

MAR 06, 2023

OPEN ACCESS

dx.doi.org/10.17504/protocol s.io.kqdq39wbzq25/v1

External link:

https://www.biorxiv.org/conte nt/10.1101/2023.02.18.5290 96v1.article-metrics

Protocol Citation: Honggui Wu, Longzhi Tan 2023. singlecell Micro-C protocol. protocols.io

https://dx.doi.org/10.17504/p rotocols.io.kqdq39wbzq25/v1

MANUSCRIPT CITATION:

https://www.biorxiv.org/conte nt/10.1101/2023.02.18.5290 96v1.article-metrics

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use. distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working We use this protocol and it's working

Created: Mar 04, 2023

Last Modified: Mar 06, 2023

single-cell Micro-C protocol

Honggui Wu¹, Longzhi Tan²

¹Biomedical Pioneering Innovation Center, Peking University, Beijing 100871. China:

²Department of Neurobiology, Stanford University, Stanford, CA 94305, **USA**



Honggui Wu

Biomedical Pioneering Innovation Center, Peking University, ...

ABSTRACT

Here we present a single-cell 3D genome mapping assay with improved spatial resolution with the help of micrococcal nuclease (MNase), termed single-cell Micro-C (scMicro-C). To achieve scMicro-C, we made three improvements. First, we titrated MNase digestion to reduce DNA loss and produce proper DNA fragments. Second, we solubilized chromatin with an ionic detergent, sodium dodecyl sulfate (SDS), which dramatically improved ligation efficiency. Third, we adopted our high-coverage transposon-based whole-genome amplification method, META (19), using Tn5 (20), to enhance the detection of chromatin "contacts." scMicro-C retains the high signalto-noise and high-resolution characteristics of bulk Micro-C, as indicated the accurate detection chromatin loops and other fine-scale chromatin structures. Furthermore, scMicro-C retains nucleosome and other chromatin-bound proteins (e.g., transcription factor) occupancy information. With our previously developed Dip-C algorithm, we confirmed that scMicro-C enables the reproducible reconstruction of 3D genome structures in single-cell at 5 kb resolution. With such kilobase 3D structures, we were able to explore the fine-scale chromatin folding in individual cells.

PROTOCOL integer ID:

78092

Keywords: single-cell 3D genome structure, single-cell Hi-C, chromosome conformation, Micro-C

Crosslink Cells for Micro-C

- **1** Prepare 1 x BSA in PBS for Washing.
 - Add 🗸 50 µL 10% BSA (MACS 130-091-376, final 1x) to 🗸 50 mL 1x PBS.
- Prepare 1% Paraformaldehyde (🗸 1 mL for 1 million cells), recover to room temperature.
 - Д 1 mL 32% PFA (EMS 15714, final 1%)
- 3 Prepare 3 mM DSG ($\angle 1 \text{ mL}$ for 1 million cells).
 - Dissolve 50 mg DSG (Thermo Fisher 20593) with \bot 511 μ L DMSO to a final concentration of 300 mM.
 - Add \angle 300 μ L 300 mM DSG to \angle 29.7 mL 1 x PBS to a final concentration of 3 mM, protected from light.
- 4 Harvest cells.
 - Collect cell growth with appropriate density and high viability.
 - Wash cells once with 1x PBS.
- **5** Fixation.
 - Resuspend cell pellets in 1% PFA, and incubate at room temperature with rotation for 10 min.
 - At the end of incubation, add 2 M Tris-HCl ph 7.5 to a final concentration of 0.75 M, and incubate at RT for 5 min.
 - Centrifuge at 3000 x g for 5 min in a swing bucket centrifuge.
- Remove the supernatant, then wash twice with \pm 5 mL ice-cold 1 x BSA in PBS.

- 7 Crosslink with DSG.
 - Resuspend in 3 mM DSG, and incubate at RT for 45 min with rotation.
 - At the end of incubation, add 2 M Tris-HCl ph 7.5 to a final concentration of 0.75 M, and incubate at RT for 5 min.
 - Centrifuge at 3000 x g for 5 min in a swing bucket centrifuge.
- 8 Count cells and aliquot.
 - Remove the supernatant, then wash twice with ∠ 5 mL ice-cold 1 x BSA in PBS.
 - Count cell number, then aliquot 1 million cells each to 1.5 mL tube.
 - Store at -80°C (up to a year).

