



# Dovetail<sup>®</sup> Micro-C Kit

USER GUIDE - Cells

**VERSION 2.1**

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## Dovetail® Micro-C Kit Components and Storage

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

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

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

Table 2. Dovetail® Micro-C Module Box 2 (PN DG-NUC-001)

| Components                          | Storage        |
|-------------------------------------|----------------|
| MNase Enzyme Mix                    | -30°C to -10°C |
| 10X Nuclease Digest Buffer          |                |
| 100 mM MgCl <sub>2</sub>            |                |
| 0.5 M EGTA                          |                |
| End Polishing Enzyme Mix            |                |
| End Polishing Buffer                |                |
| 5X Bridge Ligation Buffer           |                |
| Bridge Ligase                       |                |
| Bridge                              |                |
| Intra-Aggregate Ligation Enzyme Mix |                |
| Intra-Aggregate Ligation Buffer     |                |
| Proteinase K                        |                |
| HotStart PCR Ready Mix              |                |

\*An additional vial of HotStart PCR Ready Mix and Streptavidin Beads are supplied in a bag to support a total of 24 libraries. This is to enable customers looking to prepare 3 technical libraries from the same proximity ligation reactions.

## Optional Add-on Modules: Components and Storage

### Dovetail® Primer Module Set 1

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

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

### Dovetail® Library Module

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

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

## User Supplied Reagents, Consumables and Equipment

### Reagents

Table 5. Reagents

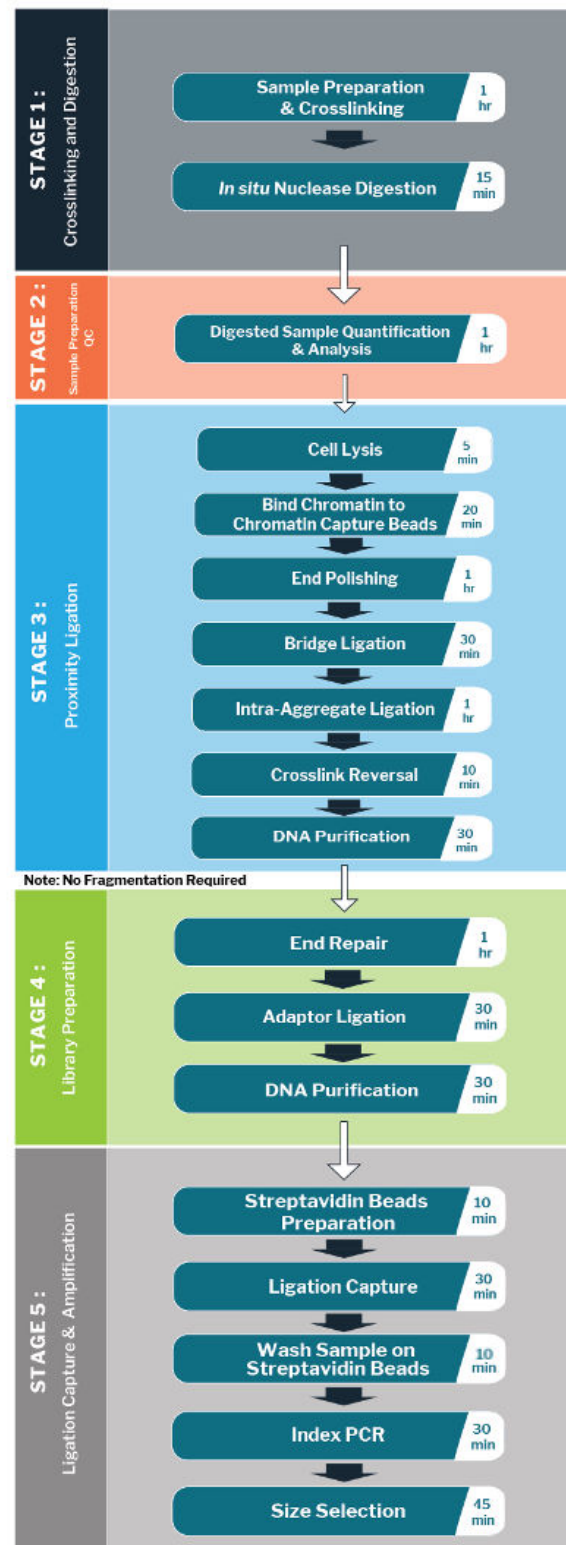
| Reagents  | Supplier                 | Part Number  |
|---|--------------------------|--------------|
| SPRIselect® Beads, 5 mL                               | Beckman Coulter          | B23317       |
| 37% Formaldehyde Solution                             | Sigma-Aldrich            | F8775        |
| 1X PBS, pH 7.4, 500 mL                                | Thermo Fisher Scientific | 10010023     |
| 100% EtOH   | Generic                  | N/A          |
| UltraPure™ DNase / RNase-Free Distilled Water, 500 mL | Thermo Fisher Scientific | 10977015     |
| TE pH 8.0   | Thermo Fisher Scientific | AM9849       |
| DSG (Disuccinimidyl Glutarate)                        | Thermo Fisher Scientific | A35392       |
| DMSO (Dimethyl Sulfoxide, Anhydrous ≥ 99.99%)         | Sigma-Aldrich            | 276855-100ML |

