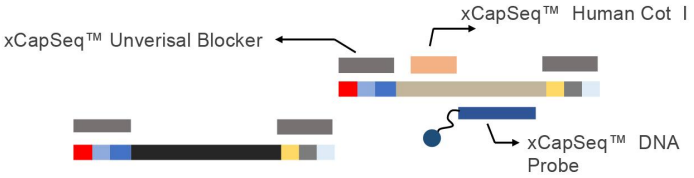
xLiBPrep DNA libraries

Add xCapSeq Unverisal Blocker
Add xCapSeq Human Got Ⅰ



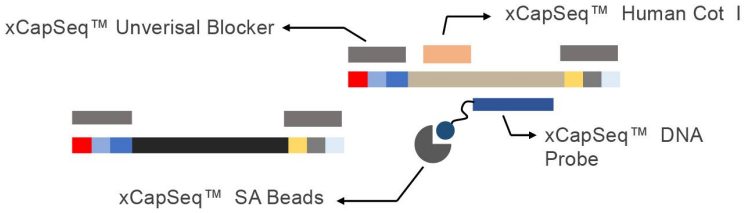
Blocking DNA libraries

Hybridize targets to captue probes



Hybridized library fragments

Incubte wit magnetic streptavidin beads



SA-Coat Beads-Bound xCapSeq™ DNA Probe

Wsah off-target DNA fragments
Isolate targets wth magnet

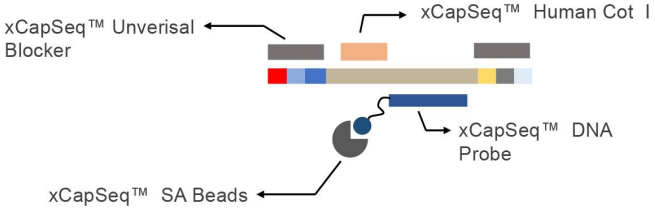


Figure 1. Desired prepared library fragments are separated from off-target fragments using hybridization capture. First, xCapSeq™ Universal Blockers are mixed with prepared library fragments to prevent adapter-to-adapter hybridization. Blocked library fragments are then annealed to the 5' biotinylated 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 ℃
xCapSeq™Hyb& Washing Buffer Kit	12 rxns	HC001-012	-30 ～ -15 ℃
	96 rxns	HC001-096	-30 ～ -15 ℃
xCapSeq™ Unverisal Blocker for illumina-TS	12 rxns	HC006-012	-30 ～ -15 ℃
	96 rxns	HC006-096	-30 ～ -15 ℃
xCapSeq™ Unverisal Blocker for MGI-SI	12 rxns	HC008-012	-30 ～ -15 ℃
	96 rxns	HC008-096	-30 ～ -15 ℃
xCapSeq™ Unverisal Blocker for MGI-DI	12 rxns	HC009-012	-30 ～ -15 ℃
	96 rxns	HC009-096	-30 ～ -15 ℃
xLIBPreP™ Universal HIFI Amplication Kit (for illumina)	24 rxns	NC004-024	-30 ～ -15 ℃
	96 rxns	NC004-096	-30 ～ -15 ℃
xLIBPreP™ Universal HIFI Amplication Kit (for MGI)	24 rxns	NC009-024	-30 ～ -15 ℃
	96 rxns	NC009-096	-30 ～ -15 ℃
xCapSeq™ SA Beads	1 mL	MB001-001	2~8 ℃
	5 mL	MB001-005	2~8 ℃
	100 mL	MB001-100	2~8 ℃
xCapSeq™ SPRI Beads	50 ml	MB002-050	2~8 ℃
	450 ml	MB002-450	2~8 ℃
QuantiFast™ HS DNA Kit (for Qubit)	500 rxns	QC001-500	2~8 ℃
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
Vacuum concentrator	Thermo Fisher Scientific SpeedVac® system or equivalent
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#(HC013-012)	96 rxns Cat#(HC013-096)	Stroage
xCap 2×Hyb Buffer	96 μL	768 μL	-30 ～ -15°C
xCap Enhancer Buffer	36 μL	288 μL	-30 ～ -15°C
xCap Human Cot I	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#(HC006-012)	96 rxns Cat#(HC006 -096)	Storage
xCapSeq™ Universal Blocker for illumina-TS	24 μL	192 μL	-20°C

Component	12 rxns Cat#(HC008 -012)	96 rxns Cat#(HC008-096)	Storage
xCapSeq™ Universal Blocker for MGI-SI	24 μL	192 μL	-20°C
xCapSeq™ Universal Blocker for MGI-DI	24 μL	192 μL	-20°C

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

Component	24 rxns Cat#(NC004 -024)	96 rxns Cat#(NC004 -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#(NC009 -024)	96 rxns Cat#(NC009 -096)	Storage
2× HiFi Master Mix	600 μL	1.2 mL*2 tube	-20°C
PCR Primer Mix for MGI SI/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 12 samples (6 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.

