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BGISEQ-500 10X library construction

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ABSTRACT

BGISEQ-500 is a desktop sequencer developed by BGI. This protocol adjusts the process in order to apply the 10X contruction to the BGISEQ-500 rather than illumina.

MATERIALS

NAME	CATALOG #	VENDOR
SPRIselect reagent kit	B23317	Beckman Coulter
Qubit® 3.0 Fluorometer	Q33216	Thermo Fisher Scientific
Buffer EB	19086	Qiagen
Ampure XP beads	A63881	Beckman Coulter
Qubit® dsDNA HS Assay kit	Q32854	Thermo Fisher Scientific
Tween 20	P1379	Sigma
TE buffer		Thermo Fisher Scientific
Nuclease-Free Water	AM9939	Thermo Fisher Scientific
Qubit assay tubes	Q32856	Thermo Fisher Scientific
200 Proof Ethanol pure	E7023	Sigma Aldrich

MATERIALS TEXT

Chromium™ Genome Reagent Kits
DynaBeads® MyOne™ Silane Beads
PEG32 beads
pfu Turbo Cx Hotstart DNA Polymerase
10X p5 primer
10X p7 primer
2X pfu Cx mix3
10X TA buffer
100mM ATP
T4 Polynucleotide Kinase
10x ILMN Splint Oligo
T4 DNA Ligase
Exo I
Exo III

1 Input HMW gDNA Quantification

1.1 Quantitate 3 µl of extracted gDNA solution (with a minimum of 2 replicates).

1.2 If the gDNA stock is >20 ng/µl, prepare an intermediate dilution of the extracted gDNA solution at <20 ng/µl in Buffer EB. Gently mix 10 times with a wide-bore pipette tip.

1.3 Quantitate 3 µl of the <20 ng/µl intermediate gDNA stock (with a minimum of 2 replicates) to verify the diluted concentration.

1.4 According to the table below, dilute gDNA with bufferEB concentration less than 20 to the corresponding final concentration. Gently mix 10 times with a wide-bore pipette tip. Quantitate 3 µl of the diluted gDNA solution (with a minimum of 2 replicates) to verify the diluted concentration.

Genome Size	diluted concentration	Volume	Acceptable concentration range
1.6-3.2GB	1ng/µl	30µl	0.8-1.2ng/µl
0.1-1.6GB	0.5ng/µl	30µl	0.4-0.6ng/µl

1.5 Verify recorded concentrations of the diluted gDNA solution are between Acceptable concentration range before proceeding to Preparing GEM Reagent Mix. If recorded concentrations are out of range, repeat from 1.4.

2 Preparing Sample Master Mix

2.1 Prepare the Sample Master Mix in a 1.5 ml or 5 ml tube on ice. Add reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly.

Sample Master Mix	1 rxn (µl)	8 rxns + 10% excess (µl)
Genome Reagent Mix	89.5	788
Additive A	3	26
Genome Enzyme Mix	5	44
Total	97.5	858

2.2 Dispense 97.5 µl Sample Master Mix per reaction into an 8-tube strip and place strip on a chilled metal block resting on ice.

2.3 Dispense 10 µl Denaturing Agent into a tube strip at room temperature.

2.4 Slowly transfer 10 µl diluted gDNA into the pre-aliquoted Denaturing Agent using a multi-channel pipette and narrow-bore pipette tips. Gently mix the combined gDNA and Denaturing Agent 10 times with a multi-channel pipette and wide-bore pipette tips.

2.5 Incubate the combined gDNA and Denaturing Agent for 5 min at room temperature.

2.6 Slowly add 2.5 µl denatured gDNA to 97.5 µl pre-dispensed Sample Master Mix with a multi-channel pipette while on ice.

2.7 After all denatured gDNA samples are added, simultaneously and gently mix all samples 10 times using a multi-channel pipette set to 90 µl with wide-bore pipette tips.