Prepare Micro-C Buffers

- 9 Prepare Micro-C Buffers
- 9.1 Prepare Micro-C Buffer 1 (Δ 300 μL per sample)
 - Δ 50 μL
 5 M NaCl (Invitrogen AM9760G; final 50 mM)
 - Д 50 µL 1 M Tris pH 7.5 (Invitrogen 15567027; final 10 mM)
 - A 25 µL 1 M MgCl2 (Invitrogen AM9530G; final 5 mM)
 - 🗸 5 µL 1 M CaCl2 (Sigma 21115; final 1 mM)
 - \blacksquare $\ \ \, \Delta$ 50 μL $\ \,$ 5% Digitonin (Sigma D141-100MG, final: 0.05%)
 - Д 50 µL 100 x protease inhibitor (Roche 4693159001) (final: 1 X)
 - 4.77 mL nuclease-free H₂O

- 9.2 Prepare Micro-C Buffer 2 (4 10 mL)
 - Δ 100 μL
 5 M NaCl (Invitrogen AM9760G; final 50 mM)
 - Δ 100 μL
 1 M Tris pH 7.5 (Invitrogen 15567027; final 10 mM)

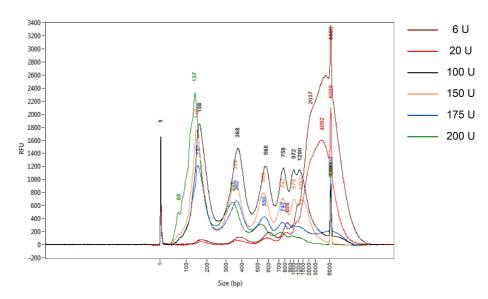
- Δ 100 μL
 1 M MgCl2 (Invitrogen AM9530G; final 10 mM)
- <u>A</u> 9.7 mL nuclease-free H₂O

- 9.3 Prepare Micro-C Buffer 3 (A 10 mL)
 - 🗸 500 µL 1 M Tris pH 7.5 (Invitrogen 15567027; final 50 mM)
 - Δ 100 μL
 1 M MgCl2 (Invitrogen AM9530G; final 10 mM)
 - 4 mL nuclease-free H₂0

MNase Digestion

- 10 Dilute MNase.
 - Make MNase Dilution Buffer (10 mM Tris-HCl ph 7.5, 50 mM NaCl, 1 mM EDTA, 50% Glycerol).
 - Aliquot 10,000 Unit MNase (NEB M0247S, 2,000 U/μL) to a final concentration of 20 U/μL.
 - Store at -80°C for several month.
- 11 Thaw 5 tubes of cell pellets on ice.
- Resuspend in $\underline{\mathbb{Z}}$ 100 μL Micro-C Buffer 1, incubate on ice for 20 min, and aspiration every 5 min.
- Centrifuge at 800 x g at 4°C for 5 min.

- Take 5 ul to check digestion efficiency, remaining centrifuge at 800 x g at 4°C for 5 min. Carefully discard the supernatant, and wash once with 500 µL Micro-C Buffer 2. Store at -80°C (stable for up to several weeks) or proceed to End Repair.
- 15 Check Digestion Efficiency
 - Take 🗓 5 µL above sample to check digestion efficiency.
 - Add 85 ul 1 x PBS, 5 ul 10% SDS (Sigma, 71736-100ML), and 5 ul 20 mg/mL ProteaseK (QIAGEN, 19131).
 - incubate at 65°C for 2 hr.
 - Purify with DNA clean column (ZYMO, D4014).
 - Run capillary electrophoresis (Fragment Analyzer/TepeStation/Bioanalyzer) to analyze digestion efficiency.



An exemplified MNase titration experiment: fragment size distribution with different MNase concentrations.

