Homologous Recombination Deficiency (HRD) Panel Assay

Instruction for Use

For Research Use Only

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Assay Product Name

Homologous Recombination Deficiency (HRD) Panel Assay

Assay Packing Specification

24 Tests/kit

Assay Contents

Table 1 Kit Contents

Reagent name and item	Content name	Volume	Storage	Remark
SGPrep DNA Library Preparation Kit (SPG-Lib001R)	End Prep Buffer	240 μL	-20±5°C	
	End Prep Enzymes	72 μL	-20±5°C	
	Ligase MM	396 μL	-20±5°C	
	Ligase Mix	72 μL	-20±5°C	
	HS 2X PCR Mix for NGS	600 μL	-20±5°C	
Adapter & UDI Primers Kit for Illumina (setA 1-24) (SPG-UDI001R)	UDI Primer (setA 1-24)	5 μL	-20±5°C	alternatively
	UDI Universal Adapter	60 μL	-20±5°C	
Adapter & UDI Primers Kit for Illumina (setB 25-48) (SPG-UDI002R)	UDI Primer (setB 25-48)	5 μL	-20±5°C	
	UDI Universal Adapter	60 μL	-20±5°C	
SGPrep DNA Library Hybrid Capture (for Illumina) Kit (SPG-Hyb002R)	2× Hyb Buffer I	68 μL	-20±5°C	
	Hyb Buffer II	21.6 μL	-20±5°C	
	Human Cot DNA	20 μL	-20±5°C	
	Blockers for Illumina	8 μL	-20±5°C	
	SG Wash Buffer	3.28 mL	-20±5°C	
	2× HiFi Mix	100 μL	-20±5°C	
	Ill-Amp Primer Mix	10 μL	-20±5°C	
	Streptavidin Beads	200 μL	2-8°C	
Homologous Recombination Deficiency (HRD) Panel (SPG-HRD001PR)	HRD Probe	8 μL	-20±5°C	
SG Pure Beads (SPG-PB003R)	SG Pure Beads	8 mL	2-8°C	

Note : The contents of different batches of reagents cannot be mix

Additional required Equipments and Materials

1. Microvolume ultraviolet-visible spectrophotometer
2. Sonication disruptor
3. Library preparation PCR apparatus: ABI9700, ABI Veriti, ABI MiniAmp
4. Fluorometer: Quantus™ Fluorometer (Promega, Cat. No. E6150), Qubit™ 4.0 Fluorometer (Thermo Fisher Scientific, Cat. No. Q33238)
5. Magnetic rack: DynaMag™-96 (Thermo Fisher Scientific, Cat.No.12331D) or DynaMag™-2 (Thermo Fisher Scientific, Cat. No.12321D)
6. Nucleic acids extraction kit
7. Quantification kit of nucleic acids: QuantiFluor® dsDNA System (Promega, Cat. No. E2670) or Qubit® dsDNA HS Assay Kit (Thermo Fisher Scientific, Cat. No. Q32851/Q32854)
8. Vacuum concentrator : Concentrator plus (Eppendorf)
9. Other reagents: Absolute ethanol (Analytical Grade), Low TE Buffer (pH 8.0), Nuclease-free water
10. Other consumables: Nuclease-free pipette tips with filter, centrifuge tubes, 8-tube strips

Module 1: SGPrep DNA Library Preparation

Product Name

SGPrep DNA Library Preparation kit

Packing Specification

24 Tests/kit

Intended Use

The kit is intended for the DNA library preparation of high-throughput sequencing platform which includes End Repair, Adapter ligation, Library amplification, and a series of library preparation required enzyme and buffer in common genome (peripheral blood, fresh tissue), cfDNA, FFPE DNA, and so on. The entire process could be completed in 2-3h.

Transportation, Stability and Storage

1. Storage Conditions: Store the kit away from light at -15℃ to -25℃, valid for 12 months. Once opened, reagents can be stored in their original packaging at -15℃ to -25℃ until the stated expiration date shown on the packaging. Repeated thawing and freezing should be avoided.
2. Transportation Conditions: The kit should be transported at low temperature, with transporting time less than one week and transporting temperature lower than 10℃.