### Consumables and Equipment

Table 6. Consumables and Equipment

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

## Dovetail® Micro-C Protocol Overview



## Good Practices

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



## Things to Consider Before You Start

### What is the impact of MNase digestion profiles on conformation analysis?

Achieving an optimal digestion profile which contains 20% - 70% mononucleosomes results in a high complexity library with enriched long-range interactions. When the chromatin is under-digested (< 20% mononucleosomes), the library complexity is lower (i.e. higher duplication rate). In this case, you may need additional libraries to support the sequencing depth needed for your application. When the chromatin is over-digested (>70% mononucleosomes), the library can have a higher fraction of inter-chromosomal information at the expense of cis long-range read pairs.

### How much coverage do you need?

The answer to “how much coverage do you need?” is highly dependent on the goal of your experiment. Below are some generalized recommendations.

| Topological feature calling | Resolution          | Coverage | Number of 2 x 150 bp paired-end reads* |
|-----------------------------|---------------------|----------|--|
| A/B compartment             | 1 Mb                | 3x       | 30 million                             |
| TADs                        | 50 kb, 25 kb, 10 kb | 30x      | 300 million                            |
| Loops                       | 10 kb, 5 kb         | 80x      | 800 million                            |

\*Assuming mapping to the human genome. These absolute values will vary dependent on species.

### How do you calculate coverage?

The general equation is:

Coverage =  $[2 \times (\text{read length in bp}) \times (\text{total number of paired-end reads})] / [(\text{genome size in bp})]$

Example: For human genomes, 30x coverage is achieved with 300 M paired-end reads of 150 bp.

$30x = [2 \times (150 \text{ bp}) \times (300 \times 10^6)] / [(3 \times 10^9 \text{ bp})]$

### How many libraries are required to achieve the desired coverage?

A Micro-C library can be sequenced up to 300 M read pairs (2 x 150 bp), depending on the library complexity as assessed by the QC analysis of the shallow sequenced library. For human genomes, 30x and 80x coverage can be achieved with one and three Micro-C libraries respectively, assuming the libraries are of high complexity.

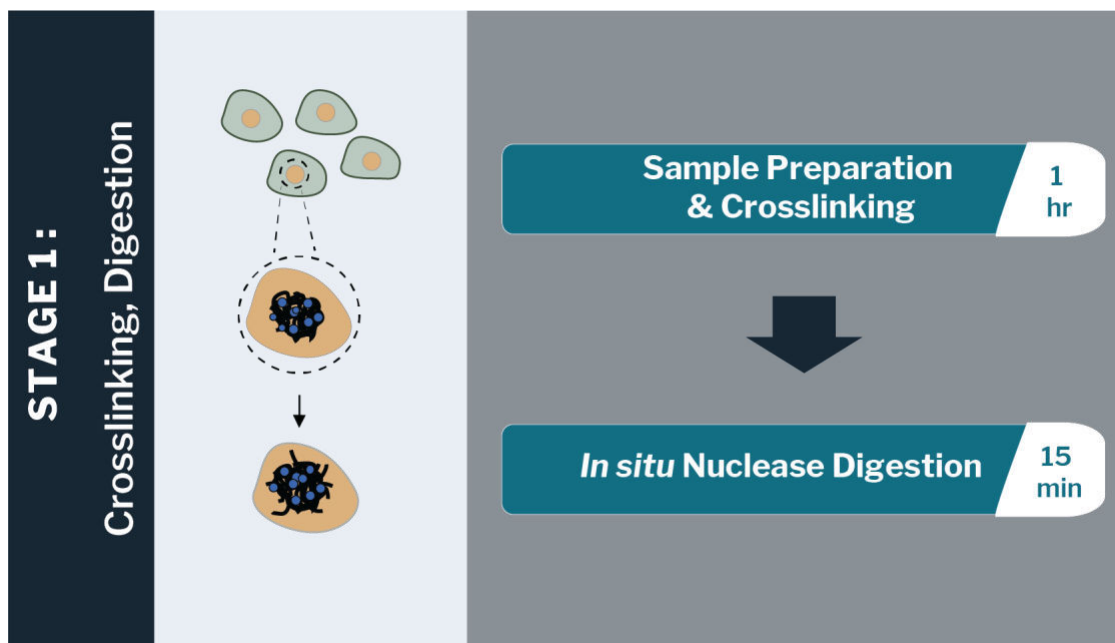
This [slide deck](#) describes in detail the different approaches one can take to prepare multiple libraries that are needed to support the desired coverage/depth.

## Stage 1: Crosslinking and Digestion

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

- Sample preparation takes ~ 1.5 hours.

