Dovetail
GENOMICS

Omni-C™ Proximity Ligation Assay

Protocol
version 1.1

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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-HiC-002)

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		
10% Triton X-100		
100 mM $MnCl_2$		
0.5 M EDTA		

Omni-C™ Kit Box 2
(PN DG-HiC-004)

Components	Color	Storage
Nuclease Enzyme Mix		-30°C to -10°C
10X Nuclease Digest Buffer		
End Polishing Enzyme Mix		
End Polishing Enzyme Buffer		
Intra-Aggregate Ligation Enzyme Mix		
Intra-Aggregate Ligation Buffer		
T4 DNA Ligase		
Bridge		
5X Bridget Ligation Buffer		
250 mM DTT		
HotStart PCR Ready Mix		
Proteinase K		

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

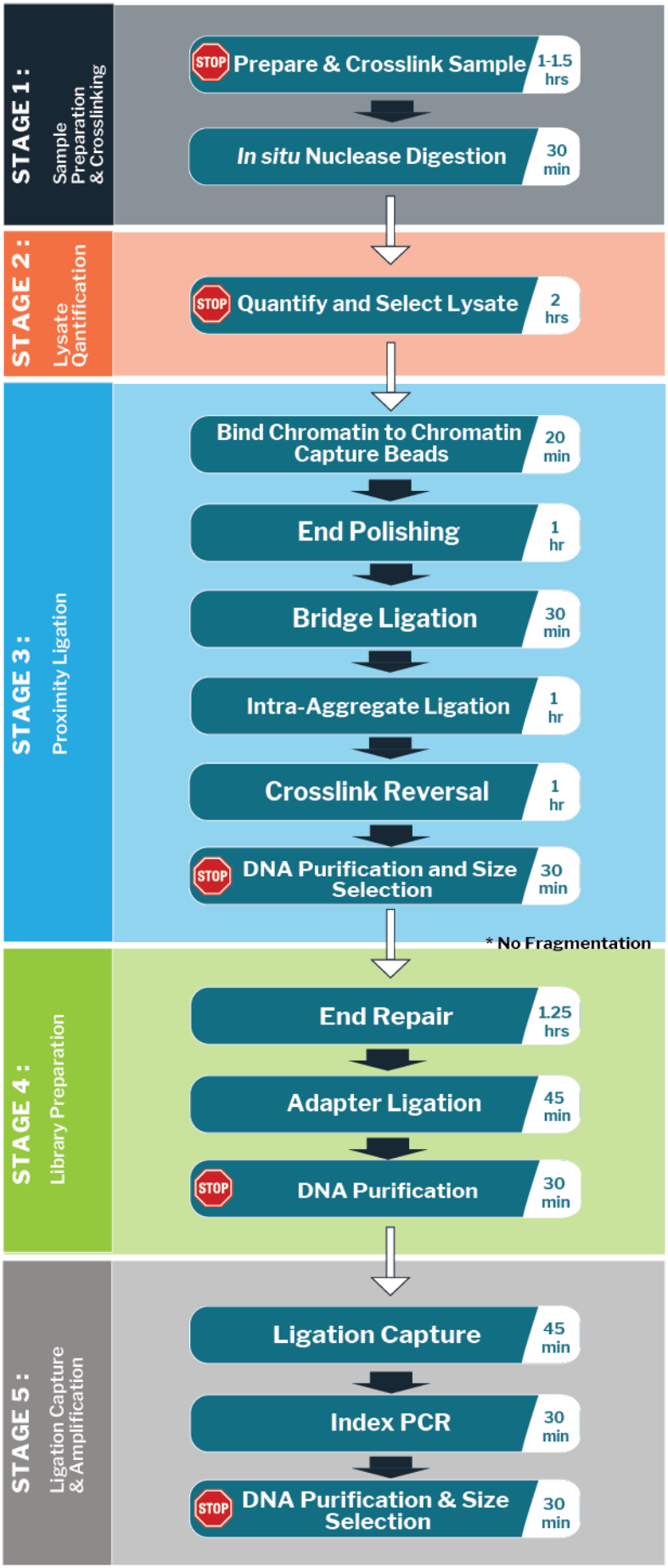
Reagents

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
Genomic DNA Clean & Concentrator™-10, 10 µg	Zymo Research	D4010

