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Micro-C protocol for frozen tissue using the Dovetail® Micro-C Kit

Eric RA Pederson[1]

[1]Uppsala University

methods

Eric RA Pederson
Uppsala University

ABSTRACT

micro-C protocol from Dovetail for the use on frozen tissue, in this case

GUIDELINES

The amount of mononuclease content must be between 30-70%

MATERIALS

Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare** **Catalog #44152105050350**

DMSO **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #472301**

Pierce DSG No-Weigh Format **Thermo Scientific** **Catalog # A35392**

Dovetail Micro-C Kit **Contributed by users** **Catalog #21006**

Dovetail Filter Set **Contributed by users** **Catalog #BOM-006**

Dovetail Hi-C Library Preparation Kit **Contributed by users** **Catalog #21004**

- 1 ml syringe

DNA Clean & Concentrator™-5 **Zymo Research** **Catalog #D4003**

SPRIselect reagent kit **Beckman Coulter** **Catalog #B23317**

16% Formaldehyde Solution (10 ml ampoules) **Canemco & Marivac** **Catalog #0173**

Equipment

Cryogrinder set

NAME

OPS Diagnostics

BRAND

CG 08-02

SKU

https://opsdiagnostics.com/CryoGrinder-Set-p340.html

LINK

Equipment

Bio RS-24 Mini-rotator

NAME

mini-rotator

TYPE

BioSan

BRAND

RS-24

SKU

https://biosan.lv/products/-bio-rs-24-mini-rotator-for-test-tubes-with-timer/

LINK

Equipment

Thermomixer C

NAME

Eppendorf

BRAND

2231000667

SKU

https://www.pipette.com/2231000667-Promotion-Eppendorf-ThermoMixer-C-with-24x1-5-mL-SmartBlock-and-ThermoTop

LINK

Equipment

new equipment	NAME
Qubit 2.0 Fluorometer instrument	BRAND
Q33226	SKU
with Qubit RNA HS Assays	SPECIFICATIONS

Equipment

4200 TapeStation System	NAME
Electrophoresis tool for DNA and RNA sample quality control.	TYPE
TapeStation Instruments	BRAND
G2991AA	SKU
https://www.agilent.com/en/product/automated-electrophoresis/tapestation-systems/tapestation-instruments/4200-tapestation-system-228263	LINK

SAFETY WARNINGS

Formaldehyde should be used only under a fume hood, while wearing goggles, gloves and a lab coat.
Be careful not to burn hands with liquid nitrogen. Also should be wearing goggles at that point.

BEFORE START INSTRUCTIONS

Complete everything in the pre-epxerimentation section

pre-experimentation

1 All steps should be performed on ice or at 4 °C.

2 Autoclave cryogrinder pieces wrapped in foil

3 pre-cool the cryogrinder pieces

4 Ensure there is enough liquid nitrogen for the protocol

pre-Step 1

1h 30m

5 Stage 1: Crosslinking, Digestion and Lysis

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

Sample preparation takes ~ 1.5 hours.

6 Before You Begin

The 10X Wash Buffer and 20% SDS might have precipitated in storage.

Incubate these solutions at 37° for 15 minutes or until the precipitate is no longer visible.

Vortex to mix prior to use.

7 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.

8 Prepare 3 M DSG in DMSO (anhydrous) by dissolving 1 mg of DSG in 10.22 µl DMSO.

DSG is water-insoluble and moisture-sensitive.

Prepare immediately before use.

Do not store DSG in solution.

DMSO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #472301**

Pierce DSG No-Weigh Format **Thermo Scientific Catalog # A35392**

9 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 µl of 1x nuclease digest buffer per sample.

To prepare 1x nuclease digest buffer (50 µl), mix the following components:

9.1

	A	B	C	D	E	F
	Reagent	1X	10% extra	3X	5X	8X
	UltraPure water	40 ul	44 ul	132 ul	220 ul	352 ul
	10x Nuclease digest buffer	5 ul	5.5 ul	16.5 ul	27.5 ul	44 ul
	100 mM MgCl2	5 ul	5.5 ul	16.5 ul	27.5 ul	44 ul
	total	50 ul		165 ul	275 ul	440 ul

10 Set the thermal mixer at 22°, shaking at 1,250 rpm.

22 °C

1250 rpm

11 Thaw 0.5 M EGTA at room temperature.

EGTA Contributed by users Catalog #E8145

Vortex to mix prior to use.

