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Dip-C (Part 1: Chromosome Conformation Capture, for Fixed Nuclei)

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

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Protocol materials

- Sodium dodecyl sulfate solution BioUltra for molecular biology 10% in H2O **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**71736
- Triton X-100, 10% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**93443
- NEBuffer 2 (10X) **New England Biolabs Catalog #**B7002S
- MboI (25,000 units/ml) - 2,500 units **New England Biolabs Catalog #**R0147M
- T4 DNA Ligase Reaction Buffer - 6.0 ml **New England Biolabs Catalog #**B0202S
- BSA, molecular biology grade, 20 mg/ml **New England Biolabs Catalog #** B9000S
- T4 DNA Ligase (1 U/µL) **Thermo Fisher Catalog #**15224025
- Corning™ Falcon™ Test Tube with 35µm Cell Strainer Snap Cap **Corning Catalog #**352235
- Falcon 40 µm Cell Strainer **Corning Catalog #**352340
- PBS, pH 7.4 **Thermo Fisher Catalog #**10010023
- PBS, pH 7.4 **Thermo Fisher Catalog #**10010023
- DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) **Thermo Fisher Catalog #**D1306
- 96 well LoBind PCR plates Semi-skirted **Eppendorf Catalog #**0030129504

Digestion

40m

1 Thaw a tube of fixed nuclei On ice .

2 Prepare 0.5% SDS (50 µL per sample; recipe below for 100 µL):

- 5 µL
  Sodium dodecyl sulfate solution BioUltra for molecular biology 10% in H2O **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**71736
  (final: [M] 0.5 Mass Percent)
- 95 µL water
- Vortex to mix

3 Resuspend nuclei in 50 µL 0.5% SDS.

4 Incubate at 62 °C for 00:10:00 . 10m

5 Add:

- 145 µL water
- 25 µL
  Triton X-100, 10% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**93443

6 Rotate at 37 °C for 00:15:00 . 15m

7 Add restriction enzyme and its buffer:

- 25 µL NEBuffer 2 (10X) **New England Biolabs Catalog #**B7002S
- 20 µL
  MboI (25,000 units/ml) - 2,500 units **New England Biolabs Catalog #**R0147M

8 Rotate at 37 °C Overnight . 15m

Ligation

40m

9 Centrifuge at 1000 x g, 4°C, 00:05:00 .

10 Make Ligation Buffer (2 mL per sample; recipe below for 1 mL):

- 100 µL
  T4 DNA Ligase Reaction Buffer - 6.0 ml **New England Biolabs Catalog #**B0202S
- 5 µL
  BSA, molecular biology grade, 20 mg/ml **New England Biolabs Catalog #** B9000S
- 865 µL water
- Vortex to mix.

11 Remove supernatant leaving ~ 50 µL .

12 Resuspend in 1 mL Ligation Buffer.

13 Centrifuge at 1000 x g, 4°C, 00:05:00 .

14 Remove supernatant leaving ~ 50 µL .

15 Resuspend in 1 mL Ligation Buffer.

16 Add 10 µL T4 DNA Ligase (1 U/µL) **Thermo Fisher Catalog #**15224025 .

17 Pipette to mix.

18 Incubate at 16 °C for 04:00:00 , occasionally inverting the tube. 4h

19 Optionally filter with Falcon 40 µm Cell Strainer **Corning Catalog #**352340 or Corning™ Falcon™ Test Tube with 35µm Cell Strainer Snap Cap **Corning Catalog #**352235 to avoid clogging the flow cytometer.

20 Aliquot if needed.

21 Centrifuge at 1000 x g, 4°C, 00:05:00 .

22 Remove supernatant.

23 Store at -80 °C .

Flow Sorting

40m

24 On the day of flow sorting, thaw a tube of ligated nuclei On ice .

25 Resuspend in 1 mL PBS, pH 7.4 **Thermo Fisher Catalog #**10010023 .

26 Make 300 uM DAPI:

- 100 µL PBS, pH 7.4 **Thermo Fisher Catalog #**10010023
- 2.1 µL 14.3 mM (5 mg/mL) DAPI (stock made by dissolving DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) **Thermo Fisher Catalog #**D1306 in 2 mL water and stored at 4 °C)
- Vortex to mix.

27 Add 1 µL 300 uM DAPI (final: [M] 0.5 nanomolar (nM)).

28 Pipette to mix.

29 Flow sort single nuclei into
96 well LoBind PCR plates Semi-skirted **Eppendorf Catalog #**0030129504 either dry or containing lysis buffer (which requires lysis by incubation before storing; see Part 2 for details).

30 Proceed directly to Part 2, or store at -80 °C.