Figure 1. Stage 1: Crosslinking and Digestion



### Before You Begin

- The 10X Wash Buffer might have precipitated in storage. Incubate these solutions at 50°C for 15 minutes or until the precipitate is no longer visible. Vortex to mix prior to use.
- Dilute 10X Wash Buffer to 1X with UltraPure™ Water. Store at room temperature. 1X Wash Buffer is stable at room temperature for 2 months. You need ~2 mL of 1X Wash Buffer per sample for the entire protocol.
- Prepare 0.3 M DSG in DMSO (anhydrous) by dissolving 1 mg of DSG in 10.22  $\mu$ L DMSO. DSG is water-insoluble and moisture-sensitive. Prepare immediately before use. Do not store DSG in solution.
- The cell centrifugation steps must be carried out in a swinging bucket rotor. Using a swinging bucket rotor reduces cell loss.

- Prepare fresh 1X Nuclease Digest Buffer and store at room temperature. 1X Nuclease Digest Buffer is stable for 1 day at room temperature. You need 50  $\mu\text{L}$  of 1X Nuclease Digest Buffer per sample. To prepare 1X Nuclease Digest Buffer (50  $\mu\text{L}$ ), mix the following components:

| Reagent                    | Volume Per Reaction | 10% Extra         |   | # Reactions |   | Final             |
|----------------------------|---------------------|-------------------|---|-------------|---|-------------------|
| UltraPure Water            | 40 $\mu\text{L}$    | 44 $\mu\text{L}$  | x | 8           | = | 352 $\mu\text{L}$ |
| 10X Nuclease Digest Buffer | 5 $\mu\text{L}$     | 5.5 $\mu\text{L}$ | x | 8           | = | 44 $\mu\text{L}$  |
| 100 mM $\text{MgCl}_2$     | 5 $\mu\text{L}$     | 5.5 $\mu\text{L}$ | x | 8           | = | 44 $\mu\text{L}$  |
| Total                      | 50 $\mu\text{L}$    |                   |   |             |   |                   |

- Set the thermal mixer at 22°C, shaking at 1,250 rpm.
- Thaw 0.5 M EGTA at room temperature. Vortex to mix prior to use.

### Follow the steps below for Crosslinking and Digestion:

1. Harvest the cells, wash with 1X PBS and count.
2. Aliquot  $1 \times 10^6$  cells into a 1.5 mL tube.
3. Spin the  $1 \times 10^6$  cell aliquot at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove and discard the supernatant.
4. Freeze the cell pellet by placing it at -80°C for at least 30 minutes.

#### □ NOTES

- Pre-freezing the cells is required to get an optimal digestion profile.
- All crosslinking reactions (steps 5 – 11) should be carried out at room temperature.

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

14. Incubate the tube at 22°C for exactly 15 minutes in an agitating thermal mixer set at 1,250 rpm. If you are working with a large number of samples, stagger the start of the digestion for each sample by 20 seconds then stop after corresponding 15 minutes.
15. Stop the reaction by adding 5 µL of 0.5 M EGTA. Pipet up and down to fully mix.
16. Continue to Stage 2: Sample Preparation QC.

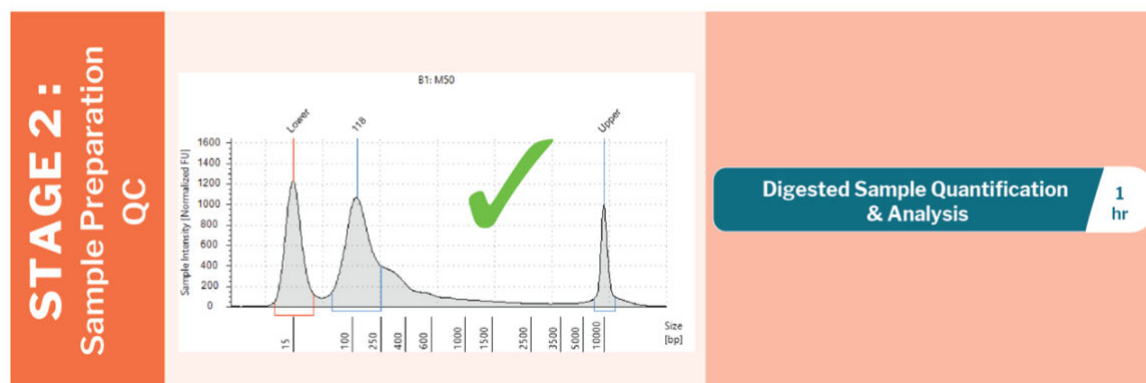
## Stage 2: Sample Preparation QC

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

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

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

Figure 2. Stage 2: Sample Preparation QC



### Before You Begin

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

| Temperature | Time       |
|-------------|------------|
| 78°C        | 10 minutes |
| 25°C        | Hold       |

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

## Follow the steps below for Sample Preparation QC:

1. Transfer 2.5 µL of your digested sample to a new 1.5 mL tube labeled QC.



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

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

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

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

| Temperature | Time       |
|-------------|------------|
| 78°C        | 10 minutes |
| 25°C        | Hold       |

