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Dip-C (Part 2: Whole-genome Amplification with Nextera)

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

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44640

Oligos

- 1 Carrier ssDNA (same as in LIANTI and META):
 - TCAGGTTTTCTGAA

- Purification: standard desalting
- Dissolve in 0.1 X TE (made from TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849**) to a final concentration of **[M] 100 micromolar (μM)** .
- Store at -20 °C .

2 Nextera i7 Index Primers:

- 701: CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGG
- 702: CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG
- 703: CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG
- 704: CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG
- 705: CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGG
- 706: CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGG
- 707: CAAGCAGAAGACGGCATACGAGATGTAGAGAGTCTCGTGGGCTCGG
- 708: CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGG
- 709: CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGG
- 710: CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGG
- 711: CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGG
- 712: CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGG
- and the following if > 96 cells need to be sequenced at the same time (e.g. NovaSeq):
- 714: CAAGCAGAAGACGGCATACGAGATTCATGAGCGTCTCGTGGGCTCGG
- 715: CAAGCAGAAGACGGCATACGAGATCCTGAGATGTCTCGTGGGCTCGG
- 716: CAAGCAGAAGACGGCATACGAGATTAGCGAGTGTCTCGTGGGCTCGG
- 718: CAAGCAGAAGACGGCATACGAGATGTAGTCCGTCTCGTGGGCTCGG
- 719: CAAGCAGAAGACGGCATACGAGATTACTACGCGTCTCGTGGGCTCGG
- 720: CAAGCAGAAGACGGCATACGAGATAGGCTCCGGTCTCGTGGGCTCGG
- 721: CAAGCAGAAGACGGCATACGAGATGCAGCGTAGTCTCGTGGGCTCGG
- 722: CAAGCAGAAGACGGCATACGAGATCTGCGCATGTCTCGTGGGCTCGG
- 723: CAAGCAGAAGACGGCATACGAGATGAGCGTAGTCTCGTGGGCTCGG
- 724: CAAGCAGAAGACGGCATACGAGATCGCTCAGTGTCTCGTGGGCTCGG
- 726: CAAGCAGAAGACGGCATACGAGATGTCTTAGGGTCTCGTGGGCTCGG
- 727: CAAGCAGAAGACGGCATACGAGATACTGATCGGTCTCGTGGGCTCGG
- Purification: standard desalting
- Dissolve in 0.1 X TE to a final concentration of **[M] 50 micromolar (μM)** .
- Dilute with 0.1 X TE to **[M] 12.5 micromolar (μM)** in PCR tubes.

3 Nextera i5 Index Primers:

- 501: AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTC
- 502: AATGATACGGCGACCACCGAGATCTACACCTCTCTATTTCGTCGGCAGCGTC
- 503: AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTC
- 504: AATGATACGGCGACCACCGAGATCTACACAGAGTAGATCGTCGGCAGCGTC
- 505: AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTC
- 506: AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTC
- 507: AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTC

- 508: AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTCGGCAGCGTC
- and the following if > 96 cells need to be sequenced at the same time (e.g. NovaSeq):
- 510: AATGATACGGCGACCACCGAGATCTACACCGTCTAATTCGTCGGCAGCGTC
- 511: AATGATACGGCGACCACCGAGATCTACACTCTCTCCGTCGTCGGCAGCGTC
- 513: AATGATACGGCGACCACCGAGATCTACACTCGACTAGTCGTCGGCAGCGTC
- 515: AATGATACGGCGACCACCGAGATCTACACTTCTAGCTTCGTCGGCAGCGTC
- 516: AATGATACGGCGACCACCGAGATCTACACCCTAGAGTTTCGTCGGCAGCGTC
- 517: AATGATACGGCGACCACCGAGATCTACACGCGTAAGATCGTCGGCAGCGTC
- 518: AATGATACGGCGACCACCGAGATCTACACCTATTAAGTCGTCGGCAGCGTC
- 520: AATGATACGGCGACCACCGAGATCTACACAAGGCTATTCGTCGGCAGCGTC
- Purification: standard desalting
- Dissolve in 0.1 X TE to a final concentration of **100 50 micromolar (μM)**.
- Dilute with 0.1 X TE to **100 12.5 micromolar (μM)** in PCR tubes.

