



Omni-C™ Proximity Ligation Assay

Protocol
version 1.2

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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.







Omni-C™ Kit Box 1 (PN DG-REF-001)		
Components	Color	Storage
TE Buffer pH 8.0	None	2°C to 8°C
10X Wash Buffer	White label	
TWB Solution		
2X NTB Solution		
LWB Solution		
NWB Solution		
Chromatin Capture Beads		
10X Crosslink Reversal Buffer		
Streptavidin Beads		
10X RBC Lysis Buffer		
20% SDS		

Omni-C™ Kit Box 2 (PN DG-REF-002)		
Components	Color	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 (T4 DNA Ligase)		
Bridge		
Intra-Aggregate Ligation Enzyme Mix		
Intra-Aggregate Ligation Buffer		
Proteinase K		
250 mM DTT		
HotStart PCR Ready Mix		

Optional Add-on Modules: Components & Storage

Omni-C™ Kit Filter Set (PN DG-HiC-005)		
Components	Color	Storage
50 µm Filters		Room Temp
200 µm Filters		

Dovetail™ Primer Set For Illumina (PN DG-PRS-001)		
Components	Color	Storage
Index Primers (x8, different)		-30°C to -10°C
Universal PCR Primer		

Dovetail™ Library Module For Illumina (PN DG-LIB-001)		
Components	Color	Storage
End Repair Enzyme Buffer		-30°C to -10°C
End Repair Enzyme Mix		
Ligation Enhancer		
Ligation Enzyme Mix		
Adaptor for Illumina		
USER Enzyme Mix		