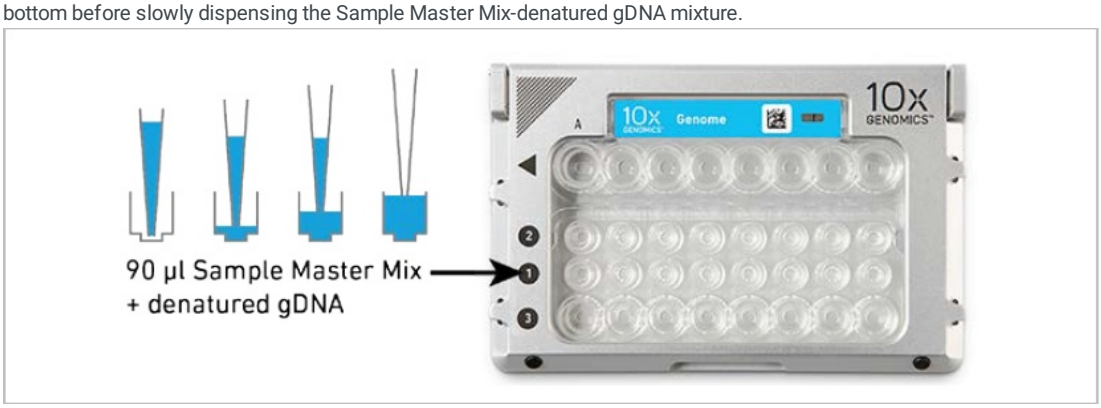
2.8 Briefly centrifuge the tube strip and return to the chilled block.

3 Loading the Genome Chip

3.1 Remove Genome Gel Bead Strip from -80°C in advance and balance at room temperature for 30 minutes. Place a Genome Chip in a 10x™ Chip Holder. Handle the chip by its edges, taking care to avoid touching its bottom surface.

3.2 If processing fewer than 8 samples per Genome Chip, first add the following volumes of 50% glycerol solution to each unused well:
i. 90 µl in the row labeled 1
ii. 85 µl in the row labeled 2
iii. 270 µl in the row labeled 3

3.3 Using a narrow-bore pipette tip, slowly transfer 90 µl Sample Master Mix-denatured gDNA mixture into the bottom of wells in the row labeled 1, taking care not to introduce bubbles. To do this, place the tips into the bottom center of the wells and raise the tips slightly above the bottom before slowly dispensing the Sample Master Mix-denatured gDNA mixture.

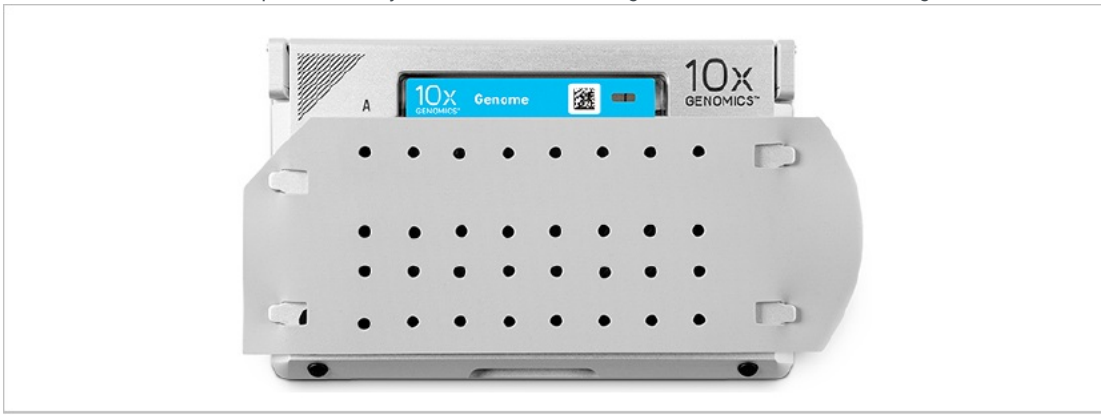


3.4 Gel Bead Preparation in Gel Bead Strips.
i. Snap the Genome Gel Bead Strip into a 10x™ Vortex Adapter. Vortex for 30 sec.
ii. Remove the Genome Gel Bead Strip and flick in a sharp, downward motion to ensure maximum recovery. Confirm that there are no bubbles at the bottom of the tube and liquid levels look even.
iii. Carefully puncture the foil seal and slowly aspirate 85 µl Genome Gel Beads.