Step 1

12 Weigh out 20 mg of frozen tissue sample.

If using muscle tissue, weigh out 50 mg.

13 Grind the sample to a fine powder (flour-like consistency) with a mortar and pestle in liquid nitrogen.

Equipment	
Cryogrinder set	NAME
OPS Diagnostics	BRAND
CG 08-02	SKU
https://opsdiagnostics.com/CryoGrinder-Set-p340.html	LINK

Ensure the sample is kept frozen while grinding.

14 Transfer the ground tissue sample to a 1.5 mL tube containing: 1mL 1XPBS, 10µL0.3 DMSG

1X PBS (Phosphate-buffered saline)

15 Rotate the tube for 10 minutes at room temperature.

Cells should not settle.

16 Add 62.5 µL 16% formaldehyde.

16% Formaldehyde Solution (10 ml ampoules) **Canemco & Marivac Catalog #0173**

17 Rotate the tube for 10 minutes at room temperature.

Cells should not settle.

18 Spin the tube at 3,000 x g for 5 minutes.

Carefully remove and discard the supernatant. Use caution, the pellet might be loose.

19 Wash the pellet with a total of 1 mL 1X Wash buffer: first add 200 µL of Wash Buffer and pipette up and down to break up clumps then add the remaining 800 µL.

Pipette up and down to fully resuspend the pellet.

20 Spin the tube at 3,000 x g for 5 minutes.

Carefully remove and discard the supernatant.

21 Repeat steps 8 and 9 once, for a total of 2 washes.

22 After removing the second wash, resuspend the pellet in 1 mL 1X Wash Buffer.

Pipette up and down to fully mix.

23 Using a 1 mL syringe, gently push the 1 mL resuspended sample through a 200 µm filter into a new 5 mL tube.

Dovetail Filter Set **Contributed by users Catalog #BOM-006**

24 If the filter clogs, replace with a new 200 µm filter and continue until all of the sample has been filtered.

25 Gently pass an additional 1 mL of 1X Wash Buffer though the 200 µm filter into the 5 mL tube.

Your tube should now contain a total volume of ~2 mL.

26 Using the same syringe but changing to a 50 µm filter, re-filter the 2 mL sample into a new 5 mL tube.

27 Gently pass an additional 1 mL of 1X Wash Buffer though the 50 µm filter into the 5 mL tube.

Your tube should now contain a total volume of ~3 mL.

28 Spin the tube at 3,000 x g for 5 minutes.

Carefully remove and discard the supernatant.

29 Resuspend the pellet in 50 µL 1X Nuclease Digest Buffer

30 Add 0.5 µL of MNase Enzyme Mix.

Pipette up and down to fully mix.

31 Incubate the tube at 22° 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

32 Stop the reaction by adding 5 µL of 0.5 M EGTA.

Pipette up and down to fully mix.

33 Add 3 µL of 20% SDS to lyse the cells.

Pipette up and down to fully mix.

34 Incubate at 22° for 5 minutes in an agitating thermal mixer set at 1,250 rpm.

35 Continue to Stage 2: Lysate QC, page 13.

pre-step 2

36 Stage 2: Lysate QC
As you prepare for Stage 2, keep the following in mind:
The Lysate QC stage takes ~ 2 hours.

This stage has 2 objectives:

Quantify the lysate to determine the volume of lysate to use in Stage 3.

Confirm that the chromatin was properly digested.

Recommended kit; D5000 HS (Tapestation)

37 Before You Begin

Verify before use that 100% ethanol was added to the DNA Wash Buffer supplied in the Zymo Research DNA Clean & Concentrator-5 Kit, as directed by the manufacturer.

38 Program the thermal mixer as follows:

38.1

A	B
Temp	Time
55	15 minutes
68	45 minutes
25	Hold

39 10X Crosslink Reversal Buffer might have precipitated in storage.

Incubate at 37° for 15 minutes or until the precipitate is no longer visible.

