Dovetail® Omni-C® Kit

USER GUIDE

VERSION 2.1

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Dovetail® Omni-C® Kit Components & Storage

Each kit contains a sufficient supply of materials to perform 8 reactions. The Omni-C® Kit comes as two boxes. Store the boxes as listed below immediately upon receipt.

Table 1. Dovetail® Proximity Ligation Core Box 1 (PN DG-REF-001)

Components	Storage
10X Wash Buffer	2°C to 8°C
NWB Solution	
Chromatin Capture Beads	
Streptavidin Beads	
20% SDS	
Crosslink Reversal Buffer	

Table 2. Dovetail® Omni-C® Module Box 2 (PN DG-REF-002)

Components	Storage
Nuclease Enzyme Mix	-30°C to -10°C
10X Nuclease Digest Buffer	
100 mM $MnCl_2$	
0.5 M EDTA	
End Polishing Enzyme Mix	
End Polishing Buffer	
5X Bridge Ligation Buffer	
Bridge Ligase	
Bridge	
Intra-Aggregate Ligation Enzyme Mix	
Intra-Aggregate Ligation Buffer	
Proteinase K	
HotStart PCR Ready Mix	

Optional Add-on Modules: Components and Storage

Dovetail® Primer Module Set 1

Table 3. Dovetail® Primer Module Set 1 (PN DG-XG-PM01)

Components	Storage
DG i5 Index 1	-30°C to -10°C
DG i5 Index 2	
DG i5 Index 3	
DG i5 Index 4	
DG i7 Index 1	
DG i7 Index 2	
DG i7 Index 3	
DG i7 Index 4	
DG i7 Index 5	
DG i7 Index 6	

Dovetail® Library Module

Table 4. Dovetail® Library Module (PN DG-LM01)

Components	Storage
End Repair Enzyme Buffer	-30°C to -10°C
End Repair Enzyme Mix	
Ligation Buffer	
Ligation Enzyme Mix	
Stubby Adaptor	

Dovetail® Filter Set

Table 5. Dovetail® Filter Set Module (PNDG-HiC-005)

Components	Storage
50 µm Filters	Room Temperature
200 µm Filters	

User Supplied Reagents, Consumables and Equipment

Reagents

Table 6. Reagents

Reagents	Supplier	Part Number
SPRIselect® Beads, 5 mL	Beckman Coulter	B23317
37% Formaldehyde Solution	Sigma-Aldrich	F8775
1X PBS, pH 7.4, 500 mL	Thermo Fisher Scientific	10010023
100% EtOH	Generic	N/A
UltraPure™ DNase / RNase-Free Distilled Water, 500 mL	Thermo Fisher Scientific	10977015
TE pH 8.0	Thermo Fisher Scientific	AM9849
DSG (Disuccinimidyl Glutarate)	Thermo Fisher Scientific	A35392
DMSO (Dimethyl Sulfoxide, Anhydrous ≥ 99.99%)	Sigma-Aldrich	276855-100ML
SepMate™-15*	Stemcell Technologies	85415
Lymphoprep™*	Stemcell Technologies	07801
10X RBC Lysis Buffer**	Thermo Fisher Scientific	00-4300
CryoStor®***	BioLife Solutions	210373

*only needed if working with Fresh Mammalian Whole Blood (≤ 24 hours post collection)
**only needed if working with Fresh Mammalian Whole Blood (24-72 hours post collection)
*** optional for cryopreservation of PBMCs

Consumables and Equipment

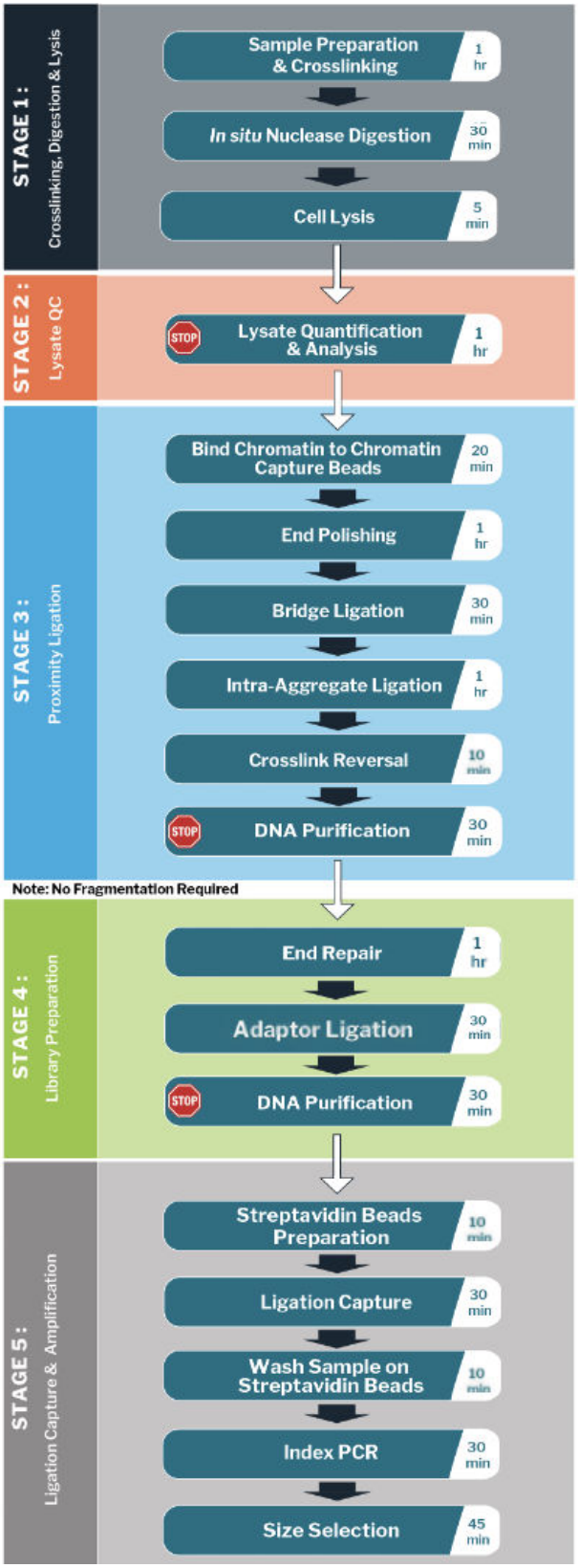
Table 7. Consumables and Equipment

Consumables/Equipment	Supplier	Part Number
1.5 mL Low binding microcentrifuge tubes	Generic	
0.2 mL PCR tubes		
5.0 mL centrifuge tubes		
Pipets and pipet tips		
Magnetic separation rack for 0.2 mL and 1.5 mL tubes		
Agitating thermal mixer		
Thermal cycler		
Vortex mixer		
Swinging Bucket Rotor		
Centrifuge for 0.2 mL, 1.5 mL and 5 mL tubes		
Hemocytometer		
Qubit® Fluorometer	Thermo Fisher Scientific	Q33226
Qubit® dsDNA HS Assay Kit	Thermo Fisher Scientific	Q32854
Qubit® Assay Tubes	Thermo Fisher Scientific	Q32856
TapeStation System (Fragment Analyzer or Bioanalyzer)	Agilent	Various
MiniStrainer 70 µm mesh	PluriSelect	43-10070-46

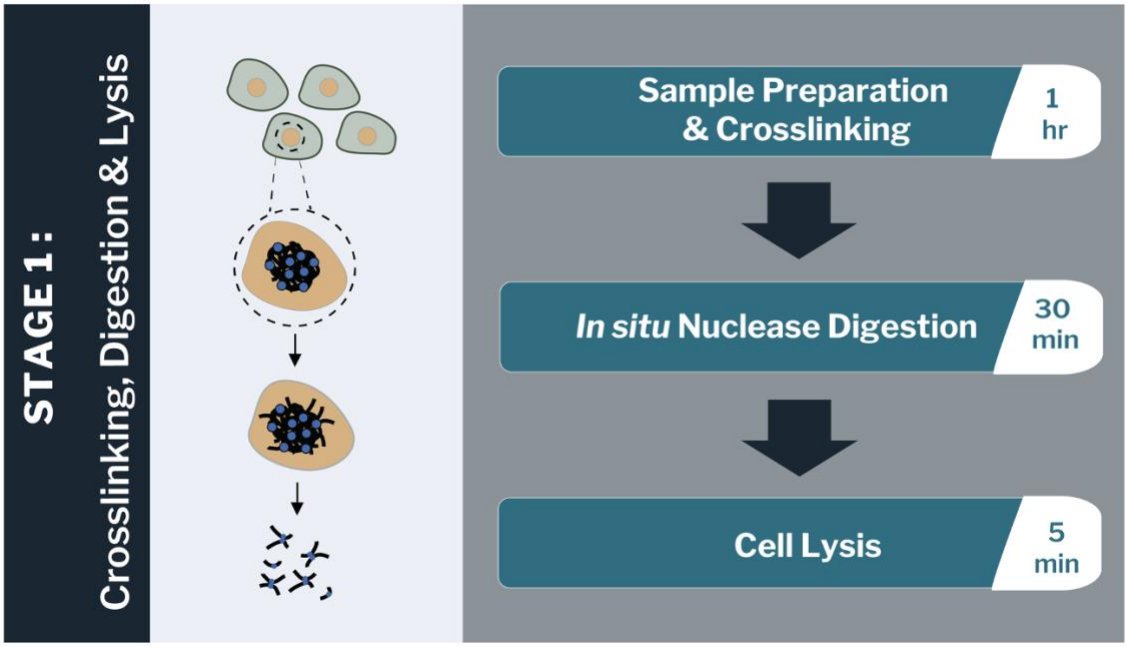
Good Practices

- Read the entire guide before use, including Before You Begin and the Notes.
- The cell input amount will influence the efficiency of the digestion reaction. To ensure an accurate cell count, use best practices such as low-speed spins (< 500 x g) using a swinging bucket rotor when harvesting the cells, and counting prior to freezing.
- To ensure efficient crosslinking, a new or recently opened solution of formaldehyde should be used. Formaldehyde solution containing white precipitates should not be used.
- Keep all enzymes and master mixes on ice during setup and use. Promptly move reagents back to the indicated storage.
- Fully thaw buffers, place on ice and thoroughly mix before use.
- When preparing master mixes, scale the volume of each reagent as appropriate, using 10% excess volume to compensate for pipetting loss.
- Always add the reagents to the master mix in the specified order as listed throughout the protocol.
- When working with beads, such as Chromatin Capture, SPRIselect and Streptavidin beads, you should:
    - Equilibrate the beads to room temperature before use.
    - Thoroughly vortex the beads immediately before use and ensure they are a homogenous slurry before use.
    - When placing the tube in the magnetic rack, always wait until the solution looks clear to allow the beads to fully separate before removing the supernatant carefully and slowly. This helps minimize bead/sample loss throughout the protocol.
    - Do not let the beads dry out during washing steps. Keep the beads in buffer until ready to resuspend them for the next step.
    - After washing the SPRIselect beads with 80% ethanol during DNA purification, do not let the beads over-dry before proceeding with elution. Over-drying the beads may result in lower recovery of DNA.