Reagents

- 4 Prepare 60 mg/mL Qiagen Protease:
 - Add **2.78 mL** water to one vial of **QIAGEN Protease (7.5 AU) Qiagen Catalog #19155**.
 - Vortex to mix.
 - Filter to sterilize.
 - Store at **4 °C**.
- 5 Prepare Lysis Buffer (**2 μL** per cell; recipe below for **1 mL**, or 4 96-well plates):
 - **20 μL** **Tris (1 M), pH 8.0, RNase-free Thermo Fisher Catalog #AM9855G** (final: **20 millimolar (mM)**)
 - **4 μL** **NaCl (5 M), RNase-free Thermo Fisher Catalog #AM9760G** (final: **20 millimolar (mM)**)
 - **15 μL** **Triton X-100, 10% solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443** (final: **0.15 % (v/v)**)
 - **25 μL** **1M DL-Dithiothreitol solution (DTT) Merck MilliporeSigma (Sigma-Aldrich) Catalog #646563** (aliquoted and stored at **-20 °C**; final: **25 millimolar (mM)**)
 - **2 μL** **EDTA (0.5 M), pH 8.0, RNase-free Thermo Fisher Catalog #AM9260G** (final: **1 millimolar (mM)**)
 - **5 μL** **100 uM Carrier ssDNA** (final: **500 nanomolar (nM)**)
 - **929 μL** water
 - Vortex to mix.

- Store at -20°C if needed.

6 Prepare Transposition Buffer ($8\ \mu\text{L}$ per cell; recipe below for $1\ \text{mL}$, or 1 96-well plate):

- $12.5\ \mu\text{L}$ $\otimes$ TAPS Buffer (1 M pH 8.5) **Boston Bioproducts Catalog #BB-2375**
(final: $12.5\ \text{mM}$)
- $6.25\ \mu\text{L}$ $\otimes$ 1M MgCl_2 **Invitrogen - Thermo Fisher Catalog #AM9530G** (final: $6.25\ \text{mM}$)
- $200\ \mu\text{L}$ $\otimes$ 50% w/v Polyethylene glycol 8000 **Hampton Research Catalog #HR2-535** (final: $10\ \text{Mass} / \%$ volume)
- $781.25\ \mu\text{L}$ water
- Vortex to mix.
- Store at -20°C if needed.

7 Prepare Transposome Removal Buffer ($2\ \mu\text{L}$ per cell; recipe below for $1\ \text{mL}$, or 4 96-well plates):

- $60\ \mu\text{L}$ $\otimes$ NaCl (5 M), RNase-free **Thermo Fisher Catalog #AM9760G** (final: $300\ \text{mM}$)
- $90\ \mu\text{L}$ $\otimes$ EDTA (0.5 M), pH 8.0, RNase-free **Thermo Fisher Catalog #AM9260G**
(final: $45\ \text{mM}$)
- $1\ \mu\text{L}$ $\otimes$ Triton X-100, 10% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443**
(final: $0.01\ \%$ (v/v) ; for ease of pipetting)
- $849\ \mu\text{L}$ water
- Vortex to mix.
- Store at -20°C if needed.

Lysis (Skip if Performing Positive Control)

2h 30m

8 Prepare Dip-C Lysis Buffer ($2\ \mu\text{L}$ per cell; recipe below for 4 96-well plate + 25%):

- $960\ \mu\text{L}$ Lysis Buffer
- $0.24\ \mu\text{L}$ 60 mg/mL Qiagen Protease (final: $15\ \mu\text{g/mL}$)
- Vortex to mix.
- Aliquot to $78\ \mu\text{L}$ in 12-strip tubes.

9 Add $2\ \mu\text{L}$ Dip-C Lysis Buffer per well to a

- $\otimes$ 96 well LoBind PCR plates Semi-skirted **Eppendorf Catalog #0030129504**

10 Seal the plate with Adhesive PCR Plate Seal **Bio-Rad Laboratories Catalog #MSB1001** and Film Sealing Roller for PCR Plates **Bio-Rad Laboratories Catalog #MSR0001**.

11 Centrifuge at 1000 x g briefly.

12 Flow sort one cell per well (see Part 1 for details).

13 Centrifuge at 1000 x g, 00:01:00.

14 Lyse the cells by running (set lid temperature to 75 °C to avoid evaporation; 2 µL volume; total: ~ 01:15:00; minimize evaporation by sealing tight and closing the PCR machine lid tight):

2h 30m

- 50 °C for 01:00:00
- 70 °C for 00:15:00
- 4 °C forever

15 Store at -80 °C if needed (stable for a few months). For longer storage at -80 °C, sort cells into dry (empty) wells.

Positive Control (Optional)

2h 30m

- 16 Prepare 100 pg/µL gDNA in a DNA LoBind Tube 1.5ml **Eppendorf Catalog #022431021** (recipe below for NEB HeLa gDNA; can be made from any human or mouse gDNA):
- 1 mL water
 - 1 µL Hela Genomic DNA - 15 ug **New England Biolabs Catalog #N4006S** (final: [M] 100 pg/µL)
 - Vortex to mix.