Consumables and Equipment

Item	Supplier
1.5 mL Low binding microcentrifuge tubes	Generic
0.2 mL PCR tubes	
5 mL centrifuge tubes	
Pipets and pipet tips	
Magnetic separation rack for 0.2 mL and 1.5 mL tubes	
Agitating thermal rotator	
Thermal cycler	
Vortex Mixer	
Centrifuge for 0.2 mL, 1.5 mL and 5 mL tubes	
Dry ice/petri dish/razor or 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 or Bioanalyzer	Agilent

Omni-C™ Protocol Overview



Getting Started:

The Omni-C™ Kit has been validated on mammalian tissues and cells. If you are using the Omni-C™ Kit on **validated** samples, please follow Stage 1 and 2 according to the protocol below. If you are using the Omni-C kit on **non-validated** samples, please follow Stage 1 and 2 according to Appendix 2. Please note that Stage 3, 4 and 5 are the same for validated and non-validated samples.

You can also choose to follow Appendix 2 to optimize the chromatin digestion specific to your sample.

Stage 1. Sample Preparation

There are two separate protocols for sample preparation depending on your sample type: cells or tissue. The lysate quantification step is the same for both sample types. Sample preparation should take 2 hours.

NOTES

- **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 minute, 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 ~15mL of 1X Wash Buffer per sample. You can also use this 1X Wash Buffer throughout the rest of the protocol. 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. You can also use this 1X Crosslink Reversal Buffer for the Proximity Ligation Protocol. 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 thawing buffers on ice and vortexing prior to use.**

A. Cells

NOTES

- **Please refer to the low input guidelines if you have less than 1 x 10^6 cells available.**

- **Please note that for 1 x 10^6 MEF cells, we recommend using 4 µL undiluted Nuclease Enzyme Mix. For other cell lines, please follow the instructions below.**
- **Before beginning, please prepare <u>fresh</u> 1X Nuclease Digest Buffer and store at room temperature. 1X Nuclease Digest Buffer is stable for 1 day at room temperature.**
  To prepare 1X Nuclease Digest Buffer, mix:

52.5µL	**UltraPure Water**
7.5 µL	**10X Nuclease Digest Buffer**
7.5 µL	**100mM $MnCl_2$**
7.5 µL	**10%Triton**

1. Harvest cells and wash in 1X PBS.
2. Count the cells and use 1 x 10^6 cell aliquot to proceed.
3. Spin 1 x 10^6 cell aliquot at 2,000 x *g* for 5 minute. Carefully remove supernatant.
4. Resuspend pellet in:

1mL	1X PBS
27µL	37% formaldehyde

5. Transfer sample to a 1 mL tube.
6. Rotate tube for 10 minutes at room temperature. Cells should not settle.
7. Spin the tube at 2,000 x *g* for 5 minute. Carefully remove supernatant. Use caution, the pellet might be loose.
8. Wash pellet with 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 tube at 2,000 x *g* for 5 minutes. Carefully remove supernatant
10. Repeat steps 8 and 9 once for a total of 2 washes.
11. After removing the second wash, resuspend pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see notes). Place tube on ice.
12. Meanwhile, make a 1:10 dilution of the Nuclease Enzyme Mix from the kit supplied tube by taking 2 µL of Nuclease Enzyme Mix into 18 µL 1X Nuclease Digest Buffer (freshly prepared, see notes).
13. Pipet up and down 10 times to mix.
14. Pre-warm the tube containing your resuspended cells and your Nuclease Enzyme Mix dilution at the same time to 30°C for 2 minutes, in an agitating thermal mixer (1,250 rpm).
15. Transfer 5 µL of pre-warmed Nuclease Enzyme Mix **dilution** to pre-warmed tube. You can discard the remainder of your pre-warmed Nuclease Enzyme Mix dilution.
16. Incubate tube for <u>exactly</u> 30 minutes at 30°C, in an agitating thermal mixer (1,250 rpm).
17. Stop the reaction by adding 5 µL of 0.5M EDTA. Mix by inversion (3 times).
18. Add 3 µL of 20% SDS to lyse the cells. Mix by inversion (3 times).
19. Incubate the tube for 5 minutes at 30°C, in an agitating thermal mixer (1,250 rpm).
20. Continue to Lysate Quantification Stage 2.