Dovetail® Omni-C® Protocol Overview



Stage 1: Sample Preparation



Before You Begin (applicable for all protocols/sample types)

- There are separate protocols for sample preparation depending on your sample type: mammalian cells, animal tissues, PBMCs, fresh mammalian whole blood, nucleated non-mammalian blood, and plants.
- The 10X Wash Buffer and 20% SDS might have precipitated in storage. Incubate these solutions at 50°C for 15 minutes or until the precipitate is no longer visible. Vortex to mix prior to use.
- Dilute 10X Wash Buffer to 1X with UltraPure™ Water. Store at room temperature. You will need ~15 mL of 1X Wash Buffer per sample. 1X Wash Buffer is stable at room temperature for 2 months.
- Prepare 0.3 M DSG in DMSO (anhydrous) by dissolving 1 mg of DSG in 10.22 µL DMSO. DSG is water-insoluble and moisture- sensitive. Prepare immediately before use. Do not store DSG in solution.
- Prepare fresh 1X Nuclease Digest Buffer and store at room temperature. 1X Nuclease Digest Buffer is stable for 1 day at room temperature. You need 50 µL Nuclease Digest Buffer per sample. To prepare 1X Nuclease Digest Buffer (50 µL), mix the following components:

Reagent	**Volume for 50 µL**
10X Nuclease Digest Buffer	5 µL
100 mM $MnCl_2$	5 µL
UltraPure Water	40 µL

- Set the thermal mixer at 30°C, shaking at 1,250 rpm.
- Thaw 0.5 M EDTA at room temperature. Vortex to mix prior to use.

A. Mammalian Cells

As you prepare for Stage 1, keep the following in mind:

- Sample preparation takes ~1.5 hours.
- Refer to the low input protocol in Appendix 1 if you have less than 1 x 10^6 cells available.
- For 1 x 10^6 MEF and fibroblast cells, we recommend using 4 µL undiluted Nuclease Enzyme Mix. For other cell lines, please follow the instructions below.
- All crosslinking reactions (steps 5 – 11) should be carried out at room temperature.
- Treat the cells **gently** throughout all the pipetting steps.

Follow the steps below for Sample Preparation:

1. Harvest cells, wash in 1X PBS and count.
2. Aliquot 1 x 10^6 cells into a 1.5 mL tube.
3. Spin the 1 x 10^6 cell aliquot at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant. The pellet might be loose.
4. Freeze the cell pellet by placing it at -80°C for at least 30 minutes.
5. Thaw your cell pellet at room temperature then resuspend the pellet in:
   - 200 µL 1X PBS
   - 2 µL 0.3 M DSG
6. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
7. Add 5.4 µL of 37% formaldehyde.
8. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
9. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant. Use caution as the pellet might be loose.
10. Wash the pellet with 200 µL of 1X Wash Buffer, pipet up and down to break up clumps and fully resuspend the pellet.
11. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant.
12. Resuspend the pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Getting Started).
13. Pre-warm the tube containing your resuspended cells to 30°C for 2 minutes in an agitating thermal mixer set at 1,250 rpm.
14. Transfer 0.5 µL of Nuclease Enzyme Mix to the pre-warmed tube. Pipet up and down to mix.
15. Incubate the tube for **exactly** 30 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
16. Stop the reaction by adding 5 µL of 0.5 M EDTA. Pipet to mix.
17. Add 3 µL of 20% SDS to lyse the cells. Pipet to mix.
18. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
19. Continue to Stage 2: Lysate QC.

B. Mammalian and Non-mammalian Animal Tissues

As you prepare for Stage 1, keep the following in mind:

- We recommend using 20 mg of tissue as starting material. If this amount of tissue sample is not available, please refer to the low input protocol in Appendix 1. The low input protocol is only applicable for **mammalian** tissue samples.
- All crosslinking reactions (steps 3 – 10) should be carried out at room temperature.

Follow the steps below for Sample Preparation:

1. Weigh out 20 mg of frozen tissue sample.
2. Disrupt the tissue by grinding it to a **fine powder** with a mortar and pestle in a **liquid nitrogen bath**.
3. Transfer the disrupted tissue sample to a 1.5 mL tube containing:
   - 1 mL 1X PBS
   - 10 µL 0.3 M DSG
4. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
5. Add 27 µL of 37% formaldehyde.
6. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
7. Spin the tube at 2,500 x g for 5 minutes. Carefully remove the supernatant. Use caution as the pellet might be loose.
8. Wash the pellet with a total of 1 mL 1X Wash buffer: first add 200 µL of Wash Buffer and pipet to break up clumps then add the remaining 800 µL. Pipet up and down to fully resuspend the pellet.
9. Spin the tube at 2,500 *x g* for 5 minutes. Carefully remove the supernatant.
10. Resuspend the pellet in 1 mL 1X Wash Buffer. Pipet up and down to fully resuspend.
11. Using a 1 mL syringe, gently push the 1 mL of resuspended sample through a 200 µm filter into a new 5 mL tube. If the filter clogs, replace with a new 200 µm filter and continue until all of the sample has been filtered.
12. Gently pass an additional 1 mL of 1X Wash Buffer though the 200 µm filter into the 5 mL tube. Your tube should now contain a total volume of ~2 mL.
13. Using the same syringe but changing to a 50 µm filter, re-filter the 2 mL sample into a new 5 mL tube.
14. Gently pass an additional 1 mL of 1X Wash Buffer though the 50 µm filter into the 5 mL tube. Your tube should now contain a total volume of ~3 mL.
15. Spin the tube at 2,500 x g for 5 minutes. Carefully remove the supernatant.
16. Resuspend the pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin).
17. Pre-warm the tube containing your resuspended cells to 30°C for 2 minutes in an agitating thermal mixer set at 1,250 rpm.
18. Transfer 0.5 µL of Nuclease Enzyme Mix to the pre-warmed tube. Pipet up and down to mix.
19. Incubate the tube for **exactly** 30 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
20. Stop the reaction by adding 5 µL of 0.5 M EDTA. Pipet to mix.
21. Add 3 µL of 20% SDS to lyse the cells. Pipet to mix.
22. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
23. Continue to Stage 2: Lysate QC.

C. Cryopreserved Peripheral Blood Mononuclear Cells (PBMCs)

As you prepare for Stage 1, keep the following in mind:

- Sample preparation takes ~ 1.5 hours.
- It is essential to work quickly and limit handling of the PBMCs once they are thawed.

Before You Begin

- **Warm 10mL of 1X Wash Buffer to 37°C for 15 minutes in a 15 mL tube.**

Follow the steps below for Sample Preparation:

1. Quickly thaw cryopreserved PBMCs stock in a 37°C water bath for approximately 2-5 minutes.
2. Transfer the thawed PBMC mix to the tube containing the 10 mL of pre-warmed 1X Wash buffer (see Before You Begin). Take care to pipet gently.
3. Spin the cells at 500 x g for 5 minutes in a swinging bucket rotor. Discard the supernatant.
4. Resuspend the pellet with 200 µL of 1X PBS, pipet up and down gently to break up clumps and resuspend the pellet.

NOTE If **cell clumping** is observed at this stage, filter the sample through the **MiniStrainer** . Place a MiniStrainer in a 1.5 mL microfuge tube. Pipet the cell mixture into the MiniStrainer. Quick spin at 500 x g for 5 seconds. The cell aggregates should be retained in the filter. The PBMCs should be in single-cell suspension in the tube.

5. Take an aliquot to count the cells. Keep the remaining cells on ice until the cells are counted. **Count quickly, this is a delicate sample.**

NOTES

- 1 x 10^6 cells are recommended per Omni-C reaction.
- Remaining cells can be cryopreserved in Cryostor® or DMSO and FBS (see Appendix 4).

6. Gently, pipette mix the PBMCs on ice. Transfer a volume equivalent to 1 x 10^6 cells to a new 1.5 mL tube.
7. Bring the volume up to 200 µL with 1X PBS, if needed.

NOTE All crosslinking reactions (steps 8 – 14) should be carried out at room temperature.

8. Add 2 µL 0.3 M DSG. Pipet to mix.
9. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
10. Add 5.4 µL of 37% formaldehyde.
11. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
12. Spin the tube at 500 x g in a swinging bucket rotor for 5 minutes. Carefully remove and discard the supernatant. Use caution, the pellet might be loose.
13. Wash the pellet with 200 µL of 1X Wash Buffer, pipet up and down to break up clumps and fully resuspend the pellet.
14. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove and discard the supernatant. Use caution, the pellet might be loose.
15. Resuspend the cell pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin).
16. Add 0.5 µL of Nuclease Enzyme Mix. Pipet up and down to fully mix.

17. Incubate the tube at 30°C for **exactly** 30 minutes in an agitating thermal mixer set at 1,250 rpm. If you are working with a large number of samples, stagger the start of the digestion for each sample by 20 seconds then stop after corresponding 30 minutes.
18. Stop the reaction by adding 5 µL of 0.5 M EDTA. Pipet up and down to fully mix.
19. Add 3 µL of 20% SDS to lyse the cells. Pipet to mix.
20. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
21. Continue to Stage 2: Lysate QC.

D. Fresh Mammalian Whole Blood (≤ 24 hours post collection)

As you prepare for Stage 1, keep the following in mind:

- Sample preparation takes ~2 hours.
- This protocol is for isolation of Peripheral Blood Mononuclear Cells (PBMCs) from mammalian whole blood ≤ 24 hours post collection.
- Fresh blood should be shipped and/or stored at **ambient temperature**. Blood samples processed within 24 hours of collection yield higher quality and quantity of PBMCs.
- If you are processing a blood sample between 24-72 hours of collection, please refer to protocol E below, as SepMate™ works best on fresh samples. We do not recommend use of blood processed >72 hours post collection.
- SepMate™-15 is designed to process 0.5 to 5 mL of blood samples. Please follow manufactures guidelines for volume recommendation.
- Typically, 0.5 to 3 x 10^6 PBMCs are isolated from 1 mL of healthy whole blood.
- It is essential to work quickly and limit handling of the PBMCs once they are isolated from whole blood.

Before You Begin

- Warm Lymphoprep™ to room temperature (15 - 25 °C) before use.
- The 1X PBS solution should be at room temperature.

Follow the steps below for Sample Preparation:

1. Place a SepMate™ tube in tube rack and keep it vertical.
2. Mix the Lymphoprep™ reagent thoroughly by inverting the bottle several times before use.
3. Add 4.5 mL of Lymphoprep™ to the SepMate™ tube by carefully pipetting it through the center hole of the column insert. The top of the Lymphoprep™ reagent will be above the insert.
4. Mix the blood sample gently by pipetting. Transfer 3 mL of blood to a new 15 mL tube.
5. Dilute the blood with 3 mL of 1X PBS. Mix gently by pipetting up and down.
6. Add the diluted blood sample to the SepMate™ tube by pipetting it down the side of the tube. Take care not to pipet the sample directly through the central hole.
7. Centrifuge at 1,200 x g for 20 minutes at room temperature, with the brake on.
8. Pour the top layer containing the PBMCs into a new 15 mL tube.

> NOTE Do not hold the tube in the inverted position for longer than 2 seconds. Some amount of red blood cells may be present in the transfer. These RBCs will not affect the Omni-C assay.

