Tagment Genomic DNA

☐1 Preheat a heat block to 55°C.

☐2 Add the following items in the order listed to a 1.7 ml microcentrifuge tube.

Item	Volume (µl)
gDNA	x µl (1 µg)
Water	76–x
Tagment Buffer Mate Pair	20
Mate Pair Tagment Enzyme	4
Total	100

☐3 Flick to mix, and then centrifuge briefly. Repeat.

☐4 Incubate at 55°C for 30 minutes.

☐5 Add 2 volumes of Zymo ChIP DNA Binding Buffer to tagmentation reaction. Pipette to mix.

☐6 Transfer up to 800 µl of mixture to a Zymo-Spin IC-XL column in a collection tube.

☐7 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.

☐8 Transfer remaining tagmentation mixture.

☐9 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.

☐10 Wash 2 times with 200 µl Zymo DNA Wash Buffer.

☐11 Centrifuge the empty column at 10,000–16,000 × g for 1 minute with lid open. Discard the flow-through and the collection tube.

☐12 Transfer column to a 1.7 ml microcentrifuge tube.

☐13 Add 30 µl RSB.

☐14 Incubate at room temperature for 1 minute.

☐15 Centrifuge at 10,000–16,000 × g for 1 minute.

☐16 To assess tagmentation, dilute 1 µl DNA with 1 µl water and run on a LabChip.

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 24 hours.

Strand Displacement

☐1 Add the following items in the order listed to the microcentrifuge tube.

Item	Volume (µl)
Tagmented DNA Sample	30
Water	10.5
10x Strand Displacement Buffer	5
dNTPs	2
Strand Displacement Polymerase	2.5
Total	50

☐2 Flick to mix, and then centrifuge briefly.

☐3 Incubate at 20°C for 30 minutes.

Purify the DNA

☐1 Add the following items in the order listed to the 1.7 ml microcentrifuge tube.

Item	Volume (µl)
Strand Displaced DNA	50
Water	50
AMPure XP Beads	40
Total	140

Success of this step depends on accurate ratio of beads to DNA (eg, 0.4x).

☐2 Flick to mix, and then centrifuge briefly.

☐3 Incubate at room temperature for 15 minutes. Flick every 2 minutes.

☐4 Centrifuge briefly.

☐5 Place on a magnetic rack for 5 minutes.

☐6 Remove and discard all supernatant.

☐7 Wash 2 times with 400 µl 70% EtOH.

☐8 Air-dry on the magnetic rack for 10–15 minutes.

☐9 Remove from the magnetic rack.

☐10 Add 30 µl RSB. Flick to mix.

☐11 Centrifuge briefly.

☐12 Incubate at room temperature for 5 minutes.

☐13 Place on the magnetic rack for 5 minutes.

☐14 Transfer all supernatant to a 1.7 ml microcentrifuge tube.

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 7 days.

Circularize DNA

☐1 Add the following items in the order listed to a 1.7 ml microcentrifuge tube.

Item	Volume (µl)
AMPure Purified or Size Selected DNA	x µl (up to 600 ng)
Water	268–x
Circularization Buffer 10x	30
Circularization Ligase	2
Total	300

☐2 Flick to mix, and then centrifuge briefly.

☐3 Incubate at 30°C overnight (12–16 hours).

Remove Linear DNA

☐1 Add 9 µl Exonuclease to the overnight circularization reaction.

☐2 Flick to mix, and then centrifuge briefly.

☐3 Incubate at 37°C for 30 minutes.

☐4 Incubate at 70°C for 30 minutes. Flick to mix.

☐5 Add 12 µl Stop Ligation Buffer.

☐6 Flick to mix, and then centrifuge briefly.

Shear Circularized DNA

☐1 Transfer the entire sample to a Covaris T6 tube (~320 µl).

☐2 Add water to fill to the top, and then cap the tube.

☐3 Make sure that no air bubbles are present.

☐4 Shear the DNA using Covaris S2 or S220 device with the following settings.

Settings	S2	S220
Peak Power Intensity	--	240
Intensity	8	--
Duty Cycle/Duty Factor	20%	20%
Cycles Per Burst	200	200
Time	40 seconds	40 seconds
Temperature	6°C	6°C

☐5 Transfer the ~320 µl sample to a 1.7 ml microcentrifuge tube.