B. Tissue

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.**
- **Before beginning, please prepare <u>fresh</u> 1X Nuclease Digest Buffer and store at room temperature. 1X Nuclease Digest buffer is stable for 1 day at room temperature.**
  To prepare 1X Nuclease Digest Buffer, mix:

52.5µL	**UltraPure water**
7.5 µL	**10X Nuclease Digest Buffer**
7.5 µL	**100mM $MnCl_2$**
7.5 µL	**10%Triton**

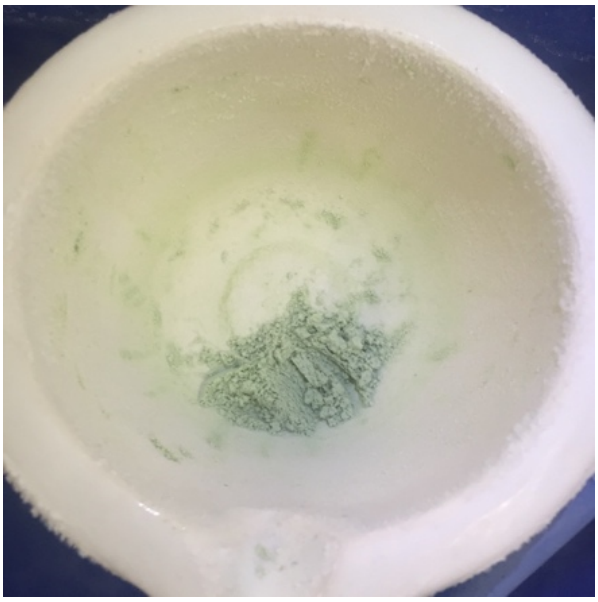
1. Weigh out 20 mg of frozen tissue sample.
2. Disrupt the tissue by grinding it to a fine powder with mortar and pestle in liquid nitrogen (see example of desired consistency below).
3. Transfer the disrupted tissue sample to a 1 mL tube which contains:
   1 mL 1X PBS
   27 µL 37% formaldehyde.
4. Rotate tube for 10 minutes at room temperature. Tissue should not settle.
5. Spin at 2,000 x *g* for 5 minutes. Carefully remove supernatant. If your tissue sample did not pellet, repeat the spin at maximum speed.
6. Wash pellet with 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 tube at 2,000 x *g* for 5 minutes. Carefully remove supernatant.
8. Repeat steps 6 and 7 once for a total of 2 washes.
9. After removing the second wash, resuspend 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 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 though 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 the filter to a 50 µm one, re-filter the 2 mL sample into a new 5 mL tube.
13. Gently pass an additional 1 mL 1X Wash Buffer though the 50 µm filter into the 5 mL tube. Your tube should now contain a total volume of ~3 mL.
14. Spin tube at 2,000 x *g* for 5 minutes. Carefully remove supernatant.

15. Resuspend pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see notes). Place tube on ice.
16. Meanwhile, make a 1:10 dilution of the Nuclease Enzyme Mix from the kit supplied tube by taking 2 µL of Nuclease Enzyme Mix into 18 µL 1X Nuclease Digest Buffer (freshly prepared, see notes above).
17. Pipet up and down 10 times to mix.
18. Pre-warm the tube containing your resuspended pellet and your Nuclease Enzyme Mix dilution at the same time to 30°C for 2 minutes, in an agitating thermal mixer (1,250 rpm).
19. Transfer 5 µL of pre-warmed Nuclease Enzyme Mix **dilution** to pre-warmed tube. You can discard the remaining of your pre-warmed Nuclease Enzyme Mix dilution.
20. Incubate tube for <u>exactly</u> 30 minutes at 30°C, in an agitating thermal mixer (1,250 rpm).
21. Stop the enzymatic reaction by adding 5 µL of 0.5M EDTA and mix by inversion (3 times).
22. Add to tube 3 µL of 20% SDS to lyse the cells; mix by inversion (3 times).
23. Incubate for 5 minutes at 30°C, in an agitating thermal mixer (1,250 rpm).
24. Continue to Lysate Quantification Stage 2.

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

A

B



Stage 2. Lysate Quantification

NOTES:

- **Lysate Quantification should take 2 hours.**
- **If you're using a Bioanalyzer® System to check your fragment size distribution, we recommend the HS DNA Kit. Please note when using a Bioanalyzer® System, the profile will appear different than the one shown below analyzed on a TapeStation® System.**
- **CDI calculation is not compatible with the Fragment Analyzer System.**
- **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) Determine the volume of sample to use to proceed to Stage 3.
(ii) Serves as QC checkpoint for the chromatin digestion based on the TapeStation® System profile.