9. Add equal volume of 1X PBS to dilute the enriched PBMCs. Invert to mix.
10. Centrifuge the PBMC containing tube at 500 x g for 5 minutes. Discard the supernatant.
11. Resuspend the pellet in 200 µL of 1X Wash Buffer, pipet up and down gently to break up clumps and resuspend the pellet.
12. Centrifuge at 500 x g for 5 minutes. Carefully discard the supernatant.
13. Resuspend the pellet with 200 µL of 1X PBS, pipet up and down gently to break up clumps and resuspend the pellet.

> NOTE **If cell clumping is observed at this stage, filter the sample through the MiniStrainer.** Place a MiniStrainer in a 1.5 mL microfuge tube. Pipet the cell mixture into the MiniStrainer. Quick spin at 500 x g for 5 seconds. The cell aggregates should be retained in the filter. The PBMCs should be in single-cell suspension in the tube.

14. Take an aliquot to count the cells. Keep the remaining cells on ice until cells are counted. **Count quickly, this is a delicate sample.**

> NOTES:
>
> - 1 x 10^6 cells are recommended per Omni-C reaction.
> - Remaining cells can be cryopreserved in CryoStor® or DMSO and FBS (see Appendix 4)

15. Gently, pipette mix the PBMCs on ice. Transfer 1 x 10^6 cells into a new 1.5 mL tube.
16. Bring the volume up to 200 µL of 1X PBS, if needed.

> NOTE All crosslinking reactions (steps 17 – 23) should be carried out at room temperature.

17. Add 2 µL of 0.3 M DSG.
18. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
19. Add 5.4 µL of 37% formaldehyde.
20. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
21. Spin the tube at 500 x g in a swinging bucket rotor for 5 minutes. Carefully remove and discard the supernatant. Use caution, the pellet might be loose.
22. Wash the pellet with 200 µL of 1X Wash Buffer, pipet up and down to break up clumps and fully resuspend the pellet.
23. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove and discard the supernatant. Use caution, the pellet might be loose.
24. Resuspend the cell pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin).
25. Add 0.5 µL of Nuclease Enzyme Mix. Pipet up and down to fully mix.
26. Incubate the tube at 30°C for exactly 30 minutes in an agitating thermal mixer set at 1,250 rpm. If you are working with a large number of samples, stagger the start of the digestion for each sample by 20 seconds then stop after corresponding 30 minutes.
27. Stop the reaction by adding 5 µL of 0.5 M EDTA. Pipet to mix.
28. Add 3 µL of 20% SDS to lyse the cells. Pipet to mix.
29. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
30. Continue to Stage 2: Lysate QC.

E. Fresh Mammalian Whole Blood (24-72 hours post collection)

As you prepare for Stage 1, keep the following in mind:

- Sample preparation takes ~ 2 hours.
- This protocol is for isolation of Peripheral Blood Mononuclear Cells (PBMCs) from mammalian whole blood 24 - 72 hours post collection.
- Fresh blood should be shipped and/or stored at **ambient temperature**. Blood samples processed within 24 hours of collection yield quality and quantity of PBMCs.
- We do not recommend use of blood processed >72 hours post collection.
- The 10X RBC Lysis Buffer contains ammonium chloride which is optimal for lysis of erythrocytes with minimal effect on lymphocytes.
- Typically, 0.5 to 3 x 10^6 PBMCs are isolated from 1 mL of healthy whole blood.
- It is essential to work quickly and limit handling of the PBMCs once they are isolated from whole blood.

Before You Begin

- Warm 10X RBC Lysis Buffer to room temperature (15 - 25 °C) before use.
- The 1X PBS solution should be at room temperature.

Follow the steps below for Sample Preparation:

1. In a 50 mL tube, prepare 30 mL of 1X RBC Lysis Buffer by adding 3mL of 10X RBC lysis buffer to room temperature 27 mL of molecular grade water. Mix by inverting.
2. Mix the blood sample gently by pipetting. Transfer 3 mL of blood to the 50 mL centrifuge tube containing 30 mL of 1X RBC Lysis Buffer. Mix by inverting.
3. Incubate for 10-15 minutes at room temperature (no more than 15 minutes).
4. Spin the cells at 500 x g for 5 minutes. Discard the supernatant.
5. Resuspend the pellet in 200 µL of 1X Wash Buffer, pipet up and down to break up clumps and resuspend the pellet.
6. Centrifuge at 500 x g for 5 minutes. Carefully discard the supernatant.
7. Resuspend the pellet with 200 µL of 1X PBS, pipet up and down gently to break up clumps and resuspend the pellet.

NOTE **If cell clumping is observed at this stage, filter the sample through the MiniStrainer.** Place a MiniStrainer in a 1.5 mL microfuge tube. Pipet the cell mixture into the MiniStrainer. Quick spin at 500 x g for 5 seconds. The cell aggregates should be retained in the filter. The PBMCs should be in single-cell suspension in the tube.

8. Take an aliquot to count the cells. Keep the remaining cells on ice until cells are counted. **Count quickly, this is a delicate sample.**

NOTES:

- 1 x 10^6 cells are recommended per Omni-C reaction.
- Remaining cells can be cryopreserved in CryoStor® or DMSO and FBS (see Appendix 4)

9. Gently, pipette mix the PBMCs on ice. Transfer 1 x 10^6 cells to a new 1.5mL tube.
10. Bring the volume up to 200 µL with 1X PBS, if needed.

> N O T E All crosslinking reactions (steps 11 – 17) should be carried out at room temperature.

11. Add 2 µL of 0.3 M DSG.
12. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
13. Add 5.4 µL of 37% formaldehyde.
14. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
15. Spin the tube at 500 x g in a swinging bucket rotor for 5 minutes. Carefully remove and discard the supernatant. Use caution, the pellet might be loose.
16. Wash the pellet with 200 µL of 1X Wash Buffer, pipet up and down to break up clumps and fully resuspend the pellet.
17. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove and discard the supernatant. Use caution, the pellet might be loose.
18. Resuspend the cell pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin).
19. Add 0.5 µL of Nuclease Enzyme Mix. Pipet up and down to fully mix.
20. Incubate the tube at 30°C for exactly 30 minutes in an agitating thermal mixer set at 1,250 rpm. If you are working with a large number of samples, stagger the start of the digestion for each sample by 20 seconds then stop after corresponding 30 minutes.
21. Stop the reaction by adding 5 µL of 0.5 M EDTA. Pipet to mix.
22. Add 3 µL of 20% SDS to lyse the cells. Pipet to mix.
23. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
24. Continue to Stage 2: Lysate QC.

F. Nucleated Blood

As you prepare for Stage 1, keep the following in mind:

- The sample preparation takes ~1.5 hours.
- This protocol for processing nucleated blood sample calls for 10 µL of whole blood as input. For each sample preparation, 2 µL of whole blood will be used; the remainder can be frozen for future assays.
- Blood samples should be collected in tubes containing EDTA. Avoid using nucleated blood samples that are coagulated.
- Good library yields were achieved with ~1 x 10^5 cells. However, counting nucleated blood cells can be tricky due to their small size and other debris in the whole blood.
- All crosslinking reactions (steps 3 – 12) should be carried out at room temperature.
- If using frozen blood, quick thaw the sample at 37°C, mix well and check for coagulation prior to starting.

Follow the steps below for Sample Preparation:

1. Add 10 µL of blood into 200 µL of 1X PBS in a 1.5 mL tube. Pipet to mix.
2. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant.
3. Resuspend the pellet in:
    - 200 µL 1X PBS
    - 2 µL 0.3 M DSG
4. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
5. Add 5.4 µL of 37% formaldehyde.
6. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
7. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant. Use caution as the pellet might be loose.
8. Wash the pellet with a total of 200 µL 1X Wash Buffer. Pipet up and down to fully resuspend the pellet.
9. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant.
10. Resuspend the pellet in 1 mL 1X Wash Buffer: first add 200 µL of Wash Buffer and pipet to break up clumps, then add the remaining 800 µL. Pipet up and down to fully resuspend the pellet.
11. Transfer 200 µL of the resuspended pellet to a new 1.5 mL tube – this will be the working sample. The remaining 800 µL of cross-linked sample can be pelleted and frozen for future use.
12. Spin your sample at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant.
13. Resuspend the pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Getting Started).
14. Pre-warm the tube containing your resuspended cells at 30°C for 2 minutes in an agitating thermal mixer set at 1,250 rpm.
15. Transfer 0.5 µL of Nuclease Enzyme Mix to the pre-warmed tube. Pipet up and down to mix.
16. Incubate the tube for **exactly** 30 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
17. Stop the reaction by adding 5 µL of 0.5 M EDTA. Pipet to mix.
18. Add 3 µL of 20% SDS to lyse the cells. Pipet to mix.
19. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
20. Continue to Stage 2: Lysate QC.

G. Plants

As you prepare for Stage 1, keep the following in mind:

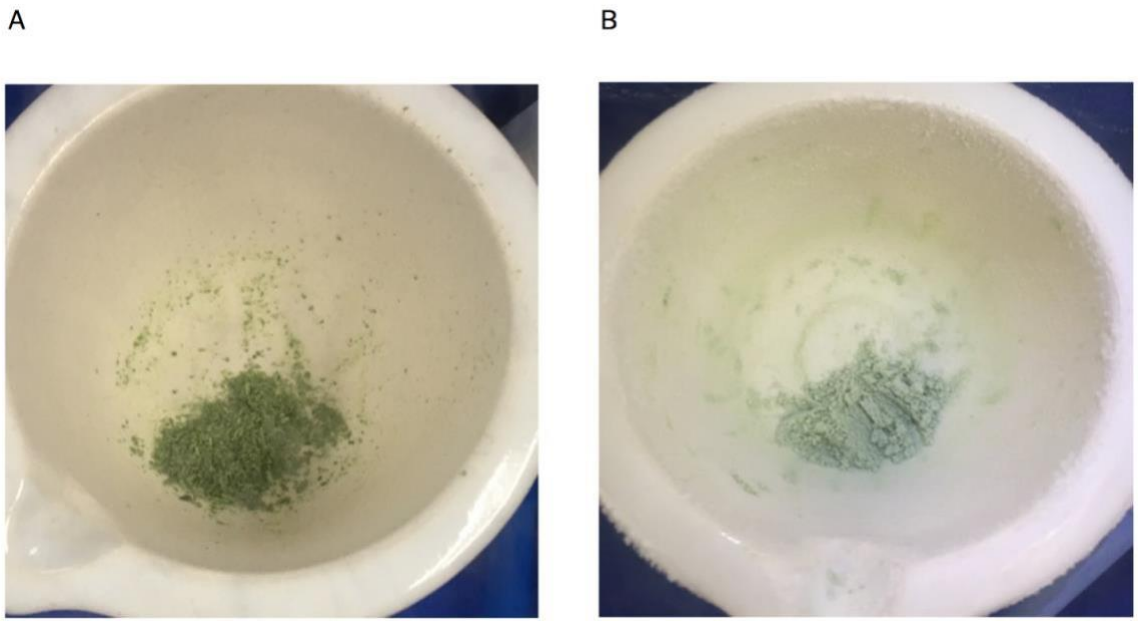
- The sample preparation takes ~2 hours.
- This protocol for processing plant leaves requires 300 mg of frozen leaves for each titration. Fresh-frozen dark-treated young leaves are optimal.
- Flash freeze harvested leaves immediately in liquid nitrogen and store at -80°C until use.
- All cross-linking reactions (steps 3 – 10) should be carried out at room temperature.