User Supplied Reagents, Consumables & Equipment

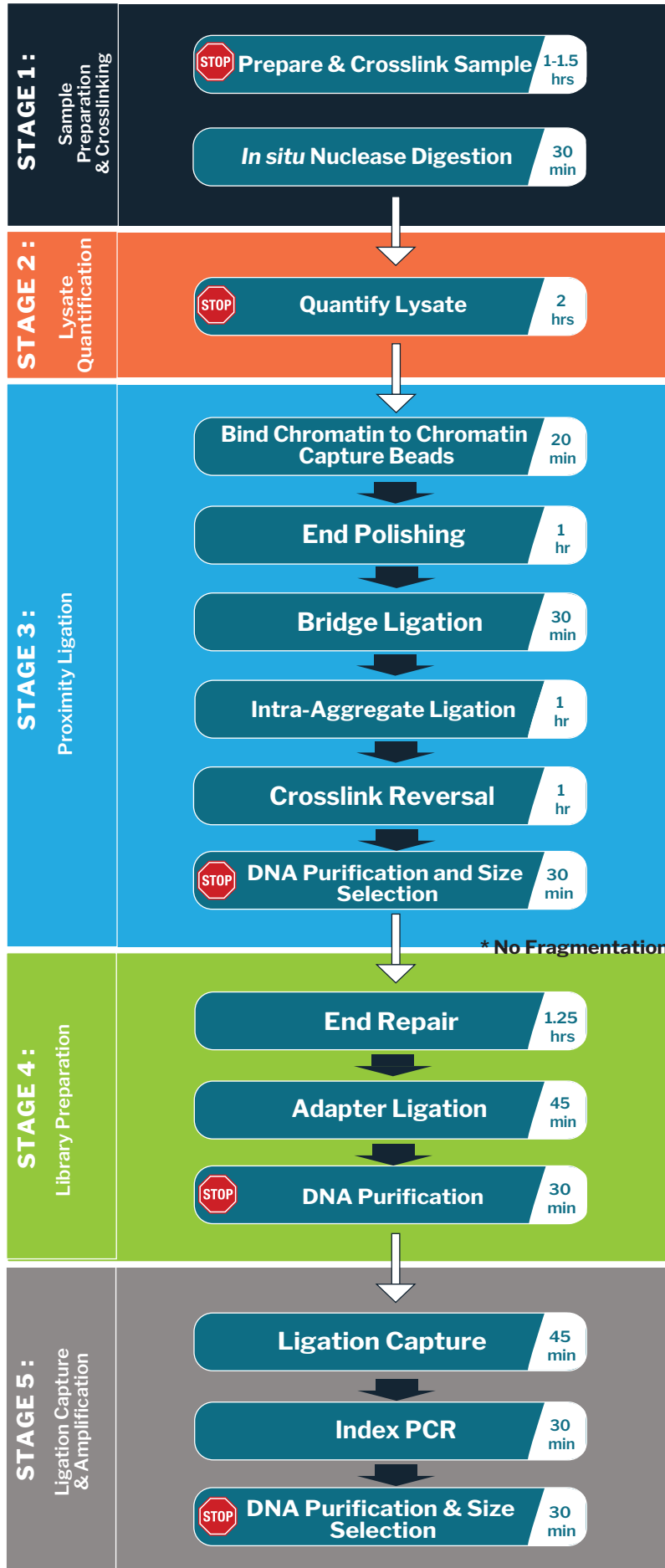
Reagents

Reagent	Supplier	PN
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	-
UltraPure™ DNase / RNase- Free Distilled Water, 500 mL	Thermo Fisher Scientific	10977015
Zymo DNA Clean & Concentrator-5	Zymo Research	D4013
DSG (Disuccinimidyl Glutarate)	Thermo Fisher Scientific	A35392
DMSO (Dimethyl Sulfoxide, Anhydrous ≥ 99.99%)	Sigma-Aldrich	276855-100ML

Consumables and Equipment

Item	Supplier
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	
Centrifuge for 0.2 mL, 1.5 mL and 5 mL tubes	
Liquid nitrogen/mortar and pestle	
Hemocytometer	
Qubit® Fluorometer	Thermo Fisher Scientific
Qubit® dsDNA HS Assay Kit	Thermo Fisher Scientific Q32854
Qubit® Assay Tubes	Thermo Fisher Scientific Q32856
TapeStation System, Fragment Analyzer, or Bioanalyzer	Agilent

Omni-C™ Protocol Overview



Stage 1: Sample Preparation

Getting Started

- There are three separate protocols for sample preparation depending on your sample type: cells, tissue, or blood.
- Sample preparation should take 2 hours
- The 10X Wash Buffer, 10X Crosslink Reversal Buffer and 20% SDS might have precipitated in storage. Please incubate the solutions at 37°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.
- Dilute 10X Crosslink Reversal Buffer to 1X with UltraPure Water. Store at room temperature. You will need ~1 mL of 1X Crosslink Reversal Buffer per sample. 1X Crosslink Reversal Buffer is stable at room temperature for 2 months.
- Prepare 0.3 M DSG in DMSO (anhydrous). DSG is water-insoluble and moisture-sensitive. Prepare immediately before use. Do not store DSG in solution.
- Agitating thermal mixer should be set at 1,250 rpm for 1.5 mL tubes.
- Use good laboratory practices including keeping enzymes on ice prior to use, thawing buffers on ice and vortexing prior to use.
- 1X Nuclease Digest Buffer should be prepared fresh and stored at room temperature. 1X Nuclease Digest Buffer is stable for 1 day at room temperature. You will need 50 µL Nuclease Digest Buffer per sample, therefore, it is recommended to make up 60 µL to account for pipetting error. To prepare 1X Nuclease Digest Buffer, mix:

Component	Volume for 50 µL	Volume for 60 µL (1.2 X)
10X Nuclease Digest Buffer	5 µL	6 µL
100 mM MnCl ₂	5 µL	6 µL
UltraPure Water	40 µL	48 µL

- In addition to preparing the buffers described above, the blood protocol requires the preparation of 1X RBC Lysis Buffer. Dilute 10X RBC Lysis Buffer to 1X with UltraPure water. You will need 10 mL of 1X RBC Lysis Buffer per 1 mL of blood. 1X RBC Lysis Buffer is stable at 4°C for 1 month.
- The provided 10X RBC Lysis Buffer has been shown to work equally well with blood collected in EDTA, Heparin, or ACD-A.

A. Mammalian Cells

NOTES

- Please refer to the low input protocol in Appendix 1 if you have less than 1×10^6 cells available.
- Please note that for 1×10^6 MEF cells, we recommend using 4 μL undiluted Nuclease Enzyme Mix. For other cell lines, please follow the instructions below.
- All crosslinking reactions (steps 4 – 11) should be carried out at room temperature.

1. Harvest cells and wash in 1X PBS.
2. Count the cells and use 1×10^6 cell aliquot to proceed.
3. Spin 1×10^6 cell aliquot at 2,000 x g for 5 minutes. Carefully remove supernatant.
4. Resuspend pellet in:
1 mL 1X PBS
10 μL 0.3 M DSG
5. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
6. Add 27 μL 37% formaldehyde.
7. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
8. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant. Use caution as the pellet might be loose.
9. 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.
10. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant.
11. Repeat steps 9 and 10 once for a total of 2 washes.
12. After removing the second wash, 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. Mix by inversion.
17. Add 3 μL of 20% SDS to lyse the cells. Mix by inversion.
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 Quantification.