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

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

- Based on the Qubit concentration, the total digested sample (ng) can be calculated as follows:  
Total digested sample (ng) = Qubit reading ng/μL x 10 μL (elution volume) x 23.4 (dilution factor)
- At the start of Stage 3, the digested sample will be lysed by SDS and will be referred to as lysate. “Total lysate” and “total digested sample” are used interchangeably when referring to the yield quantification (i.e. the ng amounts are equal). You will use in Stage 3 a volume of the lysate that corresponds to up to 1,500 ng. This volume can be calculated as follows:

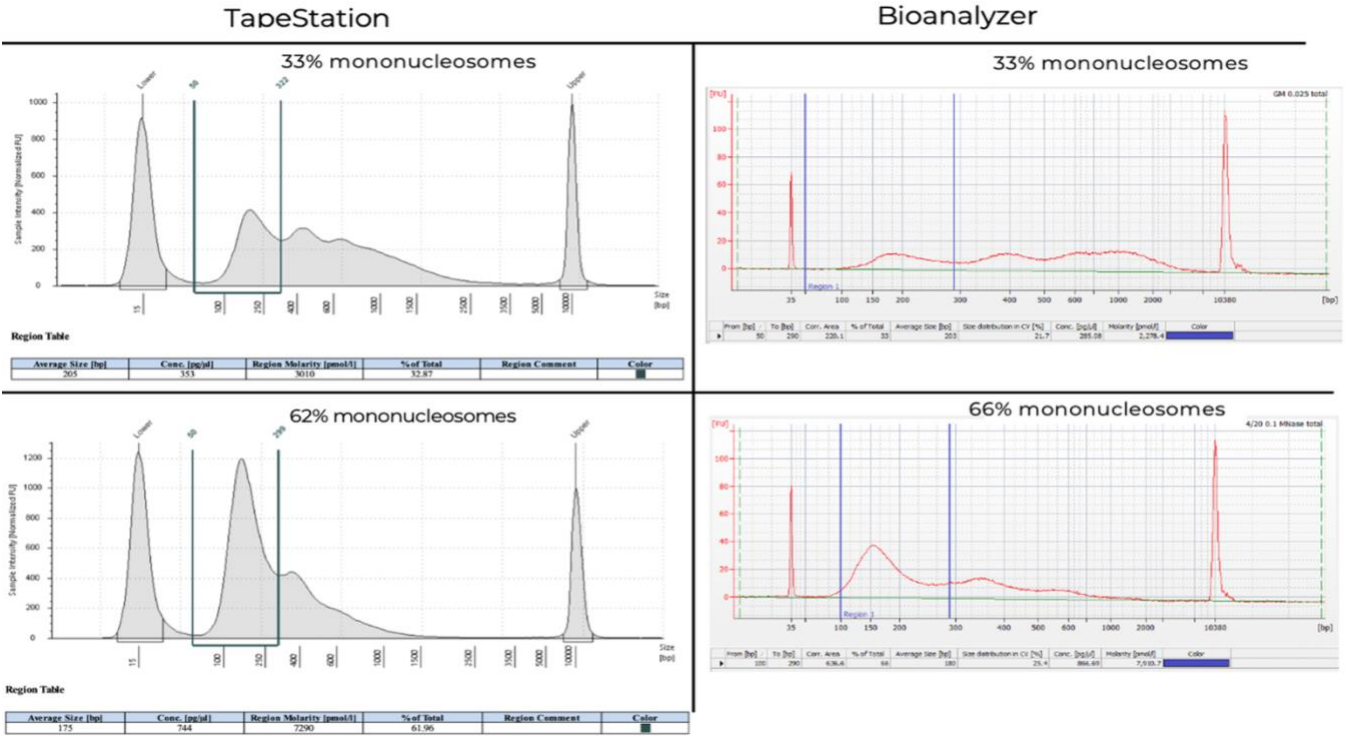
$$\text{Volume (}\mu\text{L)} = \frac{1,500 \text{ (ng)} \times 58.5 \text{ (}\mu\text{L)}}{\text{Total digested sample (ng)}}$$

- If the total lysate is < 1,500 ng, use all of the lysate in Stage 3.
- If the total lysate is > 1,500 ng, store the remainder of the lysate at -80°C.

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

- For optimal nucleosome-level resolution, the digestion profile should contain 20% - 70% mononucleosomes: the first DNA peak, typically in the size range of 50 - 300 bp for TapeStation, should account for 20% - 70% of total DNA (Figure 3). The size range of the peak may vary for other analytical instruments such as Bioanalyzer and Fragment Analyzer. If the digestion profile contains 20% - 70% mononucleosomes, proceed to Stage 3: Proximity Ligation.
- If the digestion profile contains < 20% mononucleosomes, the library complexity will be lower (i.e. higher duplication rate). In this case, you may need additional libraries to support the sequencing depth needed for your application. You have 2 options: proceed to Stage 3 knowing that additional library(ies) are likely needed **or** redigest your sample before proceeding to Stage 3 (refer to Appendix 2: Troubleshooting Guide for sample redigestion).
- If the digestion profile contains > 70%, you may proceed to Stage 3: Proximity Ligation with caution. The library may include a higher fraction of inter-chromosomal information at the expense of cis long-range read pairs. This profile is likely due to suboptimal cell counting or a significant cell loss in the washing steps after crosslinking. Alternatively, you can re-start the protocol and decrease the volume of MNase Enzyme Mix in step 13 in Stage 1: Crosslinking and Digestion. For advice on what volume to use, please contact support@cantatabio.com.