Specimen Material

1. Recommended sample types: Peripheral blood, fresh tissue, cfDNA, paraffin-embedded tissue or sections.
2. Peripheral blood: Peripheral blood should be collected with a non-heparin anticoagulant with volumes no less than 2 mL.
3. Fresh tissue samples: The diameter of fresh tissue shouldn't be less than 5 mm, and ensure that at least 20% of the collected pathological tissue is tumor lesions.
4. cfDNA: cfDNA should be collected with a non-heparin anticoagulant with volumes no less than 10 mL and nucleic acids extracted immediately.
5. Paraffin-embedded tissue or sections: It is recommended to choose FFPE samples that have not been stored for more than 2 years and at least 20% of the collected pathological tissue is tumor lesions, and use no less than 8 pieces of 5 μm section or 5 pieces of 10 μm section for nucleic acids extraction.
6. Common genome (peripheral blood, fresh tissue), FFPE DNA: Commercial kit is recommended to extract genomic DNA from the samples. Assess the quality of sample DNA with a microvolume ultraviolet-visible spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.8-2.2, quantify sample DNA with a Fluorometer, the concentration should be ≥ 4 ng/μL, the total amount of DNA should be ≥ 200 ng; once the DNA quality or quantity is not conformed with the above requirements, re-extract DNA with new and/or larger input. Library preparation is performed immediately after DNA extraction or store at -15℃ to -25℃ for no more than 12 months.
7. cfDNA: Commercial kit is recommended to extract genomic DNA from the samples. Assess the quality of sample DNA with an microvolume ultraviolet-visible spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.8-2.2, quantify sample DNA with a Fluorometer, the concentration should be ≥ 1 ng/μL, the total amount of DNA should be ≥ 20 ng; once the DNA quality or quantity is not conformed with the above requirements, re-extract DNA with new and/or larger input. Library preparation is performed immediately after DNA extraction or store at -15°C to -25°C for no more than 12 months.

Experimental Procedure

I. DNA fragmentation

1. Common genome (peripheral blood, fresh tissue), FFPE DNA recommend fragmenting using a DNA sonication disruptor with a target fragment size of 250 bp.
2. cfDNA could perform directly to end repair without fragmentation.
   The safe stopping point: The fragmentation of DNA could store at -15℃ to -25℃ for 1-2 weeks.

II. Fragmentation sorting

1. Using the following volume, perform a double sorting of the fragmented products. (Table 1.1)

Table 1.1 SG Pure Beads Fragmentation Sorting Volume

Expected Fragment Length (bp)	Sort Initial Volume (μL)	Beads Volume (V1) (μL)	Beads Volume (V2) (μL)
200	50	45	50
250	50	40	50
300	50	35	50
350	50	30	50
400	50	28	50

Note: Transfer the SG Pure Beads to room temperature and vortex thoroughly to disperse the SG Pure Beads before use; prepare fresh 80% ethanol with Nuclease-free water.

2. Transfer 50 μL fragment products (if less than 50 μL, supplement with Low TE Buffer (pH 8.0) or Nuclease-free water) into a new 0.2 mL centrifuge tube or 8-tube strips, and add V1 volume SG Pure Beads. Vortex and centrifuge briefly, then incubate 5 min at room temperature.
3. Add V2 volume (50 μL) SG Pure Beads into a new 0.2 mL centrifuge tube or 8-tube strips, and place the centrifuge tube on a magnetic rack for 5 min until the solution clears, carefully remove 40 μL supernatant without disturbing SG Pure Beads; then vortex and mix thoroughly the remaining 10 μL of SG Pure Beads and reserve at room temperature.
4. Place the centrifuge tube (step 2) on a magnetic rack for 5 min until the solution clears, followed by carefully transferred supernatant (50+V1) into 10 μL of concentrate SG Pure Beads (step 3), mix well and incubate 5 min at room temperature; never discard supernatant.
5. Keep the centrifuge tube on a magnetic rack for 5 min until the solution clears, and discard the supernatant.
6. Add 150 μL of freshly prepared 80% ethanol into the centrifuge tube, and place it on a magnetic rack for 30 s, followed discard supernatant.
7. Repeat step 6 for one time.
8. Remove all the ethanol from the centrifuge tube, and keep the centrifuge tube on a magnetic rack for 5 min to air-dry SG Pure Beads (avoid over-dry).
9. Remove the centrifuge tube from magnetic rack, add 21 μL Low TE Buffer (pH 8.0) or Nuclease-free water to each centrifuge tube, vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down multiple times), briefly centrifuge to collect the droplets, incubate the mixture for 5 min at room temperature.
10. Place the centrifuge tube on a magnetic rack for 2 min until the solution clears, carefully remove the supernatant to a new centrifuge tube (i.e. fragment sorting product).

III. End Repair

1. Prepare 0.2 mL centrifuge tubes, prepare End Repair Reaction on ice (Table 1.2).

Table 1.2 End Repair Reaction

Reagent	Volume
Fragment sorting product or cfDNA *	X μL
End Prep Buffer	10 μL
End Prep Enzymes	3 μL
Nuclease-free water	Up to 50 μL
All	50 μL

Note: *cfDNA suggests full commitment.

2. Vortex and mix thoroughly the End Repair Reaction system solution.
3. Load the 0.2 mL centrifuge tube above into the thermal cycler; then set up and run the program according to Table 1.3 with lid temperature adjusted to 75°C.

Table 1.3 End Repair Procedure

Temperature	Time
20°C	30 min
65°C	30 min
4°C	∞

IV. Adapter ligation

1. Dilute adapter with Low TE Buffer (pH 8.0) (Table 1.4), according to fragment sorting product or input of cfDNA.

Table 1.4 Adapter Dilution Recommendation

Input DNA	Dilution Factor	Concentration after Dilution
50 ng - 1 µg	no dilution	15 µM
25 ng - 49 ng	2-fold dilutions	7.5 µM
10 ng - 24 ng	5-fold dilutions	3 µM

Note: Input recommended before fragment sorting is 200 ng.