Purify the Sheared DNA

- ☐1 Shake to resuspend the beads.
- ☐2 Transfer 20 µl beads to a 1.7 ml microcentrifuge tube.
- ☐3 Place on a magnetic rack for 1 minute.
- ☐4 Remove and discard all supernatant
- ☐5 Wash 2 times with 40 µl Bead Bind Buffer.
- ☐6 Remove from the magnetic rack.
- ☐7 Add 300 µl Bead Bind Buffer.
- ☐8 Add 300 µl beads to the 300 µl sheared DNA.
- ☐9 Incubate at 20°C for 15 minutes. Flick to mix every 2 minutes.
- ☐10 Centrifuge briefly (5–10 seconds).
- ☐11 Place on a magnetic rack for 1 minute.
- ☐12 Remove and discard all supernatant.
- ☐13 Wash 4 times with 200 µl Bead Wash Buffer.
- ☐14 Wash with 200 µl RSB.
- ☐15 Repeat the RSB wash, but do not remove and discard the supernatant until the next step.

End Repair

- ☐1 Create the end repair reaction mix in a 1.7 ml microcentrifuge tube. For multiple samples, prepare a master mix.

Item	Volume (µl)
End Repair Mix	40
Water	60
Total	100

- ☐2 Remove and discard all supernatant.
- ☐3 Centrifuge briefly.
- ☐4 Place on the magnetic rack.
- ☐5 Using a 10 µl pipette, remove residual supernatant.
- ☐6 Add 100 µl end repair reaction mix.
- ☐7 Remove from the magnetic rack.
- ☐8 Flick to mix, and then centrifuge briefly. Do not allow beads to pellet.
- ☐9 Incubate at 30°C for 30 minutes.
- ☐10 Centrifuge briefly (5–10 seconds).
- ☐11 Place on a magnetic rack for 1 minute.
- ☐12 Remove and discard all supernatant.
- ☐13 Wash 4 times with 200 µl Bead Wash Buffer.
- ☐14 Wash with 200 µl RSB.
- ☐15 Repeat the RSB wash, but do not remove and discard the supernatant until the next step.

A-Tailing

- ☐1 Create the A-tailing reaction mix in a 1.7 ml microcentrifuge tube. For multiple samples, prepare a master mix.

Item	Volume (µl)
A-Tailing Mix	12.5
Water	17.5
Total	30

- ☐2 Remove and discard all supernatant.
- ☐3 Centrifuge briefly.
- ☐4 Place on the magnetic rack.
- ☐5 Using a 10 µl pipette, remove residual supernatant.
- ☐6 Add 30 µl A-tailing reaction mix.
- ☐7 Remove from the magnet rack.
- ☐8 Flick to mix, and then centrifuge briefly. Do not allow beads to pellet.
- ☐9 Incubate at 37°C for 30 minutes.

Ligate Adapters

☐1 Add the following items in the order listed to the tube that contains the A-tailing reaction mix.

Item	Volume (µl)
A-Tailing Reaction/Bead Mix	30
Ligation Mix	2.5
Water	4
DNA Adapter Index	1
Total	37.5

☐2 Flick to mix, and then centrifuge briefly. Do not allow beads to pellet.
☐3 Incubate at 30°C for 10 minutes.
☐4 Add 5 µl Ligation Stop Buffer.
☐5 Centrifuge briefly (5–10 seconds).
☐6 Place on a magnetic rack for 1 minute.
☐7 Remove and discard all supernatant.
☐8 Wash 4 times with 200 µl Bead Wash Buffer.
☐9 Wash with 200 µl RSB.
☐10 Repeat the RSB wash, but do not remove and discard the supernatant until the next step.

Amplify Libraries

☐1 Create the PCR reaction mix in a 1.7 ml microcentrifuge tube. For multiple samples, prepare a master mix.

Item	Volume (µl)
Enhanced PCR Mix	20
PCR Primer Cocktail	5
Water	25
Total	50

☐2 Remove and discard all supernatant.
☐3 Centrifuge briefly.
☐4 Place on a magnetic rack.
☐5 Using a 10 µl pipette, remove residual supernatant.
☐6 Add 50 µl PCR reaction mix. Pipette to mix.
☐7 Transfer to PCR tubes.
☐8 Place on the preprogrammed thermal cycler and run the PCR program.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at -25°C to -15°C for up to 7 days.

