Nov 18, 2020

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

Longzhi Tan[1]

[1]Stanford University

1 Works for me dx.doi.org/10.17504/protocols.io.bpt8mnrw


Longzhi Tan
Stanford University

DOI

dx.doi.org/10.17504/protocols.io.bpt8mnrw

PROTOCOL CITATION

Longzhi Tan 2020. Dip-C (Part 2: Whole-genome Amplification with Nextera). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bpt8mnrw

CREATED

Nov 18, 2020

LAST MODIFIED

Nov 18, 2020

PROTOCOL INTEGER ID

44640

Oligos

1 Carrier ssDNA (same as in LIANTI and META):
- TCAGGTTTTCCTGAA
- 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 °C** .

2 Nextera i7 Index Primers:
- 701: CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGG
- 702: CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG
- 703: CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG
- 704: CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG
- 705: CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGG
- 706: CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGG
- 707: CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGG
- 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: CAAGCAGAAGACGGCATACGAGATGTAGCTCCGTCTCGTGGGCTCGG
- 719: CAAGCAGAAGACGGCATACGAGATTACTACGCGTCTCGTGGGCTCGG
- 720: CAAGCAGAAGACGGCATACGAGATAGGCTCCGGTCTCGTGGGCTCGG
- 721: CAAGCAGAAGACGGCATACGAGATGCAGCGTAGTCTCGTGGGCTCGG
- 722: CAAGCAGAAGACGGCATACGAGATCTGCGCATGTCTCGTGGGCTCGG
- 723: CAAGCAGAAGACGGCATACGAGATGAGCGCTAGTCTCGTGGGCTCGG
- 724: CAAGCAGAAGACGGCATACGAGATCGCTCAGTGTCTCGTGGGCTCGG
- 726: CAAGCAGAAGACGGCATACGAGATGTCTTAGGGTCTCGTGGGCTCGG
- 727: CAAGCAGAAGACGGCATACGAGATACTGATCGGTCTCGTGGGCTCGG
- 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: AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTC
- 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: AATGATACGGCGACCACCGAGATCTACACCCTAGAGTTCGTCGGCAGCGTC
- 517: AATGATACGGCGACCACCGAGATCTACACGCGTAAGATCGTCGGCAGCGTC
- 518: AATGATACGGCGACCACCGAGATCTACACCTATTAAGTCGTCGGCAGCGTC
- 520: AATGATACGGCGACCACCGAGATCTACACAAGGCTATTCGTCGGCAGCGTC
- 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.

Reagents

4 Prepare 60 mg/mL Qiagen Protease:

QIAGEN Protease (7.5

- Add **2.78 mL** water to one vial of 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):

Tris (1 M), pH 8.0, RNase-free **Thermo**

- **20 µl** **Fisher Catalog #AM9855G** (final: **20 Milimolar (mM)**)

NaCl (5 M), RNase-free **Thermo**

- **4 µl** **Fisher Catalog #AM9760G** (final: **20 Milimolar (mM)**)

Citation: Longzhi Tan (11/18/2020). Dip-C (Part 2:ÃÂ Whole-genome Amplification with Nextera). https://dx.doi.org/10.17504/protocols.io.bpt8mnrw

Triton X-100, 10% solution

- **15 µl** **Sigma Catalog #93443** (final: **0.15 % (v/v)**)

1M DL-Dithiothreitol solution (DTT) **Sigma**

- **25 µl** **Aldrich Catalog #646563** (aliquoted and stored at **-20 °C** ; final: **25 Milimolar (mM)**)

EDTA (0.5 M), pH 8.0, RNase-free **Thermo**

- **2 µl** **Fisher Catalog #AM9260G** (final: **1 Milimolar (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 µl** per cell; recipe below for **1 mL** , or 1 96-well plate):

TAPS Buffer (1 M pH 8.5) **Boston**

- **12.5 µl** **Bioproducts Catalog #BB-2375** (final: **12.5 Milimolar (mM)**)

1M MgCl2 **Invitrogen - Thermo**

- **6.25 µl** **Fisher Catalog #AM9530G** (final: **6.25 Milimolar (mM)**)

50% w/v Polyethylene glycol 8000 **Hampton**

- **200 µl** **Research Catalog #HR2-535** (final: **10 Mass / % volume**)
- **781.25 µl** water
- Vortex to mix.
- Store at **-20 °C** if needed.