2. Prepare Adapter Ligation Reaction on ice (Table 1.5).

Table 1.5 Adapter Ligation Reaction

Reagent	Volume
End Repair product (step 3)	50 µL
Ligase MM	16.5 µL
Ligase Mix	3 µL
Universal Adapter (adapter is diluted with Table 1.4)	2.5 µL
All	72 µL

Note: Ligase MM and Ligase Mix can be prepared as premixes, but Universal Adapter needs to be added separately; all manipulations are performed on ice. Sequencing adapter is self-selected according to sequence platform.

3. Load the 0.2 mL centrifuge tube above into the thermal cycler; PCR reaction program is 15 min 22°C (the heating of the thermal cycler lid is turned off), followed stored at 4 °C.

V. Ligation product purification

Note: Transfer the SG Pure Beads to room temperature and vortex thoroughly to disperse the SG Pure Beads before use; prepare fresh 80% ethanol with Nuclease-free water.

1. Add 57.6 µL of SG Pure Beads to each sample, followed mix the magnetic bead suspension thoroughly and transiently centrifugation with the product and beads, and place the centrifuge tube on a magnetic rack for 5 min at room temperature.
2. Place the 0.2 mL centrifuge tube (step 1) on a magnetic rack for 5 min until the solution clears, followed by carefully transferred supernatant.
3. Add 150 µL of freshly prepared 80% ethanol into the centrifuge tube, and place it on a magnetic rack for 30 s, followed discard supernatant.
4. Repeat step 3 for one time.
5. Remove all the ethanol from the centrifuge tube, and keep the centrifuge tube on a magnetic rack for 5 min to air-dry SG Pure Beads (avoid over-dry).

6. Remove the 0.2 mL centrifuge tube from magnetic rack, add 21 μL of Low TE Buffer (pH 8.0) or Nuclease-free water to each centrifuge tube, vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down multiple times), briefly centrifuge to collect the droplets, incubate the mixture for 5 min at room temperature.
7. Place the centrifuge tube on a magnetic rack for 2 min until the solution clears, carefully remove the supernatant to a new centrifuge tube (i.e. adapter ligation product).

   The safe stopping point: The adapter ligation product after purification of DNA could store at -15°C to -25°C for 1-2 weeks.

VI. Library amplification

1. Prepare Library Amplification Reaction (Table 1.6).

Table 1.6 Library Amplification Reaction

Content	Volume
Adapter ligation product	20 μL
HS 2X PCR Mix for NGS	25 μL
UDI Primer*	5 μL

Note: * UDI Primer is only index for double-ended, self-selected according to sequencer platform; *UDI Primer has been pre-loaded in the 8-tube strips.

2. Gently remove the cap of the UDI Primer reaction strip, sequentially add 20 μL of adapter ligation product and 25 μL of HS 2X PCR Mix for NGS into the respective centrifuge tube, and replace the cap carefully, and vortex thoroughly.
3. Load the 0.2 mL centrifuge tube above into the thermal cycler; then set up and run the program according to Table 1.7 with lid temperature adjusted to 105°C.

Table 1.7 Library Amplification Procedure

Temperature	Time	Cycles
98°C	1 min	1
98°C	10 s	
60°C	30 s	According to Table 1.8
72°C	30 s	
72°C	1 min	1
4°C	∞	

Table 1.8 Library Amplification Cycle Recommendation

Input DNA (ng)	1 μg Library Yield Recommended PCR Cycle*
1000	3-4
500	4-5
200	5-6
100	6-7
50	7-8
25	8-10
10	10-12

Note: FFPE sample should increase 1-3 cycles based on Table 1.8.

VII. Library purification

Note: Transfer the SG Pure Beads to room temperature and vortex thoroughly to disperse the SG Pure Beads before use; prepare fresh 80% ethanol with Nuclease-free water.

1. Add 50 μL of SG Pure Beads to each sample, followed mix the magnetic bead suspension thoroughly and transiently centrifugation with the product and beads, and place the centrifuge tube on a magnetic rack for 5 min at room temperature.
2. Place the 0.2 mL centrifuge tube (step 1) on a magnetic rack for 5 min until the solution becomes clear, followed by carefully transferred supernatant.
3. Add 150 μL of freshly prepared 80% ethanol into the centrifuge tube, and place it on a magnetic rack for 30 s, followed discard supernatant.
4. Repeat step 3 for one time.
5. Remove all the ethanol from the centrifuge tube, and keep the centrifuge tube on a magnetic rack for 5 min to air-dry SG Pure Beads (avoid over-dry).
6. Remove the centrifuge tube from magnetic rack, add 35 μL of Nuclease-free water to each centrifuge tube, vortex thoroughly (alternatively, mix by pipetting at least half the total volume up and down multiple times), briefly centrifuge to collect the droplets, incubate the mixture for 5 min at room temperature.
7. Place the centrifuge tube on a magnetic rack for 2 min until the solution is clear, carefully remove the supernatant to a new centrifuge tube (i.e. library product).
8. Library product should store at -15°C to -25°C for the convenience of quality detecting and Sequencing.

