User Supplied Reagents, Consumables & Equipment

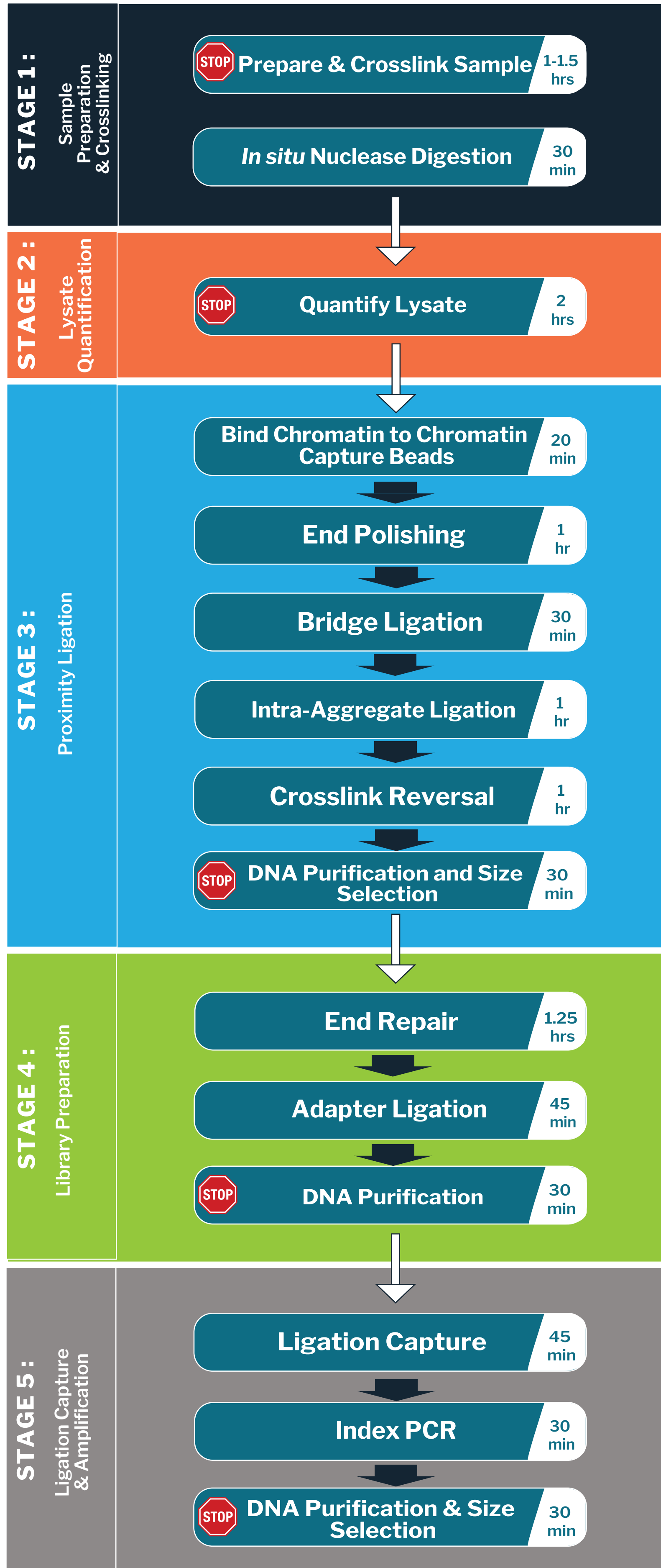
Reagents

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



*** No Fragmentation**

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.**

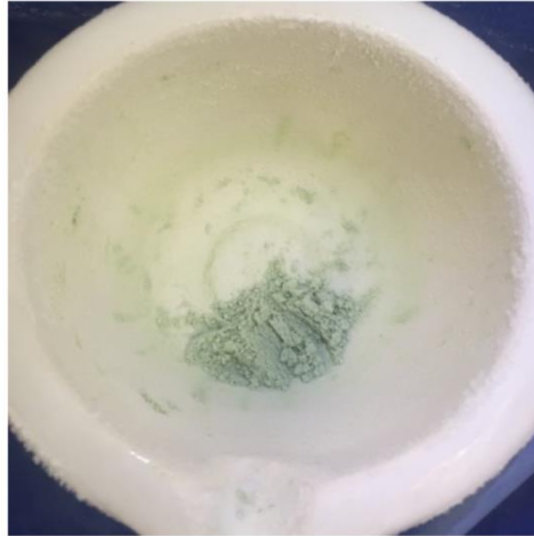
1. Aliquot 10 µL of blood into 1 mL of 1X PBS in a 1.5 mL tube.
2. Spin the tube at 2,500 x g for 5 minutes. Carefully remove the supernatant.
3. Resuspend the pellet in:
   1 mL   1X PBS
   10 µL   0.3 M DSG
4. Rotate the tube for 10 minutes at room temperature. Cells should not settle.
5. Add 27 µL 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 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.