- 17 Prepare Positive Control Solution in a **DNA LoBind Tube 1.5ml Eppendorf Catalog #022431021** (**2 μ L** per positive control; recipe below for **20 μ L**):
- **19 μ L** water
 - **1 μ L** 100 pg/ μ L gDNA (final: **5 pg/ μ L**)
 - Vortex to mix.
- 18 Add **2 μ L** Positive Control Solution per positive control well.

Transposition

2h 30m

- 19 Make Transposition Mix (**8 μ L** per cell; recipe below for 96-well plate + 10%):
- **844.8 μ L** Transposition Buffer
 - ~ **1.6 μ L** 125 nM Homemade Nextera Transposome or **TTE Mix V50 Vazyme Catalog #TD501**
 - Pipette to mix.
 - Aliquot to **69 μ L** in 12-strip tubes.
- 20 Add **8 μ L** Transposition Mix per well, avoiding touching the liquid.

- 21 Vortex and spin down.

- 22 Transpose the genome by running (**10 μ L** volume; total: ~ **00:10:00**):
- **55 °C** for **00:10:00**
 - **4 °C** forever

20m

Stop

2h 30m

- 23 Prepare Stop Mix (**2 μ L** per cell; recipe below for 96-well plate + 25%):
- **240 μ L** Transposome Removal Buffer

- 0.4 μL 60 mg/mL Qiagen Protease (final: 100 $\mu\text{g/mL}$)
- Vortex to mix.
- Aliquot to 19 μL in 12-strip tubes.

24 Add 2 μL Stop Mix per tube, avoiding touching the liquid.

25 Vortex and spin down.

26 Stop transposition by running (12 μL volume; total: ~ 01:00:00):

2h

- 50 °C for 00:40:00
- 70 °C for 00:20:00
- 4 °C forever

Amplification

2h 30m

27 Make PCR Mix (~ 11 μL per cell; recipe below for 96-well plate + 10%):

- 528 μL Q5 Reaction Buffer (included with Q5 High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0491L**)
- 528 μL Q5 High GC Enhancer (included with Q5 High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0491L**)
- 63.36 μL Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs Catalog #N04475**
- 6.336 μL 1M MgCl_2 **Invitrogen - Thermo Fisher Catalog #AM9530G**
- 26.4 μL BSA, molecular biology grade, 20 mg/ml **New England Biolabs Catalog # B9000S**
- 26.4 μL Q5 High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0491L**
- Vortex to mix.
- Aliquot to 97 μL in 12-strip tubes.

28 Add (per tube; avoid touching the liquid):

- 11 μL PCR Mix
- 1 μL 12.5 uM Nextera i5 Primer (final: 100 nM)
- 1 μL 12.5 uM Nextera i7 Primer (final: 100 nM)
- Arrange the indices so that no cells have the same index on each sequencing run.

29 Amplify by running (25 μL volume; total: ~01:00:00):

1h 14m 30s

- 4 $^{\circ}\text{C}$ for 00:03:00 (to allow the lid to pre-heat)
- 72 $^{\circ}\text{C}$ for 00:03:00
- 98 $^{\circ}\text{C}$ for 00:00:20
- 14 cycles of 98 $^{\circ}\text{C}$ for 00:00:10, 62 $^{\circ}\text{C}$ for 00:01:00, 72 $^{\circ}\text{C}$ for 00:02:00
- 72 $^{\circ}\text{C}$ for 00:05:00
- 4 $^{\circ}\text{C}$ forever

30 Store at -20 $^{\circ}\text{C}$ if needed.

Purification

2h 30m

31 Pool cells as desired and purify with

⊗ DNA Clean & Concentrator-5 (Capped) 50 Preps **Zymo Research Catalog #D4013** and
 125 μL DNA Binding Buffer per cell (a 1:5 ratio; extra buffer can be purchased as
 ⊗ DNA Binding Buffer 100 mL **Zymo Research Catalog #D4004-1-L**). Elute in 4 μL
 ⊗ TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849** per cell.
 For a 96-well plate, pooling can be done with a multi-channel pipette into a total of 12 mL
 Binding Buffer. Use 4-6 columns per plate and elute into a total of 400 μL
 ⊗ TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849**.

32 Measure concentration with

⊗ Qubit™ 1X dsDNA HS Assay Kit **Thermo Fisher Catalog #Q33231**.

33 Measure size distribution with

⊗ BioAnalyzer High Sensitivity Chip **Agilent Technologies Catalog #5067-4626**.

- 34 Remove short fragments with 0.7 X
SPRIselect 60 mL **Beckman Coulter Catalog #B23318** . Elute into
TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849** .
- 35 Measure concentration with
Qubit™ 1X dsDNA HS Assay Kit **Thermo Fisher Catalog #Q33231** .
- 36 Measure size distribution with
BioAnalyzer High Sensitivity Chip **Agilent Technologies Catalog #5067-4626** .