3.5 Slowly dispense the Genome Gel Beads into the bottom of wells in the row labeled 2, taking care not to introduce bubbles.

3.6 Pipette 270 µl Partitioning Oil into the wells in the row labeled 3. Pipette Partitioning Oil into wells one at a time. Do not use a reagent reservoir for the Partitioning Oil. Do not add Partitioning Oil to any unused input wells that already contain 50% glycerol solution.

3.7 Attach the 10x™ Gasket. The notched cut should be at the top left corner. Ensure the 10x Gasket holes are aligned with the wells. Avoid touching the smooth side of the 10x Gasket and do not press down on the top of the 10x Gasket after attachment. Keep the assembly horizontal to avoid wetting the 10x Gasket with Partitioning Oil.



4 Running the Chromium™ Controller

i. Press the button on the touchscreen of the Chromium Controller to eject the tray.

ii. Place the assembled Chip, 10x™ Chip Holder and 10x Gasket on the tray.

iii. Press the button on the touchscreen again to retract the tray. Confirm the Genome program shows on screen and press the play button to begin the run.

iv. At the completion of the run (~20 min), the Chromium Controller will chime. Proceed immediately to the next step.

5 Transferring GEMs

i. Press the eject button to eject the tray and remove the Genome Chip. Remove and discard the 10x Gasket. Press the button to retract the empty tray (or the tray will automatically close after 40 sec).

ii. Open the 10x Chip Holder and fold the lid back until it clicks to expose the wells at a 45-degree angle.

iii. Check for volume uniformity in the Gel Bead, Sample, and Partitioning Oil wells remaining in the Genome Chip.

iv. Slowly aspirate 125 µl GEMs from the lowest points of the Recovery Wells (row labeled ◄) without creating a seal between the tips and the bottom of the wells. Avoid introducing air bubbles. Withdraw pipette tips from the wells and verify that there is no air in the tips. GEMs should appear opaque and uniform across all channels.

v. Slowly dispense the GEMs into an Eppendorf twin-tec® 96-Well PCR plate on a chilled metal block resting on ice. Keep the tips above the liquid level to minimize GEMs lost on the outside of the tips.

6 GEM Isothermal Incubation

6.1 Seal the plate with pierceable foil heat seal at 185°C for 6 sec and promptly remove.

6.2 Load the sealed PCR plate into a thermal cycler that can accommodate 125 µl reaction volume and proceed with the following incubation protocol.(Lid Temperature: 75°C, Reaction Volume: 125 µl)

Step	Temperature	Time
1	30°C	3:00:00
2	65°C	10:00
3	4°C	Hold

6.3 Store in the PCR plate at 4°C for up to 72 h or at −20°C for up to 2 weeks before proceeding to Post GEM Incubation Cleanup.

7 Post GEM Incubation Cleanup – Silane DynaBeads

7.1 Remove DynaBeads MyOne Silane beads in advance and balance at room temperature for 30 minutes.

7.2 At room temperature, remove the foil seal and add 125 µl Recovery Agent to each well containing post incubation GEMs. Pipette mix thoroughly and transfer the entire volume to an 8-tube strip.

7.3 Cap the tube strip and place in a 10x™ Vortex Clip. Vortex for 15 sec.

7.4 Briefly centrifuge the tube strip. The resulting biphasic mixture contains distinct Recovery Agent/Partitioning Oil (pink) and aqueous phases (clear), with no persisting emulsion (opaque).

