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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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Protocol materials

- ☒ TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849**
- ☒ QIAGEN Protease (7.5 AU) **Qiagen Catalog #19155**
- ☒ NaCl (5 M), RNase-free **Thermo Fisher Catalog #AM9760G**
- ☒ EDTA (0.5 M), pH 8.0, RNase-free **Thermo Fisher Catalog #AM9260G**
- ☒ 1M DL-Dithiothreitol solution (DTT) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #646563**
- ☒ Triton X-100, 10% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443**
- ☒ Tris (1 M), pH 8.0, RNase-free **Thermo Fisher Catalog #AM9855G**
- ☒ TAPS Buffer (1 M pH 8.5) **Boston Bioproducts Catalog #BB-2375**
- ☒ 1M MgCl₂ **Invitrogen - Thermo Fisher Catalog #AM9530G**
- ☒ 50% w/v Polyethylene glycol 8000 **Hampton Research Catalog #HR2-535**
- ☒ 96 well LoBind PCR plates Semi-skirted **Eppendorf Catalog #0030129504**
- ☒ Film Sealing Roller for PCR Plates **Bio-Rad Laboratories Catalog #MSR0001**
- ☒ Adhesive PCR Plate Seal **Bio-Rad Laboratories Catalog #MSB1001**
- ☒ DNA LoBind Tube 1.5ml **Eppendorf Catalog #022431021**
- ☒ Hela Genomic DNA - 15 ug **New England Biolabs Catalog #N4006S**
- ☒ DNA LoBind Tube 1.5ml **Eppendorf Catalog #022431021**
- ☒ Triton X-100, 10% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443**
- ☒ NaCl (5 M), RNase-free **Thermo Fisher Catalog #AM9760G**
- ☒ EDTA (0.5 M), pH 8.0, RNase-free **Thermo Fisher Catalog #AM9260G**
- ☒ TTE Mix V50 **Vazyme Catalog #TD501**
- ☒ BSA, molecular biology grade, 20 mg/ml **New England Biolabs Catalog # B9000S**
- ☒ Q5 High-Fidelity DNA Polymerase - 500 units **New England Biolabs Catalog #M0491L**
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- ☒ Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs Catalog #N0447S**
- ☒ 1M MgCl₂ **Invitrogen - Thermo Fisher Catalog #AM9530G**
- ☒ TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849**
- ☒ DNA Clean & Concentrator-5 (Capped) 50 Preps **Zymo Research Catalog #D4013**
- ☒ DNA Binding Buffer 100 mL **Zymo Research Catalog #D4004-1-L**
- ☒ TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849**



✕ Qubit[®]; 1X dsDNA HS Assay Kit **Thermo Fisher Catalog #Q33231**

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

✕ SPRIselect 60 mL **Beckman Coulter Catalog #B23318**

✕ TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849**

✕ Qubit[®]; 1X dsDNA HS Assay Kit **Thermo Fisher Catalog #Q33231**

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

Troubleshooting

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  100 micromolar (μM) .
- Store at  $-20\text{ }^{\circ}\text{C}$.

2 Nextera i7 Index Primers:

- 701: CAAGCAGAAGACGGCATAACGAGATTGCGCTTAGTCTCGTGGGCTCGG
- 702: CAAGCAGAAGACGGCATAACGAGATCTAGTACGGTCTCGTGGGCTCGG
- 703: CAAGCAGAAGACGGCATAACGAGATTTCTGCCTGTCTCGTGGGCTCGG
- 704: CAAGCAGAAGACGGCATAACGAGATGCTCAGGAGTCTCGTGGGCTCGG
- 705: CAAGCAGAAGACGGCATAACGAGATAGGAGTCCGTCTCGTGGGCTCGG
- 706: CAAGCAGAAGACGGCATAACGAGATCATGCCTAGTCTCGTGGGCTCGG
- 707: CAAGCAGAAGACGGCATAACGAGATGTAGAGAGGTCTCGTGGGCTCGG
- 708: CAAGCAGAAGACGGCATAACGAGATCCTCTCTGGTCTCGTGGGCTCGG
- 709: CAAGCAGAAGACGGCATAACGAGATAGCGTAGCGTCTCGTGGGCTCGG
- 710: CAAGCAGAAGACGGCATAACGAGATCAGCCTCGGTCTCGTGGGCTCGG
- 711: CAAGCAGAAGACGGCATAACGAGATTGCTCTTGTCTCGTGGGCTCGG
- 712: CAAGCAGAAGACGGCATAACGAGATTCCTCTACGTCTCGTGGGCTCGG
- and the following if > 96 cells need to be sequenced at the same time (e.g. NovaSeq):
- 714: CAAGCAGAAGACGGCATAACGAGATTCATGAGCGTCTCGTGGGCTCGG
- 715: CAAGCAGAAGACGGCATAACGAGATCCTGAGATGTCTCGTGGGCTCGG
- 716: CAAGCAGAAGACGGCATAACGAGATTAGCGAGTGTCTCGTGGGCTCGG
- 718: CAAGCAGAAGACGGCATAACGAGATGTAGCTCCGTCTCGTGGGCTCGG
- 719: CAAGCAGAAGACGGCATAACGAGATTACTACGCGTCTCGTGGGCTCGG
- 720: CAAGCAGAAGACGGCATAACGAGATAGGCTCCGGTCTCGTGGGCTCGG
- 721: CAAGCAGAAGACGGCATAACGAGATGCAGCGTAGTCTCGTGGGCTCGG
- 722: CAAGCAGAAGACGGCATAACGAGATCTGCGCATGTCTCGTGGGCTCGG
- 723: CAAGCAGAAGACGGCATAACGAGATGAGCGCTAGTCTCGTGGGCTCGG
- 724: CAAGCAGAAGACGGCATAACGAGATCGCTCAGTGTCTCGTGGGCTCGG
- 726: CAAGCAGAAGACGGCATAACGAGATGTCTTAGGGTCTCGTGGGCTCGG
- 727: CAAGCAGAAGACGGCATAACGAGATACTGATCGGTCTCGTGGGCTCGG
- Purification: standard desalting
- Dissolve in 0.1 X TE to a final concentration of  50 micromolar (μM) .
- Dilute with 0.1 X TE to  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 [M] 50 micromolar (μM) .
- Dilute with 0.1 X TE to [M] 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: [M] 20 millimolar (mM))
 - 4 μL NaCl (5 M), RNase-free **Thermo Fisher Catalog #AM9760G** (final: [M] 20 millimolar (mM))
 - 15 μL Triton X-100, 10% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443**