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. 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 (Nucl A, Nucl B, and Nucl C) at 30°C for 2 minutes in an agitating thermal mixer set at 1,250 rpm.
19. Transfer:
    5 µL of pre-warmed Nucl A to pre-warmed tube A
    1 µL of pre-warmed Nucl B to pre-warmed tube B
    1 µL of pre-warmed Nucl 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:

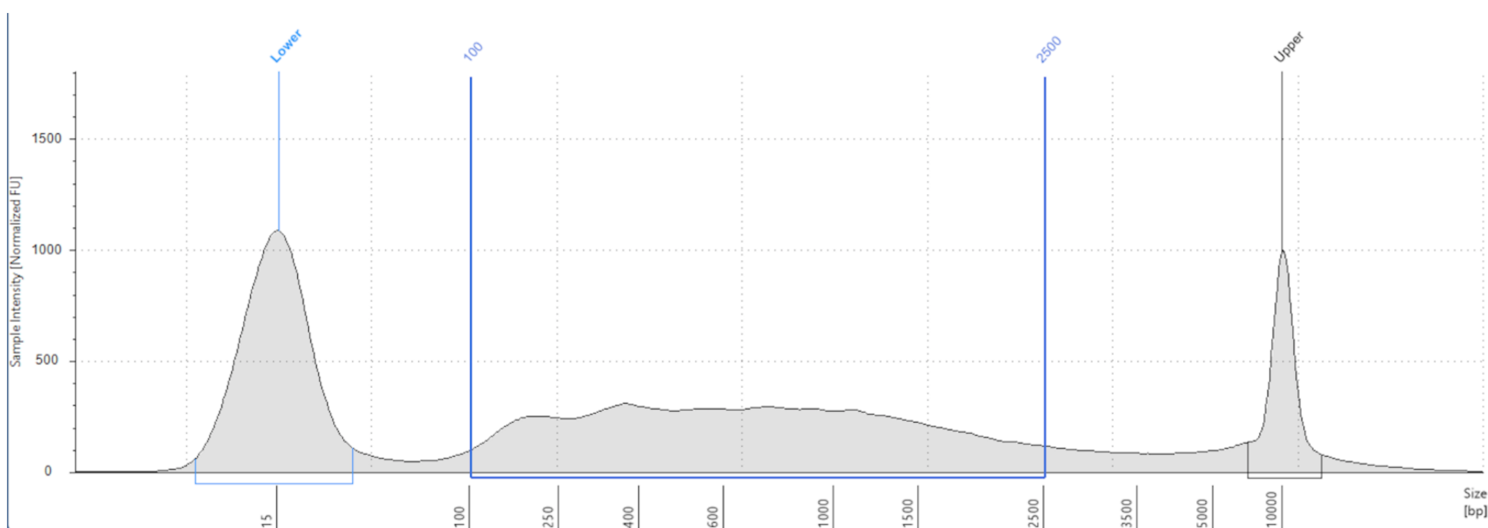
$$Total\ Lysate\ (ng) = \frac{Qubit\ (ng/\mu L)\ x\ 10\ (\mu L)}{0.04}$$

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:	$volume\ (ul) = \frac{1000\ (ng)\ x\ 58.5\ (\mu L)}{Total\ Lysate\ (ng)}$	$volume\ (ul) = \frac{500\ (ng)\ x\ 58.5\ (\mu L)}{Total\ Lysate\ (ng)}$

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.