Vortex to mix prior to use.

Step 2

40 Transfer 2.5 µL of the lysate to a new 1.5 mL tube labelled QC.

41 NOTE: Store the remainder of the lysate at -80°.

This is the lysate you will be using in Stage 3.

42 Digest Buffer (50 µL), mix the following components:

42.1

A	B	C	D	E	F
Reagent	1X	10% extra	3X	5X	8X
UltraPure water	45 ul	49 ul	147 ul	245 ul	392 ul

	A	B	C	D	E	F
	10x Crosslink Reversal buffer	5 ul	5.5 ul	16.5 ul	27.5 ul	44 ul
	Proteinase K	1.5 ul	1.65 ul	4.95 ul	8.25 ul	13.2 ul
	total	51.5 ul		165 ul	275 ul	440 ul

43 Add to the QC tube 51.5 µL from the master mix of the digest buffer

44 Pipette up and down to fully mix.

Incubate the QC tube in an agitating thermal mixer set at 1,250 rpm as follows:

44.1

	A	B
	Temp	Time
	55	15 minutes
	68	45 minutes
	25	hold

45 (make sure the samples go down to 25° as well)

46 Pipette up and down to fully mix.

Incubate the QC tube in an agitating thermal mixer set at 1,250 rpm as follows:

47 Purify the QC sample using Zymo Research DNA Clean and ConcentratorTM-5 Kit (DCC).

Quick spin your QC tube, add 200 µL of DCC DNA Binding Buffer, and mix thoroughly.

DNA Clean & Concentrator™-5 **Zymo Research Catalog #D4003**

48 Transfer the mixture to the Zymo-SpinTM Column placed in a collection tube.

49 Centrifuge for 30 seconds at 13,000 x g.

Discard the flow-through.

50 Add to the column 200 µL DCC DNA Wash Buffer

51 Centrifuge for 1 minute at 13,000 x g.

Discard the flow-through.

52 Repeat steps 7 and 8 once, for a total of 2 washes.

53 Transfer the column to a new 1.5 mL tube.

54 Add 10 µL DCC DNA Elution Buffer directly to the column and incubate for 1 minute at room temperature.

55 Centrifuge for 1 minute at 13,000 x g. Discard the column.

Your 1.5 mL tube now contains your purified QC DNA.

56 Quantify the purified QC DNA with a Qubit® Fluorometer and Qubit® dsDNA HS Kit. Based on the Qubit concentration, the total lysate amount (ng) can be calculated as follows:

Total Lysate (ng) = Qubit reading ng/µL x 10 µL (elution volume) x 23.4 (dilution factor)

You will use in Stage 3 a volume of the lysate that corresponds to 1,000 ng. This volume can be calculated as follows:

Volume (µL) = 1000 (ng) x 58.5 (µL) / Total Lysate (ng)

If the total lysate amount is < 1,000 ng, use all of the lysate in Stage 3.

57 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.

- For optimal nucleosome-level resolution, the digestion profile should contain 40% - 70% mononucleosomes: the first DNA peak, typically in the size range of 50 - 250 bp for TapeStation, should account for 40% - 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 40% - 70% mononucleosomes, proceed to Stage 3: Proximity Ligation, page 16.

- If the digestion profile contains < 40% mononucleosomes, do not proceed with the rest of the protocol. In this case, re-start the protocol and use 2 µL of MNase Enzyme Mix instead of 0.5 µL in step 14 in Stage 1: Crosslinking, Digestion and Lysis, page 11.

- If the digestion profile contains > 70%, you may proceed to Stage 3: Proximity Ligation, page 16 with caution. The library may include a reduced proportion of long-range information. This profile is likely due to suboptimal cell counting or a significant cell loss in the washing steps after crosslinking (steps 9 to 12 in Stage 1: Crosslinking, Digestion and Lysis, page 11).

pre-step 3

58 Stage 3: Proximity Ligation

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

Proximity ligation takes ~ 4.5 hours.

Follow best practices when working with beads

59 Before You Begin

10X Crosslink Reversal Buffer might have precipitated in storage.

Incubate at 37° for 15 minutes or until the precipitate is no longer visible. Vortex to mix prior to use.

