Tagment Genomic DNA

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

Item	Volume (µl)
gDNA	x µl (4 µg)
Water	308–x
Tagment Buffer Mate Pair	80
Mate Pair Tagment Enzyme	12
Total	400

☐2 Flick to mix, and then centrifuge briefly. Repeat.
☐3 Incubate at 55°C for 30 minutes.
☐4 Add 2 volumes of Zymo ChIP DNA Binding Buffer to tagmentation reaction. Pipette to mix.
☐5 Transfer up to 800 µl of mixture to a Zymo-Spin IC-XL column in a collection tube.
☐6 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.
☐7 Transfer remaining tagmentation mixture.
☐8 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.
☐9 Wash 2 times with 200 µl Zymo DNA Wash Buffer.
☐10 Centrifuge the empty column at 10,000–16,000 × g for 1 minute with lid open. Discard the flow-through and the collection tube.
☐11 Transfer column to a 1.7 ml microcentrifuge tube.
☐12 Add 30 µl RSB.
☐13 Incubate at room temperature for 1 minute.
☐14 Centrifuge at 10,000–16,000 × g for 1 minute.
☐15 To assess tagmentation, dilute 1 µl DNA with 7 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	132
10x Strand Displacement Buffer	20
dNTPs	8
Strand Displacement Polymerase	10
Total	200

☐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	200
Water	0
AMPure XP Beads	100
Total	300

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.
☐15 Proceed to *Pippin Prep Size Selection* or *Agarose Size Selection*.

SAFE STOPPING POINT

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

Pippin Prep Size Selection

- ☐1 Load 30 µl AMPure purified DNA on single lane of a Pippin Prep 0.75% agarose cassette.
- ☐2 Use the Pippin Prep Protocol range mode to define the start and end of the desired sample elution size.
- ☐3 Run for the maximum run time allowed for the cassette.
- ☐4 Transfer the elution to a new 1.7 ml microcentrifuge tube.
- ☐5 Add 5 volumes of ChIP Binding Buffer to DNA. Pipette to mix.
- ☐6 Transfer 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 Wash 2 times with 200 µl Zymo DNA Wash Buffer.
- ☐9 Discard the collection tube.
- ☐10 Transfer the column to a 1.7 ml microcentrifuge tube.
- ☐11 Add 10 µl RSB.
- ☐12 Incubate at room temperature for 1 minute.
- ☐13 Centrifuge at 10,000–16,000 × g for 30 seconds.
- ☐14 [Optional] Run 1 µl undiluted elution on a DNA 12000 LabChip.

SAFE STOPPING POINT

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

Agarose Size Selection

- ☐1 Add 6 µl 6X Loading Dye to 30 µl DNA.
- ☐2 Load over 2 consecutive lanes of the gel. Pipette 18 µl per well.
- ☐3 Load 20 µl diluted prepared 1 kb plus ladder into the lanes on either side of the sample lanes.
- ☐4 Run the gel at 100 V (constant voltage) for 120 minutes.
- ☐5 View the gel on a Dark Reader transilluminator.
- ☐6 Use a new scalpel blade and the 1 kb plus DNA ladder as a size guide to excise DNA fractions containing the desired fragment sizes.
- ☐7 Transfer agarose gel fraction to a new 3.5 ml screw cap tube.
- ☐8 Add 3 volumes of Zymo ADB to each volume of agarose excised from the gel.
- ☐9 Incubate at 50°C until the gel is dissolved (~10–15 minutes). Invert every 2 minutes to mix.
- ☐10 Transfer up to 800 µl melted agarose solution. Distribute evenly.
- ☐11 Centrifuge at 10,000–16,000 × g for 1 minute. Discard the flow-through.
- ☐12 Transfer remaining melted agarose.
- ☐13 Centrifuge at 10,000–16,000 × g for 1 minute. Discard the flow-through.
- ☐14 Wash 2 times with 200 µl Zymo DNA Wash Buffer.
- ☐15 Centrifuge the empty columns at 10,000–16,000 × g for 1 minute with the lid open.
- ☐16 Remove residual EtOH.
- ☐17 Discard the flow-through and the collection tube.
- ☐18 Transfer the columns to 1.7 ml microcentrifuge tubes.
- ☐19 Add 30 µl RSB to each column.
- ☐20 Incubate at room temperature for 1 minute.
- ☐21 Centrifuge at 10,000–16,000 × g for 1 minute.
- ☐22 Combine elutions from the 2 matching columns.
- ☐23 Run 1 µl undiluted elution on a DNA 12000 LabChip.

SAFE STOPPING POINT

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

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 High Sensitivity DNA chip. The expected library size range is 300–1500 bp, with a concentration of 1.5–20 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.