B. Mammalian Tissues

NOTES:

- **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.**
- **All crosslinking reactions (steps 3 – 10) should be carried out at room temperature.**

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 liquid nitrogen (see example of desired consistency in figure below).
3. Transfer the disrupted tissue sample to a 1.5 mL tube containing:
1 mL 1X PBS
10 μ L 0.3 M DSG
4. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
5. Add 27 μ L 37% formaldehyde.
6. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
7. Spin the tube at 3,000 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 μ L of Wash Buffer and pipet to break up clumps then add the remaining 800 μ L. Pipet up and down to fully resuspend the pellet.
9. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant.
10. Repeat steps 8 and 9 once for a total of 2 washes.
11. After removing the second wash, resuspend the pellet in 1 mL 1X Wash Buffer. Pipet up and down to fully resuspend.
12. Using a 1 mL syringe, gently push the 1 mL of resuspended sample through a 200 μ m filter into a new 5 mL tube. If the filter clogs, replace with a new 200 μ m filter and continue until all of the sample has been filtered.
13. Gently pass an additional 1 mL of 1X Wash Buffer through the 200 μ m 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 μ m filter, re-filter the 2 mL sample into a new 5 mL tube.
15. Gently pass an additional 1 mL of 1X Wash Buffer through the 50 μ m filter into the 5 mL tube. Your tube should now contain a total volume of ~3 mL.
16. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant.
17. Resuspend the pellet in 50 μ L 1X Nuclease Digest Buffer (freshly prepared, see Getting Started)
18. Pre-warm the tube containing your resuspended cells to 30°C for 2 minutes in an agitating thermal mixer set at 1,250 rpm.
19. Transfer 0.5 μ L of Nuclease Enzyme Mix to the pre-warmed tube. Pipet up and down to mix.
20. Incubate the tube for exactly 30 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.

21. Stop the reaction by adding 5 μL of 0.5 M EDTA. Mix by inversion.
22. Add 3 μL of 20% SDS to lyse the cells. Mix by inversion.
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 Quantification.

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

A



B



C. Mammalian Blood

NOTES:

- **Fresh blood samples yield higher amounts of cells. If you are using a blood sample which was flash frozen and stored at -80°C , increase the volume to 3 mL of blood for step 1 and mix with 30 mL 1X RBC Lysis Buffer.**
- **All crosslinking reactions (steps 8 – 15) should be carried out at room temperature.**

1. In a 15 mL conical tube, add 10 mL 1X RBC Lysis Buffer to 1 mL of your fresh blood sample.
2. Mix the tube by inversion. Rotate at room temperature for 10 minutes.
3. Spin the tube at $500 \times g$ for 5 minutes. Carefully remove the supernatant (please refer to your laboratory guidelines for blood disposal). We recommend using a swinging bucket rotor if available to help with pellet visualization.
4. Wash the pellet with a total of 5 mL 1X PBS: first add 200 μL of 1X PBS and pipet to break up clumps then add the remaining 4.8 mL. Pipet up and down to fully resuspend the pellet.
5. Spin the tube at $500 \times g$ for 5 minutes. Carefully remove the supernatant.
6. Resuspend the pellet in 1X PBS and count the cells. Aliquot 1×10^6 cells into a 1.5 mL tube. You can spin the remaining cells at $3,000 \times g$ for 5 minutes and store the pellet at -80°C .
7. Spin the 1×10^6 cell aliquot at $3,000 \times g$ for 5 minutes. Carefully remove the supernatant.
8. Resuspend the pellet in:
 - 1 mL 1X PBS
 - 10 μL 0.3 M DSG
9. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
10. Add 27 μL 37% formaldehyde
11. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
12. Spin the tube at $3,000 \times g$ for 5 minutes. Carefully remove the supernatant. Use caution as the pellet might be loose.
13. 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.
14. Spin the tube at $3,000 \times g$ for 5 minutes. Carefully remove the supernatant.
15. Repeat steps 13 and 14 once for a total of 2 washes.
16. After removing the second wash, resuspend the pellet in 50 μL 1X Nuclease Digest Buffer (freshly prepared, see Getting Started).
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. Mix by inversion.
21. Add 3 µL of 20% SDS to lyse the cells. Mix by inversion.
22. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250rpm.
23. Continue to Stage 2: Lysate Quantification.

Stage 2: Lysate Quantification

Getting Started

- Lysate Quantification should take 2 hours. The lysate quantification step is the same for all sample types: cells, tissue, and blood.
- 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 our recommended kits for each system.