Figure 3. Examples of QC Pass digestion profiles, as analyzed on HS D5000 ScreenTape and HS DNA Kit



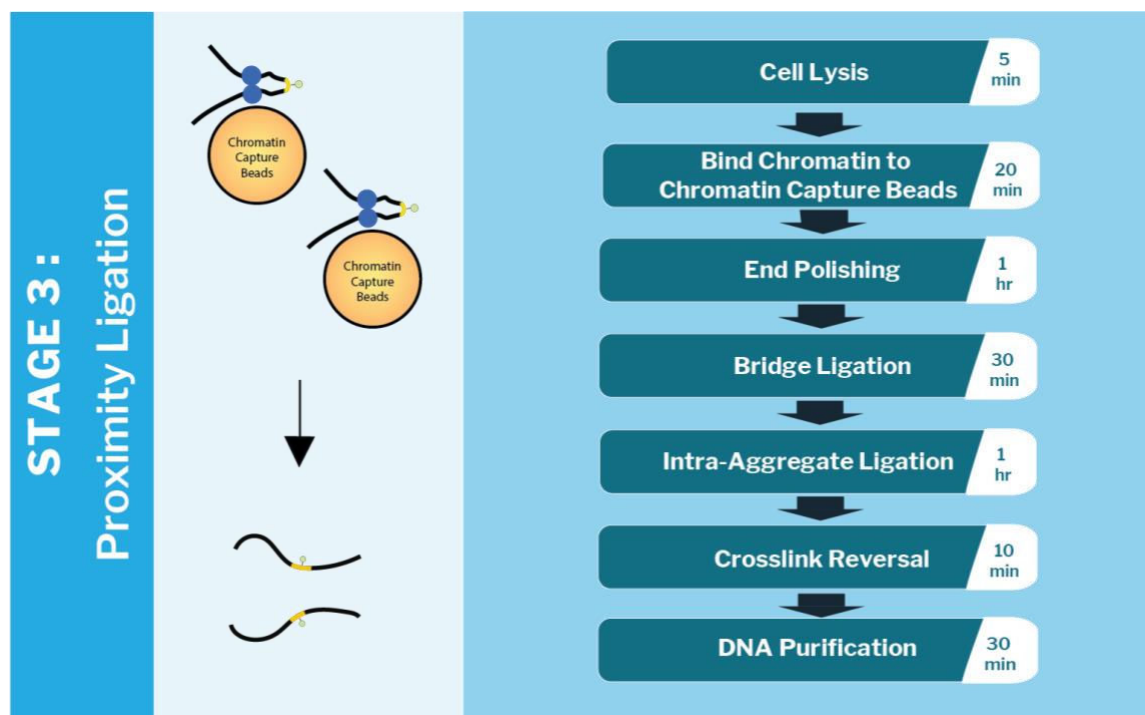


## Stage 3: Proximity Ligation

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

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

Figure 4. Stage 3: Proximity Ligation



### Before You Begin

- The Crosslink Reversal Buffer and 20% SDS might have precipitated in storage. Incubate at 50°C for 15 minutes or until the precipitate is no longer visible. Vortex to mix prior to use.
- Thaw End Polishing Buffer, 5X Bridge Ligation Buffer, Bridge, and Intra-Aggregate Ligation Buffer at room temperature. Leave on ice once thawed. Vortex to mix prior to use.
- Equilibrate Chromatin Capture Beads to room temperature.

### 3.1 Bind Chromatin to Chromatin Capture Beads

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

1. Thaw your digested sample at room temperature, if stored at -80°C (step 1 NOTE in Stage 2: Sample Preparation QC).
2. Add 3 µL of 20% SDS to the fully thawed digested sample tube. Pipet up and down to fully mix. **This is your lysate.**
3. Incubate the lysate at 22°C for 5 minutes in an agitating thermal mixer set at 1,250 rpm.
4. Equilibrate the Chromatin Capture Beads to room temperature and vortex thoroughly (>30 seconds) to resuspend.
5. Transfer 100 µL of resuspended Chromatin Capture Beads to a new 1.5 mL tube.
6. Pipet mix the lysate. Transfer 1,500 ng of the lysate to the 1.5 mL tube containing the beads. If the total amount is <1,500 ng, add all of the lysate.
7. Pipet up and down to fully mix. Incubate at room temperature, off the magnetic rack, for 10 minutes.
8. Place the tube in the magnetic rack for 5 minutes (or until the solution looks clear). Discard the supernatant.
9. Remove the tube from the magnetic rack and wash the beads with 150 µL 1X Wash Buffer. Pipet up and down to resuspend the beads, place the tube in the magnetic rack for 1 minute and discard the supernatant.
10. Repeat step 9 once, for a total of 2 washes.