60 Thaw End Polishing Buffer, 5X Bridge Ligation Buffer, Bridge, and Intra-Aggregate Ligation Buffer at room temperature.

Vortex to mix prior to use.

61 Prepare fresh 80% ethanol for DNA purification with speed beads for optimal results.

Fresh preparations of 80% ethanol will also be used in Stage 4, DNA Purification, page 24 and Stage 5, Size Selection, page 27.

You need a minimum of 1 mL for all these stages.

62 Equilibrate TE Buffer pH 8.0, Chromatin Capture Beads, and speed beads to room temperature.

Step 3

63 3.1 Bind Chromatin to Chromatin Capture Beads

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

64 Equilibrate the Chromatin Capture Beads to room temperature and vortex thoroughly (>30 seconds) to resuspend.

65 Transfer 100 µL of resuspended Chromatin Capture Beads to a new 1.5 mL tube.

66 Add 1,000 ng of the lysate that was stored at -80° (step 1 NOTE in Stage 2: Lysate QC, page 14)

to the 1.5 mL tube containing the beads.

If the total lysate amount is <1,000 ng, add all of the lysate.

67 Pipette up and down to fully mix. Incubate at room temperature, off the magnetic rack, for 10 minutes.

68 Place the tube in the magnetic rack for 5 minutes (or until the solution looks clear).

Discard the supernatant.

69 Remove the tube from the magnetic rack and wash the beads with 150 µL 1X Wash Buffer.

Pipette up and down to resuspend the beads, place the tube in the magnetic rack for 1 minute and discard the supernatant.

70 Repeat step 6 once, for a total of 2 washes.

71 3.2 End polishing
Follow the steps below for End Polishing:

Remove the tube from the magnetic rack and add to the beads 53.5 µL of a master mix containing the following reagents:

71.1

A	B	C	D	E	F
Reagents	1X	10% extra	3X	5X	8X
End Polish Buffer	50 ul	55 ul	165 ul	275 ul	440 ul
End Polishing Enzyme Mix	3.5 ul	3.85 ul	11.55 ul	19.25 ul	30.8 ul
Total	53.5 ul				

72 Pipette up and down to fully mix.

Incubate in an agitating thermal mixer set at 1,250 rpm as follows:

72.1

	A	B
	Temp	Time
	22	30 minutes
	65	30 minutes

73 Allow the tube to reach room temperature then place it in the magnetic rack for 1 minute (or until the solution looks clear).

Discard the supernatant.

74 Remove the tube from the magnetic rack and wash the beads once with 150 µL 1X Wash Buffer.

Pipette up and down to resuspend the beads, place the tube in the magnetic rack.

Do not remove and discard the supernatant at this step.

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.

75 3.3 Bridge Ligation

Follow the steps below for Bridge Ligation:

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

75.1

	A	B	C	D	E	F
	Reagents	1X	10% extra	3X	5X	8X
	UltraPure water	35 ul	38.5 ul	115.5 ul	192.5 ul	308 ul

	A	B	C	D	E	F
	5X bridge ligation buffer	10 ul	11 ul	33 ul	55 ul	88 ul
	Bridge	5 ul	5.5 ul	16.5 ul	27.5 ul	44 ul
	Total	53.5 ul				

76 Remove and discard the supernatant from step 4 in 3.2 End Polishing, page 17.

Remove the tube from the magnetic rack and add to the beads:

76.1

	A	B
	Reagent	Volume/reaction
	Bridge ligation mix	50 ul
	Bridge ligase	1 ul
	Total	51 ul

77 Pipette up and down to fully mix.

Incubate at 22° for 30 minutes in an agitating thermal mixer set at 1,250 rpm.

78 Place the tube in the magnetic rack for 1 minute (or until the solution looks clear).

Discard the supernatant.

79 Remove the tube from the magnetic rack and wash the beads once with 150 µL 1X Wash Buffer.

Pipette up and down to resuspend the beads, place the tube in the magnetic rack for 1 minute and discard the supernatant.