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

- Make sure your Zymo™ DNA Wash Buffer contains the appropriate volume of 100% Ethanol before use as specified by the manufacturer.
- This Stage has two objectives:
 - (i) Determines the volume of sample to use in Stage 3.
 - (ii) Serves as a QC checkpoint for the chromatin digestion.

1. Transfer 2.5 μL of the lysate to a new tube labeled QC. **Store the remainder of your lysate at -80°C . This is the lysate you will be using in Stage 3. It can be stored for up to 3 months.**

2. Add to the QC tube:

50 μL 1X Crosslink Reversal Buffer
1.5 μL Proteinase K

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

15 minutes at 55°C , followed by
45 minutes at 68°C
Hold at 25°C

For convenience, you can hold the QC tube at 25°C overnight in the agitating thermal mixer set at 1,250 rpm.

4. Quick spin your QC tube then clean up your QC sample using the Zymo DNA Clean & Concentrator™ -5 column by adding 200 μL of DNA Binding Buffer to your QC tube. Mix thoroughly.
5. Transfer the mixture to the Zymo-Spin™ Column placed in the collection tube.
6. Centrifuge for 30 seconds at 13,000x g. Discard the flow-through.
7. Add 200 μL Zymo™ DNA Wash Buffer to the column (see Getting Started).
8. Centrifuge for 1 minute at 13,000x g. Discard the flow-through.
9. Repeat steps 7 and 8 once, for a total of 2 washes.
10. Transfer column to a new 1.5 mL tube.
11. Add 10 μL Zymo™ DNA Elution Buffer directly to the column and incubate for 1 minute at room temperature.
12. Centrifuge for 1 minute at 13,000x g. Discard the column. Your 1.5 mL tube now contains your purified QC DNA.
13. Quantify your purified QC DNA using a Qubit® Fluorometer and Qubit® dsDNA HS Kit.

Based on your Qubit concentration, your total lysate amount (ng) can be calculated using the following equation:

$$Total\ Lysate\ (ng) = \frac{Qubit\ (ng/\ \mu L) \times 10}{0.04}$$

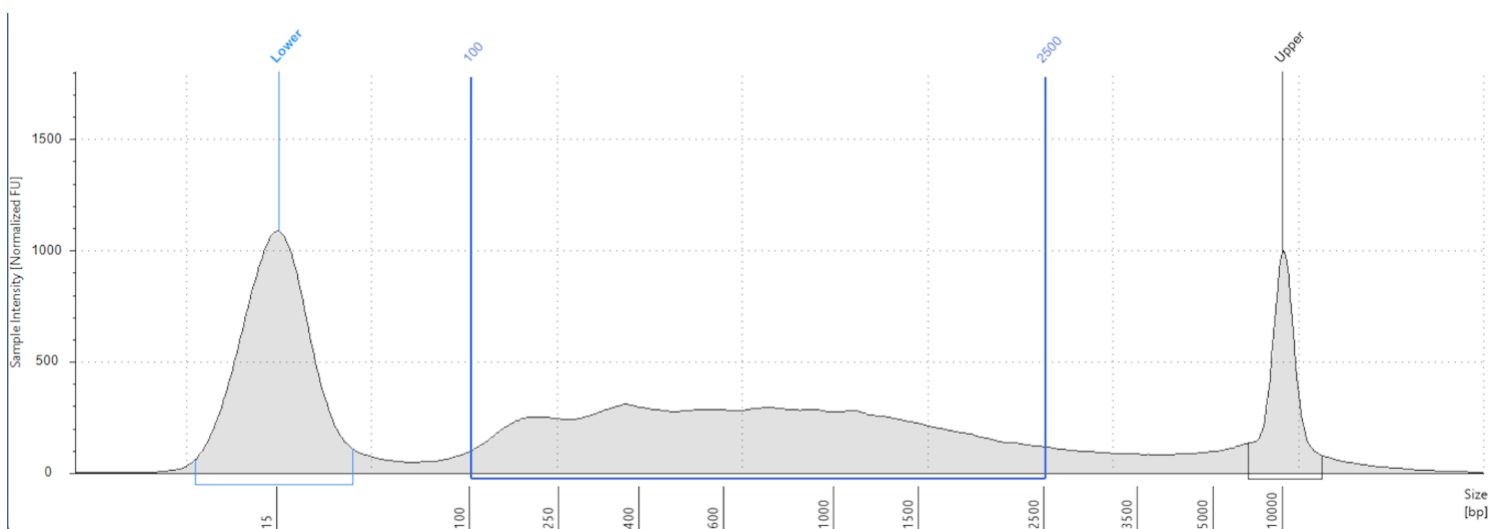
Please proceed to Stage 3 with a volume of your lysate sample that corresponds to 1,000 ng. This volume can be calculated using the following equation:

$$volume\ (ul) = \frac{1000\ (ng) \times 58.5\ (uL)}{Total\ Lysate\ (ng)}$$

If your sample has < 1,000 ng, please use all your sample to proceed to Stage 3: Proximity Ligation

14. Check the fragment size distribution of your purified QC sample on a TapeStation D5000 HS ScreenTape. Make sure your sample is diluted to 1 ng/μL.
15. 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.

