Dovetail
GENOMICS

Omni-C™ Proximity Ligation Assay

Non-mammalian Samples Protocol

version 1.2

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Table of Contents

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® Dual Index Primer Set #1 For Illumina® (PN DG-PRS-002)		
Components	**Color**	**Storage**
Unique Dual Index (UDI) Primer Pairs (x8, different)		**-30°C to -10°C**

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

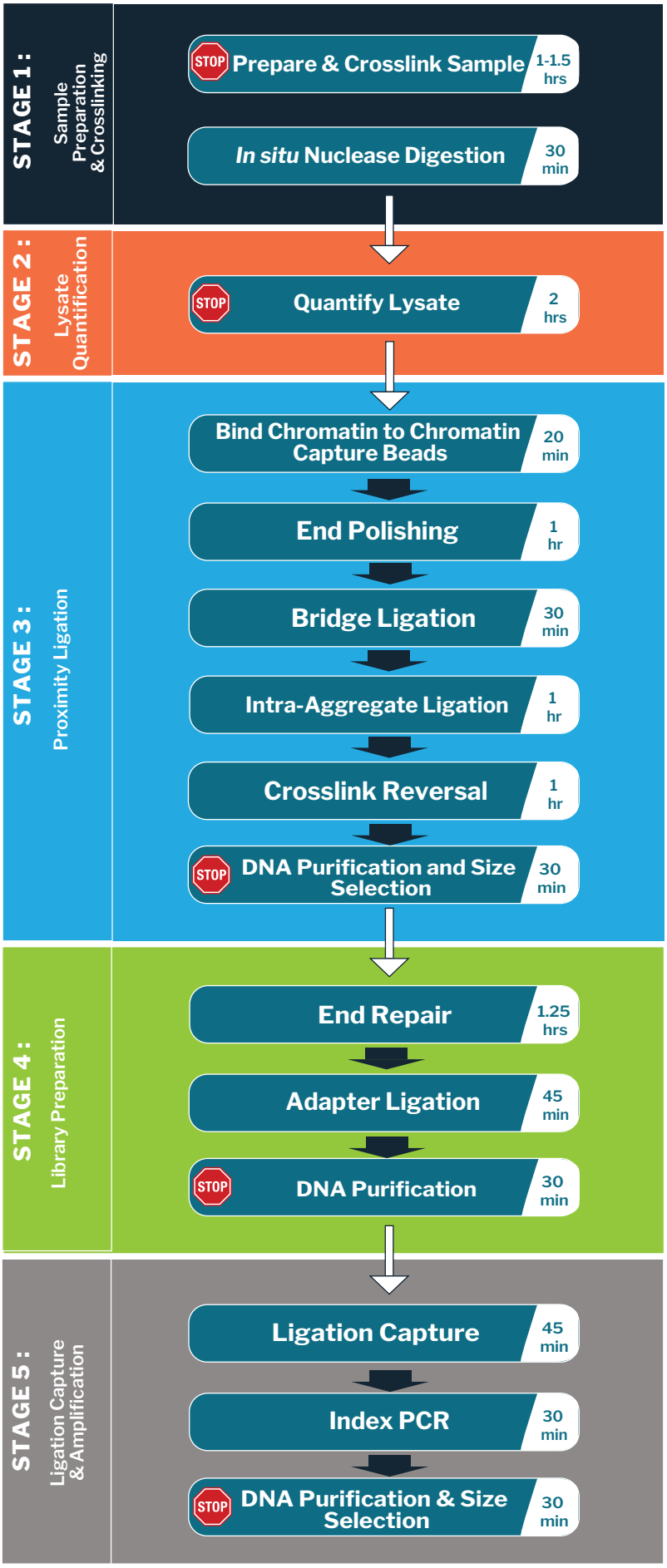
Reagents

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 4 separate protocols for sample preparation depending on your sample type: nucleated (non-mammalian) blood, animal tissue, insects/marine invertebrates, or plants.
- For sample preparation protocols requiring DSG, prepare 0.3 M DSG in DMSO (anhydrous) prepare immediately before use. DSG is water-insoluble and moisture-sensitive. Do not store DSG in solution.
- 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 ~4 to 8 mL of 1X Wash Buffer per sample depending on your sample type. 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 ~0.5 mL of 1X Crosslink Reversal Buffer per sample. 1X Crosslink Reversal Buffer is stable at room temperature for 2 months
- 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 across all sample types except for plants. For plants, you will need 163 µL 1X Nuclease Digest Buffer per sample. To prepare 50 µL of 1X Nuclease Digest Buffer, mix the following components:

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

A. Nucleated Blood

NOTES

- **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.**

1. Aliquot 10 µL of blood into 1 mL of 1X PBS in a 1.5 mL tube.
2. Spin the tube at 2,500 x g for 5 minutes. Carefully remove the supernatant.
3. Resuspend the pellet in:
   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 37% formaldehyde.
6. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