End Repair

- 16 Thaw the cell pellet (with the most appropriate fragment size) on ice.
- 17 Prepare 0.5% SDS.

- Add \bot 25 μ L 10% SDS to \bot 475 μ L Micro-C Buffer 2 to a final concentration of 0.5%.
- 18 Solubilize Chromatin.
 - Resuspend the cell pellets in 🗸 50 µL 0.5% SDS.
 - Incubate at 62°C for 10 min.
 - At the end of incubation, add \perp 50 μ L 5% Triton X-100 to quench SDS. Incubate at 37°C for 15 min.
- Centrifuge at 800 x g for 5 min, carefully remove supernatant.
- 20 Prepare End Repair Mix 1 (\pm 45 μ L each sample).
 - Δ 5 μL 10 X NEBuffer 2.1 (NEB B7202S)
 - Δ 1 μL 100 mM ATP (Thermo R0441)
 - $\boxed{ \mathbb{L} \ 2.5 \, \mu L } \ 100 \, \text{mM DTT (final: } \sim 5 \, \text{mM)}$
 - <u>A</u> 2.5 µL 10 U/uL T4 PNK (NEB M0201S) (final: ~0.5 U/uL)
 - \pm 34 μ L nuclease-free H₂O

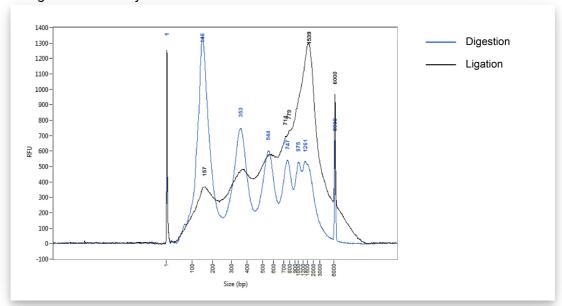
- 21 Resuspend in 🚨 45 µL End Repair Mix1, and incubate at 37°C for 15 min.
- 22 At the end of incubation, add \underline{L} 5 μL 5 U/uL Klenow Fragment (NEB M0210S), incubate at 37°C for another 15 min.
- 23 Prepare End Repair Mix2 (4 25 µL each sample)
 - 🗸 2.5 µL 10 X T4 DNA Ligase Buffer (NEB B0202S)
 - <u>A 0.5 µL</u> 10 mM (each) dNTP (NEB N0447S)

- \triangle 21.875 µL nuclease-free H₂O
- 24 After Klenow incubation, add $\underline{\mathbb{Z}}$ 25 μ L End Repair Mix2 to each tube, incubate at 23°C for 45 min (shake 30 s and pause 2 min).
- Add 5 ul 0.5 M EDTA (Invitrogen AM9260G).
- 26 Incubate at 65°C for 20 min.
- Wash once with A 1 mL Micro-C Buffer 3.

Proximity Ligation

- 28 Prepare Ligation Mix ($\mathbb{Z}_{250 \, \mu L}$ each sample).
 - Δ 25 μL
 10 X T4 DNA Ligase Buffer (NEB B0202S)
 - <u>I 1.25 μL</u> 20 mg/mL BSA (NEB B9000S)
 - <u>A 12.5 µL</u> 400 U/uL T4 DNA Ligase (NEB M0202S)
 - Z 211.25 µL nuclease-free H₂O
- Resuspend the cell pellet in $\ \underline{\ \ }\ 250\ \mu L$ Ligation Mix.

- Take \angle 25 μ L as ligation efficiency control.
- 31 Check Ligation Efficiency
 - Take \bot 25 μ L ligation product above sample to check ligation efficiency.
 - Add 65 ul 1 x PBS, 5 ul 10% SDS (Sigma, 71736-100ML), and 5 ul 20 mg/mL ProteaseK (QIAGEN, 19131).
 - incubate at 65°C for 2 hr.
 - Purify with DNA clean column (ZYMO, D4014).
 - Run capillary electrophoresis (Fragment Analyzer/TepeStation/Bioanalyzer) to analyze ligation efficiency.