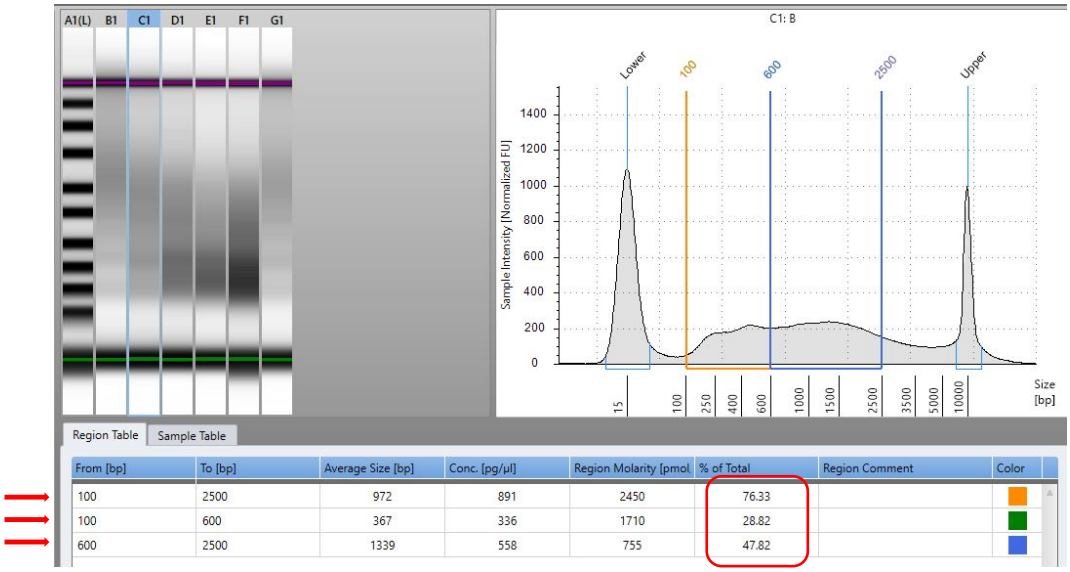
1. Transfer 2.5 µL of the lysate to a new tube labeled QC. **STORE the remainder of your lysate at -80°C. This is the lysate you will be using to proceed to Stage 3. It can be stored for up to 3 months.**
2. Add to the QC tube:
   50 µL 1X Crosslink Reversal Buffer
   1.5 µL Proteinase K
3. Pipet up and down to mix. Incubate in an agitating thermal mixer (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 (1,250 rpm).
4. Quick spin your QC tube then clean up your QC sample using the Zymo Genomic DNA Clean & Concentrator™-10 column by adding 200 µL of ChIP DNA Binding Buffer to your QC tube. Mix thoroughly.
5. Transfer the mixture to the 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, 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 13,000 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.

14. Enter the concentration you obtained in the spreadsheet labelled ‘Lysate QC Workbook’ provided with this protocol.
15. 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.**
16. On the TapeStation System, create 3 regions as follows:

- Region 1: 100 – 2,500 bp
- Region 2: 100 – 600 bp
- Region 3: 600 – 2,500 bp

Creating these regions will automatically generate a “percent of total” value. Copy these values into the Lysate QC Workbook.

TapeStation trace showing the 3 regions described above



Based on the concentrations you input into the Lysate QC Workbook, the volume of your sample that corresponds to 1,000 ng will be automatically calculated.

Based on the percent of total values you input into Lysate QC Workbook, Chromatin Digestion Efficiency (CDE) and Chromatin Digestion Index (CDI) values will be automatically calculated. The metrics are explained in the Lysate QC Workbook.

If your lysate does not meet the CDE/CDI metrics (i.e. your sample is color coded in red), please refer to Appendix 2 and repeat sample preparation (Stage 1), this time including a Nuclease Enzyme Titration step.

Stage 3. Proximity Ligation

NOTES:

- **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.**
- **You need to prepare fresh Bridge Ligation Mix (50 µL) for Step III. Store tube on ice prior to use. The Bridge Ligation Mix should be made fresh and used the same day. To prepare 50 µL Bridge Ligation Mix, mix:**

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

- **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 µL 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 by the lysate QC Workbook. 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 magnet.
5. Place tube on the magnet 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, wash beads with 150 µL 1X Wash Buffer: pipet up and down 10 times to resuspend the beads then place tube on the magnet 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 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 (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 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, wash beads once with 150 µL 1X Wash Buffer: pipet up and down 10 times to resuspend the beads then place tube on the magnet for 1 minute. Remove supernatant.

