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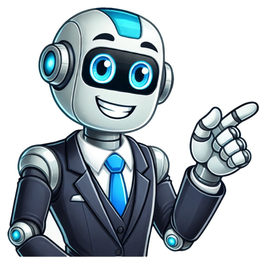

While phenol-chloroform extraction remains a popular method for cleaning up RNA or DNA, its use still has its advantages. In our previous article on how phenol extraction works, we touched upon the basics of how organic solvents can remove proteins from an aqueous solution. Proteins have both hydrophobic and hydrophilic residues, allowing them to balance their solubility in water with their affinity for non-polar environments. When exposed to phenol or chloroform, these molecules can adopt a configuration that facilitates their removal from the aqueous phase. ###ARTICLEAcidic buffer-saturated phenol plays a crucial role in RNA purification protocols due to its acidic nature, which helps to destabilize and separate RNA molecules from other contaminants. However, when DNA phenol extractions fail to yield results, the pH of the phenol can be called into question, making it challenging to measure accurately using traditional methods. To address this issue, a specific method has been developed that involves diluting 1 ml of buffer-saturated phenol with 9 ml of 45% methanol, mixing well, and then measuring the pH using a standard pH meter. An alternative approach is to replace the aqueous phase on top of the phenol solution with a fresh aliquot of ~100 mM buffered water (usually Tris pH 7.9 for DNA work), mix the phases well, and let the bottle settle until the phases are well separated again. The key to successful phenol-chloroform extractions lies in achieving equilibrium between the two phases. Increasing the surface area between the organic and aqueous phases can speed up this process, which can be achieved by vortexing the phases for a couple of minutes. However, not all samples can tolerate vigorous mixing, so gentle handling is essential. Some protocols may recommend protein denaturation and digestion with Proteinase K before phenol-chloroform extraction to reduce material trapped in the interphase and improve yield. While denaturation has no negative effect on DNA or RNA recovery, digestion can reduce purity due to varying partition numbers of peptides. Phase Lock Gel is a novel solution that collects between the aqueous layer and organic phase, separating them and preventing interphase formation during phenol-chloroform extraction. The use of Phase Lock Gel has been shown to consistently yield high-quality DNA samples with 260/280 ratios above 1.8 and recovery rates exceeding 98%. Another innovative approach involves using silicone lubricant (vacuum grease) to aid in nucleic acid recovery, resulting in a tight interphase allowing for complete recovery of the aqueous phase. Phenol–chloroform extraction is a liquid-liquid extraction technique in molecular biology used to separate nucleic acids from proteins and lipids. Aqueous samples, lysed cells, or homogenised tissue are mixed with equal volumes of a phenol:chloroform mixture. This mixture is then centrifuged. Because the phenol:chloroform mixture is immiscible with water, the centrifuge will cause two distinct phases to form: an upper aqueous phase, and a lower organic phase. The phenol-chloroform extraction method utilizes the differential solubility of DNA, proteins, and lipids in these solvents to isolate DNA from other cellular components. Phenol is used to denature and extract proteins, while chloroform is used to extract lipids. This method can be applied to various sample types, including cells, tissues, and some bodily fluids. It is compatible with both small-scale and large-scale DNA extractions. However, the phenol-chloroform extraction method has several disadvantages in forensic use. It is time-consuming and uses hazardous reagents. Also, because it is a two-step process involving transfer of reagents between tubes, it is at a greater risk of contamination. The extraction protocol begins with sample preparation, where biological samples are homogenized for complete cell lysis. For cells, this involves resuspending them in an appropriate volume of lysis buffer, while tissues require grinding in liquid nitrogen followed by resuspension in lysis buffer. Cell lysis is then achieved through incubation at 55°C for 1-2 hours or until the sample is completely lysed, with optional addition of proteinase K to the lysis buffer if necessary. Phenol-chloroform extraction involves adding one volume of phenol:chloroform:isoamyl alcohol (25:24:1) to the lysed sample and thoroughly vortexing or shaking by hand for approximately 20 seconds. The mixture is then centrifuged at room temperature for 5 minutes at 16,000 × g, resulting in phase separation where the upper aqueous phase contains the genomic DNA. The phenol chloroform isoamyl alcohol (PCI) DNA isolation technique relies heavily on the optimal composition and handling of its chemical components. Phenol acts as a critical agent in this process, requiring careful preparation and equilibration with Tris-HCl to achieve the desired pH range. The addition of 8-hydroxyquinoline helps prevent oxidation of phenol, while Tris maintains the solution's pH. During phenol preparation, it is essential to handle the chemical with caution due to its volatility and skin-burning potential. A pinch of beta-mercaptoethanol is added to the collected phenol as a stabilizer, and an overlay of 0.1M Tris helps protect the phenol from light degradation. The PCI solution's composition is equally crucial, with the precise ratio of phenol, chloroform, and isoamyl alcohol affecting DNA purity and yield. The recommended ratios for these components vary depending on the step in the extraction process: 25:24:1 for initial use, 24:1 for subsequent steps. In the protocol provided, 5 ml of blood is processed using Solution-I containing Nonidet P40, followed by centrifugation and successive additions of phenol, PCI solution, and chloroform-isoamyl alcohol. The final step involves precipitating DNA with chilled ethanol or sodium acetate, washing, and dissolving it in ddH2O or TE buffer. The PCI method has been standardized and widely accepted for its efficiency in achieving high DNA purity and yield. However, the protocol emphasizes the need to prepare each solution fresh and according to specific requirements, ensuring optimal results. The use of different DNA extraction methods has been a subject of interest in recent years, particularly among researchers Stephanie Bougel and Jean Benhatter. They conducted an experiment using various techniques to extract DNA from 10 unrelated samples, with the goal of comparing their efficacies. According to the results shown in the graph, it is clear that the Phenol-Chloroform method produced a higher yield of DNA compared to the Maxwell 16 method among all 10 samples. However, it's worth noting that this yield was still lower than that obtained using the Qiagen DNeasy Blood and Tissue kit. ###ARTICLE