A typical fragment size distribution after digestion and ligation.

Single-cell Amplification

32 Primers

META 16 sequence

A	В
META-16-1	GGCACCG AAAA

A	В
META-16-2	CTCGGCGA TAAA
META-16-3	GGTGGAGC ATAA
META-16-4	CGAGCGCA TTAA
META-16-5	AGCCCGGT TATA
META-16-6	TCGGCACC AATA
META-16-7	GCCTGTGG ATTA
META-16-8	GCGACCCT TTTA
META-16-9	GCATGCGG TAAT
META-16-10	GCGTTGCC ATAT
META-16-11	GGCCGCAT TTAT
META-16-12	ACCGCCTC TATT
META-16-13	CCGTGCCA AAAT
META-16-14	TCTCCGGG AATT
META-16-15	CCGCGCTT ATTT
META-16-16	CTGAGCTCG TTTT

META Transposon

	A	В
META Transposon 5'-[META sequence]-AGATGTGTATAAGAGAG		5'-[META sequence]-AGATGTGTATAAGAGACAG-3'
	19 bp ME	5'-/phos/-CTGTCTCTTATACACATCT-3'

■ First Step PCR Primers

A	В
First PCR primers	5'-[META sequence]-AGATGTGTATAAG-3'

Here we use 12 x 8 barcode combinations to distinguish individual cells.

■ META16-ADP1 cell barcode

A	В
1-1	GATATG
1-2	ATACG
1-3	ссвтств

A	В
1-4	TGCG
1-5	GAACTCG
1-6	ATGTAG
1-7	CCCG
1-8	TATGT
1-9	GAGTAAG
1-10	ATCG
1-11	CCTAG
1-12	TGACCG

■ META16-ADP2 cell barcode

A	В
2-1	ACTCTA
2-2	AGAGCAT
2-3	GGTATG
2-4	TCGATGC
2-5	CTACTAG
2-6	TATGCA
2-7	CACACGA
2-8	GTCGAT

Second Step Barcoded PCR Primers

A	В
META16-ADP1	5'-CTTTCCCTACACGACGCTCTTCCGATCT-[cell barcode]-[META sequence]-AGATGTGTATAAG-3'
META16-ADP2	5'-GAGTTCAGACGTGTGCTCTTCCGATCT-[cell barcode]-[META sequence]-AGATGTGTATAAG-3'

33 Prepare Reagents.

- **33.1** Prepare 60 mg/mL Protease.
 - Dissolve 1 vial QIAGEN protease (QIAGEN 19155) in 2.78 mL 50% glycerol.
 - Aliquot and store at -80°C (stable for up half a year).
- 33.2 Cell Triton Lysis Buffer (A 1 mL).
 - \pm 10 μ L 1 M Tris pH 8.0 (Invitrogen AM9855G, final 10 mM)
 - 4 µL 5 M NaCl (Invitrogen AM9760G, final 20 mM)
 - A 2 µL 0.5 M EDTA (Invitrogen AM9260G, final 1 mM)
 - Δ 10 μL
 10% Triton X-100 (Sigma 93443, final 0.1%)
 - <u>A</u> 25 µL 1 M DTT (Sigma 646563, final 25 mM)
 - Δ 5 μL 100 μM Carrier ssDNA (final 100 nM)
 - Add H₂O to 1 mL

A	В
Carrier ssDNA	TCAGGTTTTCCTGAA

- 2 X Transposition Buffer (4 1 mL
 - \pm 20 μ L 1 M Tris-HCl pH 8.0 (Invitrogen AM9855G, final 20 mM)
 - \blacksquare $\ \ \, 4.10\ \mu L$ $\ \ \, 1\ M\ MgCl_2$ (Thermo Fisher AM9530G, final 10 mM)
 - 400 ul 40% (w/w) Polyethylene glycol 8000 (Sigma P1458, final 16% (w/w))
 - \bot 570 μ L nuclease-free H₂O

Store at -20°C if needed

Pick single cells.

Cell lysis.
Incubate as:
50°C, 1 hr

65°C, 1 hr 70°C, 15 min

Stored at -80°C or proceed to transposon-based amplification.