The safe stopping point: The adapter ligation product after purification of DNA could store at -15°C to -25°C for 1-2 weeks.

Module 2: SGPrep DNA Library Hybrid Capture

Product Name

SGPrep DNA Library Hybrid Capture (for Illumina) kit

Packing Specification

4 Tests/kit

Intended Use

SGPrep DNA Library Hybrid Capture (for Illumina) kit is used for Illumina high-throughput sequencing platform which includes rapidly hybrid capture and optimized elution step, by targeting and enriching target DNA with biotinylated DNA probes, followed high-throughput sequencing. This kit includes hybrid, elution buffer, universal blockers, human genome common repetitive sequences blocking reagent, capture beads, PCR-amplified reagent, amplification primers and so on.

Universal blockers are applied for universal seal sequence for Illumina sequencing platform, and could bind adapter sequence of library to avoid decrease effectively tandem structures between different libraries for adapter annealing, thereby decreasing unspecific hybridization between adapter to increasing incidence of target and degree of enrichment in capture reaction, but it's only applied for. blocking 8 bases double-ended unique index, and cannot be mixed with others.

Transportation, Stability and Storage

1. Storage Condition: Store the kit away from light at -15℃ to -25℃, valid for 18 months. Once opened, reagents can be stored in their original packaging at -15℃ to -25℃ until the stated expiration date shown on the packaging. Repeated thawing and freezing should be avoided.
2. Storage Condition: Store the streptavidin Beads away from light at 2℃ to 8℃, valid for 18 months. Once opened, reagents can be stored in their original packaging at 2℃ to 8℃ until the stated expiration date shown on the packaging. Frozen storage should be avoided.
3. Transportation Condition: The kit should be transported at low temperature, with transporting time less than one week and transporting temperature lower than 10℃.

Precautions and Warning

1. Transfer the streptavidin and SG Pure Beads to room temperature half an hour earlier, and vortex thoroughly to disperse the SG Pure Beads before, otherwise, maybe cause yield decrease.
2. Ensure reagents competently are melted, without precipitation before use, and briefly centrifugation.2×Hyb Buffer I must be fully melted into completely no crystals in the 65℃ and vortexed and mixed, if is no melted in the room temperature.
3. Care must be taken to set reaction and lid temperature needed pre-setup for temperature-controlled equipment, such as vacuum concentrator, , thermal cycler and so on.
4. Because the temperature is important for experimental success and sequencing data, require operation at the 65℃ in hybrid, capture, elution.

Experimental Procedure

I. Library hybrid

1. Thaw the 2×Hyb Buffer I and Hyb Buffer II from SGPrep DNA Library Hybrid Capture (for Illumina) kit at room temperature until no ice is present in the centrifuge tube before use. 2×Hyb Buffer I must be completely melted no crystals.

Note: 2×Hyb Buffer I must be sufficiently melted into completely no crystals in the 65℃ with vortexed and mixed, if is no melted in the room temperature.

2. Open vacuum concentrator to preheat with 60℃ before use.
3. Place Blockers for Illumina and Human Cot DNA on room temperature to dissolve, vortex slightly to mix; for the multi-library capture hybridization, according to Table 2.1.

Table 2.1 Library Mixed

Content Name	Input
Library	500 ng/library

Human Cot DNA	5 μL
Blockers for Illumina	2 μL

Note: Each library recommended input is 500 ng. If input ≤ 6 ug, volume of Blockers for Illumina is 2 μL.

Blockers for Illumina only applied for blocking 8 bases double-ended unique index library.

It's recommended 4-6 library for each hybrid reaction to maintain library complexity, and ensure each library input more than 50% in hybrid.

4. Mix each content in a 1.5 mL Low-adhesion centrifuge tube according to Table 2.1, vortex slightly to mix, and then centrifuge briefly.
5. Place the 1.5 mL low-adhesion centrifuge tube into vacuum concentrator preheated to 60°C.
6. Wait the liquid is evaporated and dried, followed sealed for ready.

Note: The centrifuge tube are sealed, and stored at -15℃ to -25℃ or room temperature overnight.

7. Place HRD Probes on ice for natural melting, it should be divided into small packages according to needed after first use.
8. Prepare Library Hybrid Reaction according to the Table 2.2, vortex slightly to mix, transfer to bottom of the centrifuge tube have concentrated to dryness, followed mix slightly by pipetting at least half the total volume up and down 15-20 times, centrifuge briefly, and then incubate at 25℃ with 5-10 min.

Table 2.2 Library Hybrid Reaction

Content name	Input
2×Hyb Buffer I	8.5 μL
Hyb Buffer II	2.7 μL
HED Probes	2-4 μL
Nuclease-free water	1.8-3.8 μL
Total	17 μL

Note: The information of panel probes is detailed in the appendix Table 2.

