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A rapid and convenient method for isolation of nuclear, cytoplasmic and total cellular RNA

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The following is a rapid procedure for preparing nuclear, cytoplasmic, and total cellular RNA from moderate to large numbers (10^7-10^9) of eukaryotic cells. The procedure involves lysing the cells in detergent, isolation of the cytoplasmic RNA by a simple extraction procedure, and isolation of nuclear RNA by lysis of the nuclei in guanidinium buffer and ultracentrifugation over a cesium chloride cushion. The guanidinium/cesium chloride procedure (modified from the procedure described in ref. 1) can also be used to prepare total cellular RNA. The maximum number of cells which can be processed per 15ml conical tube is between 5 x 10^7 to 3 x 10^8 cells (depending on the cell type). Isolation of RNA from more cells requires use of multiple tubes; it is not recommended to use larger tubes and volumes since this often results in considerable turbidity during phenol/chloroform extraction.

Wash the cells two times in ice-cold tris-saline [25mM tris (pH 7.4), 130mM NaCl, 5mM KCl]. Transfer the cells to a 15ml conical tube and resuspend in 4ml of ice-cold tris-saline. Add 1ml of ice-cold NDD buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.02% dextran sulphate made up in tris-saline) and quickly, but gently invert the tube ten times. Centrifuge at 2500g for 5min at 4°C. Transfer the supernatant (cytoplasmic fraction) to a 15ml conical tube containing 5ml of phenol:chloroform (1:1), add 0.25ml 20% SDS and 150μl of 5M NaCl, vortex for 15sec, and centrifuge at 2500g for 10min at room temperature. Repeat the phenol:chloroform extraction procedure at least three times, then extract one time with chloroform:iso-amyl alcohol (24:1). Precipitate the cytoplasmic RNA with 10ml of absolute ethanol. The critical step in this procedure is the lysis of the cells. The method, as described, provides quantitative yield of cytoplasmic RNA from lymphoid cells, but for some cell types it may be necessary to alter the detergent concentrations and/or dounce homogenize the cells.

To prepare nuclear RNA, add 1ml of guanidinium lysis buffer [4M guanidinium isothiocyanate, 1M 2-mercaptoethanol, and 25mM sodium acetate (pH 5.2)] to the nuclear pellet (previously kept on ice for a minimum amount of time) and vortex for 15 sec to completely lyse the nuclei. This guanidinium lysis buffer is easier to prepare than the recipe described in (1) and the RNA pellet which is obtained after centrifugation (see below) is generally easier to resuspend. The nuclear lysate dissolved in this buffer may be stored for several days at 4°C. Dilute the nuclear lysate with lysis buffer so that it can be layered over 5.7M cesium chloride/2mM EDTA in an ultracentrifuge tube at a volume ratio of 1.5:1 (lysate:CsCl). Because of the extreme viscosity of DNA, it is suggested that the concentration of mammalian nuclei in the lysis buffer be no more than 3 x 10^7 nuclei/ml. Centrifuge in SW50.1 or SW60 rotors at 45K rpm for at least 12hr at 20°C. For the Beckman TLS-55 rotor (for use in the benchtop ultracentrifuge), add 0.5g of cesium chloride per ml of lysate and centrifuge at 55K rpm for 3hr (2). After centrifugation, collect the DNA band (if preparation of DNA is desired), aspirate the remaining supernatant, and invert the tube. Cut off the bottom of the tube with a razor, resupend the RNA pellet (often not visible) in 400μl (4 x 100μl) of water, and transfer to a microfuge tube containing 400μl of chloroform:1-butanol (4:1). Extract twice in chloroform:butanol; add 50μl of 3M sodium acetate (pH 5.2) and 1ml of absolute ethanol to precipitate the RNA. A typical yield of nuclear RNA is between 2-30μg of RNA per 10^7 cells, depending on the cell type. Cytoplasmic RNA yields are normally 10 times more.

References

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