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

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1 Works for me dx.doi.org/10.17504/protocols.io.bpt7mnrn


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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 **Sigma Aldrich Catalog #71736**

  (final: **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

  Triton X-100, 10% solution

- **25 µl** **Sigma Catalog #93443**

6 Rotate at **37 °C** for **00:15:00** . 15m

7 Add restriction enzyme and its buffer:

NEBuffer 2 (10X) **New England**

- **25 µl** **Biolabs Catalog #B7002S**

MboI (25,000 units/ml) - 2,500 units **New England**

- **20 µl** **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**):

T4 DNA Ligase Reaction Buffer - 6.0 ml **New England**

- **100 µl** **Biolabs Catalog #B0202S**

BSA, molecular biology grade, 20 mg/ml **New England**

- **5 µl** **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** .

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15 Resuspend in **1 mL** Ligation Buffer.

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

17 Pipette to mix.

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

19 Falcon 40 µm Cell
Optionally filter with 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 PBS, pH 7.4 **Thermo**
Resuspend in **1 mL** **Fisher Catalog #10010023** .

26 Make 300 uM DAPI:
PBS, pH 7.4 **Thermo**
- **100 µl** **Fisher Catalog #10010023**

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- **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: **0.5 Nanomolar (nM)**).

28 Pipette to mix.

29 Flow sort single nuclei into skirted **Eppendorf Catalog #0030129504** 96 well LoBind PCR plates Semi- 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** .

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