III. Bridge Ligation

1. Remove the tube from the magnetic rack then add to beads:
   50 µL Bridge Ligation Mix (freshly made, see notes)
   1 µL 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 (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, wash beads once with 150 µL 1X Wash Buffer: pipet up and down 10 times to resuspend the beads then place tube on the magnet for 1 minute. Remove supernatant.

IV. Intra-Aggregate Ligation

1. Remove the tube from the magnetic rack then add to 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 (1,250 rpm). For convenience, this ligation reaction can proceed overnight, at 22°C, in an agitating thermal mixer.
3. Place tube on 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 (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 (1,250 rpm).

3. Place tube on 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 second 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 magnet.
4. Place tube on the magnet for 5 minutes or until the solution looks clear and the beads have fully separated. Remove supernatant.
5. Leave tube on the magnet, and wash 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 magnet, resuspend beads in 52 µL TE Buffer pH 8.0.
9. Vortex briefly, quick spin and incubate for 5 minutes at room temperature off magnet.
10. Quick spin the tube and place on the magnet 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

NOTES:

- **The library preparation protocol does not require fragmentation.**
- **The library preparation protocol takes 2 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 58.5 µL of end-repaired sample:
   - 2.5 µL Adaptor for Illumina
   - 1 µL Ligation Enhance
   - 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 SPRIselects 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 magnet.
4. Quick spin the tube and place on the magnet for 5 minutes. Remove supernatant.
5. Leave tube on the magnet, and wash 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 on the magnet for 1 minute. Use a pipet with a fine tip to remove the last EtOH traces.
7. Air dry the beads for 5 minutes on the magnet until no residual EtOH remains. Do not over dry.
8. Off magnet, resuspend beads in 100 µL TE Buffer pH 8.0.
9. Vortex briefly, quick spin and incubate for 5 minutes at room temperature off magnet.
10. Quick spin the tube and place on the magnet for 1 minute.
11. Transfer 95 µL of the SUPERNATANT to a new tube. Discard Beads.

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

Stage 5. Ligation Capture & Amplification

NOTE: 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 µL of the resuspended Streptavidin Beads to a new 1.5 mL tube.
2. Place tube containing the Streptavidin Beads on the magnet 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 on the magnet 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 (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 on the magnet for 1 minute and remove supernatant (remove all of the supernatant between each wash; residual supernatant can interfere with the downstream PCR).

1. Quick spin tube and place on magnet 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™ 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 2 for list of primers)
    15 µL UltraPure DNase and 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 minute	---
98˚C	20 second	12 cycles
65˚C	30 second	
72˚C	30 second	
72˚C	1 minute	---
12˚C	Hold	

V. Size Selection

1. Quick spin the PCR tube and place on the magnet 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 magnet.
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 magnet.
11. Quick spin the tube and place on the magnet for 5 minutes. Remove supernatant.
12. Leave tube on the magnet, and wash beads twice with 200 µL 80% EtOH. Do not resuspend the beads for these washes.
13. Quick spin the tube and place on the magnet for 1 minute. Use a 10 µL pipet tip to remove traces of EtOH.
14. Air dry beads for 5 minutes on the magnet until no residual EtOH remains. Do not over dry.
15. Off magnet, 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 magnet.
17. Quick spin the tube and place on the magnet 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. If you recovered < 60 ng, discard the library and repeat from Stage 3 with your remaining lysate.
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 1000 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.

When using this low input protocol, please make sure to use the Lysate QC Workbook **Low Input** tab for Stage 2.

A. Cells

Depending on the amount of cells available to you; proceed with the sample preparation protocol at Stage 1 step 2 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.

1. Transfer volume X µL (based on input amount, see table below) of pre-warmed Nuclease Enzyme Mix dilution to pre-warmed tube. You can discard the remaining of your pre-warmed Nuclease Enzyme Mix dilution.

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

2. Incubate tube for exactly 30 minutes at 30°C, in an agitating thermal mixer (1,250 rpm).
3. Stop the reaction by adding 5 µL of 0.5M EDTA. Mix by inversion.
4. Add 3 µL of 20% SDS to lyse the cells. Mix by inversion.
5. Incubate the tube for 5 minutes at 30°C in an agitating thermal mixer (1,250 rpm).
5. Continue to Lysate Quantification Stage 2.

