Nuclei Isolation

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This protocol is based on the following paper:

Cell type-specific chromatin immunoprecipitation from multicellular complex samples using BiTS-ChIP.
Bonn S, Zinzen RP, Perez-Gonzalez A, Riddell A, Gavin AC, Furlong EE.
Nat Protoc. 2012 Apr 26;7(5):978-94. doi: 10.1038/nprot.2012.049.

MATERIALS

HB buffer:
This solution can be stored at 4 °C for 1 year when filter-sterilized.
15 mM Tris-HCl (pH 7.4)
0.34 M sucrose
15 mM NaCl
60 mM KCl
0.2 mM EDTA
0.2 mM EGTA
Proteinase inhibitors (Roche Complete) should be added shortly before use

PBTB buffer:
0.1% (vol/vol) Triton X-100 in PBS
The solution can be stored at room temperature or at 4 °C for 1 year.
5% (wt/vol) BSA
This solution should be freshly prepared on the day of use and kept at 4 °C.
Proteinase inhibitors should be added shortly before use (Roche Complete) and the solution must be filtered through a 0.22-µm-pore filter, as undissolved albumin particulates may affect sorting.

100µm cell strainers. Corning Life Sciences DL No.:352360.

20µm cell strainers. EMD Millipore Steriflip 20 µm SCNY00020.

Dounce tissue grinder set- 2 mL complete from sigma, SKU-Pack Size: D8938-1SET, price: 119.00$.

PROCEDURE

1. Embryos were dechorionated by pronase treatment.

2. Embryos were rinsed twice in fish water.

3. Embryos were deyolked in calcium free Ringer and passed several times through a 200µL pipet tip.

4. Embryos were rinsed twice in fish water.

5. Add 2 ml of chilled HB buffer on embryo pellet on ice.

6. Dissociate the embryos by pipetting up and down with a 10-ml serological pipette.

7. Dounce 10 times with a loose pestle and 15 times with a tight pestle.

   [*This step frees the nuclei from the rest of the material, and thus care should be taken to get a homogenous solution. If the embryos are not dounced sufficiently, the nuclear yield will be decreased.*]

8. Filter the lysate through 100µm cell strainers.

9. Spin at 3,500g for 5 min to pellet the nuclei, and then carefully pour off the supernatant.

10. Wash the nuclei in 10 ml of HB buffer, pipette up and down with a 10-ml serological pipette to dissociate the nuclei, and transfer them to a new 15-ml conical tube. Pellet the nuclei at 3,500g for 5 min.

11. Resuspend the nuclei in 2 or 3 ml of PBTB buffer and transfer them to a 15-ml conical tube.

12. Dissociate the nuclei by passing them ten times through a 21-G needle using a 3-ml syringe.

    [*Do not apply too much pressure when syringing to avoid shearing and excessive foaming*.]

13. Filter the lysate through 20μm cell strainers.

14. Estimate the total number of nuclei and verify their effective dissociation by microscopy. You can stain your nuclei with DAPI or Hoechst.