9. Vortex slightly to mix hybrid reaction, and then briefly centrifuge, followed transfer all of hybrid reaction to a new low-adhesion centrifuge tube, load it on thermal cycler with 100℃ lid temperature according to Table 2.3.

Table 2.3 Hybrid Procedure

Temperature	Time
95℃	30 s
65℃	1-12 h
65℃	Hold

II. Library elution

Note: Transfer the Streptavidin beads to room temperature for 30 min and vortex thoroughly to disperse the SG Pure Beads.

1. Thaw other reagents from SGPrep DNA Library Hybrid Capture (for Illumina) kit at room temperature, vortex slightly to mix.

Note: SG Wash Buffer could be sufficiently melted into completely no crystals in the 65℃ with vortexed and mixed, if is no melted in the room temperature.

2. Prepare Bead Suspensions according to Table 2.4.

Table 2.4 Bead Suspensions

Content name	Input
2×Hyb Buffer I	8.5 μL
Hyb Buffer II	2.7 μL

Nuclease-free water	5.8 μL
Total	17 μL

3. Wash streptavidin SG Pure Beads.
   (1) Vortex slightly to mix Streptavidin beads 15 s, ensure thoroughly are mixed, followed pitched 50 μL of beads into a low-adhesion centrifuge 0.2 mL or 1.5 mL centrifuge tube.
   (2) Add 100 μL of SG buffer placed on room temperature to the centrifuge tube, mix slightly by pipetting at least half the total volume up and down 10 times, briefly centrifuge, and place the centrifuge tube on the magnetic rack for 1-2 min until the solution becomes clear, carefully remove and discard the supernatant, followed remove the centrifuge tube from magnetic rack.
   (3) Repeat step (1) one more time.
   (4) Add 17 μL of SG buffer to the centrifuge tube, mix slightly by pipetting at least half the total volume up and down, followed transfer all magnetic bead suspension to a new 0.2 mL low-adhesion centrifuge tube.
   (5) Place the 0.2 mL centrifuge tube into thermal cycler with incubating at 65°C for 5 min.
4. Streptavidin beads capture
   (1) Add magnetic bead suspension prepared above to hybrid suspension after 1-12 h, and mix slightly by pipetting at least half the total volume up and down.

   Note: This operation should rapid and avoid temperature decrease, which could affect the outcome of experiment.

   (2) Load the 0.2 mL low-adhesion centrifuge tube into the thermal cycler with 100°C lid temperature; then set up and run the program according to Table 2.5.

Table 2.5 Elution Procedure

Temperature	Time
65°C	45 min

   (3) Mix slightly by pipetting at least half the total volume up and down 5-8 times each 10-12 min, ensure beads are fully resuspended.
   (4) SG Wash Buffer is subpackaged to two parts with 160 μL, and incubated at 65°C for more than 15 min.
5. 65°C elution
   (1) Open the cap in the thermal cycler after mix is incubated at 65°C for 45 min, add 150 μL of 65°C SG Wash Buffer, mix slightly by pipetting at least half the total volume up and down 10-15 times.
   (2) Place the centrifuge tube on a magnetic rack for 30 s until the solution becomes clear, carefully remove and discard the supernatant.
   (3) Transfer the centrifuge tube to thermal cycler, add 150 μL of 65°C SG Wash Buffer, mix slightly by pipetting at least half the total volume up and down 10-15 times, followed incubate at 65°C for 5 min.
6. Elution at room temperature
   (1) Place the centrifuge tube on the magnetic rack for 30 s until the solution becomes clear, carefully remove and discard the supernatant, briefly centrifuge, followed remove and discard the remaining SG Wash Buffer by 10 μL tips.
   (2) Add 150 μL of SG Wash Buffer placed at room temperature, mix slightly by pipetting at least half the total volume up and down 10-15 times (vortex is avoided), and transfer all reaction to a new 0.2 mL low-adhesion centrifuge tube, followed is incubated for 2 min at room temperature, and vortexed, mixed, settled of 30 s each, ensure fully mixed.

   Note: After removing and discarding the 65°C SG Wash Buffer, must be discard fully remaining buffer by small pipette tips; pipette at least half the total volume up and down SG Wash Buffer placed at room temperature, must be transfer reaction to a new centrifuge tube, otherwise, may be affect quality of sequencing data.

   (3) Place the centrifuge tube on the magnetic rack for 30 s until the solution becomes clear, carefully remove and discard the supernatant, briefly centrifuge, followed remove and discard the remaining SG Wash Buffer by 10 μL tips, add 150 μL of SG Wash Buffer placed at room temperature, mix slightly by pipetting at least half the total volume up and down 10-15 times(vortex is avoided),and transfer all reaction to a new 0.2 mL low-adhesion centrifuge tube, followed incubate for 2 min at room temperature , and vortexed ,mixed , settled of 30 s each ,ensure fully mixed.

