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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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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: 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

- 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 μ M DAPI:
- 100 μ L PBS, pH 7.4 **Thermo Fisher Catalog #10010023**
 - 2.1 μ L 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 $^{\circ}$ C)
 - Vortex to mix.
- 27 Add 1 μ L 300 μ M 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 $^{\circ}$ C .