7. Spin the tube at 5,000 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 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 5,000 x g for 5 minutes. 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 5,000 x g for 5 minutes. 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 <u>exactly</u> 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. Animal Tissues (other than insects & marine invertebrates)

NOTES:

- **All cross-linking 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:

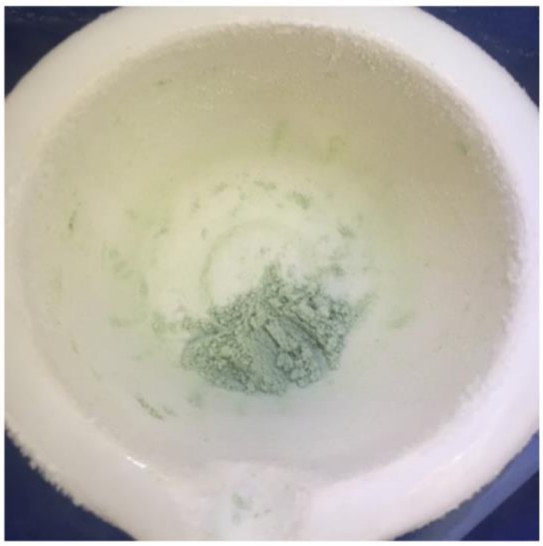
   1 mL 1X PBS
   10 µL 0.3 M DSG
4. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
5. Add 27 µL 37% formaldehyde.
6. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
7. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant. Use caution as the pellet might be loose.
8. Wash the pellet with a total of 1 mL 1X Wash Buffer: first add 200 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 3,000 x g for 5 minutes. Carefully remove the supernatant.
10. Repeat steps 8 and 9 once.
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 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.
13. Gently pass an additional 1 mL of 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 of 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. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant.
17. Resuspend the pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Getting Started).
18. Pre-warm the tube containing your resuspended cells at 30°C for 2 minutes in an agitating thermal mixer set at 1,250 rpm.
19. Transfer 0.5 µL of Nuclease Enzyme Mix to the pre-warmed tube. Pipet up and down to mix.
20. Incubate the tube for <u>exactly</u> 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. Insects & marine invertebrates

NOTES:

- **We recommend degutting adults prior to freezing. Alternatively, they may be starved for a few days prior to freezing. Flash freeze the individuals in liquid nitrogen, either in bulk for inbred species or individually for outbred species, and store at -80°C prior to use.**
- **All crosslinking reactions (steps 3 – 8) 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.
3. Transfer the disrupted tissue sample to a 1.5 mL tube containing:

   1 mL 1X PBS
   27 µL 37% Formaldehyde

4. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
5. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant. Use caution as the pellet might be loose.
6. 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.
7. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant.
8. Repeat steps 6 and 7 once.
9. After removing the second wash, resuspend the pellet in 1 mL 1X Wash Buffer. Pipet up and down to fully resuspend.
10. 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.
11. Gently pass an additional 1 mL of 1X Wash Buffer through the 200 µm filter into the 5 mL tube. Your tube should now contain a total volume of ~2 mL.
12. Using the same syringe but changing to a 50 µm filter, re-filter the 2 mL sample into a new 5 mL tube.
13. Gently pass an additional 1 mL of 1X Wash Buffer through the 50 µm filter into the 5 mL tube. Your tube should now contain a total volume of ~3 mL.
14. Spin the tube at 3,000 x g for 5 minutes. Carefully remove the supernatant.
15. Resuspend the pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Getting Started).
16. Pre-warm the tube containing your resuspended cells at 30°C for 2 minutes in an agitating thermal mixer set at 1,250rpm.
17. Transfer 0.5 µL of Nuclease Enzyme Mix to the pre-warmed tube. Pipet up and down to mix.
18. Incubate the tube for <u>exactly</u> 30 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
19. Stop the reaction by adding 5 µL of 0.5 M EDTA. Mix by inversion.
20. Add 3 µL of 20% SDS to lyse the cells. Mix by inversion.
21. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer set at 1,250 rpm.
22. Continue to Stage 2: Lysate Quantification

D. Plants

NOTES:

- **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 – 8) should be carried out at room temperature.**

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 liquid nitrogen (see example of desired consistency below).
3. Transfer the ground sample to a 5 mL tube which contains:

   4 mL 1X PBS
   108 µL 37% Formaldehyde
4. Rotate the tube for 10 minutes at room temperature. The tissue sample should not settle.
5. 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.
6. 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 1500 µL. Vortex to fully resuspend the pellet.
7. Spin the tube at 5,000 x g for 5 minutes. Carefully remove the supernatant.
8. Repeat steps 6 and 7 once.
9. After removing the second wash, resuspend the pellet in 1 mL 1X Wash Buffer. Vortex to fully resuspend.
10. 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.
11. 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.
12. Using the same syringe but changing to a 50 µm filter, re-filter the 2 mL sample into a new 5 mL tube.
13. 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.
14. Resuspend well and aliquot evenly into 3 separate tubes (A, B, and C); each corresponds to 100 mg of input plant tissue.
15. Spin tubes A, B and C at 2,000 x g for 5 minutes. Carefully remove the supernatant.
16. Resuspend the pellet in tubes A, B and C in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Getting Started). Leave the tubes on the bench.
17. 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

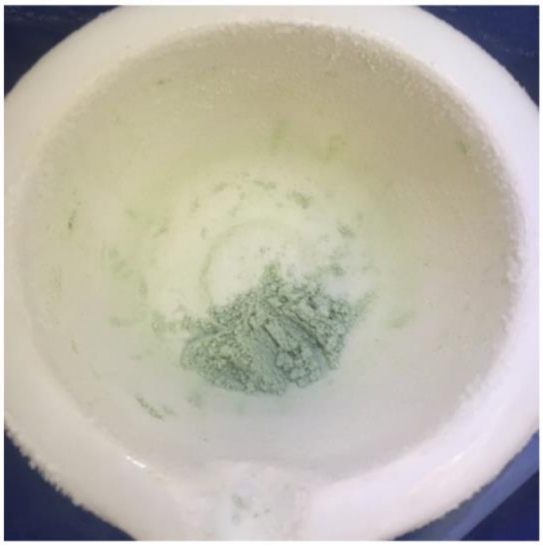
    Mix each dilution well and quick spin before aliquoting.

