A Single-step Method for the Simultaneous Preparation of DNA, RNA, and Protein from Cells and Tissues

Source : https://github.com/jaeeponde/Systems-Bio-Medicine-/blob/main/IsolationOfDNARNAProtein.pdf

1. Prepare cells and tissue samples :

    A. Why drop tissues in liquid nitrogen and then store at -70 : At a temperature of −196°C, that of liquid nitrogen, all chemical reactions, biological processes and physical intra and extracellular activities are suspended. Theoretically, a **cryopreserved** organ can be kept indefinitely.

    B. Polypropylene snap cap tubes are very small vials where tissues are stored after pulverisation.

    C. The use of **monophasic lysis reagent**, is a rapid method for isolating high-quality total RNA from multiple samples. Monophasic lysis reagents contain phenol, which is poisonous, and guanidine thiocyanate, which is an irritant. When working with monophasic lysis reagent, use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical fume hood; avoid breathing vapour.

    D. For instance, in industry talk, a **Polytron homogenize**r indicates a specific brand of immersion dispersers, which uses a rotating blade to dissolve solid particles in a liquid substance. Thus, “polytron homogenizer” is often used to reference a homogenizer being used with plant and animal matter in a lab setting.

    E. The smaller the particle size, the higher the **centrifugation speed**. For example, bacterial cells are pelleted at higher speeds (2000–10,000 x g) than mammalian cells (500–2000 x g). Furthermore, lower centrifugation speeds may be used with more fragile samples

    F. **Aspiration** means to draw in or out using a sucking motion

    G. **Phosphate-buffered saline** is a buffer solution commonly used in biological research. It is a water-based salt solution containing disodium hydrogen phosphate, sodium chloride and, in some formulations, potassium chloride and potassium dihydrogen phosphate. The buffer helps to maintain a constant pH.

2. **Why do we use chloroform?** After solubilization, the addition of chloroform causes phase separation, where protein stays in the bottom organic phase, DNA resolves as the interface, and RNA is extracted to the top aqueous phase. Hence, RNA is purified from the sample.

3. Role of **isopropanol in RNA isolation:** After the salt concentration has been adjusted, RNA may be precipitated by adding 2.5 volumes of ethanol or 1 volume of isopropanol and mixing thoroughly, followed by chilling for at least 15 minutes at –20° C. The role of isopropanol in RNA isolation is to precipitate RNA.

4. **Why is DNA and Protein in Organic phase while RNA in Aqueous Phase** : In the acidic conditions, total RNA will remain in the upper aqueous phase of the whole mixture, while DNA and proteins remain in the interphase or lower organic phase. Recovery of total RNA is then done by precipitation with isopropanol

5. Usually, about 70 percent of ethanol solution is used during the DNA washing steps. This allows the salts to dissolve while minimising DNA solubility.

6. DEPC-treated (and therefore RNase-free) water is used in handling of RNA in the laboratory to reduce the risk of RNA being degraded by RNases. Water is usually treated with 0.1% v/v DEPC for at least 2 hours at 37 °C and then autoclaved (at least 15 min) to inactivate traces of DEPC.