7.5 Slowly remove 135 µl Recovery Agent/Partitioning Oil (pink) from the bottom of the tubes and discard. Be careful not to aspirate any of the clear aqueous sample. A small volume of Recovery Agent/Partitioning Oil will remain. Do not aspirate the aqueous solution during Recovery Agent/Partitioning Oil removal. Should aspiration of the aqueous solution occur, return the solution to the tube strip, reduce removal volume by 5 µl, and reattempt removal.

7.6 Vortex DynaBeads MyOne Silane beads until fully resuspended. Prepare the DynaBeads Cleanup Mix by adding reagents in the order shown below. Vortex mix thoroughly and use immediately.

DynaBeads Cleanup Mix	1 rxn (µl)	8 rxns + 10% excess (µl)
Buffer Sample Clean Up 1	136	1197
DynaBeads MyOne Silane	8	70
Additive A	6	53
Total	150	1320

7.7 Immediately add 150 µl DynaBeads Cleanup Mix to each sample. Pipette mix thoroughly and incubate at room temperature for 10 min. Do not attempt to cap the tube strip as the liquid volume is high.

7.8 Prepare Elution Solution I by adding reagents in the order shown below. Vortex mix thoroughly and centrifuge briefly.

Elution Solution I	1 rxn (µl)	8 rxns + 25% excess (µl)
Buffer EB	89	890
10% Tween 20	1	10
Additive A	10	100

Total	100	1000

7.9 After the 10 min incubation step is completed, place the tube strip into a 10x™ Magnetic Separator in the High position until the solution is clear (>2 min). Carefully remove and discard the supernatant.

7.10 Add 250 µl freshly prepared 80% ethanol to the pellet while on the magnet and stand for 30 sec.

7.11 Carefully remove and discard the ethanol wash.

7.12 Add 200 µl 80% ethanol to the pellet and stand for 30 sec and carefully remove and discard the ethanol wash.

7.13 Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the Low position until the solution is clear.

7.14 Remove and discard any remaining ethanol. Remove the tube strip from the magnet and immediately add 51µl Elution Solution I from a reservior with a multi-channel pipette.

7.15 Incubate 30s before resuspending the pellet in Elution Solution I. Pipette mix thoroughly until beads are fully resuspended. Silane Dynabeads can be difficult to resuspend due to residual reagents from the GEM reaction. Mix thoroughly with a pipette set to 40µl to avoid introducing air bubbles.

7.16 Incubate the tube strip at room temperature for 5min.

7.17 Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the Low position until the solution is clear.Transfer 50µl sample to a new tube strip.

8 Post GEM Incubation Cleanup – SPRIselect

8.1 Remove SPRIselect beads in advance and balance at room temperature for 30 minutes.

8.2 Prepare Elution Solution II by adding appropriate volume of reagents in the order shown below. Vortex mix thoroughly and centrifuge briefly.

Elution Solution II	1 rxn (µl)	8 rxns + 25% excess (µl)
Buffer EB	98	980
Additive A	2	20
Total	100	1000

8.3 Vortex the SPRIselect Reagent until fully resuspended. Add 35 µl SPRIselect Reagent (0.7X) to each sample in the tube strip. Pipette mix thoroughly and centrifuge briefly.

8.4 Incubate the tube strip at room temperature for 5 min.

8.5 Place the tube strip in a 10x™ Magnetic Separator in the High position until the solution is clear (>2 min).

8.6 Carefully remove and discard the supernatant.

8.7 Add 125 µl 80% ethanol to the pellet and stand for 30 sec.

8.8 Carefully remove and discard the ethanol wash.

8.9 Repeat steps 8.7 and 8.8 for a total of 2 washes.

8.10 Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the Low position. Remove and discard any remaining ethanol. Remove the tube strip from the 10x Magnetic Separator and immediately add 52 µl Elution Solution II from a reservoir with a multi-channel pipette.
SPRIselect beads dry very quickly at this step and may clump if Elution Solution II is not added immediately after removal of residual ethanol.