B. Tissues:

NOTES:

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

Proceed with the sample preparation protocol at Stage 1, step 1 with 5 mg of frozen tissue. Follow the sample preparation protocol through step 17. Upon completing step 17, continue with the steps below which are customized for low input samples.

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

Appendix 2: Sample Preparation (Stage 1) And Lysate Quantification (Stage 2), When **Including** Nuclease Enzyme Titration.

Stage 1. Sample Preparation

There are two separate protocols for sample preparation depending on your sample type: cells or tissue. The lysate quantification step is the same for both sample types. Sample preparation should take 2 hours.

NOTES

- **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, 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. You can also use this 1X Wash Buffer throughout the rest of the protocol. 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. You can also use this 1X Crosslink Reversal Buffer for the Proximity Ligation Protocol. 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 thawing buffers on ice and vortexing prior to use.**

A. Cells

NOTES

- Before beginning, please prepare fresh 1X Nuclease Digest Buffer and store at room temperature. 1X Nuclease Digest Buffer is stable for 1 day at room temperature.

To prepare 1X Nuclease Digest Buffer, mix:

122.5 µL	UltraPure Water
17.5 µL	10X Nuclease Digest Buffer
17.5 µL	100mM $MnCl_2$
17.5 µL	10%Triton

1. Harvest cells and wash in 1X PBS.
2. Count the cells and use 3 x 10^6 cell aliquot to proceed.
3. Spin 3 x 10^6 cell aliquot at 2000 x *g* for 5 minute. Carefully remove supernatant.
4. Resuspend pellet in: 1mL 1X PBS
   27µL 37% formaldehyde
5. Transfer sample to a 1 mL tube.
6. Rotate tube for 10 minutes at room temperature. Cells should not settle.
7. Spin the tube at 2,000 x *g* for 5 minutes. Carefully remove supernatant. Use caution, the pellet might be loose.
8. Wash pellet with 1 mL 1X Wash Buffer: first add 200 µL of Wash Buffer and pipet to break up clump. Then add the remaining 800 µL. Pipet up and down to fully resuspend the pellet.
9. Spin tube at 2,000 x *g* for 5 minutes. Carefully remove supernatant
10. Repeat steps 8 and 9 once for a total of 2 washes.
11. After removing the second wash, resuspend the pellet in 325 µL 1X Wash Buffer. Pipet up and down to fully resuspend the pellet.
12. Transfer 100 µL of the cell suspension into 3 separate tubes: A, B, and C. This is the equivalent of ~1,000,000 cells.
13. Spin A, B and C tubes at 2,000 x *g* for 5 minutes. Carefully remove supernatant.
14. Resuspend pellet in tubes A, B and C in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see notes above).
15. Pre-warm tubes A, B and C to 30°C for 2 minutes, in an agitating thermal mixer (1,250 rpm).
16. Transfer 7.5 µL of Nuclease Enzyme Mix from the kit supplied tube to a new 1.5 mL tube. Pre-warm to 30°C for 2 minutes in an agitating thermal mixer (1,250 rpm; this is to avoid pre-warming the entire enzyme tube). **Pre-warm A, B and C tubes and nuclease enzyme mix aliquot at the same time.**
17. Transfer:
    a. 0.5 µL of pre-warmed Nuclease Enzyme Mix to pre-warmed tube A
    b. 2.0 µL of pre-warmed Nuclease Enzyme Mix to pre-warmed tube B
    c. 4.0 µL of pre-warmed Nuclease Enzyme Mix to pre-warmed tube C
18. Incubate tubes for <u>exactly</u> 30 minutes at 30°C in an agitating thermal mixer (1,250 rpm).
19. Stop the reaction by adding 5 µL of 0.5M EDTA. Mix by inversion.
20. Add 3 µL of 20% SDS to lyse the cells. Mix by inversion.
21. Incubate the tubes for 5 minutes at 30°C, in an agitating thermal mixer (1,250 rpm).
22. Continue to Lysate Quantification Stage 2.