Follow the steps below for Sample Preparation:

1. Weigh out 300 mg of frozen leaves.
2. Disrupt the leaves by grinding them into a fine powder with a mortar and pestle in a liquid nitrogen bath (see example of desired consistency below).
3. Transfer the ground sample to a 5 mL tube which contains:
    - 4 mL 1X PBS
    - 40 µL 0.3 M DSG
4. Rotate the tube for 10 minutes at room temperature. The tissue sample should not settle.
5. Add 108 µL of 37% Formaldehyde.
6. Rotate the tube for 10 minutes at room temperature. The tissue sample should not settle.
7. Spin at 5,000 x g for 5 minutes. Carefully remove the supernatant. If your tissue sample did not pellet, repeat the spin at maximum speed.
8. Wash the pellet with a total of 2 mL 1X Wash Buffer: first add 500 µL of Wash Buffer and pipet to break up clumps, then add the remaining 1,500 µL. Vortex to fully resuspend the pellet.
9. Spin the tube at 5,000 x g for 5 minutes. Carefully remove the supernatant.
10. Repeat steps 8 and 9 once for a total of two washes.
11. After removing the second wash, resuspend the pellet in 1 mL 1X Wash Buffer. Vortex to fully resuspend.
12. Using an attachable 1 mL syringe, gently push the 1 mL resuspended sample through a 200 µm filter into a new 5 mL tube. If the filter clogs, replace with a new 200 µm filter and continue until all the sample has been filtered.
13. Gently pass an additional 1 mL 1X Wash Buffer through the 200 µm filter into the 5 mL tube. Your tube should now contain a total volume of ~2 mL.
14. Using the same syringe but changing to a 50 µm filter, re-filter the 2 mL sample into a new 5 mL tube.
15. Gently pass an additional 1 mL 1X Wash Buffer through the 50 µm filter into the 5 mL tube. Your tube should now contain a total volume of ~3 mL.
16. Resuspend well and aliquot evenly into 3 separate tubes (A, B, and C); each corresponds to 100 mg of input plant tissue.
17. Spin tubes A, B and C at 2,000 x g for 5 minutes. Carefully remove the supernatant.
18. Resuspend the pellet in tubes A, B and C in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin). Leave the tubes on the bench.
19. Meanwhile, prepare serial dilutions of the Nuclease Enzyme Mix as follows:
    Nucl A: 1 µL of Nuclease Enzyme Mix in 9 µL of 1X Nuclease Digest Buffer
    Nucl B: 2 µL of Nucl A dilution in 2 µL of 1X Nuclease Digest Buffer
    Nucl C: 2 µL of Nucl B dilution in 2 µL of 1X Nuclease Digest Buffer
    Mix each dilution well and quick spin before aliquoting.

20. Pre-warm simultaneously tubes labeled A, B and C containing the cells resuspended in Nuclease Digest Buffer and the dilutions of Nuclease Enzyme Mix (NucI A, NucI B, and NucI C) at 30°C for 2 minutes in an agitating thermal mixer set at 1,250 rpm.
21. Transfer:
    5 µL of pre-warmed NucI A to pre-warmed tube A
    1 µL of pre-warmed NucI B to pre-warmed tube B
    1 µL of pre-warmed NucI C to pre-warmed tube C
22. Incubate all three tubes for **exactly** 30 min at 30°C, in an agitating thermal mixer set at 1,250 rpm.
23. Stop the enzymatic reaction by adding 5 µL of 0.5M EDTA. Pipet to mix.
24. Add to each tube 3 µL of 20% SDS to lyse the cells. Pipet to mix.
25. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
26. Continue to Stage 2: Lysate QC.

Examples of insufficient (A) and sufficient (B) tissue grinding.



Stage 2: Lysate QC

As you prepare for Stage 2, keep the following in mind:

- The Lysate QC stage takes ~ 1 hour.
- This stage has 2 objectives:
    - Quantify the total lysate to determine the sample volume to use in Stage 3.
    - Confirm that the chromatin was properly digested.
- The protocol below is written for the TapeStation; however, it is also compatible with the Bioanalyzer System and Fragment Analyzer. Please refer to the table below for the recommended kits for each system.

System	Recommended Kits
TapeStation	HS D5000
Bioanalyzer System	HS DNA
Fragment Analyzer	DNF-488 HS Genomic DNA

Figure 2. Stage 2: Lysate QC

Before You Begin

- Prepare fresh 80% ethanol for DNA purification with SPRIselect beads for optimal results. Fresh preparations of 80% ethanol will also be used in the remaining stages 3,4, and 5. You need a minimum of 2 mL for all these stages.
- Program the thermal mixer as follows:

Temperature	Time
78°C	10 minutes
25°C	Hold

- The Crosslink Reversal Buffer might have precipitated in storage. Incubate at 50°C for 15 minutes or until the precipitate is no longer visible. Vortex to mix prior to use.

Follow the steps below for Lysate QC:

1. Transfer 2.5 µL of the lysate to a new 1.5 mL tube labeled QC.

> NOTE Store the remainder of your lysate on ice. This is what you will be using in Stage 3. If you are not going to proceed with Stage 3 on the same day, store the remainder of the sample at **-80°C**.

2. Add to the QC tube 51.5 µL of a master mix containing the following reagents **in the order listed:**

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
Crosslink Reversal Buffer	50 µL	55 µL	x	8	=	440 µL
Proteinase K	1.5 µL	1.7 µL	x	8	=	13.6 µL
Total	51.5 µL					

3. Pipet up and down to fully mix. Incubate the QC tube in an agitating thermal mixer set at 1,250 rpm as follows:

Temperature	Time
78°C	10 minutes
25°C	Hold

4. Quick spin the QC tube after incubation.
5. Vortex the SPRIselect beads thoroughly (>30 seconds) to resuspend. Add 90 µL of resuspended SPRIselect beads to the 1.5 mL tube containing your sample.
6. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
7. Incubate the tube at room temperature, off the magnetic rack, for 10 minutes.
8. Quick spin the tube and place it in the magnetic rack for 5 minutes. Discard the supernatant.
9. Leave the tube in the magnetic rack and wash the beads twice with 200 µL fresh 80% ethanol. Do not resuspend the beads for these washes. Add the ethanol, wait for 1 minute then discard the ethanol supernatant.
10. After the last wash, quick spin the tube and place it in the magnetic rack for 1 minute. Use a 10 µL pipet tip to remove traces of ethanol.
11. Air dry the beads in the magnetic rack for 5 minutes until no residual ethanol remains. Do not over dry the beads.
12. Off the magnetic rack, resuspend the beads in 10 µL TE Buffer pH 8.0.
13. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
14. Incubate at room temperature, off the magnetic rack, for 5 minutes.
15. Quick spin the tube and place it in the magnetic rack for 1 minute (or until the solution looks clear).
16. **Transfer** 8 µL of the **SUPERNATANT** (purified DNA) to a new tube. This new tube contains your purified QC DNA. Discard the beads.

17. Quantify the purified QC DNA with a Qubit® Fluorometer and Qubit® dsDNA HS Kit.

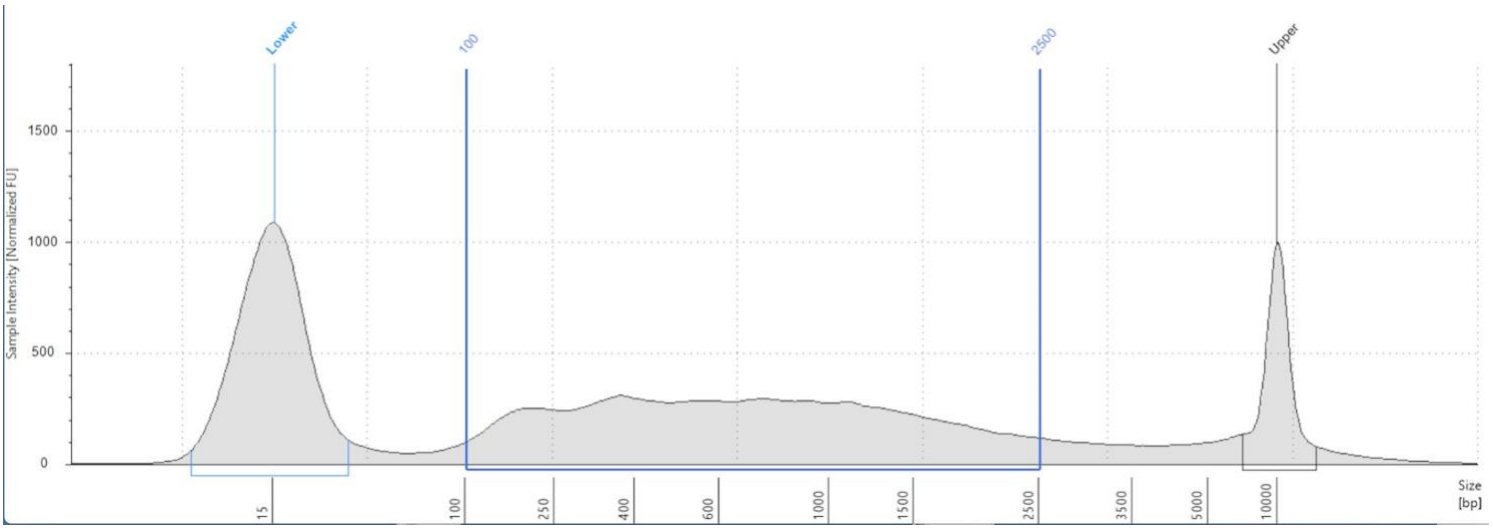
- Based on the Qubit concentration, the total lysate (ng) can be calculated as follows:
  Total lysate (ng) = Qubit reading ng/µL x 10 µL (elution volume) x 23.4 (dilution factor)
- You will use in Stage 3 a volume of the lysate that corresponds to 1,000 ng or 500 ng depending on your sample type (see table below). This volume can be calculated as follows:

Sample Type	Lysate amount (ng) to use in Stage 3	Volume of lysate (µL) to use in Stage 3
Mammalian cells, animal tissues (except insects and marine invertebrates), PBMCs, Mammalian whole blood, non-mammalian nucleated blood	1,000 ng	Volume (µL) = $\frac{1{,}000\ (ng) \times 58.5\ (\mu L)}{\text{Total lysate (ng)}}$
Insects, marine invertebrates	500 ng	Volume (µL) = $\frac{500\ (ng) \times 58.5\ (\mu L)}{\text{Total lysate (ng)}}$
Plants	500 ng	Volume of lysate **A** = $\frac{500\ (ng) \times 63\ (\mu L)}{\text{Total lysate (ng)}}$ Volume of lysate **B** = $\frac{500\ (ng) \times 59\ (\mu L)}{\text{Total lysate (ng)}}$ Volume of lysate **C** = $\frac{500\ (ng) \times 59\ (\mu L)}{\text{Total lysate (ng)}}$

- If the total lysate is less than the recommended amount, use all of the lysate in Stage 3.
- If the total lysate is more than the recommended amount, store the remainder of the lysate at -80°C.