8.11 Pipette mix thoroughly and incubate at room temperature for 5 min.

8.12 Centrifuge the tube strip briefly and place it in a 10x Magnetic Separator in the Low position until the solution is clear.

8.13 Transfer 52 µl of sample to a new tube strip. Store the samples in a tube strip at 4°C for up to 72 h or at −20°C for up to 2 weeks before proceeding to Library Construction.

8.14 Take 1 µl sample for Qubit dsDNA HS quantification. Concentration > 1ng/µl.

9 End Repair & A-tailing

9.1 Vortex the End Repair and A-tailing Buffer. Verify there is no precipitate before proceeding.

9.2 Prepare End Repair and A-tailing Mix by adding the reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly.

End Repair and A-tailing Mix	1 rxn (µl)	8 rxns + 10% excess (µl)
Nuclease-Free Water	2.5	22
End Repair and A-tailing Buffer	7.5	66
End Repair and A-tailing Enzyme	15	132
Total	25	220

9.3 Add 25 µl End Repair and A-tailing Mix to each tube containing 50 µl sample from Post GEM Incubation Cleanup. Pipette mix thoroughly and centrifuge briefly.

Incubate in a thermal cycler with the following protocol.(Lid Temperature: 85°C, Reaction Volume: 75 µl)

9.4

Step	Temperature	Time
End Repair	20°C	30:00
A-tailing	65°C	30:00
Hold	4°C	Hold

9.5 Proceed immediately to the next step.

10 Adaptor Ligation

10.1 Prepare the Adaptor Ligation Mix by adding the reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly.

Adaptor Ligation Mix	1 rxn (µl)	8 rxns + 10% excess (µl)
Ligation Buffer	22	194
DNA Ligase	11	97
Adaptor Mix	2.5	22
Total	35.5	313

10.2 Add 35.5 µl Adaptor Ligation Mix to each tube containing 75 µl of sample from the End Repair and A-tailing step. Pipette mix thoroughly and centrifuge briefly.

10.3 Incubate in a thermal cycler with the following protocol.(Lid Temperature: 30°C, Reaction Volume: 110 µl)

Step	Temperature	Time
1	20°C	15:00

10.4 Proceed immediately to the next step.

11 Post Ligation Cleanup – SPRIselect

11.1 Vortex the SPRIselect Reagent until fully resuspended. Add 88 µl SPRIselect Reagent(0.8X) to each sample in the tube strip. Pipette mix thoroughly.
Do not attempt to cap the tube strip as the liquid volume is high.

11.2 Incubate the tube strip at room temperature for 5 min.

11.3 Place the tube strip in a 10x™ Magnetic Separator in the High position until the solution is clear (>2 min). Carefully remove and discard the supernatant.

11.4 Add 250 µl 80% ethanol to the pellet and stand for 30 sec.

11.5 Carefully remove and discard the ethanol wash.

11.6 Repeat steps 11.4 and 11.5 for a total of 2 washes.

11.7 Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the Low position. Remove and discard any remaining ethanol. Remove the tube strip from the 10x Magnetic Separator and immediately add 40 µl Buffer EB.

11.8 Pipette mix thoroughly and incubate at room temperature for 5 min.

11.9 Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the Low position until the solution is clear.

1.10 Transfer 40 µl of sample to a new tube strip and proceed immediately to the next step.

12 Sample Index PCR

12.1 Prepare the Sample Index PCR Mix by adding the reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly.

Sample Index PCR Mix	1 rxn (µl)	8 rxns + 10% excess (µl)
Amplification Master Mix	50	440
Forward PCR Primer	5	44
Total	55	484

12.2 Add 55 µl Sample Index PCR Mix to each tube containing 40 µl purified Post Ligation sample. Add 5 µl of an individual Chromium i7 Sample Index to each well and record their assignment. Pipette mix thoroughly and centrifuge briefly.