18. 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.
19. 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
20. Incubate all three tubes for <u>exactly</u> 30 min at 30°C, in an agitating thermal mixer set at 1,250 rpm.
21. Stop the enzymatic reaction by adding 5 µL of 0.5M EDTA. Mix by inversion.
22. Add to each tube 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



Stage 2: Lysate Quantification (for all sample types except plants)

Getting Started

- Lysate Quantification should take 2 hours.
- 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

- Verify before use that the Zymo™ DNA Wash Buffer contains the appropriate volume of 100% Ethanol, 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

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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. DO NOT DISCARD. 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 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. Clean up your QC sample using Zymo DNA Clean & Concentrator™-5 kit: start by adding 200 µL of DNA Binding Buffer to your QC tube. Mix thoroughly.
5. Transfer the mixture to a Zymo-Spin™ Column placed in a collection tube.
6. Centrifuge for 30 seconds at 13,000 x g. Discard the flow-through.
7. Add to the column 200 µL Zymo™ DNA Wash Buffer (see notes).
8. Centrifuge for 1 minute at 13,000 x g. Discard the flow-through.
9. Repeat steps 7 and 8 once.
10. Transfer the 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 13,000 x 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:

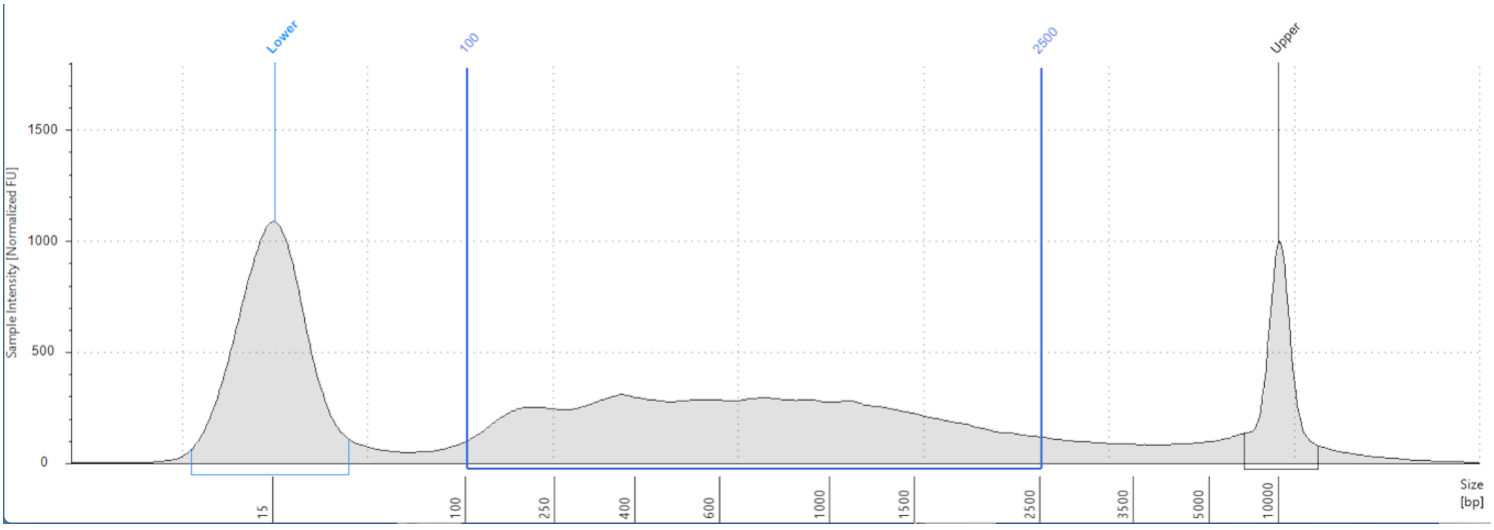
$$Total\ Lysate\ (ng) = \frac{Qubit\ (ng/\mu L)\ x\ 10\ (\mu L)}{0.04}$$

In Stage 3, you should use a volume of your lysate that corresponds to either 1,000 ng or 500 ng depending on your sample type (see table below). If your sample has less than the recommended amount, please use all your sample in Stage 3.