18. Check the fragment size distribution of your purified QC sample on a TapeStation HS D5000 ScreenTape. Make sure your sample is diluted to 1 ng/µL when using the TapeStation HS D5000 tape. On the TapeStation System, create a region from 100 – 2,500 bp (see figure below). Creating this region will automatically generate a "percent of total" value. This value corresponds to the Chromatin Digestion Efficiency (CDE) metric and should be ≥ 50%. If your CDE < 50%, do not proceed with the rest of the protocol. Instead, please refer to Appendix 2: Troubleshooting Guide.

Figure 3. TapeStation trace showing the 100 - 2,500 bp region described above. When using a Bioanalyzer or a Fragment Analyzer, the profile will be different than the one shown below. The CDE in this example is 81.19% and passes QC.



Region Table | Sample Table

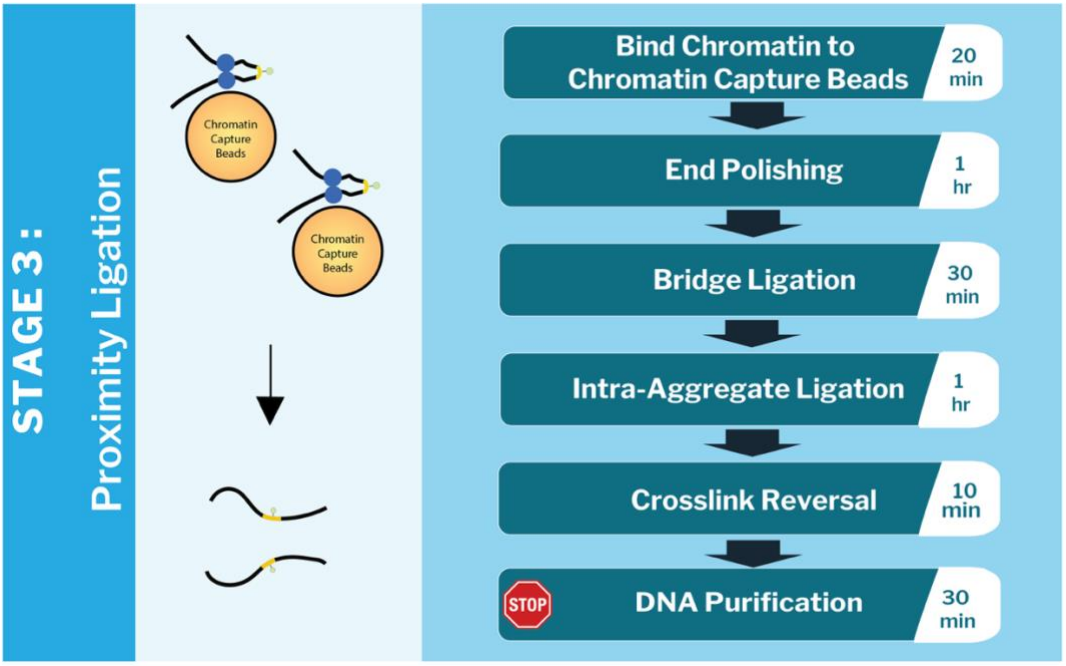
From [bp]	To [bp]	Average Size [bp]	Conc. [pg/µl]	Region Molarity [pmol/l]	% of Total	Region Comment	Color
100	2500	818	1160	3920	81.19		

Stage 3: Proximity Ligation

As you prepare for Stage 3, keep the following in mind:

- Proximity ligation takes ~ 4 hours.
- Follow best practices when working with beads (see Good practices).

Figure 4. Stage 3: Proximity Ligation



Before You Begin

- The Crosslink Reversal Buffer might have precipitated in storage. Incubate at 50°C for 15 minutes or until the precipitate is no longer visible. Vortex to mix prior to use.
- Thaw End Polishing Buffer, 5X Bridge Ligation Buffer, Bridge, and Intra-Aggregate Ligation Buffer at room temperature. Leave on ice once thawed. Vortex to mix prior to use.
- Equilibrate Chromatin Capture Beads to room temperature.

3.1 Bind Chromatin to Chromatin Capture Beads

Follow the steps below for Bind Chromatin to Chromatin Capture Beads:

1. Equilibrate the Chromatin Capture Beads to room temperature and vortex thoroughly (>30 seconds) to resuspend.
2. Transfer 100 µL of resuspended Chromatin Capture Beads to a new 1.5 mL tube.
3. Thaw the lysate at room temperature, if stored at -80°C (see Step 1 Note in Stage 2: Lysate QC). Pipet mix the lysate.
4. Transfer 1,000 ng of the lysate **(or 500 ng if working with insects, marine invertebrates, and plants)** to the 1.5 mL tube containing the beads.
5. Pipet up and down to fully mix. Incubate at room temperature, off the magnetic rack, for 10 minutes.
6. Place the tube in the magnetic rack for 5 minutes (or until the solution looks clear). Discard the supernatant.
7. Remove the tube from the magnetic rack and wash the beads with 150 µL 1X Wash Buffer. Pipet up and down to resuspend the beads, place the tube in the magnetic rack for 1 minute and discard the supernatant.
8. Remove the tube from the magnetic rack and resuspend the beads in 150 µL 1X Wash Buffer. Pipet up and down to resuspend the beads. **Leave the tube on the bench while you prepare for the next reaction.**

3.2 End Polishing

Follow the steps below for End Polishing:

1. Prepare 53 µL of End Polishing master mix containing the following reagents:

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
End Polishing Buffer	50 µL	55 µL	x	8	=	440 µL
End Polishing Enzyme Mix	3 µL	3.3 µL	x	8	=	26.4 µL
Total	53 µL					

2. Place the tube from step 8 in Stage 3.1 in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant and immediately proceed to the next step (**do not let the beads dry out**).
3. Remove the tube from the magnetic rack and immediately add to the beads 53 µL of the End Polishing master mix.
4. Pipet up and down to fully mix. Incubate in an agitating thermal mixer set at 1,250 rpm as follows:

Temperature	Time
22°C	30 minutes
65°C	30 minutes

5. Allow the tube to reach room temperature then quick spin and place it in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.
6. Remove the tube from the magnetic rack and resuspend the beads in 150 µL 1X Wash Buffer. Pipet up and down to resuspend the beads. **Leave the tube on the bench while you prepare for the next reaction**.

3.3 Bridge Ligation

Follow the steps below for Bridge Ligation:

1. Prepare and place on ice fresh 50 µL Bridge Ligation Mix by mixing the following reagents:

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
UltraPure Water	35 µL	38.5 µL	x	8	=	308 µL
5X Bridge Ligation Buffer	10 µL	11 µL	x	8	=	88 µL
Bridge	5 µL	5.5 µL	x	8	=	44 µL
Total	50 µL					

2. Place the tube from step 6 in Stage 3.2 in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant and immediately proceed to the next step (**do not let the beads dry out**).
3. Remove the tube from the magnetic rack and immediately add to the beads:

Reagent	Volume Per Reaction
Bridge Ligation Mix	50 µL
Bridge Ligase	1 µL
Total	51 µL

4. Pipet up and down to fully mix. Incubate at 22°C for 30 minutes in an agitating thermal mixer set at 1,250 rpm.
5. Quick spin the tube, then place it in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.
6. Remove the tube from the magnetic rack and resuspend the beads in 150 µL 1X Wash Buffer. Pipet up and down to resuspend the beads. **Leave the tube on the bench while you prepare for the next reaction.**

3.4 Intra-Aggregate Ligation

Follow the steps below for Intra-Aggregate Ligation:

1. Prepare 52 µL of Intra-Aggregate Ligation master mix containing the following reagents:

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
Intra-Aggregate Ligation Buffer	50 µL	55 µL	x	8	=	440 µL
Intra-Aggregate Ligation Enzyme Mix	2 µL	2.2 µL	x	8	=	17.6 µL
Total	52 µL					

2. Place the tube from step 6 in Stage 3.3 in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant and immediately proceed to the next step (**do not let the beads dry out**).
3. Remove the tube from the magnetic rack and immediately add to the beads 52 µL of the Intra-Aggregate Ligation master mix.
4. Pipet up and down to fully mix. Incubate at 22°C for 1 hour in an agitating thermal mixer set at 1,250 rpm.

> ☐ SAFE STOP For convenience, this ligation reaction can proceed overnight at 22°C in an agitating thermal mixer set at 1,250 rpm.

5. Quick spin the tube, then place it in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.

3.5 Crosslink Reversal

Follow the steps below for Crosslink Reversal:

1. Remove the tube from the magnetic rack and add to the beads 51.5 µL of a master mix containing the following reagents in the order listed:

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
Crosslink Reversal Buffer	50 µL	55 µL	x	8	=	440 µL
Proteinase K	1.5 µL	1.7 µL	x	8	=	13.6 µL
Total	51.5 µL					

2. Pipet up and down to fully mix. Incubate in an agitating thermal mixer set at 1,250 rpm as follows:

Temperature	Time
78°C	10 minutes
25°C	Hold

> ☐ SAFE STOP For convenience, you can hold at 25°C overnight in an agitating thermal mixer set at 1,250 rpm.

3. Quick spin the tube, then place it in the magnetic rack for 1 minute. **Transfer** 50 µL of the **SUPERNATANT** to a new 1.5 mL tube. Discard the beads.

3.6 DNA Purification

Follow the steps below for DNA Purification on SPRIselect Beads:

1. Vortex the SPRIselect beads thoroughly (>30 seconds) to resuspend.
2. Add 35 µL of resuspended SPRIselect beads to the 1.5 mL tube containing your sample.
3. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
4. Incubate the tube at room temperature, off the magnetic rack, for 10 minutes.
5. Quick spin the tube and place it in the magnetic rack for 5 minutes. Discard the supernatant.
6. Leave the tube in the magnetic rack and wash the beads **twice** with 200 µL **fresh** 80% ethanol. Do not resuspend the beads for these washes. Add the ethanol, wait for 1 minute then discard the ethanol supernatant.
7. After the last wash, quick spin the tube and place it in the magnetic rack for 1 minute. Use a 10 µL pipet tip to remove traces of ethanol.
8. Air dry the beads for 5 minutes in the magnetic rack until no residual ethanol remains. **Do not over dry the beads.**
9. Off the magnetic rack, resuspend the beads in 52 µL TE Buffer pH 8.0.
10. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
11. Incubate at room temperature, off the magnetic rack, for 5 minutes.
12. Quick spin the tube and place it in the magnetic rack for 1 minute.
13. **Transfer** 50 µL of the **SUPERNATANT** (purified DNA) to a new tube. Discard the beads.
14. Quantify the purified DNA using a Qubit Fluorometer and Qubit dsDNA HS Kit. You will use 150 ng of your purified DNA per library prep for Stage 4 in a 50 µL volume. If needed, you can bring up the volume to 50 µL using TE Buffer pH 8.0.
    - If you recovered < 150 ng, use all of the purified DNA to proceed to Stage 4.
    - If you recovered > 150 ng, use 150 ng to proceed to Stage 4 and keep the remaining purified DNA stored at -20°C. You can use the remaining DNA to prepare additional libraries if your application requires more complexity or coverage.