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

NaCl (5 M), RNase-free **Thermo**

- **60 µl** **Fisher Catalog #AM9760G** (final: **300 Milimolar (mM)**)

EDTA (0.5 M), pH 8.0, RNase-free **Thermo**

- **90 µl** **Fisher Catalog #AM9260G** (final: **45 Milimolar (mM)**)

Triton X-100, 10% solution

- **1 µl** **Sigma Catalog #93443** (final: **0.01 % (v/v)** ; for ease of pipetting)
- **849 µl** 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 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 µg/mL**)

Citation: Longzhi Tan (11/18/2020). Dip-C (Part 2:ÃÂ Whole-genome Amplification with Nextera). https://dx.doi.org/10.17504/protocols.io.bpt8mnrw

- 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 Adhesive PCR Plate Seal **Bio-rad** Seal the plate with **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** ; 2h 30m minimize evaporation by sealing tight and closing the PCR machine lid tight):
- **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 DNA LoBind Tube 1.5ml Prepare 100 pg/uL gDNA in a **Eppendorf Catalog #022431021** (recipe below for NEB HeLa gDNA; can be made from any human or mouse gDNA):
- **1 mL** water
- Hela Genomic DNA - 15 ug **New England Biolabs Catalog #N4006S** **1 µl** (final: **100 pg/µL**)
- Vortex to mix.

17 DNA LoBind Tube 1.5ml Prepare Positive Control Solution in a **Eppendorf Catalog #022431021** (**2 µl** per positive control; recipe below for **20 µl**):

Citation: Longzhi Tan (11/18/2020). Dip-C (Part 2:ÃÂ Whole-genome Amplification with Nextera). https://dx.doi.org/10.17504/protocols.io.bpt8mnrw

- **19 µl** water
- **1 µl** 100 pg/uL 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 V50 **Vazyme Catalog #TD501**

TTE Mix

- 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 µg/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

Citation: Longzhi Tan (11/18/2020). Dip-C (Part 2:ÃÂ Whole-genome Amplification with Nextera). https://dx.doi.org/10.17504/protocols.io.bpt8mnrw

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**)
- ☒ Deoxynucleotide Solution Mix - 8 umol of each **New England Biolabs Catalog #N0447S**
- **63.36 µl** ☒ 1M MgCl2 **Invitrogen - Thermo Fisher Catalog #AM9530G**
- **6.336 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**
- **26.4 µl**
- 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.

1h 14m 30s

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

- **4 °C** for **00:03:00** (to allow the lid to pre-heat)
- **72 °C** for **00:03:00**
- **98 °C** for **00:00:20**
- 14 cycles of **98 °C** for **00:00:10** , **62 °C** for **00:01:00** , **72 °C** for **00:02:00**
- **72 °C** for **00:05:00**
- **4 °C** forever

30 Store at **-20 °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

Citation: Longzhi Tan (11/18/2020). Dip-C (Part 2:ÃÂ Whole-genome Amplification with Nextera). https://dx.doi.org/10.17504/protocols.io.bpt8mnrw

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 Qubit™ 1X dsDNA HS Assay Kit **Thermo**

Measure concentration with **Fisher Catalog #Q33231** .

33 Measure size distribution with BioAnalyzer High Sensitivity Chip **Agilent Technologies Catalog #5067-4626** .

34 SPRIselect 60 mL **Beckman**

Remove short fragments with 0.7 X **Coulter Catalog #B23318** . Elute into TE, pH 8.0, RNase-free **Thermo Fisher Catalog #AM9849** .

35 Qubit™ 1X dsDNA HS Assay Kit **Thermo**

Measure concentration with **Fisher Catalog #Q33231** .

36 Measure size distribution with BioAnalyzer High Sensitivity Chip **Agilent Technologies Catalog #5067-4626** .

Citation: Longzhi Tan (11/18/2020). Dip-C (Part 2:ÃÂ Whole-genome Amplification with Nextera). https://dx.doi.org/10.17504/protocols.io.bpt8mnrw