Sample Type	Nucleated blood or animal tissues	Insects or marine invertebrates
Amount of lysate to use in Stage 3	1,000 ng	500 ng
Volume calculated:	$volume\ (ul) = \frac{1000\ (ng)\ x\ 58.5\ (\mu L)}{Total\ Lysate\ (ng)}$	$volume\ (ul) = \frac{500\ (n\,g)\ x\ 58.5\ (\mu L\ )}{Total\ Lysate\ (\ ng)}$

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%, it passes QC. Please proceed to Stage 3: Proximity Ligation.
    - If your CDE < 50%, it does not pass QC. Do not proceed to Stage 3. Instead, please refer to Appendix 1: 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.



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 2: Lysate Quantification (for plants)

Getting Started

- Lysate Quantification should take 2 hours.
- 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

- Verify before use that the Zymo™ DNA Wash Buffer contains the appropriate volume of 100% Ethanol, as specified by the manufacturer.
- This Stage has two objectives:
  (i) Determines the volume of sample to use in Stage 3
  (ii) Determines which of the lysate tubes A, B, or C to use to proceed to Stage 3 based on the chromatin digestion profile

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1. Prepare three 1.5 mL tubes labelled QC-A, QC-B, and QC-C
2. Transfer:
   - 2.5 µL of lysate A to the tube labeled QC-A
   - 2.5 µL of lysate B to the tube labeled QC-B
   - 2.5 µL of lysate C to the tube labeled QC-C

   Store the remainder of your lysate intubes A, B and C at -80°C. You will be using one of these tubes to proceed to Stage 3. DO NOT DISCARD. The lysates can be stored for up to 3 months.
3. Add to each QC tube:
   50 µL 1X Crosslink Reversal Buffer
   1.5 µL Proteinase K
4. Pipet up and down to mix. Incubate the QC tubes in an agitating thermal mixer set at 1,250 for:
   15 minutes at 55°C, followed by
   45 minutes at 68°C
   Hold at 25°C
   For convenience, you can hold the QC tubes at 25°C overnight in the agitating thermal mixer set at 1,250 rpm.
5. Quick spin the QC tubes. Clean up your QC samples using Zymo DNA Clean & Concentrator™-5 kit: start by adding 200 µL of DNA Binding Buffer to each QC tube. Mix thoroughly.
6. For each QC tube, transfer the mixture to a Zymo-Spin™ Column placed in a collection tube.
7. Centrifuge for 30 seconds at 13,000 x g. Discard the flow-through.
8. Add to the column 200 µL Zymo™ DNA Wash Buffer (see notes).
9. Centrifuge for 1 minute at 13,000 x g. Discard the flow-through.

10. Repeat steps 8 and 9 once.
11. Transfer the column to a new 1.5 mL tube. You should have three 1.5 mL tubes.
12. Add 10 µL Zymo™ DNA Elution Buffer directly to the column and incubate for 1 minute at room temperature.
13. Centrifuge for 1 minute at 13,000 x g. Discard the column. Your 1.5 mL tubes now contain your purified QC-A, QC-B and QC-C DNA.
14. Quantify each of 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:

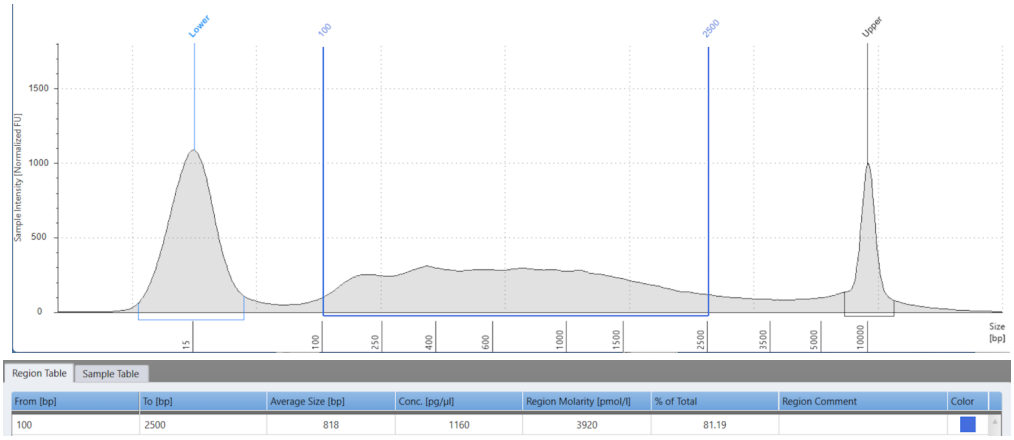
$$Total\ Lysate\ (ng) = \frac{Qubit\ (ng/\mu L)\ x\ 10\ (\mu L)}{0.04}$$