B. Tissue

NOTES:

- **Before beginning, please prepare <u>fresh</u> 1X Nuclease Digest Buffer and store at room temperature. 1X Nuclease Digest Buffer is stable for 1 day at room temperature.**
  To prepare 1X Nuclease Digest Buffer, mix:

122.5 µL	**UltraPure Water**
17.5 µL	**10X Nuclease Digest Buffer**
17.5 µL	**100mM MnCl2**
17.5 µL	**10%Triton**

1. Weigh out at least 60 mg of frozen tissue sample.
2. Disrupt the tissue by grinding it to a fine powder with mortar and pestle in liquid nitrogen (see example of desired consistency below).
3. Transfer the disrupted tissue sample to a 1 mL tube which contains:
   1mL 1X PBS
   27 µL 37% formaldehyde.
4. Rotate tube for 10 minutes at room temperature. Tissue should not settle.
5. Spin at 2,000 x *g* for 5 minutes. Carefully remove supernatant. If your tissue sample did not pellet, repeat the spin at maximum speed.
6. Wash pellet with 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 tube at 2,000 x *g* for 5 minutes. Carefully remove supernatant.
8. Repeat steps 6 and 7 once for a total of 2 washes.
9. After removing the second wash, resuspend 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 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 though the used 200 µm filter(s) into the 5 mL tube. Your tube should now contain a total volume of ~2 mL.
12. Using the same syringe but changing the filter to a 50 µm one, re-filter the 2 mL sample into a new 5 mL tube.
13. Gently pass an additional 1 mL 1X Wash Buffer though the used 50 µm filter into the 5 mL tube. Your tube should now contain a total volume of ~3 mL.
14. Aliquot into 3 separate tubes (A, B, and C) a volume of the resuspended sample that corresponds to 20 mg of tissue. For example, if your starting material was 68 mg of tissue now contained in a total volume of 3 mL, you

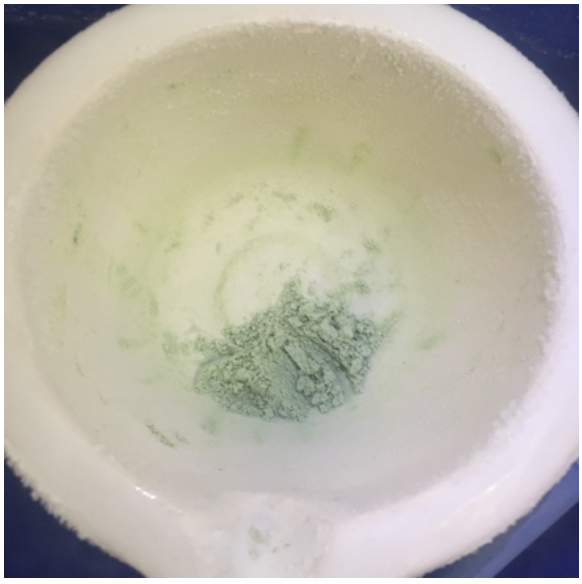
should aliquot in each tube 882 µL of the resuspended sample. The remaining sample can be pelleted and stored at -80°C.

15. Spin tubes A, B and C at 2,000 x *g* for 5 minutes. Carefully remove supernatant.
16. Resuspend pellet in tubes A, B and C in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see notes above).
17. Pre-warm tubes A, B and C to 30°C for 2 minutes, in an agitating thermal mixer (1,250 rpm).
18. Transfer 7.5 µL of Nuclease Enzyme Mix from your kit supplied tube to a new 1.5 mL tube and pre-warm to 30°C for 2 minutes, in an agitating thermal mixer (1250 rpm). (This is to avoid pre-warming the entire enzyme tube). **Prewarm the A, B and C tubes and nuclease enzyme mix aliquot at the same time.**
19. Transfer:
    0.5 µL of pre-warmed Nuclease Enzyme Mix to pre-warmed tube A
    2.0 µL of pre-warmed Nuclease Enzyme Mix to pre-warmed tube B
    4.0 µL of pre-warmed Nuclease Enzyme Mix to pre-warmed tube C
20. Incubate all three tubes for exactly 30 minute at 30°C, in an agitating thermal mixer (1250 rpm).
21. Stop the enzymatic reaction by adding 5 µL of 0.5M EDTA and mix by inversion.
22. Add to tubes 3 µL of 20% SDS to lyse the cells; mix by inversion.
23. Incubate for 5 minutes at 30°C, in an agitating thermal mixer (1,250 rpm).
24. Continue to Lysate Quantification Stage 2.