80 3.4 Intra-Aggregate Ligation

Follow the steps below for Intra-Aggregate Ligation:

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

80.1

	A	B	C	D	E	F
	Reagents	1X	10% extra	3X	5X	8X
	Intra-Aggregate Ligation Buffer	50 ul	55 ul	165 ul	275 ul	440 ul
	Intra-Aggregate Ligation enzyme mix	2 ul	2.2 ul	6.6 ul	11 ul	17.6 ul
	Total	53.5 ul				

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

SAFESTOP For convenience, this ligation reaction can proceed overnight at 22° in an agitating thermal mixer set at 1,250 rpm.

82 Place the tube in the magnetic rack for 1 minute (or until the solution looks clear).

Discard the supernatant.

83 3.5 Crosslink Reversal

Follow the steps below for Crosslink Reversal:

1. Remove the tube from the magnetic rack and add to the beads 51.5 µL of a master mix containing the following reagents (in order):
2.

83.1

	A	B	C	D	E	F
	Reagents	1X	10% extra	3X	5X	8X

	A	B	C	D	E	F
	UltraPure water	45 ul	49.5 ul	148.5 ul	247.5 ul	396 ul
	10% crosslink reversal buffer	5 ul	5.5 ul	165 ul	275 ul	44 ul
	Proteinase K	1.5 ul	1.65 ul	4.95 ul	8.25 ul	13.2 ul
	Total	51.5 ul				

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

84.1

	A	B
	Temp	Time
	55	15 minutes
	68	45 minutes
	25	hold

84.2 SAFESTOP; For convenience, you can hold at 25° overnight in an agitating thermal mixer set at 1,250 rpm.

85 Place the tube in the magnetic rack for 1 minute. Transfer 50 µL of the SUPERNATANT to a new 1.5 mL tube.

Discard the beads.

86 3.6 DNA Purification
Follow the steps below for DNA Purification on SPRIselect Beads:

Vortex the SPRIselect beads thoroughly (>30 seconds) to resuspend.

SPRIselect reagent kit **Beckman Coulter Catalog #B23317**

87 Add 90 µL of resuspended SPRIselect beads to the 1.5 mL tube containing your sample.

88 Vortex to mix thoroughly.

Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

89 Incubate the tube at room temperature, off the magnetic rack, for 5 minutes.

90 Quick spin the tube and place it in the magnetic rack for 5 minutes. Discard the supernatant.

91 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.

Simply add the ethanol, wait for 1 minute then discard the ethanol supernatant.

92 After the last wash, quick spin the tube and place it in the magnetic rack for 1 minute.

Use a 10 µL pipette tip to remove traces of ethanol

93 Air dry the beads for 5 minutes in the magnetic rack until no residual ethanol remains.

Do not over dry the beads.

94 Off the magnetic rack, resuspend the beads in 52 µL TE Buffer pH 8.0.

95 Vortex to mix thoroughly.

Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

96 Incubate at room temperature, off the magnetic rack, for 5 minutes.

97 Quick spin the tube and place it in the magnetic rack for 1 minute.

98 Transfer 50 µL of the SUPERNATANT (purified DNA) to a new tube. Discard the beads.

99 Quantify the purified DNA using a Qubit Fluorometer and Qubit dsDNA HS Kit.

You should recover a minimum of 50 ng to proceed to Step 4: Library Preparation

100 You will use 150 ng of your purified DNA for Stage 4 in a 50 µL volume. If needed, you can bring up the volume to 50 µL 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° You can use the remaining

SAFESTOP ; Purified DNA sample can be stored at -20° for up to 6 months.

pre-step 4

101 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 ~ 2.5 hours.

Dovetail Hi-C Library Preparation Kit **Contributed by users Catalog #21004**

102 Before You Begin

The End Repair Buffer may have precipitated in storage.

Incubate for at least 10 minutes at 37° until there is no visible precipitate.

103 Equilibrate TE Buffer pH 8.0 and SPRIselect beads to room temperature.

104 Thaw 250 mM DTT and Adaptor for Illumina at room temperature.

Vortex to mix prior to use.

Step 4

105 4.1 End Repair

Follow the steps below for End Repair:

Place 50 µL of purified DNA (150 ng) in a 0.2 mL PCR tube.