   Note: If use table vortex, the rotation speed should be 1500 rpm, ensure it is fully dissolved, otherwise, may be affect quality of

sequencing data.

(4) Place the centrifuge tube on the magnetic rack for 30 s until the solution becomes clear, carefully remove and discard the supernatant, briefly centrifuge, followed remove and discard the remaining SG Wash Buffer by 10 µL tips.

(5) Remove the centrifuge tube from magnetic rack, add 22.5 µL Nuclease-free water, fully vortex and mix, briefly centrifuge.

(6) Move the capture products with beads to next step procedure PCR amplification.

Note: In this step, do not discard the SG Pure Beads; it is necessary to capture the product and carry out PCR amplification together with the SG Pure Beads.

7. PCR amplification

(1) Thaw 2×HiFi Mix and Ill-Amp Primer Mix on ice until no ice is present in the tubs, vortex fully, briefly centrifuge.

(2) Prepare PCR Amplification Reaction on ice in 0.2 mL centrifuge tube, according to Table 2.6.

Table 2.6 PCR Amplification Solution

Content name	Input
2×HiFi Mix	25 µL
Ill-Amp Primer Mix	2.5 µL
Beads with capture product	22.5 µL
Total	50 µL

Note: Ill-Amp Primer Mix only applied for 8 bases double-ended unique index library.

(3) Load the centrifuge tube into the thermal cycler with 105℃ lid temperature; then set up and run the program according to Table 2.7.

Table 2.7 PCR Amplification Procedure

Temperature	Time	Cycle
98℃	45 s	1
98℃	15 s	
60℃	30 s	according to Table 2.8
72℃	30 s	
72℃	1 min	1
4℃	Hold	1

Table 2.8 PCR Cycle Recommended

Panel size	Hybrid time	PCR cycle recommended			
		Library input			
		1 library (500 ng)	4 libraries (2 µg)	8 libraries (4 µg)	12 libraries (6 µg)
>10 Mb	2-16 h	12-15	10-13	9-12	8-11
1 Mb-10 Mb	2-16 h	15-18	13-16	12-15	11-14
50 Kb-1 Mb	2-16 h	16-19	14-17	13-16	12-15
1 Kb-50 Kb	2-16 h	17-20	15-18	14-17	13-16

Note: Adjust PCR cycle according to sample type, panel size, hybrid time and library input.

8. Library purification and quantification

Note: Transfer the SG Pure Beads (or others) to room temperature for 30 min and vortex thoroughly to disperse the SG Pure Beads

before use; prepare fresh 80% ethanol with Nuclease-free water.

(1) Place the 0.2 mL low-adhesion centrifuge tube on a magnetic rack for 2 min until the solution clears, followed by carefully transferred supernatant to a new 0.2 mL centrifuge tube.

(2) Add 75 μL of SG Pure Beads to the centrifuge tube, and vortex thoroughly, followed is incubate at 25°C for 5-10 min.

(3) After briefly centrifuge, place the 0.2 mL low-adhesion centrifuge tube on a magnetic rack for 5 min until the solution clears, discard supernatant;

(4) Add 150 μL of freshly prepared 80% ethanol into the centrifuge tube, and place it on a magnetic rack for 30 s, followed discard supernatant without disturbing SG Pure Beads.

(5) Repeat step 4 for one time.

(6) Keep the centrifuge tube on a magnetic rack and remove all the ethanol from the centrifuge tube by 10 μL tips without disturbing SG Pure Beads.

(7) Open the cap of centrifuge tube, and wait 2-5 min to the ethanol volatilized completely.

Note: Do not dry excessively, otherwise the final yield will be affected.

(8) Remove the centrifuge tube from magnetic rack, add 24 μL of Low TE Buffer (pH 8.0), vortex thoroughly, incubate the mixture for 5-10 min at 25°C.

(9) Place the centrifuge tube on a magnetic rack for 1-2 min until the solution clears, carefully remove the supernatant to a new low-adhesion centrifuge 0.2 mL or 1.5 ml centrifuge tube without disturbing SG Pure Beads.

(10) The library should store at -15°C to -25°C for the convenience of quality detecting and sequence.

Note: The PCR product after purification could store at 15°C to -25°C for one week.

Appendix Table1: UDI information

Product Name

Adapter & UDI Primers Kit for Illumina (setA 1-24)

Adapter & UDI Primers Kit for Illumina (setB 25-48)

Packing Specification

24 Tests/kit

Intended Use

Adapter & UDI Primers Kit for Illumina (setA 1-24) and Illumina (setB 25-48) are intended for the DNA library preparation of Illumina high-throughput sequencing platform, and the 8 bases double-ended unique index (UDI) is contained in both ends of adapter. UDI is double-ended unique marker for the samples, so that both ends of the libraries index is unique and one-to-one correspondence, which is absent for index sharing between samples, and could avoid effectively cross-talk data between samples. Two kits contain UDI Universal Adapter and 24 kinds different UDI respectively which could support 24 samples mixed sequencing, and could be used with most library preparation kit.