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

A

B



Stage 2. Lysate Quantification

NOTES:

- **Lysate Quantification should take 2 hours.**
- **If you are using a Bioanalyzer to check your fragment size distribution, we recommend the HS DNA kit. Please note when using a Bioanalyzer, the profile will appear different than the one shown below analyzed on a TapeStation.**
- **CDI calculation is not compatible with the Fragment Analyzer system.**
- **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:

(iii) Determine the volume of sample to use to proceed to Stage 3.
(iv) Determine which of the lysate tubes A, B and C to use to proceed to Stage 3.

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 tubes 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.

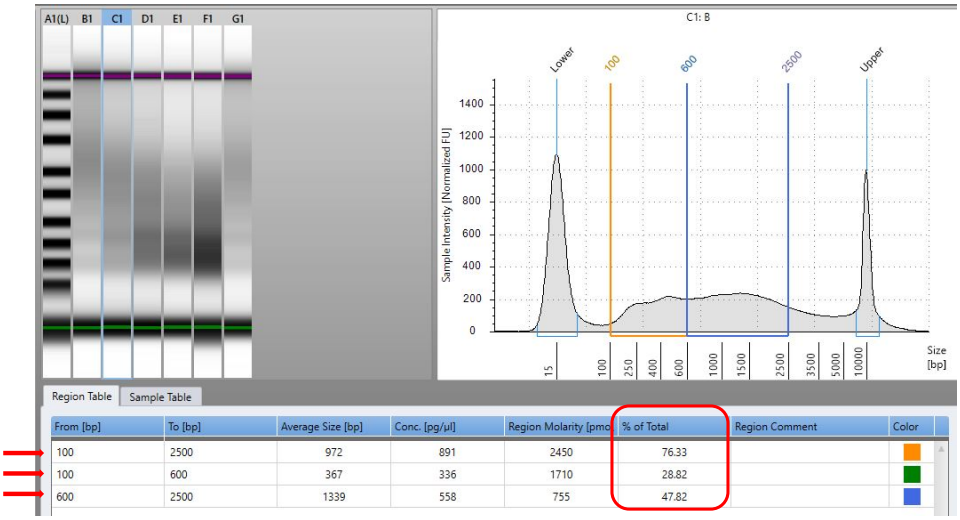
3. Add to each of the three QC tubes QC-A, QC-B and QC-C:
    50 µL 1X Crosslink Reversal Buffer
    1.5 µL Proteinase K
4. Pipet up and down to mix. Incubate all tubes in an agitating thermal mixer (1,250 rpm) for: 15 minutes at 55°C followed by:
    45 minutes at 68°C
    Hold at 25°C
    For convenience, you can hold at 25°C overnight in an agitating thermal mixer (1,250 rpm).
5. Quick spin your three QC tubes then clean up your QC samples using the Zymo Genomic DNA Clean & Concentrator™-10 Column by adding 200 µL of ChIP DNA Binding Buffer to each QC tube. Mix thoroughly.
6. For each QC tube, transfer the mixture to the 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 7 and 8 once, for a total of 2 washes.
11. Transfer 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 minutes 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.
15. Write down the concentration you obtained in the spreadsheet labelled 'Lysate QC Workbook Appendix 2_Titration' provided with this protocol.
16. Check the fragment size distribution of each QC sample on a TapeStation D5000 HS ScreenTape. **Make sure your samples are diluted to 1 ng/µL.**
17. On the TapeStation System, create 3 regions as follows:

- Region 1: 100 – 2500 bp
- Region 2: 100 – 600 bp
- Region 3: 600 – 2500 bp

Creating these regions will automatically generate a "percent of total" value. Copy these values into the Lysate QC Workbook Appendix 2_Titration.

TapeStation trace showing the 3 regions described above



Based on the concentrations you input into the Lysate QC Workbook, the volume of your sample that corresponds to 1000 ng will be automatically calculated.

Based on the percent of total values you input into Lysate QC Workbook, CDE (Chromatin Digestion Efficiency) and CDI (Chromatin Digestion Index) values will be automatically calculated. The tube for which CDE and CDI values pass metrics should be selected for Stage 3. The metrics are explained in the Lysate QC Workbook.

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 *or* 5 and 19
3	2, 7 and 19 *or* either of the 2-plex options plus any other Index Primer
4	5, 6, 12 and 19 *or* either of the 3-plex options plus any other Index Primer