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.



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		Blue

Stage 3: Proximity Ligation

Getting Started

- Proximity ligation should take 5.5 hours.
 - Agitating thermal mixer should be set at 1,250 rpm for 1.5 mL tubes.
 - When placing the sample on the magnet, always wait until the solution is clear to allow the beads to fully separate before removing the supernatant.
 - 80% ethanol should be freshly prepared for DNA purification with SPRIselect Beads.
-

I. Bind Chromatin to Chromatin Capture Beads

1. Allow Chromatin Capture Beads to reach room temperature. Vortex prior to use.
2. Transfer 100 μ L Chromatin Capture Beads to a new 1.5 mL tube.
3. Add to the 1.5 mL tube 1,000 ng of your sample from Stage 1 as calculated in Stage 2. If your sample has less than 1,000 ng, please use all of your sample.
4. Pipet up and down 10 times to fully mix. Incubate for 10 minutes at room temperature off the magnetic rack.
5. Place the tube in the magnetic rack for 5 minutes or until the solution looks clear and the beads have fully separated. Remove supernatant.
6. Remove the tube from the magnetic rack and wash the beads with 150 μ L 1X Wash Buffer: pipet up and down 10 times to resuspend the beads then place the tube in the magnetic rack for 1 minute. Remove supernatant.
7. Repeat step 6 once for a total of 2 washes.

II. End Polishing

1. Remove the tube from the magnetic rack then add to beads:
 - 50 μ L End Polishing Buffer
 - 3.5 μ L End Polishing enzyme mix
2. Pipet up and down 10 times to fully mix. Incubate in an agitating thermal mixer set at 1,250 rpm for:
 - 30 minutes at 22°C followed by,
 - 30 minutes at 65°C.

3. Allow the tube to reach room temperature then place the tube in the magnetic rack for 1 minute or until the solution looks clear and the beads have fully separated. Remove supernatant.
4. Remove the tube from the magnetic rack and wash the beads once with 150 μ L 1X Wash Buffer: pipet up and down 10 times to resuspend the beads then place the tube in the magnetic rack for 1 minute. Remove supernatant.

III. Bridge Ligation

NOTES: The Bridge Ligation Mix (50 μ L) should be made fresh and used the same day. Store tube on ice prior to use. To prepare 50 μ L Bridge Ligation Mix, mix:

10 μ L	5X Ligation Buffer
5 μ L	Bridge
35 μ L	UltraPure Water

1. Remove the tube from the magnetic rack then add to beads:
50 μ L Bridge Ligation Mix (freshly made, see Notes)
1 μ L Bridge Ligase (T4 DNA Ligase)
2. Pipet up and down 10 times to fully mix. Incubate for 30 minutes at 22°C, in an agitating thermal mixer set at 1,250 rpm.
3. Place tube on the magnetic rack for 1 minute or until the solution looks clear and the beads have fully separated. Remove supernatant.
4. Remove the tube from the magnetic rack and wash the beads once with 150 μ L 1X Wash Buffer: pipet up and down 10 times to resuspend the beads then place the tube in the magnetic rack for 1 minute. Remove supernatant.

IV. Intra-Aggregate Ligation

1. Remove the tube from the magnetic rack then add to beads:
50 μ L Intra-Aggregate Ligation Buffer
2 μ L Intra-Aggregate Ligation Enzyme Mix
2. Pipet up and down 10 times to fully mix. Incubate for 1 hr at 22°C, in an agitating thermal mixer set at 1,250 rpm. For convenience, this ligation reaction can proceed overnight, at 22°C, in an agitating thermal mixer set at 1,250 rpm.
3. Place the tube in the magnetic rack for 1 minute or until the solution looks clear and the beads have fully separated. Remove supernatant.