### 3.2 End Polishing

Follow the steps below for End Polishing:

1. Remove the tube from the magnetic rack and add to the beads 53 µL of a master mix containing the following reagents:

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

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

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

3. Allow the tube to reach room temperature then quick spin and place it in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.
4. Remove the tube from the magnetic rack and wash the beads once with 150 µL 1X Wash Buffer. Pipet up and down to resuspend the beads, place the tube in the magnetic rack. Do not remove and discard the supernatant at this step yet. Keep the tube in the magnetic rack and the beads in buffer to ensure they do not dry out while you prepare for the next reaction.

### 3.3 Bridge Ligation

Follow the steps below for Bridge Ligation :

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

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

2. Aspirate and discard the supernatant from step 4 in 3.2 End Polishing. Remove the tube from the magnetic rack and add to the beads:

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

3. Pipet up and down to fully mix. Incubate at 22°C for 30 minutes in an agitating thermal mixer set at 1,250 rpm.
4. Quick spin the tube, then place it in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.
5. Remove the tube from the magnetic rack and wash the beads once with 150 µL 1X Wash Buffer. Pipet up and down to resuspend the beads, place the tube in the magnetic rack for 1 minute and discard the supernatant.

### 3.4 Intra-Aggregate Ligation

Follow the steps below for Intra-Aggregate Ligation :

1. Remove the tube from the magnetic rack and add to the beads 52 µL of a master mix containing the following reagents:

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

- Pipet up and down to fully mix. Incubate at 22°C for 1 hour in an agitating thermal mixer set at 1,250 rpm.

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

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

### 3.5 Crosslink Reversal

Follow the steps below for Crosslink Reversal:

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

| Reagent                   | Volume Per Reaction | 10% Extra |   | # Reactions |   | Final   |
|---------------------------|---------------------|-----------|---|-------------|---|---------|
| Crosslink Reversal Buffer | 50 µL               | 55 µL     | x | 8           | = | 440 µL  |
| Proteinase K              | 1.5 µL              | 1.65 µL   | x | 8           | = | 13.2 µL |
| Total                     | 51.5 µL             |           |   |             |   |         |

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

| Temperature | Time       |
|-------------|------------|
| 78°C        | 10 minutes |
| 25°C        | Hold       |

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

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

### 3.6 DNA Purification

Follow the steps below for DNA Purification on SPRIselect Beads:

- Vortex the SPRIselect beads thoroughly (>30 seconds) to resuspend.
- Add 90 µL of resuspended SPRIselect beads to the 1.5 mL tube containing your sample.
- Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

4. Incubate the tube at room temperature, off the magnetic rack, for 10 minutes.
5. Quick spin the tube and place it in the magnetic rack for 5 minutes. Discard the supernatant.
6. Leave the tube in the magnetic rack and wash the beads **twice** with 200  $\mu$ L **fresh** 80% ethanol. Do not resuspend the beads for these washes. Add the ethanol, wait for 1 minute then discard the ethanol supernatant.
7. After the last wash, quick spin the tube and place it in the magnetic rack for 1 minute. Use a 10  $\mu$ L pipet tip to remove traces of ethanol.
8. Air dry the beads for 5 minutes in the magnetic rack until no residual ethanol remains. **Do not over dry the beads.**
9. Off the magnetic rack, resuspend the beads in 52  $\mu$ L TE Buffer pH 8.0.
10. Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).
11. Incubate at room temperature, off the magnetic rack, for 5 minutes.
12. Quick spin the tube and place it in the magnetic rack for 1 minute.
13. Transfer 50  $\mu$ L of the **SUPERNATANT** (purified DNA) to a new tube. Discard the beads.
14. Quantify the purified DNA using a Qubit Fluorometer and Qubit dsDNA HS Kit.
  - >> If you are using the Micro-C Kit for genome-wide analysis (such as calling A/B compartments, TADs, and loops), you will use 150 ng of your purified DNA per library prep for Stage 4 in a 50  $\mu$ L volume. If needed, you can bring up the volume to 50  $\mu$ L using TE Buffer pH 8.0.
    - ☐ If you recovered < 150 ng, use all of the purified DNA to proceed to Stage 4.
    - ☐ If you recovered > 150 ng, use 150 ng to proceed to Stage 4 and keep the remaining purified DNA stored at -20°C. You can use the remaining DNA to prepare additional libraries if your application requires more complexity or coverage (see Things to Consider Before You Start).
  - > If you are using the Micro-C Kit together with Dovetail® Pan Promoter Enrichment Kit to study the chromatin topology anchored at gene promoter sites, we recommend using **all of the purified DNA (up to 1,000 ng)** for Stage 4. Increasing the amount of DNA going into library preparation increases the complexity of the **enriched** library (i.e. lowers the duplication rate). Please note, if less than 150 ng of DNA were recovered and used for library preparation, the enriched library will likely be of less than desired complexity.