- Transposition (A 8 µL for each well, below recipe for 1 96-well plate).
 - 1. Prepare Transposition Mix
 - 🗸 440 µL 2 X Transposition Buffer
 - Add META transposome* to a final concentration of 0.3 nM
 - Add nuclease-free H₂O to 🗸 880 µL
 - * Transposome: META transposome is optional. Alternatively, Nextera transposome can be used (if using Nextera procedure, please refer to "Dip-C (Part 2: Whole-genome Amplification with Nextera)") on protocol.io.
 - 2. Add A gul Transposition Mix to each well with a multi-channel pipette, vortex to mix.
 - 3. Incubate at 55°C for 10 min.
- **37** Tn5 Stop.
 - Prepare Tn5 Removal Mix (250 mM NaCl, 37.5 mM EDTA, 2 mg/mL QIAGEN protease).
 - Add 🗸 2 µL Tn5 Removal Mix to each tube, and mix thoroughly.
 - Incubate as: 50°C, 30 min 70°C, 15 min
- 38 Pre-amplification.
- 38.1 Prepare Preamplification Mix (A 16 µL each well, below recipe for 1 96-well plate)
 - Δ 1540 μL
 2 X Q5 High-fidelity Master Mix (NEB M0492L)
 - \pm 98.6 μ L 50 μ M First Step PCR Primers (final 0.1 μ M each)
 - <u>A</u> 55 µL 100 mM MgCl₂
 - \bot 66.4 μ L nuclease-free H₂O
- 38.2 Add \pm 16 μ L Preamplification Mix each well with a multi-channel pipette, and vortex to mix.

38.3 Amplified as:

72°C, 5 min

98°C, 30 s

12 cycles of [98°C for 10 s, 62°C for 30 s, 72°C, 2 min] 65°C, 5 min.

39 Cell Barcoding PCR.

- Add \square 1 μ L individual 50 μ M barcoded META16-ADP1 Primer and \square 1 μ L individual 50 μ M indexed META16-ADP2 Primer Mix to each well with a multi-channel pipette.
- Incubate as:

98°C, 30 s

3 cycles of [98°C for 10 s, 62°C for 30 s, 72°C, 2 min] 65°C, 5 min

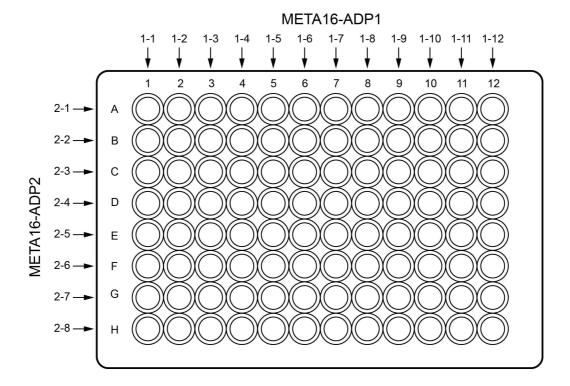


Illustration of barcode addition.

40 Purification.

- Pool a 96-well plate to purify.
- Purify with clean column (ZYMO, D4014).
- Quantify with Qubit Fluorometer.

41 Library preparation.

■ Take 400 ng amplified product for each plate as input.

- Prepare Library Preparation Mix (\pm 50 μ L 2 X Q5 High-fidelity Mater Mix, template (400 ng), \pm 5 μ L 10 μ M Indexed i5 primer, \pm 5 μ L 10 μ M Indexed i7 primer (Vazyeme N321/N322), nuclease-free H₂O to \pm 100 μ L)
- Amplified as:
 98°C, 30 s
 2 cycles of [98°C, 10 s, 68°C, 30 s, 72°C, 2 min]
 72°C, 5 min
- Purify with PCR clean column (ZYMO D4014), then purify with 0.75 x AMPUre XP beads to select > 300 bp fragments for sequencing.

42 Sequencing.

META library only has 20% valid fragments having both P7 and P5 on either side, need to RUN qPCR quantify before loading.