(final: [M] 0.15 % (v/v))

- 25 μL

⊗ 1M DL-Dithiothreitol solution (DTT) Merck MilliporeSigma (Sigma-Aldrich) Catalog #646563

(aliquoted and stored at $-20\text{ }^{\circ}\text{C}$; final: [M] 25 millimolar (mM))

- 2 μL

⊗ EDTA (0.5 M), pH 8.0, RNase-free Thermo Fisher Catalog #AM9260G (final:

[M] 1 millimolar (mM))

- 5 μL 100 μM Carrier ssDNA (final: [M] 500 nanomolar (nM))

- 929 μL water

- Vortex to mix.

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

6 Prepare Transposition Buffer (8 μL per cell; recipe below for 1 mL , or 1 96-well plate):

- 12.5 μL ⊗ TAPS Buffer (1 M pH 8.5) Boston Bioproducts Catalog #BB-2375

(final: [M] 12.5 millimolar (mM))

- 6.25 μL ⊗ 1M MgCl_2 Invitrogen - Thermo Fisher Catalog #AM9530G (final:

[M] 6.25 millimolar (mM))

- 200 μL

⊗ 50% w/v Polyethylene glycol 8000 Hampton Research Catalog #HR2-535

(final: [M] 10 Mass / % volume)

- 781.25 μL water

- Vortex to mix.

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

7 Prepare Transposome Removal Buffer (2 μL per cell; recipe below for 1 mL , or 4 96-well plates):

- 60 μL ⊗ NaCl (5 M), RNase-free Thermo Fisher Catalog #AM9760G (final:

[M] 300 millimolar (mM))

- 90 μL

⊗ EDTA (0.5 M), pH 8.0, RNase-free Thermo Fisher Catalog #AM9260G (final:

[M] 45 millimolar (mM))



- 1 μL



Triton X-100, 10% solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #93443

(final: 0.01 % (v/v) ; for ease of pipetting)

- 849 μL water
- Vortex to mix.
- Store at $-20\text{ }^{\circ}\text{C}$ if needed.

Lysis (Skip if Performing Positive Control)

2h 30m

- 8 Prepare Dip-C Lysis Buffer (2 μL per cell; recipe below for 4 96-well plate + 25%):

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

- 9 Add 2 μL Dip-C Lysis Buffer per well to a

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 $75\text{ }^{\circ}\text{C}$ to avoid evaporation;

2h 30m

2 μL volume; total: ~ 01:15:00 ; minimize evaporation by sealing tight and closing the PCR machine lid tight):

- $50\text{ }^{\circ}\text{C}$ for 01:00:00
- $70\text{ }^{\circ}\text{C}$ 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: 100 pg/μL)
 - Vortex to mix.
- 17 Prepare Positive Control Solution in a DNA LoBind Tube 1.5ml Eppendorf Catalog #022431021 (2 μL per positive control; recipe below for 20 μL):
- 19 μL water
 - 1 μL 100 pg/μL gDNA (final: 5 pg/μL)
 - Vortex to mix.
- 18 Add 2 μL Positive Control Solution per positive control well.

Transposition

2h 30m

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



21 Vortex and spin down.

22 Transpose the genome by running ( 10 μL volume; total: ~  00:10:00):

20m

-  55 °C for  00:10:00
-  4 °C forever

Stop

2h 30m

23 Prepare Stop Mix ( 2 μL per cell; recipe below for 96-well plate + 25%):

-  240 μL 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 μmol of each **New England Biolabs Catalog #N0447S**

- 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: 500 nanomolar (nM))
- 1 μL 12.5 uM Nextera i7 Primer (final: 500 nanomolar (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):

- 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

1h 14m
30s

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 μ L 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 μ L 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 μ L 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** .