V. Crosslink Reversal

1. Remove the tube from the magnetic rack then add to beads:
50 μ L 1X Crosslink Reversal Buffer
1.5 μ L Proteinase K
2. Pipet up and down 10 times to fully mix. Incubate in an agitating thermal mixer set at 1,250 rpm for:
15 minutes at 55°C **followed** by
45 minutes at 68°C
Hold at 25°C

For convenience, you can hold at 25°C overnight in an agitating thermal mixer set at 1,250 rpm.
3. Place the tube in the magnetic rack for 1 minute. Transfer 50 μ L of the SUPERNATANT to a new 1.5 mL tube. Discard beads.

VI. DNA Purification on SPRIselect™ Beads

1. Vortex SPRIselect™ Beads for > 30 seconds to resuspend.
2. Add 35 μ L of resuspended SPRIselect Beads to the 1.5 mL tube containing your sample.
3. Vortex to resuspend, quick spin and incubate for 5 minutes at room temperature off the magnetic rack.
4. Place the tube in the magnetic rack for 5 minutes or until the solution looks clear and the beads have fully separated. Remove supernatant.
5. Leave the tube in the magnetic rack, and wash the beads twice with 150 μ L 80% EtOH. Do not resuspend the beads for these washes. Simply add the EtOH, wait for 1 minute then remove the EtOH wash.
6. After the second wash, quick spin the tube and place on the magnet for 1 minute. Use a pipet with a fine tip to remove the last EtOH traces.
7. Air dry beads for 5 minutes on the magnet until no residual EtOH remains on the side of the tube. Do not over dry.
8. Off the magnetic rack, resuspend beads in 52 μ L TE Buffer pH 8.0.
9. Vortex briefly, quick spin and incubate for 5 minutes at room temperature off the magnetic rack.
10. Quick spin the tube and place in the magnetic rack for 1 minute. Transfer 50 μ L of the SUPERNATANT to a new 1.5 mL tube. Discard beads.
11. Quantify the sample using a Qubit Fluorometer and Qubit dsDNA HS Kit. You should recover a minimum of 150 ng to proceed to Stage 4: Library Preparation.
12. You will use 150 ng of your purified DNA for library preparation (Stage 4) in a 50 μ L volume. You can bring up the volume to 50 μ L using TE Buffer pH 8.0.



The purified DNA sample can be stored at -20°C for up to 6 months.

Stage 4: Library Preparation

Getting Started

- The library preparation protocol does not require fragmentation.
 - The library preparation protocol takes two hours.
 - You can use the remainder of your purified DNA from the end of Stage 3 to carry out an additional library preparation, if your application requires more complexity.
-

I. End Repair (Box 2 & Dovetail™ Library Module for Illumina®)

NOTES:

- **The End Repair Buffer may have precipitated in storage. Incubate for at least 10 minutes at 37 °C until there is no visible precipitate.**
- **Pipet up and down to fully mix 250 mM DTT prior to use.**

1. Place in a 0.2 mL PCR tube:
 - 50 µL Purified Sample (150 ng)
 - 7 µL End Repair Buffer
 - 3 µL End Repair Enzyme Mix
 - 0.5 µL 250 mM DTT
2. Pipet up and down 10 times to mix. Quick spin the tube.
3. Incubate in a thermal cycler for:
 - 30 minutes at 20°C, followed by
 - 30 minutes at 65°C,
 - Hold at 12°C.Proceed immediately.

II. Adapter Ligation & USER Digest (Dovetail™ Library Module for Illumina)

1. Add to the 0.2 mL PCR tube containing 60.5 µL of end-repaired sample:
 - 2.5 µL Adaptor for Illumina
 - 1 µL Ligation Enhancer
 - 30 µL Ligation Enzyme Mix
2. Pipet up and down 10 times to mix. Quick spin the tube.
3. Incubate for 15 minutes at 20°C in a thermal cycler. Hold at 12°C.
3. Following incubation, add 3 µL of USER Enzyme Mix to the PCR tube.
4. Pipet up and down 10 times to mix. Quick spin the tube.
5. Incubate for 15 minutes at 37°C in a thermal cycler. Hold at 12°C.

III. DNA Purification (Box 1)

1. Vortex SPRIselect Beads for 30 seconds to resuspend.
2. Add 80 μ L of the resuspended SPRIselect Beads to the PCR tube.
3. Vortex to resuspend, quick spin and incubate for 5 minutes at room temperature off the magnetic rack.
4. Quick spin the tube and place in the magnetic rack for 5 minutes. Remove supernatant.
5. Leave the tube in the magnetic rack, and wash the beads twice with 150 μ L 80%EtOH. Do not resuspend the beads for these washes.
6. After the second wash, quick spin the tube and place in the magnetic rack for 1 minute. Use a pipet with a fine tip to remove the last EtOH traces.
7. Air dry the beads for 5 minutes in the magnetic rack until no residual EtOH remains. Do not over dry.
8. Off the magnetic rack, resuspend beads in 100 μ L TE Buffer pH 8.0.
9. Vortex briefly, quick spin and incubate for 5 minutes at room temperature off the magnetic rack.
10. Quick spin the tube and place in the magnetic rack for 1 minute.
11. Transfer 95 μ L of the SUPERNATANT to a new tube. Discard the beads.