12.3 Index the library DNA in a thermal cycler for a total of 5 cycles.(Lid Temperature: 105°C,Reaction Volume: 100µl)

Step	Temperature	Time
1	98°C	0:45
2	98°C	0:20
3	54°C	0:30
4	72°C	0:20
5	Go to step 2, 9X (for 5 cycles in total)	
6	72°C	1:00
7	4°C	Hold

12.4 Store the tube strip at 4°C for up to 72 h or proceed directly to Post Sample Index PCR Cleanup.

13 Post Sample Index Double Sided Size Selection – SPRI Select

13.1 Transfer 96 µl of the indexed PCR samples to a new tube strip. If the sample is less than 96 µl, bring the total volume to 96 µl with Buffer EB.

13.2 Vortex the SPRIselect Reagent until fully resuspended. Add 48 µl SPRIselect Reagent (0.5X) to each 96 µl sample in the tube strip. Pipette mix thoroughly.

13.3 Incubate the tube strip at room temperature for 5 min.

13.4 Place the tube strip in a 10x™ Magnetic Separator in the High position until the solution is clear (>2 min).
DO NOT discard supernatant.

13.5 Transfer 135 µl supernatant to a new tube strip and discard previous tube strip.

13.6 Vortex the SPRIselect Reagent until fully resuspended. Add 18 µl SPRIselect Reagent (0.7X final) to the supernatant in the new tube strip. Pipette mix thoroughly.

13.7 Incubate the tube strip at room temperature for 5 min.

13.8 Place the tube strip in a 10x Magnetic Separator in the High position until the solution is clear. Carefully remove and discard the supernatant.

13.9 Add 200 µl 80% ethanol to the pellet and stand for 30 sec.

3.10 Carefully remove and discard the ethanol wash.

3.11 Repeat steps 13.9 and 13.10 for a total of 2 washes.

3.12 Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the Low position. Remove and discard any remaining ethanol. Remove the tube strip from the 10x Magnetic Separator and immediately add 20 µl Buffer EB.

3.13 Pipette mix thoroughly and incubate at room temperature for 5 min.

3.14 Centrifuge the tube strip briefly and return it to a 10x Magnetic Separator in the Low position until the solution is clear.

3.15 Transfer 20 µl of sample to a new tube strip.

3.16 Store the tube strip at 4°C for up to 72 h or at −20°C for long-term storage.

14 pfuCx PCR

14.1 Divide the product from last step into four portions, 4.5 µl per serving. Prepare the pfuCx PCR Mix by adding the reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly.(The samples were divided into four parts for system amplification.)

The name of reagent	Dosage of single pipe(μl)
Sample DNA	4.5
2x pfxCx Mix3	37.5
Trubo pfuCx	15
10X P5 primer(100μM)	0.5
10X P7 primer(100μM)	0.5
NF-Water	30.5
Total	75

14.2 PfuCx PCR the library DNA in a thermal cycler for a total of 5 cycles.(Lid Temperature: 105°C,Reaction Volume: 75μl)

Step	Temperature	Time
1	95°C	3:00
2	95°C	0:30
3	54°C	0:30
4	72°C	1:00
5	Go to step 2 to 4X (for 5 cycles in total)	
6	72°C	10:00
7	4°C	Hold

14.3 pfuCx PCR product purification

i. Remove Ampare XP beads in advance and balance at room temperature for 30 minutes.

ii. Merge 4 PCR products into a 1.5 mL centrifuge tube and add 300 μl XP beads. Pipette mix thoroughly.

iii. Incubate the tube at room temperature for 10 min.

iv. Place the tube in a Magnetic Separator until the solution is clear. Carefully remove and discard the supernatant.

v. Add 600 μl 80% ethanol to the pellet and stand for 30 sec. Carefully remove and discard the ethanol wash.

vi. Repeat steps 14.3 for a total of 2 washes.

vii. Centrifuge the tube briefly and return it to a Magnetic Separator. Remove and discard any remaining ethanol. Remove the tube from the Magnetic Separator and immediately add 35 μl Buffer TE. Pipette mix thoroughly and incubate at room temperature for 5 min.

viii. Centrifuge the tube briefly and return it to a Magnetic Separator until the solution is clear. Transfer 35 μl of sample to a new 1.5mL centrifuge tube.