Kit Contents

Two kits contain 24 different UDIs and UDI Universal Adapters respectively (Table S1.1 and S1.2).

Table S1.1 Adapter & UDI Primers kit for Illumina (setA 1-24) Contents

Reagent Name	Content Name	Volume	Quantity
UDI Primer (setA 1-24)	UDI Primer (10 µM)	5 µL	24 centrifuge tubes
UDI Universal Adapter	Adapter (15 µM)	60 µL	1 centrifuge tube

Table S1.2 Adapter & UDI Primers kit for Illumina (setB 25-48) Contents

Reagent Name	Content Name	Volume	Quantity
UDI Primer (setB 25-48)	UDI Primer (10 µM)	5 µL	24 centrifuge tubes
UDI Universal Adapter	Adapter (15 µM)	60 µL	1 centrifuge tube

Transportation, Stability and Storage

1. Storage Condition: Store the kit away from light at -15℃ to -25℃, valid for 12 months. Once opened, reagents can be stored in their original packaging at -15℃ to -25℃ until the stated expiration date shown on the packaging. Repeated thawing and freezing should be avoided.
2. Transportation Condition: The kit should be transported at low temperature, with transporting time less than one week and transporting temperature lower than 10℃.

Precautions and Warning

1. All reagent operations should be in super clean bench to avoid contamination.
2. Reagents should be thoroughly mixed and centrifuged before capping.
3. To avoid adapter self-linked, do not pre-incubated UDI Universal Adapter, Ligation Buffer and Ligase.
4. To avoid ligation efficiency decrease, do not store kit at the ambient above room temperature.
5. The concentration of UDI Universal Adapter according to initial amounts of input, which be diluted as show in Table S1.3 (adapters diluted with Low TE Buffer (pH 8.0)).

Table S1.3 Dilution of Adapter

Input DNA	Dilution factor	Concentration
50 ng-1 µg	no dilution	15 µM
25 ng-49 ng	2-fold dilutions	7.5 µM
10 ng-24 ng	5-fold dilutions	3 µM

UDI Sequence Information

Table S1.4 UDI Information of Split Sequences (setA 1-24)

UDI number	i7 Sequence	i5 Sequence for NovaSeq, MiSeq	i5 Sequence for iSeq,MiniSeq, NextSeq
UDI 1	TCCTCCAT	TGGCCACA	TGTGGCCA
UDI 2	GAAGAATA	GCATGTTC	GAACATGC
UDI 3	CGTATTGG	AATATCAG	CTGATATT
UDI 4	ATGCGGCC	CTCGAGGT	ACCTCGAG
UDI 5	AACACCTT	CTATGAAT	ATTCATAG
UDI 6	TTATAAGC	TCGCCTCC	GGAGGCGA
UDI 7	CGTCGGCA	GATGTGTG	CACACATC
UDI 8	GCGGTTAG	AGCAACGA	TCGTTGCT
UDI 9	GACATGCA	TTCTGCGT	ACGCAGAA
UDI 10	CGGCGAAT	AGGATGAC	GTCATCCT
UDI 11	ATATATGG	CATGCATG	CATGCATG
UDI 12	TCTGCCTC	GCACATCA	TGATGTGC
UDI 13	AGTGACCG	CACGGATA	TATCCGTG
UDI 14	GCGATAAT	GGTCACAC	GTGTGACC
UDI 15	TTCTGTTA	TCATCGCT	AGCGATGA
UDI 16	CAACCGGC	ATGATTGG	CCAATCAT
UDI 17	GTCCATTC	GGTCTTAG	CTAAGACC
UDI 18	CGAATAAT	AAGACGGA	TCCGTCTT
UDI 19	AATTGGCG	CCAGGACT	AGTCCTGG
UDI 20	TCGGCCGA	TTCTACTC	GAGTAGAA
UDI 21	AGAGCCGC	CGAATCCT	AGGATTCG
UDI 22	CTCTGAAT	TTCCAGGA	TCCTGGAA
UDI 23	GCTAATTA	AATGCTTC	GAAGCATT
UDI 24	TAGCTGCG	GCGTGAAG	CTTCACGC

Table S1.5 UDI Information of Split Sequences (setB 25-48)

