

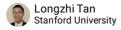
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© Dip-C (Part 1: Chromosome Conformation Capture, for Fixed Nuclei)

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



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Digestion

40m

- Thaw a tube of fixed nuclei § On ice.
- 2 Prepare 0.5% SDS ($\Box 50 \mu I$ per sample; recipe below for $\Box 100 \mu I$):
 - 35 μl

Sodium dodecyl sulfate solution BioUltra for molecular biology 10% in H2O Sigma

Aldrich Catalog #71736

(final: [M] 0.5 Mass Percent)

- **95** µI water
- Vortex to mix
- 3 Resuspend nuclei in **30 μl** 0.5% SDS.

4 Incubate at & 62 °C for © 00:10:00.

10m

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5
      Add:
       ■ 145 μI water
                 ■ 25 μl Sigma Catalog #93443
                                                                                            15m
   6
      Rotate at § 37 °C for © 00:15:00.
      Add restriction enzyme and its buffer:
                 ⊠NEBuffer 2 (10X) New England
       ■ 25 µl Biolabs Catalog #B7002S

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

       ■ 20 µl Biolabs Catalog #R0147M
                                                                                            15m
      Ligation
                 40m
   9
      Centrifuge at @1000 x g, 4°C, 00:05:00.
      Make <u>Ligation Buffer</u> ( □2 mL per sample; recipe below for □1 mL ):
                  ⊠T4 DNA Ligase Reaction Buffer - 6.0 ml New England
       ■ 100 μl Biolabs Catalog #B0202S
                ■ 5 μl Biolabs Catalog # B9000S
       ■ 365 µl water
       Vortex to mix.
 11
      Remove supernatant leaving ~ $\bullet$50 \mu I .
 12
      Resuspend in 1 mL Ligation Buffer.
      Centrifuge at 31000 x g, 4°C, 00:05:00.
      Remove supernatant leaving ~ $\square$50 \mu$.
```

```
15
        Resuspend in 11 mL Ligation Buffer.
   16
                     Add 10 µl Fisher Catalog #15224025
        Pipette to mix.
   17
                                                                                                        4h
        Incubate at § 16 °C for © 04:00:00, occasionally inverting the tube.
   19
                          Optionally filter with Strainer Corning Catalog #352340
                                                                              or
         ⊠ Corning<sup>™</sup> Falcon<sup>™</sup> Test Tube with 35μm Cell Strainer Snap
         Cap Corning Catalog #352235
                                                                                           to avoid clogging
        the flow cytometer.
        Aliquot if needed.
   20
   21
        Centrifuge at 31000 x g, 4°C, 00:05:00.
   22
        Remove supernatant.
   23
        Store at 8-80 °C.
 Flow Sorting
                       40m
       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
        Make 300 uM DAPI:
   26
                     ⊠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 11 µl 300 uM DAPI (final: [M] 0.5 Nanomolar (nM)). 28 Pipette to mix. 29 ₩96 well LoBind PCR plates Semi-Flow sort single nuclei into 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 8 -80 °C.