For optimal results, use a SpeedVac® system (Savant) for concentrating DNA.

Note: To multiplex a high quantity of samples, we recommend using a SpeedVac® system; however, if you require a quicker turnaround, you may also consider preparing the DNA samples following the instructions in Appendix B: xCapSeq™ SPRI Beads DNA concentration protocol.

Important:The xCapSeq™ SPRI Beads DNA concentration protocol (Appendix B) requires 10 µL of xCap Human Cot I .

Tube Protocol

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

Guidelines

During the incubation time, the tube needs to be sealed properly to avoid evaporation. Excessive evaporation during the hybridization can lead to capture failure.

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.

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.

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

Perform hybridization reaction

1. Reagent

xCapSeq™ Universal Blocker:

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

xCapSeq™ Human Cot-I:

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

xCapSeq™ 2X 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 : 2X Hyb_buffer will appear to crystallize, it needs to be heated to 65°C about 2 mins then keep at room temperature until it completely dissolves.

2. Adding the following component in 1.5 mL microtube (low-bind).

Component	Amount or Volume
Single or pooled barcoded library	500 ng *
xCapSeq Human Cot-I	5 μL
xCapSeq Universal Blocker	2 μL

①The input of each DNA library is recommended for 500 ng/library, Previous experiments have shown that library input could be as low as 187.5 ng satisfy hybridization requirements, but duplication rate will increase and uniformity will slight drop down;

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

③for 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.

4. Dry down the mixture in a pre-warm 60°C SpeedVac system.

Safe Stop: Be sure to seal the sample tube. Store the sample at RT overnight, or -20°C for longer.

5. Add the following component to the 1.5 mL microtube (low-bind).

Component	Volume
2X Hyb_Buffer	8 μL
Enhancer	3 μL
Nuclease-Free Water	1 μL
Probe*	4 μL

* Different xCapSeq Panel have different input volume for each reaction. Please according to **APPENDIX A.**

6. Mix by pipetting or shaking with a vortex shaker and leave at room temperature for 5~10 min; pipet or shake again and centrifuge instantaneously. Transfer to a 0.2 mL PCR tube.

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

7. 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℃.The beads need to be at room temperature for a minimum of 30 min before performing the washes.

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℃ 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℃ 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℃ 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 beads have equilibrated to room temperature.
6) Mix the beads thoroughly by vortexing for 15 sec.
7) Aliquot 50 µL of streptavidin 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 16 µL of the Hybridization mix to the Streptavidin 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 washes.

Perform Wash

1. Heated washes

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 10times 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 washes

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 washes, 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 ℃) 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	Input ddH20 Volume / reaction
xCapSeq™ Exome Panel,V1	HC004	ssDNA	4 μL	1 μL
xCapSeq™ 160 PanCancer Panel	HC001	ssDNA	2 μL	3 μL
xCapSeq™ TMB 680 Panel	HC003	ssDNA	2 μL	3 μL
xCapSeq™ 120 Hotspot Panel	HC005	ssDNA	2 μL	3 μL
xCapSeq™ MRD Custom-design Panel	Various	ssDNA	2 μL	3 μL

APPENDIX B

xCapSeq™ SPRI Beads DNA concentration protocol.

Important: This protocol requires 10 μL of Human Cot DNA.

To order additional Human Cot DNA, go to the order@wisgen.cn.

1. Add 500 ng of library to the sample well. If multiplexing, pool 500 ng of each library into the sample well (maximum of 12 samples).

Note: This could be a large volume requiring either 1.5 mL tubes, or a deep well plate.

2. Add 10 μL of xCap Human Cot I.

3. Add 2X volume of xCapSeq™ SPRI Beads.

4. If using plates, securely seal the plate with a Microseal B seal.

5. Vortex thoroughly to mix. If using plates, adjust the settings to prevent any splashing onto the seal.

6. Incubate for 10 min at room temperature.

7. Incubate the plate or tube on the magnet for at least 2 min or until supernatant is clear.

8. Remove and discard the supernatant. Keeping the tube on the magnet, add 80% ethanol to cover the surface of the beads. Incubate for 30 sec without disturbing the beads.

9. Remove and discard the supernatant, then repeat another ethanol wash.

10. Allow the beads to air dry for approximately 2 min. Do not over-dry.

11. Add these components to the tube to make the Hybridization Reaction Mix:

Hybridization Reaction Mix	Volume
2X Hyb_Buffer	8 µL
Enhancer	3 µL
xCapSeq Universal Blocker	2 µL
Probe*	x µL
Nuclease-Free Water	4-x µL

Note: The Hybridization Reaction Mix elutes the DNA from the xCapSeq™ SPRI beads.

12. Vortex to mix. Ensure that the beads are fully resuspended.

13. Incubate for 5 min at room temperature.

14. After incubation, place on a magnet for 5 min or until the supernatant is clear.

15. Transfer 16 µL of the supernatant to the sample plate, or tube, where the hybridization will occur.

Important: Make sure to avoid bead carryover during the transfer process.

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