UDI 编号	i7 Sequence	i5 Sequence for NovaSeq,	i5 Sequence for iSeq,MiniSeq,

		MiSeq	NextSeq
UDI 25	GATGGACT	ATGCCGAC	GTCGGCAT
UDI 26	TTCCATAA	TATGTCTG	CAGACATA
UDI 27	CGAATGTG	GGCAGTCT	AGACTGCC
UDI 28	ACGTCCGC	CCATAAGA	TCTTATGG
UDI 29	TATGCCGC	GATCACCG	CGGTGATC
UDI 30	ATGTAACA	TTAGGAAC	GTTCCTAA
UDI 31	CGCCTTAT	AGCTTGTT	AACAAGCT
UDI 32	GCAAGGTG	CCGACTGA	TCAGTCGG
UDI 33	ACAAGCGC	GAGGTCGC	GCGACCTC
UDI 34	GGTCCAAT	TGTTCTCA	TGAGAACA
UDI 35	CTGGATTA	ACCAAGAT	ATCTTGGT
UDI 36	TACTTGCG	CTACGATG	CATCGTAG
UDI 37	GAGTTGGC	CGTAAGGA	TCCTTACG
UDI 38	CTTGCACA	GTCTCTAC	GTAGAGAC
UDI 39	ACCAATAT	ACGCTACG	CGTAGCGT
UDI 40	TGACGCTG	TAAGGCTT	AAGCCTTA
UDI 41	GAGGTGTG	TCACACTC	GAGTGTGA
UDI 42	TCTTCAAC	GTTGGTGA	TCACCAAC
UDI 43	ATAAGTGT	CGCTTGAG	CTCAAGCG
UDI 44	CGCCACCA	AAGACACT	AGTGTCTT
UDI 45	GGTACTTA	TCGCACCA	TGGTGCGA
UDI 46	TAGTAAGG	AGTGCGTT	AACGCACT
UDI 47	ACAGGCAC	CTATGTAG	CTACATAG
UDI 48	CTCCTGCT	GACATAGC	GCTATGTC

Appendix Table2: HRD probe information

Product Name

Homologous Recombination Deficiency (HRD) Panel

Packing Specification

4 Tests/kit

Intended Use

Homologous Recombination Deficiency (HRD) panel contains 36 Homologous recombination repair (HRR), 5 Mismatch repair (MMR), single nucleotide variations (SNVs) and small insertions/deletions (InDels) of 15 common driver genes (TP53, PTEN, PIK3CA, CDH1, ARID1A, BRAF, KRAS, CDKN2A, CTNNB1, EGFR, POLE, ATRX, STK11, SMAD4 and NRAS), capture probes target regions cover 5.5 Mb. Based on 4.25 ten thousand SNP sites detect Genomic Instability Score (GIS) of loss-of-heterozygosity (LOH), telomeric allelic imbalance (TAI), large-scale state transition (LST) for assessing HRD states (see Gene List).

Kit Contents

Table S2.1 Kit Contents

Content Name	4 Tests/kit
Homologous Recombination Deficiency (HRD) panel probes	8 μL

Transportation, Stability and Storage

1. Storage Conditions: Store the kit away from light at -15℃ to -25℃, valid for 24 months. Once opened, reagents can be stored in their original packaging at -15℃ to -25℃ until the stated expiration date shown on the packaging. Repeated thawing and freezing should be avoided.
2. Transportation Conditions: The kit should be transported at low temperature, with transporting time less than one week and transporting temperature lower than 10℃.

Product use instructions

It's recommended to use 2 μL of Homologous Recombination Deficiency (HRD) panel probes for each hybridization reaction, according to the Hybridization Capture Kit instructions.

Precautions and Warning

1. All reagent operations should be in super clean bench to avoid contamination.
2. Reagents should be thoroughly mixed and centrifuged before capping, avoid repeated freezing and thawing, and can be dispensed in small quantities.

Symbols

Symbol	Symbol definition
	Indicates the need for the user to consult the instructions for use.
	Indicates the date when the medical device is manufactured.
LOT	Indicates the manufacturer's batch code so that the batch or lot can be identified.

	Indicates the temperature limitation.
	Indicates the date after which the medical device is not to be used.
	This is the correct upright position of the distribution packages for transport or storage.
	Indicates a medical devices should be kept dry.
	Indicates a medical device that needs protection from light sources.
	Indicates the medical device manufacturer.

Manufacturer: XAMEN SPACEGEN CO., LTD.
Address: 4th floor, No.2041 Xizhou Road, Xike Town, Tong'an District, Xiamen 361100, P. R. China
Tel: +86 592 7578317 Fax: +86 592 7578319
Email: spacegen@ispacegen.com
Website: http://www.sspacegen.com/

Gene List

Table S2.2 Gene List

Gene List							
HRR							
BRCA1	BRCA2	ATM	ATR	BARD1	BRIP1	PALB2	NBN
CHEK1	CHEK2	FANCL	RAD51C	RAD51D	FANCM	FANCD2	RAD51B
MRE11	RAD50	CDK12	FANCA	BAP1	FANCC	BLM	FAM175A
FANCE	FANCF	FANCG	FANCI	RAD51	RAD52	RAD54B	RAD54L
RPA1	XRCC2	XRCC3	PPP2R2A				
MMR							
PMS2	MSH6	EPCAM	MLH1	MSH2			
Other Driver Genes							
TP53	PTEN	PIK3CA	CDH1	ARID1A	SMAD4	STK11	CDKN2A
ATRX	EGFR	POLE	CTNNB1	KRAS	BRAF	NRAS	

Note: EGFR、POLE、CTNNB1、KRAS、BRAF、NRAS genes cover mutational hot spot, other genes cover whole CDS.