In Stage 3, you should use a volume of your lysate that corresponds to 1,000 ng. This volume can be calculated according to the table below. If your sample has < 1,000 ng, please use all your sample in Stage 3.

Volume (µL) of lysate A to use in Stage 3 (if lysate A is selected) =	Volume (µL) of lysate B to use in Stage 3 (if lysate B is selected) =	Volume (µL) of lysate C to use in Stage 3 (if lysate C is selected) =
$\frac{1000\ (ng)\ x\ 63\ (uL)}{Total\ Lysate\ (ng)}$	$\frac{1000\ (ng)\ x\ 59\ (uL)}{Total\ Lysate\ (ng)}$	$\frac{1000\ (ng)\ x\ 59\ (uL)}{Total\ Lysate\ (ng)}$

15. Check the fragment size distribution of each purified QC sample on a TapeStation D5000 HS ScreenTape. Make sure your samples are diluted to 1 ng/µL.
16. 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%.
    - The lysate tube for which CDE passes QC (≥ 50%) should be selected for Stage 3.
    - If none of your lysates passes QC (all your lysates have CDE < 50%), please contact our technical support team at support@dovetail-genomics.com for the most up to date guidance.

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.



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 The Chromatin to Chromatin Capture Beads

1. Allow the Chromatin Capture Beads to reach room temperature. Vortex prior to use.
2. Transfer 100 µL Chromatin Capture Beads to a new 1.5 mL tube.
3. Add to the 1.5 mL tube 1,000 ng of your lysate (or 500 ng for insects and marine invertebrates) as calculated in Stage 2. If your sample has less than 1,000 ng (or 500 ng for insects and marine invertebrates), 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 µL 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 and add to the beads:
   50 µL End Polishing Buffer
   3.5 µL 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 µL 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 µL) should be made fresh and used the same day. Store tube on ice prior to use. To prepare 50 µL Bridge Ligation Mix, mix:

10 µL 5X Ligation Buffer
5 µL Bridge
35 µL UltraPure Water

1. Remove the tube from the magnetic rack and add to the beads:
   50 µL Bridge Ligation Mix (freshly made, see Notes)
   1 µL 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 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 µL 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 and add to the beads:
   50 µL Intra-Aggregate Ligation Buffer
   2 µL 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 µL 1X Crosslink Reversal Buffer
   1.5 µL 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 µL 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 µL 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 µL 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 µL 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 µL 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 µL volume. You can bring up the volume to 50 µL 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 µL 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 µL 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 µL 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 µL 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.
- Dovetail® Dual Index Primer Set #1 For Illumina® Module supplies premixed unique dual index primer pairs.

I. Streptavidin Beads Preparation (Box 1)

NOTE: This step does not involve any DNA sample.

1. Vortex Streptavidin Beads thoroughly to resuspend. Transfer 25 µL 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 µL 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 µL 2X NTB (Yellow Label). Pipet up and down 10 times to mix.

II. Ligation Capture (Box 1)

1. Transfer 95 µL of the purified DNA to the 1.5 mL tube containing the Streptavidin Beads resuspended in 100 µL 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 and 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 µL LWB (Green Label).
3. Wash beads twice with 200 µL NWB (Blue Label).
4. Wash beads twice with 200 µL 1X Wash Buffer.

