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Cell Fractionation/Isolation of Nuclei

1. Grow cells in 10-cm cell culture dishes until they are ~90% confluent (~10^6 cells/dish).
   Alternatively, pool three wells of a 6-well dish to achieve a similar cell density.
2. On the day of isolation, place the culture dish on ice, aspirate the culture medium, and then wash the cells with ice-cold PBS. Aspirate the PBS.
3. Scrape the cells off the plate using 1 mL of PBS. Transfer the cells to a 1.5 mL microcentrifuge tube on ice.
4. Centrifuge briefly for 5-10 sec at 10,000 RPM (i.e., perform a *pop spin* to pellet the cells).
5. Aspirate the supernatant and resuspend the cell pellet of ~100-200 µL in 900 µL of ice-cold PBS containing 0.1% Triton-X 100.
   If examining proteins that are sensitive to proteolysis, include protease inhibitors and PMSF (1 mM) in this 0.1% Triton-X 100 solution. If examining protein phosphorylation, also include phosphatase inhibitors (see lysis buffer recipe for details).
6. Triturate the cell suspension five times on ice with a P1000 micropipette tip.
7. If desired, transfer 300 µL of the lysed cell suspension to a new tube. This is the whole-cell lysate. Add 100 µL of 4× Laemmli sample buffer and mix. Keep on ice until the sonication step.
   This aliquot of whole-cell lysate contains ~25% of the total protein in the sample.
   If protein quantification by BCA is needed, take an aliquot before adding the 4× Laemmli sample buffer. The aliquot may be stored at -80°C without sample buffer if protease inhibitors were added to the 0.1% Triton-X 100 solution in Step 5.
   In the case of immunoprecipitation, keep the whole cell lysate on ice until the sonication step.
8. Centrifuge the remaining lysed cell suspension (700–800 µL) for 5–10 sec at 10,000 RPM.
9. If desired, transfer 300 µL of the supernatant to a new tube (this is the cytoplasmic fraction). Add 100 µL 4× Laemmli sample buffer and mix. Boil for 1 min. Return to ice or store at -80°C.
   This aliquot contains ~25% of the total protein in the cytoplasmic fraction of the sample.
10. Discard the remaining supernatant. Resuspend the pellet in 1 mL of PBS containing 0.1% Triton-X 100.
    If examining proteins that are sensitive to proteolysis, include protease inhibitors and PMSF (1 mM) in this 0.1% Triton-X 100 solution. If examining protein phosphorylation, also include phosphatase inhibitors (see lysis buffer recipe for details).
11. Centrifuge for 5-10 sec at 10,000 RPM. Discard the supernatant.
    The pelleted nuclei should appear white, compared with yellowish pellets of whole cells. At this stage, the pellet can be stored for months at −80˚C.
    The isolated nuclei are suitable for multiple applications (e.g., western blotting, 2D gel electrophoresis, or immunoprecipitation). To prepare the sample for SDS–PAGE (e.g., in preparation for western blotting), proceed to Step 12. To prepare the sample for immunoprecipitation, resuspend the pellet in 500–1000 µL of RIPA–SDS buffer with optional protease inhibitors, sonicate on ice (for sonication details, see Step 13), centrifuge in a microcentrifuge at 4˚C for 1 min, and transfer the supernatant (which will be used for immunoprecipitation) to a fresh microcentrifuge tube. If necessary, store the supernatant at −20˚C before carrying out the immunoprecipitation procedure. Freezing supernatant for coimmunoprecipitation study is not recommended.
    To isolate histones and chromatin from the nuclei, 0.3 M KCl works well to remove bound proteins. For more details on this procedure, refer to Schnitzler (2001).
12. Resuspend the pellet in 150 µL of 1× Laemmli sample buffer. This is the nuclear fraction.
    This sample contains ~75% of the nuclear proteins.

13. Sonicate the whole-cell lysate (from Step 7) and the nuclear fraction briefly while on ice.
    Sonication settings can be varied depending on the equipment. In case of a sonicator with the frequency of 20 kHz, 2 pulses, 8 sec each is enough. [A microtip probe should be used that has very high amplitude value and can vibrate up to 150 µm.] Keep the samples on ice after each pulse to cool down before the next pulse. Vortex sample to ensure it has been sufficiently disrupted. If it appears viscous, then repeat sonication until sample has a liquid consistency.

14. Boil the nuclear fraction and whole cell lysate for 1 min.

15. Store the samples at -80°C until use.
    If comparing the relative amounts of particular proteins in the whole-cell lysate, cytoplasmic fraction, and nuclear fraction, the ratio of volumes to load onto the gel is ~18:18:6 (i.e., 18 µL of the whole-cell lysate, 18 µL of the cytoplasmic fraction, and 6 µL of the nuclear fraction). When performing western blotting, bring samples to desired volume by adding 10X DTT and 1X Laemmli sample buffer.