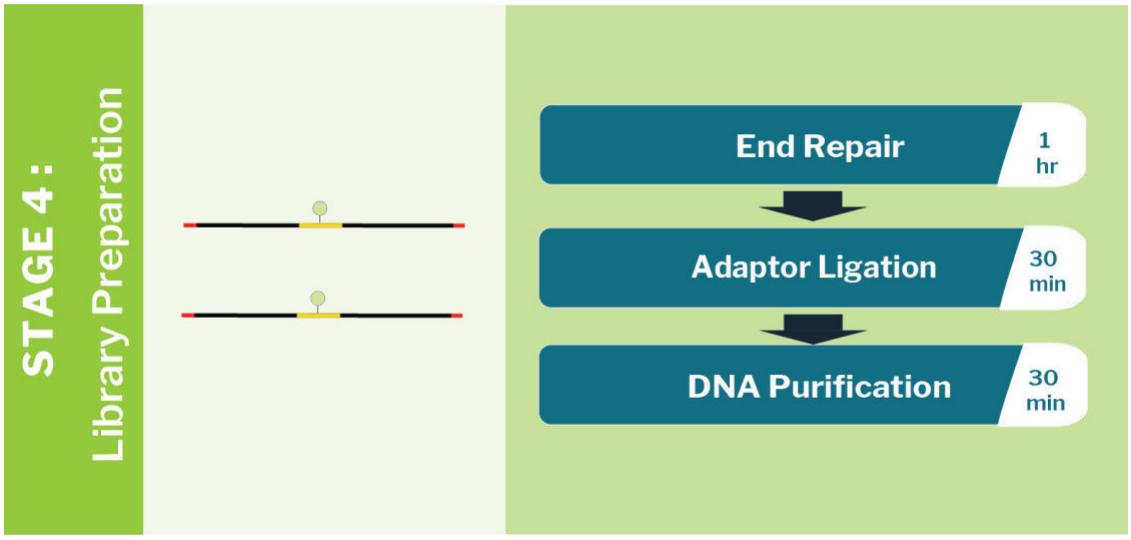
☐ SAFE STOP Purified DNA sample can be stored at -20°C for up to 6 months.

Stage 4: Library Preparation

As you prepare for Stage 4, keep the following in mind:

- The library preparation protocol does not require fragmentation.
- The library preparation protocol takes ~ 2 hours.
- Follow best practices when working with beads (see Good Practices).

Figure 5. Stage 4: Library Preparation



Before You Begin

- The End Repair Buffer may have precipitated in storage. Incubate for at least 10 minutes at 37˚C until there is no visible precipitate.
- Thaw Stubby Adaptor at room temperature. Vortex to mix prior to use.

4.1 End Repair

Follow the steps below for End Repair:

1. To a new 0.2 mL PCR tube, transfer 150 ng of the purified DNA and bring up the volume to 50 µL with TE Buffer pH 8.0, if needed.
2. Add to the PCR tube 10 µL of a master mix containing the following reagents:

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
End Repair Buffer	7 µL	7.7 µL	x	8	=	61.6 µL
End Repair Enzyme Mix	3 µL	3.3 µL	x	8	=	26.4 µL
Total	10 µL					

3. Pipet up and down to fully mix. Quick spin the tube.
4. Place in a thermal cycler, with the heated lid set to ≥ 75°C, and run the following program:

Temperature	Time
20°C	30 minutes
65°C	30 minutes
12°C	Hold

4.2 Adaptor Ligation

Follow the steps below for Adaptor Ligation:

1. Add to the PCR tube containing the end-repaired sample the following reagents in order:

Reagent	Volume Per Reaction
Stubby Adaptor	2.5 µL
Ligation Buffer	25 µL
Ligation Enzyme Mix	5 µL
Total	32.5 µL

> NOTE The Ligation Enzyme Mix and Ligation Buffer can be mixed and used as a master mix. We do not recommend adding the Stubby Adaptor to the master mix. If using a master mix, add the Stubby Adaptor first then add 30 µL of the Ligation master mix.

2. Pipet up and down to fully mix. Quick spin the tube.
3. Incubate at 20°C for 15 minutes in a thermal cycler with the heated lid off. Hold at 12°C.

4.3 DNA Purification

Follow the steps below for DNA Purification:

1. Vortex the SPRIselect beads thoroughly (>30 seconds) to resuspend.
2. Add 85 µL of resuspended SPRIselect beads to the PCR tube containing the adaptor-ligated sample.
3. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
4. Incubate the tube at room temperature, off the magnetic rack, for 10 minutes.
5. Quick spin the tube and place it in the magnetic rack for 5 minutes. Discard the supernatant.
6. Leave the tube in the magnetic rack and wash the beads **twice** with 200 µL **fresh** 80% ethanol. Do not resuspend the beads for these washes. Add the ethanol, wait for 1 minute then discard the ethanol supernatant.
7. Quick spin the tube and place it in the magnetic rack for 1 minute. Use a 10 µL pipet tip to remove traces of ethanol.
8. Air dry the beads for 5 minutes in the magnetic rack until no residual ethanol remains. **Do not over dry the beads.**
9. Off the magnetic rack, resuspend the beads in 22 µL TE Buffer pH 8.0.
10. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
11. Incubate at room temperature, off the magnetic rack, for 5 minutes.
12. Quick spin the tube and place it in the magnetic rack for 1 minute (or until the solution looks clear).
13. **Transfer** 20 µL of the **SUPERNATANT** (purified adaptor-ligated DNA) to a new tube. Discard the beads.

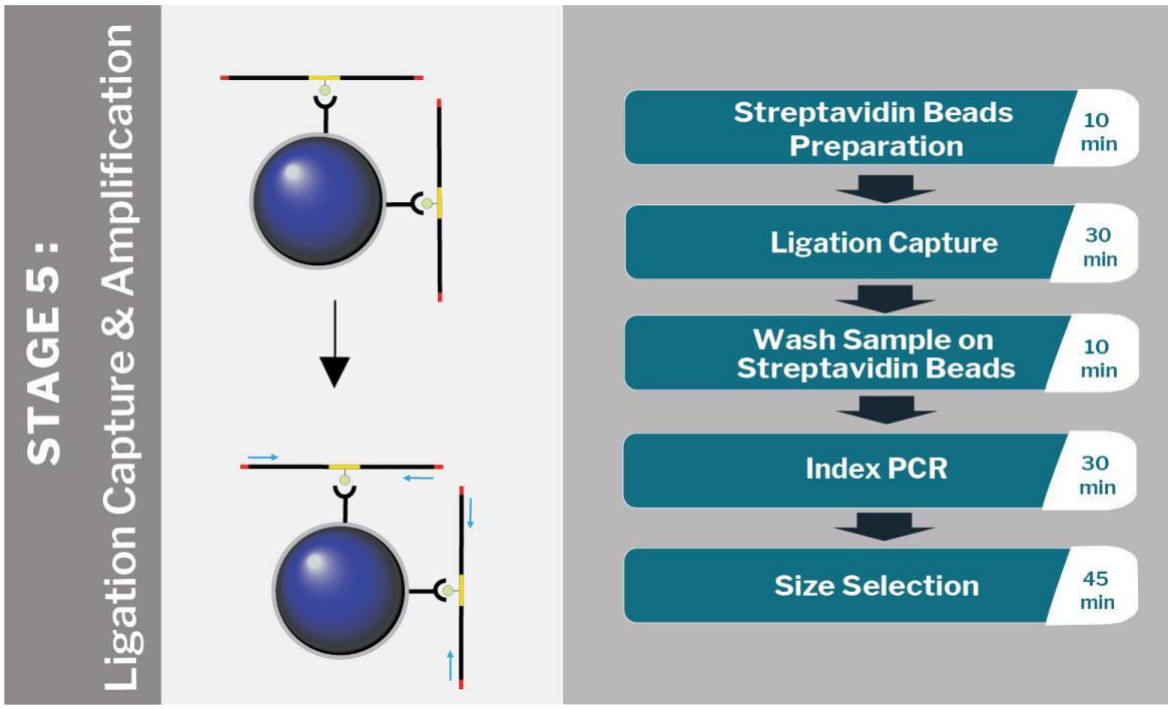
☐ SAFE STOP Purified DNA sample can be stored at -20°C overnight.

Stage 5: Ligation Capture and Amplification

As you prepare for Stage 5, keep the following in mind:

- The Ligation Capture and Amplification protocol takes ~ 2 hours.
- Follow best practices when working with beads (see Good Practices).

Figure 6. Stage 5: Ligation Capture and Amplification



Before You Begin

- ☐ Thaw the index primers and HotStart PCR Ready Mix and **keep on ice** while in use. Vortex to mix prior to use.
- ☐ Equilibrate Streptavidin Beads and NWB at room temperature.

5.1 Streptavidin Beads Preparation

> NOTE This step does not involve any DNA sample.

Follow the steps below for Streptavidin Beads Preparation:

1. Vortex the Streptavidin Beads vial thoroughly (> 30 seconds) to resuspend the beads. Transfer 25 µL of resuspended Streptavidin beads to a new 1.5 mL tube.
2. Place the 1.5 mL tube containing the beads in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.
3. Remove the tube from the magnetic rack and wash the beads with 200 µL of 1X Wash Buffer: pipet up and down to resuspend the beads and place the tube in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.
4. Repeat step 3 once, for a total of 2 washes.
5. After the second wash, resuspend the beads in 100 µL **NWB**. Pipet up and down to fully mix.

5.2 Ligation Capture

Follow the steps below for Ligation Capture:

1. Transfer the 100 µL of prepared Streptavidin beads (from Step 5, Stage 5.1) to the 0.2 mL PCR tube containing the 20 µL of purified adaptor-ligated DNA (from step 13 in 4.3 DNA Purification).
2. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
3. Incubate at 25˚C for 30 minutes in an agitating thermal mixer set at 1,250 rpm (or on the bench with no shaking, if an agitating thermal mixer for 0.2 mL tubes is not available).

5.3 Wash Sample on Streptavidin Beads

> NOTE For each of the washes below, remove the tube from the magnetic rack, add the indicated buffer to the beads, pipet up and down to resuspend the beads, place the tube in the magnetic rack for 1 minute (or until the solution looks clear), and discard the supernatant. Remove all of the supernatant between each wash; residual supernatant can interfere with the downstream PCR.

Follow the steps below for Wash Sample on Streptavidin Beads:

1. Quick spin the tube and place it in the magnetic rack for 1 minute. Discard the supernatant.
2. Wash the beads **twice** with 200 µL NWB.
3. Wash the beads **twice** with 200 µL 1X Wash Buffer.

5.4 Index PCR

> NOTE Not all PCR enzymes and master mixes are compatible for amplification in the presence of Streptavidin beads. Please use the HotStart PCR Ready Mix supplied in your Dovetail® Kit (Box 2).