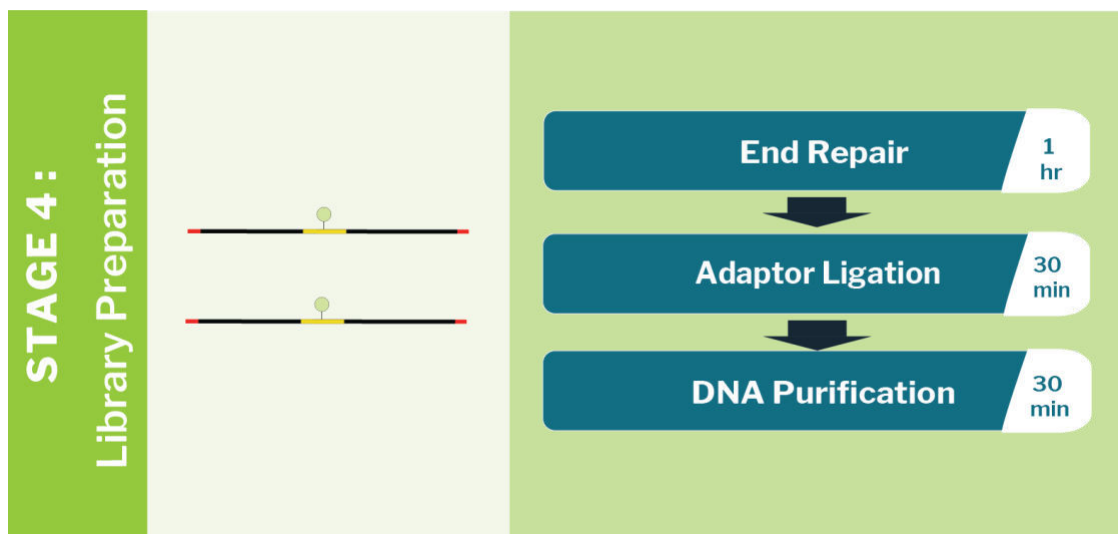
☐ **SAFE STOP** Purified DNA sample can be stored at -20°C for up to 6 months.

## Stage 4: Library Preparation

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

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

Figure 5. Stage 4: Library Preparation



### Before You Begin

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

## 4.1 End Repair

Follow the steps below for End Repair:

1. Place 50  $\mu\text{L}$  of purified DNA (150 ng, or up to 1,000 ng if running the Pan Promoter Enrichment) in a 0.2 mL PCR tube.
2. Add to the PCR tube 10  $\mu\text{L}$  of a master mix containing the following reagents:

| Reagent               | Volume Per Reaction | 10% Extra         |   | # Reactions |   | Final              |
|-----------------------|---------------------|-------------------|---|-------------|---|--------------------|
| End Repair Buffer     | 7 $\mu\text{L}$     | 7.7 $\mu\text{L}$ | x | 8           | = | 61.6 $\mu\text{L}$ |
| End Repair Enzyme Mix | 3 $\mu\text{L}$     | 3.3 $\mu\text{L}$ | x | 8           | = | 26.4 $\mu\text{L}$ |
| Total                 | 10 $\mu\text{L}$    |                   |   |             |   |                    |

3. Pipet up and down to fully mix. Quick spin the tube.
4. Place in a thermal cycler, with the heated lid set to  $\geq 75^{\circ}\text{C}$ , and run the following program:

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

## 4.2 Adaptor Ligation

Follow the steps below for Adaptor Ligation:

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

| Reagent             | Volume Per Reaction |
|---------------------|---------------------|
| Stubby Adaptor      | 2.5 $\mu$ L         |
| Ligation Buffer     | 25 $\mu$ L          |
| Ligation Enzyme Mix | 5 $\mu$ L           |
| Total               | 32.5 $\mu$ L        |



**NOTE** The Ligation Enzyme Mix and Ligation Buffer can be mixed and used as a master mix. We do not recommend adding the Stubby Adaptor to the master mix. If using a master mix, first add to the PCR tube containing the end-repaired sample the Stubby Adaptor then add 30  $\mu$ L of the Ligation mastermix.

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



## 4.3 DNA Purification

Follow the steps below for DNA Purification :