IV. Index PCR (Box 2, Dovetail® Library Module for Illumina® & Dovetail® Dual Index Primer Set #1 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).**
- **Keep the UDI Primer Pair on ice while in use.**

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 UDI Primer Pair (unique to each sample; see Appendix 2 for list of primers)
    20µL 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 µL 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 µL 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 µL 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 µL 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.

Stage 6: Sequencing & QC Analysis of Omni-C Libraries

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). We recommend to shallow sequence the library to run a QC analysis prior to deep sequencing. The QC analysis requires 1 to 2 million (2 x 150 bp) read pairs. Dovetail® provides all kit users access to the QC analysis pipeline available on readthedocs (https://omni-c.readthedocs.io/en/latest/#)

Appendix 1: Troubleshooting Guide

Chromatin Digestion Efficiency (CDE) Out Of Range

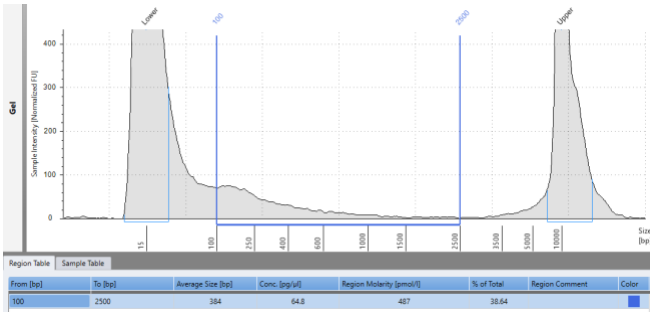
You are following this guide because your CDE is < 50%.

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%).



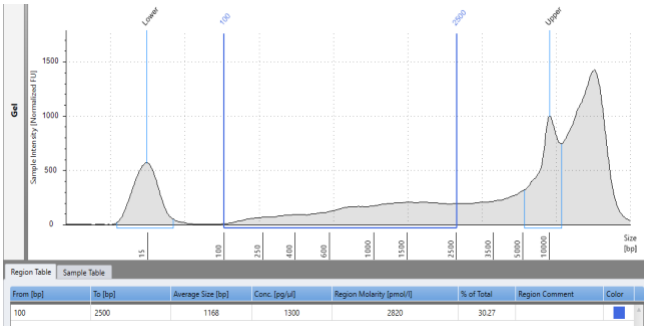
Solution:

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).
- 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 2: Dual Index Primers

Dovetail® Dual Index Primer Set #1 For Illumina® includes the following eight UDI Primer Pairs:

Primer Name	HiSeq® 2000/2500, MiSeq®, NovaSeq® systems i5 index	HiSeq® 3000, 4000, X, NextSeq®, MiniSeq®, iSeq® systems i5 index	All systems i7 index
UDI Primer Pair 1	ATATGCGC	GCGCATAT	CTGATCGT
UDI Primer Pair 2	TGGTACAG	CTGTACCA	ACTCTCGA
UDI Primer Pair 3	AACCGTTC	GAACGGTT	TGAGCTAG
UDI Primer Pair 4	TAACCGGT	ACCGGTTA	GAGACGAT
UDI Primer Pair 5	GAACATCG	CGATGTTC	CTTGTCGA
UDI Primer Pair 6	CCTTGTAG	CTACAAGG	TTCCAAGG
UDI Primer Pair 7	TCAGGCTT	AAGCCTGA	CGCATGAT
UDI Primer Pair 8	GTTCTCGT	ACGAGAAC	ACGGAACA

The indexes on the UDI Primer Pairs are color-balanced in sets of four (1-4, 5-8). Indexes within each group of four are fully color balanced and can be pooled for sequencing. You can multiplex less than four samples, but verify color balance before pooling.