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:

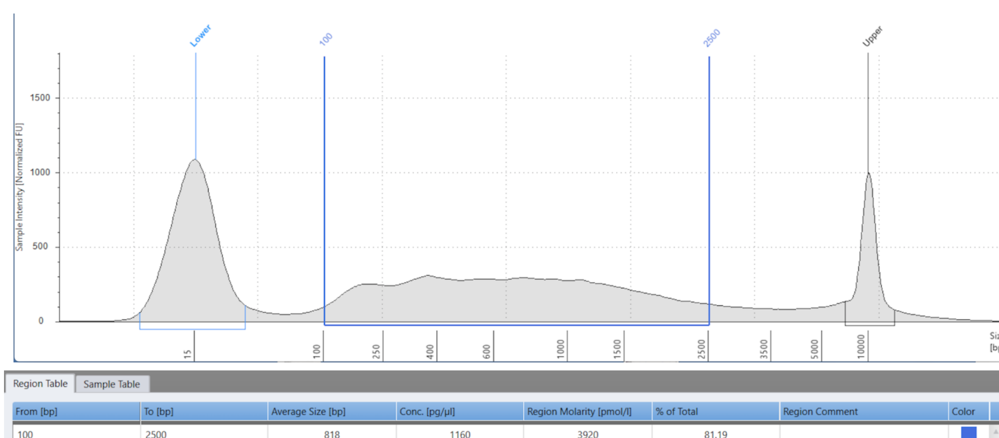
$$Total\ Lysate\ (ng) = \frac{Qubit\ (ng/\mu L)\ x\ 10\ (\mu L)}{0.04}$$

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.



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		

Appendix 1: Troubleshooting Guide

Chromatin Digestion Efficiency (CDE) Out Of Range

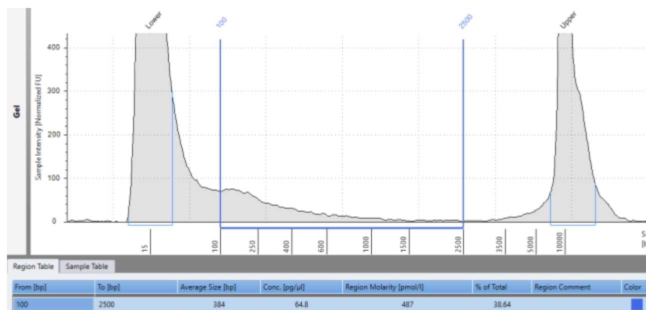
You are following this guide because your CDE is < 50%.

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%).



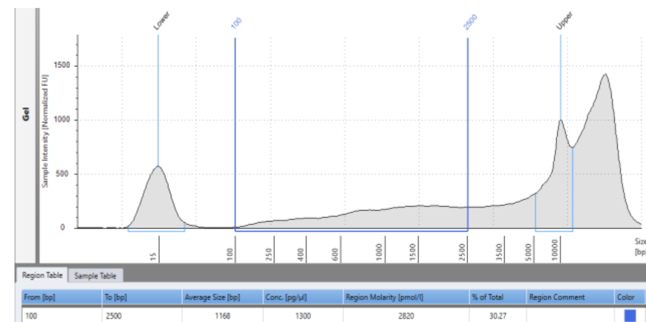
Solution:

Repeat the Sample Preparation and Lysate Quantification stages modifying <u>only</u> 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 <u>only</u> the amount of nuclease enzyme used as follows:

Transfer 2 µL of Nuclease Enzyme Mix (UNDILUTED) to the pre-warmed sample tube.

Appendix 2: ual Index Primers

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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.