Follow the steps below for Index PCR:

1. After the last wash, remove the tube from the magnetic rack and add to the beads 40 µL of a master mix containing the following reagents:

Reagent	Volume Per Reaction	10% Extra		# Reactions		Final
UltraPure Water	15 µL	16.5 µL	x	8	=	132 µL
HotStart PCR Ready Mix	25 µL	27.5 µL	x	8	=	220 µL
Total	40 µL					

2. Add 5 µL of DG i5 Index Primer to the PCR reaction. (see Appendix 3: Index Primers).
3. Add 5 µL of DG i7 Index Primer to the PCR reaction. (see Appendix 3: Index Primers).
4. Pipet up and down to fully mix.
5. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle). Place the tube into the thermal cycler and run the following program:

Step	Temperature	Time	Cycles
Enzyme Activation	98°C	3 minutes	1
Denature	98°C	20 seconds	12
Anneal	65°C	30 seconds	
Extend	72°C	30 seconds	
Extend	72°C	1 minute	1
	12°C	Hold	

5.5 Size Selection

Follow the steps below for Size Selection:

1. Quick spin the PCR tube and place it in the magnetic rack for 1 minute.
2. **Transfer** 47 µL of the **SUPERNATANT** to a new 1.5 mL tube. Discard the beads.
3. Add 53 µL of TE Buffer pH 8.0 to the 1.5 mL tube to bring the volume of the sample in the tube to 100 µL.
4. Vortex the SPRIselect beads thoroughly (>30 seconds) to resuspend.
5. Add 50 µL of resuspended SPRIselect beads to the 1.5 mL tube containing your sample.

6. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
7. Incubate the tube at room temperature, off the magnetic rack, for 10 minutes.
8. Quick spin the tube and place it in the magnetic rack for 5 minutes.
9. **Transfer** 145 µL of the **SUPERNATANT** to a new 1.5 mL tube. Discard the beads.
10. Add 30 µL of resuspended SPRIselect beads to the 1.5 mL tube containing your sample.
11. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
12. Incubate the tube at room temperature, off the magnetic rack, for 10 minutes.
13. Quick spin the tube and place it in the magnetic rack for 5 minutes. Discard the supernatant.
14. Leave the tube in the magnetic rack and wash the beads **twice** with 200 µL fresh 80% ethanol. Do not resuspend the beads for these washes. Add the ethanol, wait for 1 minute then discard the ethanol supernatant.
15. Quick spin the tube and place it in the magnetic rack for 1 minute. Use a 10 µL pipet tip to remove traces of ethanol.
16. Air dry the beads for 5 minutes in the magnetic rack until no residual ethanol remains. **Do not over dry the beads**.
17. Off the magnetic rack, resuspend the beads in 30 µL TE Buffer pH 8.0.
18. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
19. Incubate the tube at room temperature, off the magnetic rack, for 5 minutes.
20. Quick spin the tube and place it in the magnetic rack for 1 minute (or until the solution looks clear).
21. **Transfer** 28 µL of the **SUPERNATANT** to a new 1.5 mL tube. The supernatant is your size selected library. Discard the beads.
22. Quantify your size selected library using a Qubit Fluorometer and Qubit dsDNA HS Kit. We expect a yield ≥ 60 ng.
23. Use a TapeStation or Bioanalyzer to verify the size distribution of your size selected library. The size range is expected to be between 350 bp and 1,000 bp.

☐ SAFE STOP The library can be stored at -20°C for up to 6 months.

Sequencing & QC Analysis of Dovetail® Omni-C Libraries

Dovetail® Omni-C libraries are sequenced via Illumina® sequencers in paired-end mode. Each Omni-C library can be deep sequenced up to 300 M read pairs (2 x 150 bp) on any Illumina platform. We recommend shallow sequencing the library to run a QC analysis prior to deep sequencing. The QC analysis requires 1 to 2 million read pairs (2 x 150 bp) generated from **MiniSeq** or **MiSeq**. If you don't have access to a MiSeq or MiniSeq, you can shallow sequence the library on an alternative Illumina platform to assess mappability and long-range information. However, you may not be able to accurately assess the duplication rate. Cantata Bio provides all kit users with access to QC analysis pipeline available on readthedocs (https://omni-c.readthedocs.io/en/latest/#).

Appendix 1: Low-Input Sample Preparation Guide

Use this guide when the recommended input amount is not available to you. Please note that a lower input may result in a lower final library complexity.

A. Mammalian Cells

Depending on the number of cells available to you; proceed with either 100,000 or 500,000 cells. Follow the sample preparation protocol through step 13. Upon completing step 13, continue with the steps below which are customized for low input samples.

14. Make a 1:10 dilution of the Nuclease Enzyme Mix from the kit supplied tube by transferring 2 µL of Nuclease Enzyme Mix into 18 µL 1X Nuclease Digest Buffer (freshly prepared). Pipet to mix.
15. Transfer volume X µL based on input amount (see table below) of Nuclease Enzyme Mix (DILUTED) to pre-warmed tube. You can discard the remainder of your Nuclease Enzyme Mix (DILUTED).

Number of input cells	Volume of DILUTED Nuclease Enzyme Mix
100,000 cells	0.5 µL
500,000 cells	1 µL

16. Incubate the tube for **exactly** 30 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
17. Stop the reaction by adding 5 µL of 0.5 M EDTA. Pipet to mix.
18. Add 3 µL of 20% SDS to lyse the cells. Pipet to mix.
19. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
20. Continue to Stage 2: Lysate QC.

B. Mammalian Tissues:

NOTES:

- The low input tissue protocol requires 5 mg of tissue.
- The low input tissue protocol is not compatible with muscle tissue.

Proceed with the sample preparation protocol with 5 mg of frozen tissue. Follow the sample preparation protocol through step 17. Upon completing step 17, continue with the steps below which are customized for low input samples.

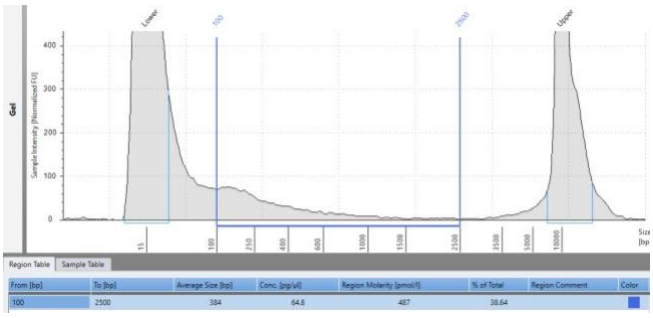
18. Make a 1:10 dilution of the Nuclease Enzyme Mix from the kit supplied tube by transferring 2 µL of Nuclease Enzyme Mix into 18 µL 1X Nuclease Digest Buffer (freshly prepared). Pipet to mix.
19. Transfer 1 µL of Nuclease Enzyme Mix (DILUTED) to the prewarmed tube. You can discard the remainder of your Nuclease Enzyme Mix (DILUTED).
20. Incubate the tube for **exactly** 30 minutes at 30°C in an agitating thermal mixer at 1,250 rpm.
21. Stop the enzymatic reaction by adding 5 µL of 0.5 M EDTA and pipet to mix.
22. Add 3 µL of 20% SDS to the tube to lyse the cells; pipet to mix.
23. Incubate for 5 minutes at 30°C in an agitating thermal mixer at 1,250 rpm.
24. Continue to Stage 2: Lysate QC.

Appendix 2: Troubleshooting Guide

Chromatin Digestion Efficiency (CDE) Out Of Range
You are following this guide because your CDE is < 50%.
CDE ≥ 50% indicates that your chromatin is sufficiently digested. CDE < 50% indicates your chromatin is either:

- Over-digested *or*
- Under-digested

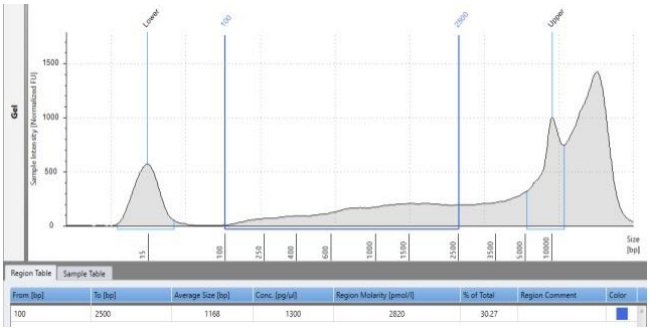
Scenario 1: Over-Digested
Your chromatin is over-digested if the majority of your DNA is less than 600 bp (see figure below; in this example, CDE is 38.64%).



Solution:
Repeat the Sample Preparation and Lysate Quantification stages modifying **only** the amount of nuclease enzyme used as follows:

- Make a 1:10 dilution of the Nuclease Enzyme Mix from the kit supplied tube by transferring 2 µL of Nuclease Enzyme Mix into 18 µL 1X Nuclease Digest Buffer (freshly prepared). Pipet to mix.
- Transfer 1 µL of Nuclease Enzyme Mix **(DILUTED)** to the pre-warmed sample tube.

Scenario 2: Under-Digested
Your chromatin is under-digested if the majority of your DNA is greater than 2,500 bp (see figure below; in this example, CDE is 30.27%).



Solution:
Repeat Sample Preparation and Lysate Quantification stages modifying **only** the amount of nuclease enzyme used as follows:
Transfer 2 µL of Nuclease Enzyme Mix **(UNDILUTED)** to the pre-warmed sample tube.

Appendix 3: Index Primers

Dovetail® Primer Module Set 1 includes four i5 index primers and six i7 index primers. **Verify that the indexes selected for pooling have the appropriate color balance for your sequencing instrument.**

Dovetail® Primer Module Set 1 contains sufficient index primers to support multiplexing up to 24 libraries, where each library is prepared by combining a unique i5 index primer and a unique i7 index primer in steps 2 and 3 of Stage 5.4 Index PCR. For example, 6 libraries can be prepared by setting up 6 individual PCR reactions as follows:

- Library 1: i5 Index Primer 1 and i7 Index Primer 1
- Library 2: i5 Index Primer 1 and i7 Index Primer 2
- Library 3: i5 Index Primer 1 and i7 Index Primer 3
- Library 4: i5 Index Primer 1 and i7 Index Primer 4
- Library 5: i5 Index Primer 1 and i7 Index Primer 5
- Library 6: i5 Index Primer 1 and i7 Index Primer 6

Table 8. Index Primer

i5 Index Name	Bases in Adapter	Bases for Sample Sheet in Forward Orientation	Bases for Sample Sheet in Reverse Complement Orientation
DG i5 Index 1	ATATGCGC	ATATGCGC	GCGCATAT
DG i5 Index 2	TGGTACAG	TGGTACAG	CTGTACCA
DG i5 Index 3	AACCGTTC	AACCGTTC	GAACGGTT
DG i5 Index 4	TAACCGGT	TAACCGGT	ACCGGTTA

i7 Index Name	Bases in Adapter	Bases for Sample Sheet
DG i7 Index 1	ACGATCAG	CTGATCGT
DG i7 Index 2	TCGAGAGT	ACTCTCGA
DG i7 Index 3	CTAGCTCA	TGAGCTAG
DG i7 Index 4	ATCGTCTC	GAGACGAT
DG i7 Index 5	TCGACAAG	CTTGTCGA
DG i7 Index 6	CCTTGGAA	TTCCAAGG

Appendix 4: Cryopreservation

A. Cryopreserve Peripheral Blood Mononuclear Cells (PBMCs) using CryoStor®

As you prepare for cryopreservation, keep the following in mind:

- At any point in the protocol if there are cell aggregations observed, it is essential to filter out the aggregates using MiniStrainer. Place a MiniStrainer in a 1.5 mL microfuge tube. Pipet the cell mixture into the MiniStrainer. Quick spin at 500 x g for 5 seconds. The cell aggregates should be retained in the filter. The PBMCs should be in single-cell suspension in the tube.