106 Add to the PCR tube 10.5 µL of a master mix containing the following reagents:

106.1

	A	B	C	D	E	F

	A	B	C	D	E	F
	Reagents	1X	10% extra	3X	5X	8X
	End Repair Buffer	7 ul	7.7 ul	23.1 ul	38.5 ul	61.6 ul
	End repair enzyme mix	3 ul	3.3 ul	9.9 ul	16.5 ul	26.4 ul
	250 mM DTT	0.5 ul	0.55 ul	1.65 ul	2.75 ul	4.4 ul
	Total	10.5 ul				

107 Pipette up and down to fully mix.

Quick spin the tube.

108 Incubate in a thermal cycler as follows:

108.1

	A	B
	Temp	Time
	20	30 minutes
	65	30 minutes
	12	hold

109 4.2 Adapter Ligation and USER Digest

Follow the steps below for Adapter Ligation and USER Digest:

Add to the PCR tube containing the end-repaired sample the following reagents:

109.1

	A	B
	Reagent	Volume/reaction

	A	B
	Adaptor for Illumina	2.5 ul
	Ligation Enzyme mix	30 ul
	Ligation Enhancer	1 ul
	Total	33.5 ul

109.2 NOTE The Ligation Enzyme Mix and Ligation Enhancer can be mixed ahead of time.

The master mix is stable for 8 hours at 4°

We do not recommend adding the Adaptor for Illumina to the master mix.

110 Pipette up and down to fully mix.
Quick spin the tube.

111 Incubate at 20° for 15 minutes in a thermal cycler.

Hold at 12°

112 Following incubation, add 3 µL of USER Enzyme Mix to the PCR tube.

113 Pipette up and down to fully mix.

Quick spin the tube.

114 Incubate at 37 ° for 15 minutes in a thermal cycler.

Hold at 12 °

115 4.3 DNA Purification

Follow the steps below for DNA Purification:

Vortex the speed beads thoroughly (>30 seconds) to resuspend.

116 Add 80 µL of resuspended speed beads to the PCR tube containing the adaptor-ligated sample.

117 Vortex to mix thoroughly.

Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

118 Incubate the tube at room temperature, off the magnetic rack, for 5 minutes.

119 Quick spin the tube and place it in the magnetic rack for 5 minutes. Discard the supernatant.

120 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.

Simply add the ethanol, wait for 1 minute then discard the ethanol supernatant.

121 Quick spin the tube and place it in the magnetic rack for 1 minute. Use a 10 µL pipette tip to remove traces of ethanol

122 Air dry the beads for 5 minutes in the magnetic rack until no residual ethanol remains. Do not over dry the beads.

123 Off the magnetic rack, resuspend the beads in 100 µL TE Buffer pH 8.0.

124 Vortex to mix thoroughly.

Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

125 Incubate at room temperature, off the magnetic rack, for 5 minutes.

126 Quick spin the tube and place it in the magnetic rack for 1 minute.

127 Transfer 95 µL of the SUPERNATANT (purified adaptor-ligated DNA) to a new tube. Discard the beads.

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

Pre-Step 5

128 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.

Dovetail Hi-C Library Preparation Kit **Contributed by users** **Catalog #21004**

129 Before You Begin

Thaw Universal PCR Primer, Index Primer, and HotStart PCR Ready Mix at room temperature.

Vortex to mix prior to use.

130 Equilibrate TE Buffer pH 8.0, speed beads, Streptavidin Beads, TWB, 2X NTB, LWB, and NWB to room temperature.

Step 5

131 5.1 Streptavidin Beads Preparation
NOTE This step does not involve any DNA sample.

Follow the steps below for Ligation Capture and Amplification:

Vortex the Streptavidin Beads vial thoroughly (> 30 seconds) to resuspend the beads.

Transfer 25 µL of resuspended Streptavidin beads to a new 1.5 mL tube.

132 Place the 1.5 mL tube containing the beads in the magnetic rack for 5 minutes.

Discard the supernatant.

133 Remove the tube from the magnetic rack and wash the beads with 200 µL TWB: pipette up and down to resuspend the beads and place the tube in the magnetic rack for 1 minute.

Discard the supernatant.

