ONCOseeker™ HS Quick Guide

Preparation for Assay

- Before starting, spin down all reagents briefly to collect components clinging under the lid and keep them on ice until ready for use.
- In the case of frozen reagents, they are fully thawed and mixed using a vortex mixer. Briefly spin down and keep on ice until ready for use.
- AMPure XP beads & Streptavidin beads must be sufficiently vortexed before use. It should appear homogenous and consistent in color.
- The 80% ethanol used for size selection is prepared and used immediately before the washing process.
- Using a filter tip during all experimental processes is recommended.

Step 1. End repair

1-1) Thermal cycler program (Lid temperature: 70°C)

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

1-2) Add a DNA sample and each reagent in a 0.2ml PCR tube under the conditions shown in the table below.

Reagent		Volume per Sample	
Master Mix	Low EDTA TE	3 µL	10 µL
	● Buffer E1	4.7 µL	
	● Enzyme E2	1 µL	
	● Enzyme E3	1 µL	
	● Enzyme E4	0.3 µL	
cfDNA			50 µL
		Total Volume	**60 µL**

1-3) Perform vortex and spin down.

1-4) Place the tube in the thermal cycler and advance the program of (Step 1).

Step 2. Adapter Ligation

2-1) Thermal cycler program (Lid temperature: OFF)

Temperature	Time
20 °C	15 min

2-2) Add 29 µL of the adapter ligation master mix to the End repair sample from (Step 1).

Reagent		Volume per Sample	
Master Mix	● Buffer L1	18 µL	24 µL
	● Enzyme L2	6 µL	
(plate) Full-Length Adapter / (UDI-UMI index/ each)			5 µL
End Repair Sample			60 µL
		Total Volume	**89 µL**

2-3) Perform vortex and spin down.

2-4) Place the tube in the thermal cycler and advance the program of (Step 1).

(Optional) Repair of deaminated bases

① Thermal cycler program (Lid temperature: 47°C)

Temperature	Time
37 °C	15 min

② Add 1 µL of UDG to the Adapter ligation sample.

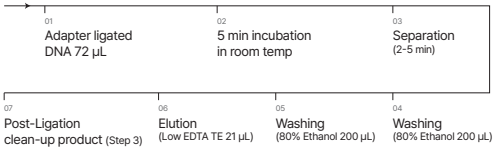
Reagent	Volume per Sample
● UDG	1 µL
Adapter Ligation sample	89 µL
Total Volume	**90 µL**

③ Perform vortex and spin down

④ Place the tube in the thermal cycler and advance the program of (Step 1).

Step 3. Post-Ligation clean-up

3-1) Add 72 µL(0.8Xvolume) of AMPure XP beads to 89 µL of adapter ligated DNA and mix 10 times with a pipette.



Step 4. PCR amplification

4-1) Thermal cycler program (Lid temperature: 105°C)

Temperature	Time	Cycles
98 °C	2 min	1
98 °C	20 sec	8-10
60 °C	30 sec	
72 °C	30 sec	
72 °C	1 min	1
4 °C	Hold	Hold

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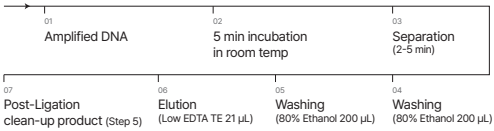
4-2) Referring to the below, add the index into the ligated DNA and add the PCR Master Mix 25 µL to each sample tube.

Reagent		Volume per Sample	
Master Mix	● PCR Master Mix	25 µL	30 µL
	● Reagent P2	5 µL	
Ligation DNA (purified DNA from Step 3)			20 µL
		Total Volume	50 µL

Step 5. Post-PCR clean-up

5-1) Add 75 µL (1.5Xvolume) of AMPure XP beads to the amplified DNA and mix 10 times with a pipette.



5-2) Measure the concentration with Qubit®4.0 Fluorometer and check the size using TapeStation or Bioanalyzer.

5-3) With the quantitative value measured by the Qubit®4.0 Fluorometer, mix the samples to be pooled in equal amounts to make a library mixture of 500 to 1500 ng in total.

Step 6. Combine blocker Mix, Cot DNA, library, and dry

6-1) Referring to the table below, mix the barcoded library pool of (Step 5), Cot DNA and Mix the Blockers Mix in a DNA LoBind 1.5 mL tube.

Reagent	Volume per Sample
Barcoded library pool	500-1500 ng
● Blockers Mix	2 µL
● Cot DNA	5 µL

6-2) Dry down the mixture using a vacuum concentrator.
* Keep the temperature of the vacuum concentrator below 70 °C

Step 7. Hybridization reaction

7-1) Thermal cycler program (Lid temperature: 100°C)

Temperature	Time
95 °C	30 sec
65 °C	4-16 h
65 °C	Hold

7-2) Prepare the Hybridization Master Mix by referring to the table below.

Reagent	Volume per Sample
○ 2X Hyb Buffer	8.5 µL
● Hyb Buffer Enhancer	2.7 µL
● Target Probe	4 µL
Nuclease-Free Water	1.8 µL
	Total Volume 17 µL

* Check the tube of 2X Hyb buffer for crystallization. If crystals are present, heat the tube at 65 °C, until the buffer is completely solubilized.

7-3) Add 17 µL of Hybridization Master Mix to the library pool concentra ted from (Step 6), mix by pipetting, and incubated at room temperature for 5-10 minutes.