Before You Begin

- ☐ Ensure CryoStor® is stored on ice.

Follow the steps below for Cryopreservation:

1. Wipe down the outside of the CryoStor® CS10 container with 70% ethanol before opening the bottle.
2. Centrifuge cells at 500 x g for 5 minutes.
3. Carefully pipette out the supernatant, leaving a small amount of liquid to ensure the cell pellet is not disturbed.
4. Resuspend the cell pellet by gently flicking the tube.
5. Add cold (2 - 8°C) CryoStor® CS10, pipet up and down to mix thoroughly.
6. Transfer the suspension to a cryovial.

NOTE It is recommended to freeze isolated PBMCs at a concentration of 2 – 10 x 10^6 cells/mL.

7. Incubate the tube at 2 - 8°C for 10 minutes.
8. If using Nalgene® Mr. Frosty, transfer the tube in the Mr. Frosty and place in a -80°C freezer overnight.

NOTE An alternative method could be used for rate-controlled cooling to approximately -1°C /minute in a controlled-rate freezer.

9. For long-term storage, transfer vials of frozen PBMCs from the freezer to vapor phase liquid nitrogen (below -135°C).

B. Cryopreserve Peripheral Blood Mononuclear Cells (PBMCs) using 90% FBS/10% DMSO

As you prepare for cryopreservation, keep the following in mind:

- At any point in the protocol if there are cell aggregations observed, it is essential to filter out the aggregates using MiniStrainer. Place a MiniStrainer in a 1.5 mL microfuge tube. Pipet the cell mixture into the MiniStrainer. Quick spin at 500 x g for 5 seconds. The cell aggregates should be retained in the filter. The PBMCs should be in single-cell suspension in the tube.

Before You Begin

- ☐ Ensure DMSO is at room temperature.
- ☐ Fetal Bovine Serum (FBS) should be stored on ice.

Follow the steps below for Cryopreservation:

1. Prepare 1 mL of 10% DMSO in FBS in a 1.5 mL tube. Keep on ice.
2. Ensure PBMCs are in single-cell suspension.
3. Spin the cells at 500 x g for 5 minutes at room temperature.
4. Discard the supernatant, leaving a small amount of buffer to ensure the cell pellet is not disturbed.
5. Resuspend the pellet in 10% DMSO-FBS mix. Pipet up and down to break up clumps and resuspend the pellet.

NOTE It is recommended to freeze isolated PBMCs at a concentration of 2 – 10 x 10^6 cells/mL.

6. Transfer the suspension to a cryovial.
7. If using Nalgene® Mr. Frosty, transfer the tube in the Mr. Frosty and place in a -80°C freezer overnight.

NOTE An alternative method could be used for rate-controlled cooling to approximately -1°C /minute in a controlled-rate freezer.

8. For long-term storage, transfer vials of frozen PBMCs from the freezer to vapor phase liquid nitrogen (below -135°C).

Appendix 5: Sample Preparation For Plant Nuclei

The standard sample preparation protocol for plants (Stage 1G) starts with plant leaves. However, some plants are high in secondary metabolites which can inhibit the proximity ligation reactions and result in low final library yield. In this case, we recommend isolating the nuclei from these plants and using nuclei as starting material for the Omni-C assay. This guide walks you through the steps for isolating nuclei from plant leaves following a protocol adapted from Workman et al. 2018 (Recalcitrant plant species for third generation sequencing) and for carrying out Stage 1 of the Omni-C assay: Sample Preparation starting with nuclei as input material. The nuclei isolation step requires user supplied reagents listed below.

User Supplied Reagents For Nuclei Isolation
Miracloth (Millipore Sigma, 475855-1R)
14 M β-mercaptoethanol (Sigma-Aldrich, M3148-100ML)
Triton X-100 (Sigma-Aldrich, X100-100ML)
Trizma base (Sigma-Aldrich, T4661-100G)
Potassium chloride (Sigma-Aldrich, P9541-500G)
0.5 M EDTA pH 8.0 (ThermoFisher, 15575020)
Spermidine trihydrochloride (Sigma-Aldrich, S2501-5G)
Spermine tetrahydrochloride (Sigma-Aldrich, S1141-5G)
Sucrose, molecular biology grade (Sigma-Aldrich, S0389-1KG)
10 N NaOH (Sigma-Aldrich, 72068-100ML)
PVP 360K (Sigma-Aldrich, PVP360-100G)
200 proof ethanol

Step 1: Nuclei Isolation

Before You Begin

- Prepare **10X** HB (homogenization buffer) stock (100 mL) and store at 4°C. 10X HB Buffer is stable for 1 year at 4°C in a glass bottle. Prepare 10X HB Buffer by mixing the following components and adjust pH to 9-9.4 with 10 N NaOH drops.

Reagent	Volume
Trizma base	1.21 g
KCl	5.96 g
0.5 M EDTA	20 mL
Spermidine	0.255 g
Spermine	0.348 g
ddH2O	Fill to 100 mL

- Prepare **1X** HB (homogenization buffer) stock (500 mL) and store at 4°C. 1X HB Buffer is stable for 3 months at 4°C in a glass bottle. Prepare 1X HB Buffer by mixing the following components:

Reagent	Volume
10X HB	50 mL
Sucrose	85.6 g
ddH2O	Fill to 300 mL, stir until dissolved then bring to 500 mL

- Prepare **Triton X-100 mix** (20% vol/vol, 100 mL) and store at 4°C. Triton X-100 mix is stable for 1 year at 4°C in a glass bottle. Prepare Triton X-100 mix by mixing the following components:

Reagent	Volume
Triton X-100	20 mL
10X HB	10 mL
Sucrose	17.15 g
ddH2O	Fill to 60 mL. Stir until dissolved then bring to 100 mL

- Prepare NIB (nuclear isolation buffer) the **day of isolation.** Make 100 mL NIB by mixing the following components:

Reagent	Volume
1X HB	97.5 mL
Triton X-100 mix	2.5 mL
B-mercaptoethanol (add immediately before use)	250 µL
PVP	1 g

1. Grind 1 gram of snap frozen leaves into fine powder in liquid nitrogen with a mortar and pestle. Immediately transfer ground tissue to capped 250 mL bottle containing 10 mL cold NIB. Cap bottle and attach to end over end mixer, rotating at max speed for 15 min at 4°C. Alternatively, transfer ground tissue to a beaker containing 10 mL cold NIB. Cap the beaker with foil and mix on stir plate with stir bar at 4°C for 15 minutes.

NOTE

- We recommend leaves that were flash frozen in liquid nitrogen upon collection and stored at -80°C until ready to use.
- We recommend the following tissues, ordered from most preferred to least:
    1. Leaves of plants at the one- or two-leaf seedling stage.
    2. Very young leaves from more mature plants.
    3. Leaves collected from plants that are pretreated in the dark for 2–3 days.
- Keep the preparation as cold as possible.

2. Using a funnel, gravity filter homogenate through 5 layers of Miracloth into a 50 mL conical tube. Cap tube and centrifuge at 4°C for 20 min.

NOTE Spin speed is dependent on the genome size. For example, spin at 1,900 x g for 30G genomes, 2,500 x g for 2.5 Gb and 2,900 x g for 1 Gb.

3. Decant the supernatant and add 1 mL cold NIB to pellet. Carefully resuspend pellet with paint brush presoaked in NIB. Alternatively, carefully resuspend pellet by pipetting up and down.
4. Transfer 1 mL nuclei suspension to a 15 mL conical.
5. Bring volume up to 15 mL with ice-cold NIB. Centrifuge at same speed and temperature as used in step 2 for 10 minutes.
6. After centrifugation, if the supernatant is clear, remove supernatant and take pellet into step 7. If coloration remains in the supernatant after centrifugation, remove supernatant, resuspend nuclei pellet in 15 mL ice-cold NIB and repeat centrifugation under conditions used in step 5. Repeat the wash up to 3 times until the supernatant is clear.
7. Remove supernatant and resuspend in 1 mL cold 1X HB.
8. Divide the sample resuspended in 1X HB equally into two 1.5 mL tubes
9. Spin down the tube at 6,000 x g for 5 minutes. Discard the supernatant.
10. Flash freeze the pellets. Proceed with only one pellet to Step 2: Omni-C sample preparation and store the other pellet at -80°C. If not proceeding with Omni-C sample preparation on the same day, store both pellets at -80°C.

Step 2. Omni-C Sample Preparation

Before You Begin:

- The 10X Wash Buffer and 20% SDS might have precipitated in storage. Incubate these solutions at 50C for 15 minutes or until the precipitate is no longer visible. Vortex to mix prior to use.
- Dilute 10X Wash Buffer to 1X with UltraPure™ Water. Store at room temperature. You will need ~15 mL of 1X Wash Buffer per sample. 1X Wash Buffer is stable at room temperature for 2 months.
- Prepare 0.3 M DSG in DMSO (anhydrous) by dissolving 1 mg of DSG in 10.22 µL DMSO. DSG is water-insoluble and moisture- sensitive. Prepare immediately before use. Do not store DSG in solution.
- Prepare fresh 1X Nuclease Digest Buffer and store at room temperature. 1X Nuclease Digest Buffer is stable for 1 day at room temperature. You need 60 µL Nuclease Digest Buffer per sample. To prepare 1X Nuclease Digest Buffer (60 µL), mix the following components:

Reagent	**Volume for 50 µL**
10X Nuclease Digest Buffer	6 µL
100 mM $MnCl_2$	6 µL
UltraPure Water	48 µL

- Set the thermal mixer at 30°C, shaking at 1,250 rpm.
- Thaw 0.5 M EDTA at room temperature. Vortex to mix prior to use.

1. Thaw your nuclei pellet at room temperature if frozen, then resuspend the pellet in:
    - 1 mL 1X PBS
    - 10 µL 0.3 M DSG
2. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
3. Add 27 µL of 37% formaldehyde.
4. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
5. Spin the tube at 1,000 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant. Use caution as the pellet might be loose.
6. Wash the pellet with 500 µL of 1X Wash Buffer, pipetting up and down to break up clumps and fully resuspend the pellet.
7. Spin the tube at 1,000 x g for 5 minutes in a swinging bucket rotor. Carefully remove the supernatant.
8. Resuspend the pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin).
9. Pre-warm the tube containing your resuspended cells to 30°C for 2 minutes in an agitating thermal mixer set at 1,250 rpm.
10. Prepare 1:10 dilution of Nuclease Enzyme Mix by mixing 1 µL of Nuclease Enzyme Mix with 9 µL of 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin). Pipet up and down to mix.
11. Transfer 1 µL of 1:10 Nuclease Enzyme Mix to the pre-warmed tube. Pipet up and down to mix.
12. Incubate the tube for **exactly** 30 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
13. Stop the reaction by adding 5 µL of 0.5 M EDTA. Pipet to mix.
14. Add 3 µL of 20% SDS to lyse the cells. Pipet to mix.
15. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
16. Continue to Stage 2: Lysate QC.