Tagment Genomic DNA

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gDNA</td>
<td>x µl (4 µg)</td>
</tr>
<tr>
<td>Water</td>
<td>308–x</td>
</tr>
<tr>
<td>Tagment Buffer Mate Pair</td>
<td>80</td>
</tr>
<tr>
<td>Mate Pair Tagment Enzyme</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
</tr>
</tbody>
</table>

- Flick to mix, and then centrifuge briefly. Repeat.
- Incubate at 55°C for 30 minutes.
- Add 2 volumes of Zymo ChIP DNA Binding Buffer to tagmentation reaction. Pipette to mix.
- Transfer up to 800 µl of mixture to a Zymo-Spin IC-XL column in a collection tube.
- Centrifuge at 10,000–16,000 x g for 30 seconds. Discard the flow-through.
- Transfer remaining tagmentation mixture.
- Centrifuge at 10,000–16,000 x g for 30 seconds. Discard the flow-through.
- Wash 2 times with 200 µl Zymo DNA Wash Buffer.
- Centrifuge the empty column at 10,000–16,000 x g for 1 minute with lid open. Discard the flow-through and the collection tube.
- Transfer column to a 1.7 ml microcentrifuge tube.
- Add 30 µl RSB.
- Incubate at room temperature for 1 minute.
- Centrifuge at 10,000–16,000 x g for 1 minute.
- 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

- Add the following items in the order listed to the microcentrifuge tube.

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tagmented DNA Sample</td>
<td>30</td>
</tr>
<tr>
<td>Water</td>
<td>132</td>
</tr>
<tr>
<td>10x Strand Displacement Buffer</td>
<td>20</td>
</tr>
<tr>
<td>dNTPs</td>
<td>8</td>
</tr>
<tr>
<td>Strand Displacement Polymerase</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
</tr>
</tbody>
</table>

- Flick to mix, and then centrifuge briefly. Incubate at 20°C for 30 minutes.

Purify the DNA

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strand Displaced DNA</td>
<td>200</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
</tr>
<tr>
<td>AMPure XP Beads</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
</tr>
</tbody>
</table>

- Flick to mix, and then centrifuge briefly.
- Incubate at room temperature for 15 minutes. Flick every 2 minutes.
- Centrifuge briefly.
- Place on a magnetic rack for 5 minutes.
- Remove and discard all supernatant.
- Wash 2 times with 400 µl 70% EtOH.
- Air-dry on the magnetic rack for 10–15 minutes.
- Remove from the magnetic rack.
- Add 30 µl RSB. Flick to mix.
- Centrifuge briefly.
- Incubate at room temperature for 5 minutes.
- Place on the magnetic rack for 5 minutes.
- Transfer all supernatant to a 1.7 ml microcentrifuge tube.
- 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMPure Purified or Size Selected DNA</td>
<td>x µl (up to 600 ng)</td>
</tr>
<tr>
<td>Water</td>
<td>268–x</td>
</tr>
<tr>
<td>Circularization Buffer 10x</td>
<td>30</td>
</tr>
<tr>
<td>Circularization Ligase</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Settings</th>
<th>S2</th>
<th>S220</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Power Intensity</td>
<td>--</td>
<td>240</td>
</tr>
<tr>
<td>Intensity</td>
<td>8</td>
<td>--</td>
</tr>
<tr>
<td>Duty Cycle/Duty Factor</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>Cycles Per Burst</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Time</td>
<td>40 seconds</td>
<td>40 seconds</td>
</tr>
<tr>
<td>Temperature</td>
<td>6°C</td>
<td>6°C</td>
</tr>
</tbody>
</table>

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.
☐ 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>End Repair Mix</td>
<td>40</td>
</tr>
<tr>
<td>Water</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

☐ 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.
☐ 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Tailing Mix</td>
<td>12.5</td>
</tr>
<tr>
<td>Water</td>
<td>17.5</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Tailing Reaction/Bead Mix</td>
<td>30</td>
</tr>
<tr>
<td>Ligation Mix</td>
<td>2.5</td>
</tr>
<tr>
<td>Water</td>
<td>4</td>
</tr>
<tr>
<td>DNA Adapter Index</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>37.5</strong></td>
</tr>
</tbody>
</table>

- **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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced PCR Mix</td>
<td>20</td>
</tr>
<tr>
<td>PCR Primer Cocktail</td>
<td>5</td>
</tr>
<tr>
<td>Water</td>
<td>25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
</tr>
</tbody>
</table>

- **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.

**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.

**SAFE STOPPING POINT**

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