14.4 Post Library Construction QC

Take 1 μl sample for Qubit dsDNA HS quantification. Concentration > 7ng/μl.

15 Post Library PNK

15.1 Adjust sample dosage to 1.5 pmol according to the concentration of sample(DNA input(670ng) = 1.5 pmol * 650g/mol * size(690bp)). If the sample is less than 1.5pmol, Adjust sample dosage to 1 pmol according to the formula above; if the sample is less than 1 pmol, use all the library.

15.2 Prepare the PNK Mix by adding the reagents in the order shown below. Pipette mix thoroughly and centrifuge briefly

PNK Mix	1 rxn(μl)
DNA	X
10X TA buffer	5
100mM ATP	0.5
T4 Polynucleotide Kinase	6
NF-Water	38.5-X
Total	50

15.3 Incubate in a thermal cycler with the following protocol.

Temperature	Time
37°C	30:00
65°C	15:00
4°C	Hold

15.4 Proceed immediately to the next step.

16 Post Library ssDNA circilarization

16.1 Add 15 µl TE buffer and 5 µl 20 µM 10X ILMN Splint Oligo to the sample after PNK.

16.2 95°C for 3 min, put sample on ice immediately.

16.3 Prepare the Ligase Mix by adding the reagents in the order shown below.

Ligase Mix	1 rxn(µl)
10X TA Buffer	12
100mM ATP	1.2
T4 DNA Ligase(600U/µl)	0.4
NF-Water	35.4
Total	50

16.4 Add 50 µl Ligase Mix to the sample, gently mix 10 times by pipette.
Don't mix sample by votex.

16.5 Incubate in a thermal cycler with the following protocol.

Temperature	Time
37°C	1:00:00
4°C	Hold

17 Medium-Scale ExoI and ExoIII

17.1 Prepare the Mix by adding the reagents in the order shown below.

ExoI/III Mix	1rxn(µl)
10X TA buffer	0.8
ExonucleaseI	3.9
Exonuclease III	1.3
NF-Water	2.0
Total	8.0

17.2 Add 8 µl ExoI/III Mix to the sample after cyclization(128µl), gently mix 10 times by pipette.

17.3 Remove PEG32 beads in advance and balance at room temperature for 30 minutes.
Incubate in a thermal cycler with the following protocol.

Temperature	Time
37°C	30:00

17.4 Add 6 µl 0.5M EDTA to the sample immediately and mix. Incubate the tube at room temperature for 3 min. Transfer all the sample into a new 1.5mL centrifuge tube.

17.5 Add 170 µl PEG32 beads and Pipette mix thoroughly. Incubate the tube at room temperature for 10 min.

17.6 Place the tube in a Magnetic Separator until the solution is clear. Carefully remove and discard the supernatant.

17.7 Add 400 µl 80% ethanol to the pellet and stand for 30 sec. Carefully remove and discard the ethanol wash.

17.8 Repeat steps 17.7 for a total of 2 washes.

17.9 Centrifuge the tube briefly and return it to a Magnetic Separator. Remove and discard any remaining ethanol. Remove the tube from the Magnetic Separator and immediately add 32 µl Buffer TE. Pipette mix thoroughly and incubate at room temperature for 5 min.

7.10 Centrifuge the tube briefly and return it to a Magnetic Separator until the solution is clear. Transfer 32 µl of sample to a new 1.5mL centrifuge tube.

18 Quality standard and evaluation
Take 2 µl sample for Qubit ssDNA HS quantification. Quality standard has been shown below.

Quality standard name	Quality standard
Concentration	>1.5ng/µl
efficiency	>12%