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

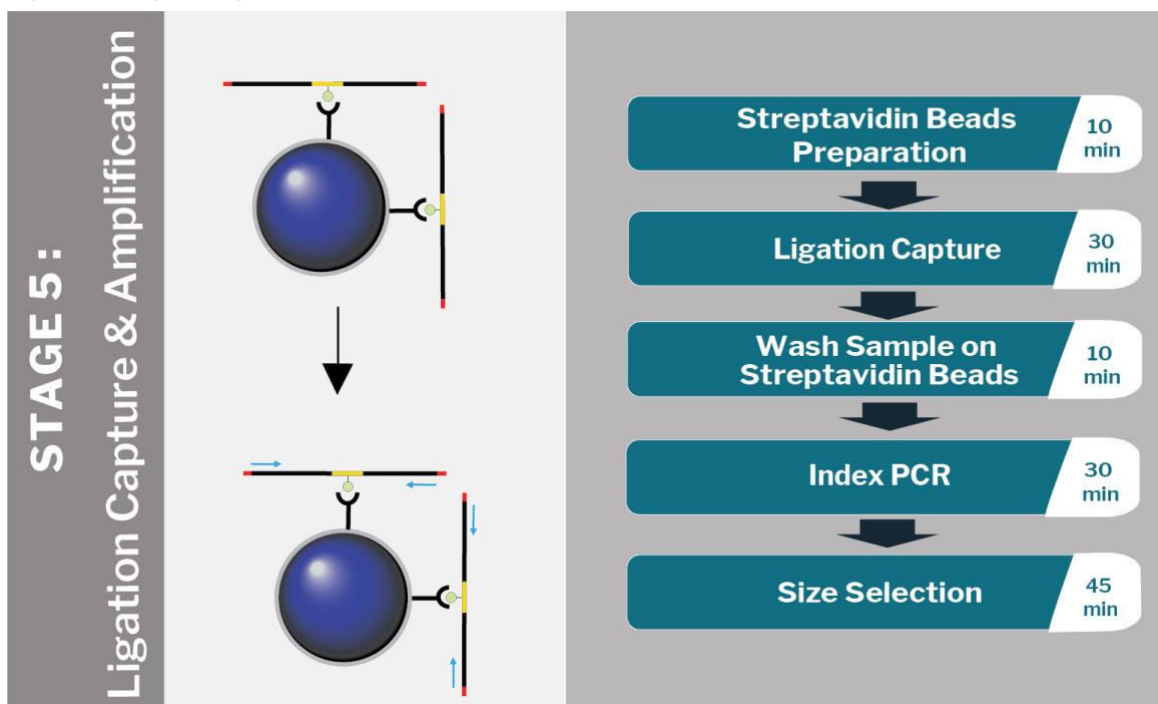
☐ **SAFE STOP** Purified DNA sample can be stored at -20°C overnight.

## Stage 5: Ligation Capture and Amplification

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

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

Figure 6. Stage 5: Ligation Capture and Amplification



### Before You Begin

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

## 5.1 Streptavidin Beads Preparation

☐ **NOTE** This step does not involve any DNA sample.

Follow the steps below for Streptavidin Beads Preparation:

1. Vortex the Streptavidin Beads vial thoroughly (> 30 seconds) to resuspend the beads. Transfer 25  $\mu$ L of resuspended Streptavidin beads to a new 1.5 mL tube.
2. Place the 1.5 mL tube containing the beads in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.
3. Remove the tube from the magnetic rack and wash the beads with 200  $\mu$ L of 1X Wash Buffer: pipet up and down to resuspend the beads and place the tube in the magnetic rack for 1 minute (or until the solution looks clear). Discard the supernatant.
4. Repeat step 3 once, for a total of 2 washes.
5. After the second wash, resuspend the beads in 100  $\mu$ L NWB. Pipet up and down to fully mix.
6. Transfer to a 0.2 mL PCR tube.

## 5.2 Ligation Capture

Follow the steps below for Ligation Capture:

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

## 5.3 Wash Sample on Streptavidin Beads

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

Follow the steps below for Wash Sample on Streptavidin Beads:

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

## 5.4 Index PCR

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

### Follow the steps below for Index PCR:

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

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

2. Add 5 µL of DG i5 Index Primer to the PCR reaction. (see Appendix 1: Index Primers).
3. Add 5 µL of DG i7 Index Primer to the PCR reaction. (see Appendix 1: Index Primers).
4. Pipet up and down to fully mix.
5. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle). Place the tube into the thermal cycler and run the following program:

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

## 5.5 Size Selection

### Follow the steps below for Size Selection :

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

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



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

## Sequencing & QC Analysis of Dovetail® Micro-C Libraries

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

## Appendix 1: Index Primers

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

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

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

Table 7. Index Primer

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

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

## Appendix 2: Troubleshooting Guide

Refer to this troubleshooting guide if the percentage of mononucleosomes of your sample is < 20% (i.e. chromatin is under-digested). The steps below allow you to re-digest the sample.

1. Thaw the digested sample at room temperature, if stored at -80°C (step 1 NOTE in Stage 2: Sample Preparation QC).
2. Pipet mix the digested sample then spin at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove and discard the supernatant. Use caution, the pellet might be loose.
3. Wash the pellet with 200 µL of 1X Wash Buffer, pipet up and down to break up clumps and fully resuspend the pellet.
4. Spin the tube at 500 x g for 5 minutes in a swinging bucket rotor. Carefully remove and discard the supernatant.
5. Resuspend the cell pellet in 50 µL 1X Nuclease Digest Buffer (freshly prepared, see Before You Begin).
6. Add 2 µL of MNase Enzyme Mix. Pipet up and down to fully mix.
7. Incubate the tube at 22°C for exactly 15 minutes in an agitating thermal mixer set at 1,250 rpm. If you are working with a large number of samples, stagger the start of the digestion for each sample by 20 seconds then stop after corresponding 15 minutes.
8. Stop the reaction by adding 5 µL of 0.5 M EGTA. Pipet up and down to fully mix.
9. Continue to Stage 2: Sample Preparation QC.