7-4) Perform vortex and spin down. Transfer to a new 0.2 mL PCR tube.

7-5) Then incubate the samples in a thermal cycler at 65 °C for 4 to 16 hours.

Step 8. Prepare wash buffers

8-1) 1X working solution diluted with the table.

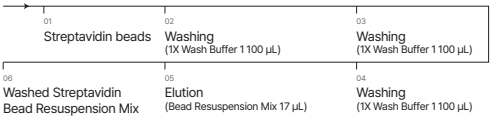
Reagent	Nuclease-Free Water	Buffer	Total	Storage
○ 2X Beads Wash Buffer	160 µL	160 µL	320 µL	RT
◎ 10X Wash Buffer 1	252 µL	28 µL	280 µL	110 µL: 65 °C 170 µL: RT
◎ 10X Wash Buffer 2	144 µL	16 µL	160 µL	RT
◎ 10X Wash Buffer 3	144 µL	16 µL	160 µL	RT
◎ 10X Stringent Wash Buffer	288 µL	32 µL	320 µL	160 µL (2tube) : 65 °C

8-2) Prepare Bead Resuspension Mix by referring to the table below.

Reagent	Volume per Sample
○ 2X Hyb Buffer	8.5 µL
● Hyb Buffer Enhancer	2.7 µL
Nuclease-Free Water	5.8 µL
	Total Volume 17 µL

Step 9. Wash streptavidin beads

9-1) Transfer 50 µL of Streptavidin beads per capture reaction to a 1.5 mL LoBind tube.



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Step 10. Bead capture

10-1) When the HYB program (4-16 h) of (Step 7) is finished, take the tube out of the thermal cycler.

10-2) After taking out the tube, set the thermal cycler under the conditions shown in the table below and immediately start the WASH program. (Lid temperature: 70°C)

Temperature	Time
65 °C	Hold

10-3) Add 17 µL of resuspension mix containing beads from (Step 9) to the tube finished with HYB from (Step 7).

10-4) Perform vortex and spin down briefly.

10-5) Place the sample into the thermal cycler where the WASH program is running and incubate at 65 °C for 45 minutes.

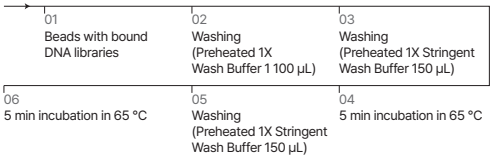
10-6) Every 10-12 minutes, remove the tube from the thermal cycler and gently vortex to ensure the sample is fully resuspended.

10-7) At the end of the 45 minutes, take the sample off the thermal cycler. Proceed immediately to Heated washes of (Step 11).

Step 11. Wash

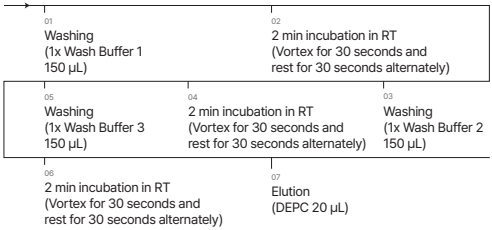
11-1) Heated washes.

- Add 100 µL preheated 1X Wash Buffer 1 to the tube from (Step 10), slow the pipette 10 times, and spin down briefly.
- Carefully pipette to minimize bubble formation.
- Transfer the beads mixture to a new 1.5 mL LoBind tube.



11-2) Room temperature washes.

- Add 150 µL of 1X Wash Buffer 1 stored at room temperature.



* Do not throw away the beads. 20 µL beads containing captured DNA are used in the Post-capture PCR step.

Step 12. Post-capture PCR

12-1) Transfer the 20 µL of beads with a captured DNA sample from (Step 11) to a new 0.2 mL PCR tube.

12-2) Referring to the table below, add 30 µL of the Amplification Reaction Mix to the 0.2 mL tube containing the sample.

Reagent	Volume per Sample
Beads with captured DNA (Step 11)	20 µL
● 2X HotStart Mix	25 µL
● P5 primer	2.5 µL
● P7 primer	2.5 µL
	Total Volume 50 µL

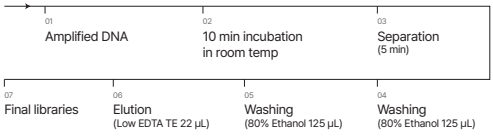
12-3) Perform vortex and spin down.

12-4) Preset the following thermal cycler program and proceed with the Post-capture PCR run. (Lid temperature: 105°C)

		Temperature	Time	Cycles
Polymerase activation		98 °C	45 sec	1
Amplification	Denaturation	98 °C	15 sec	11
	Annealing	60 °C	30 sec	
	Extension	72 °C	30 sec	
Final extension		72 °C	60 sec	1
Hold		4 °C	Hold	Hold

Step 13. Purify post-capture PCR fragments

13-1) Add 75 µL (1.5X volume) of AMPure XP beads to the PCR reaction from (Step 12) and mix well by pipetting 10 time.



Step 14. Library QC

14-1) Measure the concentration with Qubit®4.0 Fluoromete rand check the size using TapeStation or Bioanalyzer.

14-2) After converting to molarity using the quantitative value measured by the Qubit 4.0 Fluorometer and the size of the TapeStation result, dilute to 4nM using Nuclease-Free Water.

Step 15. Sequencing

15-1) Please refer to the manufacturer's instructions and recommendations.

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