134 Repeat step 3 once, for a total of 2 washes.

135 After the second wash, resuspend the beads in 100 µL 2X NTB.

Pipette up and down to fully mix.

136 5.2 Ligation Capture
Follow the steps below for Ligation Capture:

Transfer the 95 µL of purified adaptor-ligated DNA (from step 13 in 4.3 DNA Purification, page

24) to the 1.5 mL tube containing the Streptavidin beads resuspended in 100 µL of 2X NTB.

137 Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

138 Incubate at 25° for 30 minutes in an agitating thermal mixer set at 1,250 rpm.

139 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, pipette up and down to resuspend the beads, place the tube in the magnetic rack for 1 minute, 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:

Quick spin the tube and place it in the magnetic rack for 1 minute. Discard the supernatant.

140 Wash the beads once with 200 µL LWB.

141 Wash the beads twice with 200 µL NWB.

142 Wash the beads twice with 200 µL 1X Wash Buffer.

143 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:

After the last wash, remove the tube from the magnetic rack and add to the beads 45 µL of a master mix containing the following reagents:

143.1

	A	B	C	D	E	F
	Reagents	1X	10% extra	3X	5X	8X
	UltraPure water	15 ul	16.5 ul	49.5 ul	82.5 ul	132 ul
	HotStart PCR Ready Mix	25 ul	27.5 ul	82.5 ul	137.5 ul	220 ul
	Universal PCR primer	5 ul	5.5 ul	16.5 ul	27.5 ul	44 ul
	Total	45 ul				

144 Add 5 µL Index Primer to the PCR reaction.

Use one Index Primer per PCR reaction

145 Pipette up and down to fully mix then transfer to a new 0.2 mL PCR tube.

146 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:

146.1

	A	B	C	D
	Step	Temp	Time	Cycles
	Enzyme activation	98	3 minutes	1

	A	B	C	D
	Denature	98	20 seconds	12
	Anneal	65	30 seconds	
	Extend	72	30 seconds	
	Extend	72	1 minute	1
		12	hold	1

147 5.5 Size Selection

Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare Catalog #44152105050350**

Follow the steps below for Size Selection:

Quick spin the PCR tube and place it in the magnetic rack for 1 minute.

148 Transfer 47 µL of the SUPERNATANT to a new 1.5 mL tube.

Discard the beads.

149 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.

150 Vortex the speed beads thoroughly (>30 seconds) to resuspend.

151 Add 50 µL of resuspended speed beads to the 1.5 mL tube containing your sample.

Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare Catalog #44152105050350**

152 Vortex to mix thoroughly. Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

153 Incubate the tube at room temperature, off the magnetic rack, for 10 minutes.

154 Quick spin the tube and place it in the magnetic rack for 5 minutes.

155 Transfer 145 µL of the SUPERNATANT to a new 1.5 mL tube. Discard the beads.

156 Add 30 µL of resuspended Speed beads to the 1.5 mL tube containing your sample.

Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare Catalog #44152105050350**

157 Vortex to mix thoroughly.

Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

158 Incubate the tube at room temperature, off the magnetic rack, for 10 minutes.

159 Quick spin the tube and place it in the magnetic rack for 5 minutes.

Discard the supernatant.

160 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.

Simply add the ethanol, wait for 1 minute then discard the ethanol supernatant.

161 Quick spin the tube and place it in the magnetic rack for 1 minute. Use a 10 µL pipette tip to

remove traces of ethanol.

162 Air dry the beads for 5 minutes in the magnetic rack until no residual ethanol remains. Do not over dry the beads.

163 Off the magnetic rack, resuspend the beads in 30 µL TE Buffer pH 8.0.

164 Vortex to mix thoroughly.

Quick spin the tube (make sure to spin down only briefly and to stop before the beads start to settle).

165 Incubate the tube at room temperature, off the magnetic rack, for 5 minutes.

166 Quick spin the tube and place it in the magnetic rack for 1 minute.

167 Transfer 28 µL of the SUPERNATANT to a new 1.5 mL tube. The supernatant is your size selected library. Discard the beads.

168 Quantify your size selected library using a Qubit Fluorometer and Qubit dsDNA HS Kit.

169 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.

SAFESTOP The library can be stored at -20° for up to 6 months.