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

Stage 5: Ligation Capture & Amplification

Getting Started

- The Ligation Capture & Amplification protocol should take 2 hours.
-

I. Streptavidin Beads Preparation (Box 1)

NOTE: This step does not involve any DNA sample.

1. Vortex Streptavidin Beads thoroughly to resuspend. Transfer 25 μ L of the resuspended Streptavidin Beads to a new 1.5 mL tube.
2. Place the tube containing the Streptavidin Beads in the magnetic rack for 5 minutes. Remove supernatant.
3. Remove the tube from the magnetic rack, wash Streptavidin beads with 200 μ L TWB (Red Label): pipet up and down 10 times to resuspend the beads then place tube in the magnetic rack for 1 minute. Remove supernatant.
4. Repeat step 3 for a second wash.
5. After removing the second wash, resuspend the Streptavidin Beads in 100 μ L 2X NTB (Yellow Label). Pipet up and down 10 times to mix.

II. Ligation Capture (Box 1)

1. Transfer 95 μ L of the purified DNA to the 1.5 mL tube containing the Streptavidin Beads resuspended in 100 μ L of 2X NTB.
2. Vortex for 10 seconds to thoroughly mix. Quick spin tube.
3. Incubate for 30 minutes at 25°C, in an agitating thermal mixer set at 1,250 rpm.

III. Wash Sample on Streptavidin Beads (Box 1)

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 10 times to resuspend the beads then place tube in the magnetic rack for 1 minute before removing the supernatant (remove all of the supernatant between each wash; residual supernatant can interfere with the downstream PCR).

1. Quick spin tube and place in the magnetic rack for 1 minute. Remove supernatant.
2. Wash beads once with 200 μ L LWB (Green Label).
3. Wash beads twice with 200 μ L NWB (Blue Label).
4. Wash beads twice with 200 μ L 1X Wash Buffer.

IV. Index PCR (Box 2, Dovetail™ Library Module for Illumina® & Dovetail™ Primer Set for Illumina®)

NOTE: Not all PCR enzymes and master mixes are compatible for amplification in the presence of streptavidin beads. Please use the PCR ready mix supplied in your Dovetail Kit (Box 2).

1. After the last wash has been aspirated, remove the tube from the magnetic rack then add to beads:
 - 25 µL HotStart PCR Ready Mix
 - 5 µL Universal PCR Primer
 - 5 µL Index Primer (unique to each sample, see Appendix 3 for list of primers)
 - 15 µL UltraPure DNase/RNase-Free Distilled Water
2. Pipet up and down 10 times to resuspend then transfer to a new 0.2 mL PCR tube.
3. Quick spin the tube and place it into the thermal cycler. Run the following program:

Temperature	Time	Cycles
98°C	3 minutes	---
98°C	20 seconds	12 cycles
65°C	30 seconds	
72°C	30 seconds	
72°C	1 minute	---
12°C	Hold	

V. Size Selection

1. Quick spin the PCR tube and place in the magnetic rack for 1 minute.
2. Transfer 47 µL of the SUPERNATANT to a new 1.5 mL tube. Discard beads.
3. Bring the volume of the sample in the 1.5 mL tube to 100 µL using TE Buffer pH 8.0.
4. Vortex SPRIselect™ Beads for 30 seconds to resuspend.
5. Add 50 µL of resuspended SPRIselect™ Beads to the 1.5 mL tube containing your sample.
6. Vortex to resuspend, quick spin and incubate for 10 minutes at room temperature off the magnetic rack.
7. Quick spin the tube and place on the magnet for 5 minutes.
8. Transfer 145 µL of the SUPERNATANT to a new 1.5 mL tube. Discard beads.
9. Add 30 µL of resuspended SPRIselect Beads to the 1.5 mL tube.