Clean Up Libraries

☐1 Place PCR tubes on a magnetic rack for 1 minute.
☐2 Transfer 45 µl supernatant to a 1.7 ml microcentrifuge tube.
☐3 Add 30 µl AMPure XP beads to PCR mix.
☐4 Flick to mix, and then centrifuge briefly.
☐5 Incubate at room temperature for 5 minutes.
☐6 Place a magnetic rack for 5 minutes.
☐7 Remove and discard all supernatant.
☐8 Wash 2 times with 200 µl 70% EtOH.
☐9 Air dry on the magnetic rack for 10–15 minutes.
☐10 Remove from the magnetic rack.
☐11 Add 20 µl RSB. Flick to mix.
☐12 Incubate at room temperature for 5 minutes.
☐13 Place on the magnetic rack for 5 minutes.
☐14 Transfer supernatant to a 1.7 ml microcentrifuge tube.

Check Libraries

- ☐1 Load 1 µl undiluted library on a 7500 or 12000 High Sensitivity DNA chip. The expected library size range is 300–1500 bp, with a concentration of 5–50 nM.
- ☐2 Calculate concentration of library using qPCR or Bioanalyzer analysis.
- ☐3 Normalize libraries to 2 nM by diluting with Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.
- ☐4 Make sure that all libraries have been accurately quantified and normalized to 2 nM.
- ☐5 Combine 10 µl of each library in a 1.7 ml microcentrifuge tube.
- ☐6 Vortex to mix, and then centrifuge briefly.
- ☐7 Proceed to cluster generation and sequencing. To prepare, see the Denature and Dilute Libraries guide for the Illumina sequencing system you are using.

Purify the Tagmentation Reaction [Alternative Procedure]

- ☐1 Incubate tagmentation reaction at 55°C for 30 minutes.
- ☐2 Add 25 µl neutralize tagment buffer. Pipette to mix.
- ☐3 Incubate at room temperature for 5 minutes.
- ☐4 Add 125 µl AMPure XP beads. Flick the tube for 5 seconds.
- ☐5 Incubate at room temperature for 15 minutes. Flick to mix every 2 minutes.
- ☐6 Place a magnetic rack for 5 minutes.
- ☐7 Remove and discard all supernatant.
- ☐8 Wash 2 times with 200 µl 70% EtOH.
- ☐9 Air-dry on the magnetic rack for 10–15 minutes.
- ☐10 Remove from the magnetic rack.
- ☐11 Add 30 µl RSB. Flick to mix.
- ☐12 Incubate at room temperature for 5 minutes.
- ☐13 Place on the magnetic rack for 5 minutes.
- ☐14 Transfer supernatant to a 1.7 ml microcentrifuge tube.
- ☐15 [Optional] Dilute 1 µl DNA with 1 µl water and run on a DNA 12000 LabChip.

Shear Circularized DNA - Nebulizer Procedure [Alternative Procedure]

- ☐1 Remove nebulizer from packaging. Remove blue lid.
- ☐2 Using gloves, remove a piece of tubing from packaging and slip it over the central atomizer tube. Push it to the inner surface of the lid.
- ☐3 Transfer the DNA to the nebulizer.
- ☐4 Add 550 µl nebulization buffer. Pipette to mix.
- ☐5 Attach the blue lid to the nebulizer (finger tight).
- ☐6 Set aside on ice.
- ☐7 Connect the compressed air source to the nebulizer with the tubing. Ensure a tight fit.
- ☐8 Bury the nebulizer in an ice bucket and place in a fume hood.
- ☐9 Make sure that the compressed air is 32 psi.
- ☐10 Nebulize for 6 minutes.
- ☐11 Centrifuge at 450 × g for 2 minutes.
- ☐12 Collect the droplets from the side of the nebulizer.
- ☐13 Measure the recovered volume (~400 µl).
- ☐14 Add 5 volumes (~2000 µl) of Zymo DNA Binding Buffer to tagmentation reaction. Pipette to mix.
- ☐15 Transfer up to 750 µl of mixture to a Zymo-Spin column in a collection tube.
- ☐16 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.
- ☐17 Transfer remaining tagmentation mixture.
- ☐18 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.
- ☐19 Wash 2 times with 200 µl Zymo Wash Buffer.
- ☐20 Add 50 µl RSB.
- ☐21 Incubate at room temperature for 1 minute.
- ☐22 Transfer to a 1.7 ml microcentrifuge tube.
- ☐23 Centrifuge at 10,000–16,000 × g for 30 seconds.
- ☐24 Add 250 µl RSB.