ONCOseeker™ SOLID 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) Set 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 0.2 mL 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	
Fragmented DNA or 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) Set thermal cycler program

Temperature	Time
20 °C	15 min

2-2) Adapter dilution (Mix the Reagent L3 before use.)

DNA Input	Reagent L3 (Adapter)
≥ 50 ng	No Dilution
10 ng	10-fold (1:10)
1 ng	20-fold (1:20)

2-3) 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	29 μL
	Enzyme L2	6 μL	
	Reagent L3	5 μL	
End Repair Sample			60 μL
		Total Volume	89 μL

2-4) Vortex and spin down.

2-5) 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.

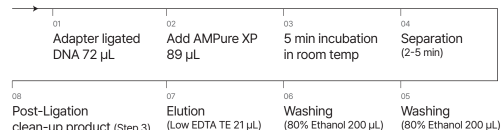
Reagent	Volume per Sample
UDG	1 μL
Adapter Ligation sample	89 μL
	Total Volume 90 μL

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(0.8X volume) 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	3-10*
60 °C	30 sec	
72 °C	30 sec	
72 °C	1 min	1
4 °C	Hold	Hold

DNA Input	Minimum recommended cycles
1 ng	3
100 ng	3
10 ng	6-7
1 ng	9-10

* Number of PCR cycles according to the amount of input DNA

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

Reagent	Volume per Sample
Ligation DNA (Purified DNA from Step 3)	20 μL
(Plate) Index 01-12 (each)	5 μL
● PCR Master Mix	25 μL
	Total Volume 50 μL

Step 5. Post-PCR clean-up, Library QC/Pooling

01 PCR product 50 μL
02 Add AMPure XP 75 μL
03 5 min incubation in room temp
04 Separation (2-5 min)
05 Washing (80% Ethanol 200 μL)
06 Washing (80% Ethanol 200 μL)
07 Elution (Low EDTA TE 21 μL)
08 Post-Ligation clean-up product (Step 5)

5-1) Measure the concentration with Qubit®4.0 Fluoromete rand check the size using TapeStation or Bioanalyzer.

5-2) Mix the samples to be pooled in equal amounts to make a library mixture of 500 to 1,500ng 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) 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.

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

7-3) Add 17 μL of Hybridization Master Mix to the library pool concentrated 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.

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

01 Streptavidin beads 50 μL
02 Washing (1X Wash Buffer 1 100 μL)
03 Washing (1X Wash Buffer 1 100 μL)
04 Washing (1X Wash Buffer 1 100 μL)
05 Elution (Bead Resuspension Mix 17 μL)
06 Washed Streptavidin Bead Resuspension Mix

Step 10. Bead capture

10-1) When the HYB program (4-16 h) of (Step 7) is finished, 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

10-2) Add 17 μL of resuspension mix containing beads from (Step 9) to the tube finished with HYB from (Step 7).

10-3) Perform vortex and spin down briefly.

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10-4) Place the sample into the thermal cycler where the WASH program is running and incubate at 65 °C for 45 minutes.

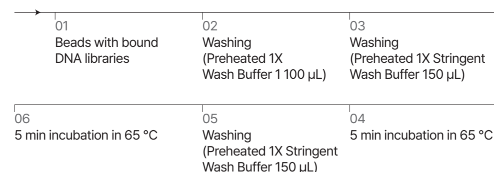
10-5) Every 10-12 minutes, remove the tube from the thermal cycler and gently vortex to ensure the sample is fully resuspended.

10-6) 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

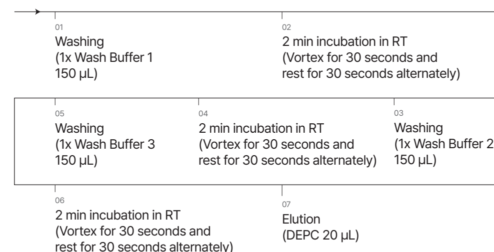
11-1) Heated washes.

- 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

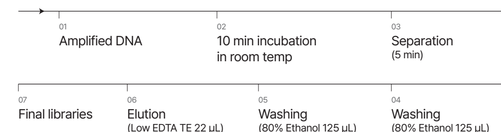
12-3) Perform vortex and spin down.

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 Fluorometerand 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 4 nM using Nuclease-Free Water.

Step 15. Sequencing

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

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