10. Vortex to resuspend, quick spin and incubate for 10 minutes at room temperature off the magnetic rack.
11. Quick spin the tube and place in the magnetic rack for 5 minutes. Remove supernatant.
12. Leave tube in the magnetic rack, and wash beads twice with 200 μ L 80% EtOH. Do not resuspend the beads for these washes.
13. Quick spin the tube and place in the magnetic rack for 1 minute. Use a 10 μ L pipet tip to remove traces of EtOH.
14. Air dry beads for 5 minutes in the magnetic rack until no residual EtOH remains. Do not over dry.
15. Off the magnetic rack, resuspend beads in 30 μ L TE Buffer pH 8.0.
16. Pipet up and down 10 times to resuspend. Quick spin and incubate for 2 minutes at room temperature off the magnetic rack.
17. Quick spin the tube and place in the magnetic rack for 1 minute.
18. **Transfer 28 μ L of the SUPERNATANT to a new 1.5 mL tube. The tube containing the supernatant is your size selected library.** Discard the beads.
19. Quantify your size selected library using a Qubit Fluorometer and Qubit dsDNA HS Kit. You should recover at least 60 ng of DNA.
20. 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.



You can store the library at -20°C for up to 6 months.

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).
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. Mix by inversion.
18. Add 3 μL of 20% SDS to lyse the cells. Mix by inversion.
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 Quantification.

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 18. Upon completing step 18, continue with the steps below which are customized for low input samples.

19. 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).
20. Transfer 1 μL of Nuclease Enzyme Mix (DILUTED) to the prewarmed tube. You can discard the remainder of your Nuclease Enzyme Mix (DILUTED).
21. Incubate the tube for exactly 30 minutes at 30°C in an agitating thermal mixer at 1,250 rpm.
22. Stop the enzymatic reaction by adding 5 μL of 0.5 M EDTA and mix by inversion.
23. Add 3 μL of 20% SDS to the tube to lyse the cells; mix by inversion.
24. Incubate for 5 minutes at 30°C in an agitating thermal mixer at 1,250 rpm.
25. Continue to Stage 2: Lysate Quantification.

Appendix 2: Troubleshooting Guide

Chromatin Digestion Efficiency (CDE) Out Of Range

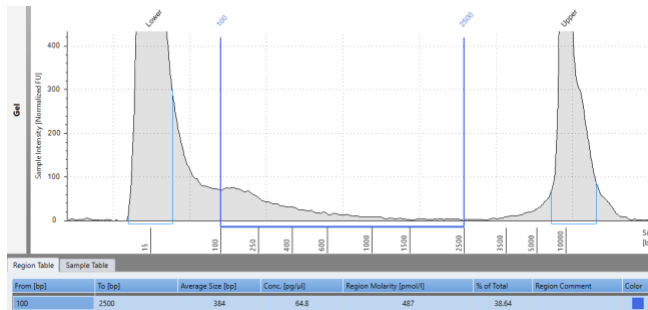
You are following this guide because your CDE is < 50%.

CDE \geq 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%).



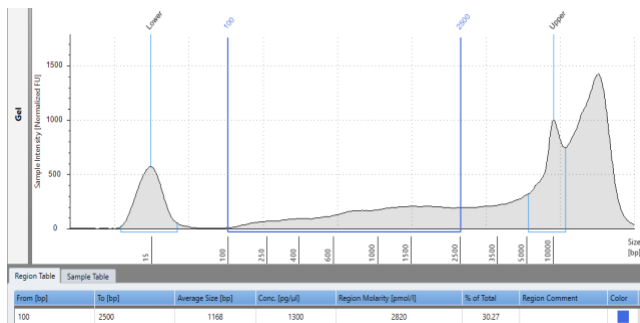
Solution:

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 μ L of Nuclease Enzyme Mix into 18 μ L 1X Nuclease Digest Buffer (freshly prepared).
- Transfer 1 μ L 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 μ L of Nuclease Enzyme Mix (**UNDILUTED**) to the pre-warmed sample tube.

Appendix 3: Index Primers

Omni-C™ Primer Set for Illumina includes the following eight index primers.

Index Primer	Sequence
Index Primer 2	CGATGT
Index Primer 4	TGACCA
Index Primer 5	ACAGTG
Index Primer 6	GCCAAT
Index Primer 7	CAGATC
Index Primer 8	ACTTGA
Index Primer 12	CTTGTA
Index Primer 19	GTGAAA

To choose which index primers to use for multiplexing, please refer to the table below:

Number of Libraries	Index Primer Combination
2	6 and 12 <i>or</i> 5 and 19
3	2, 7 and 19 <i>or</i> either of the 2-plex options plus any other Index Primer
4	5, 6, 12 and 19 <i>or</i> either of the 3-plex options plus any other Index Primer