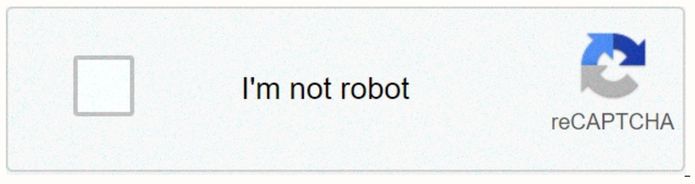

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Phenol chloroform extraction rna protocol

Volume 5, 2018, Pages 599-608Propolol-chloroform RNA ExtractionPutenido-chloroformView Abstract Home Protocols Purification ofd RNA (E2040) In general, Undemended RNA transcriptions of standard RNA synthesis can be purified by extracting phenol-chloroform and ethanol precipitation or by a spin column-based method (e.g. Monarch RNA cleaning kits, neb #T2040 NEB or #T2050). For limited RNA synthesis, radiolabelled RNA, or specific high-activity radiolabelled RNA probes, spin column chromatography is the preferred method. If absolute full-length RNA (e.g. RNA probe is required for the RNase protection test), we recommend gel purification. Phenol-chloroform extraction and ethanol precipitation For the elimination of proteins and most free nucleotides, phenol: chloroform extraction and ethanol precipitation from RNA transcriptions is the preferred method. Adjust the reaction volume to 180 ol by adding 160 l nuclease-free water. Add 20 sl of sodium acetate of 3 M, pH 5.2 or 20 l of ammonium acetate of 5 M, mix thoroughly. Extract with an equal volume of 1:1 phenol/chloroform mixture, followed by two extractions with chloroform. Collect the aqueous phase and transfer to a new tube. Precipite the RNA by adding 2 volumes of ethanol. Incubate at -20oC for at least 30 minutes and collect the pellet by centrifugation. Remove the overnatant and rinse the pellet with 500 ol of 70% cold ethanol. Resuspend RNA at 50 l of 0.1 mM EDTA. Store the ARN at -20oC or less. Spin Column Chromatography Spin columns will remove nucleotides, proteins, and uninspotioned salts. Adjust the volume of the reaction mixture to 100 ol by adding 80 l nuclease-free water, mix well. Purify the RNA according to the manufacturer's instructions. Each reaction could produce up to 180 g of RNA that can exceed the capacity of the column, requiring additional columns. Gel purification When high purity RNA transcription is desired, we recommend gel purification of the transcription product. Monarch RNA cleaning kits (nebigar #T2030, #T2040, or #T2050) can be used for the extraction of RNA gel (see the protocol included in the NEB #T2030, #T2040 or #T2050). While there are many more methods to choose from to clean up your RNA or DNA than there used to be, sometimes phenol/chloroform extraction is still the best way to do it. Here I will discuss some of the practical aspects of using this technique. Nick introduced the issue of phenol/chloroform extraction in a previous article, touching on some of the ideas on how organic extraction will remove proteins from an aqueous solution. In short, proteins consist of hydrophobic and hydrophilic residues, and through protein folding, achieve a with water to stay soluble. However, when given the opportunity to move to an environment that can accommodate both polar and non-polar residues (i.e. phenol or phenol/chloroform) without required commitment (i.e. folding), they happily move to that phase. Phase. more highly polar molecules, such as carbohydrates and nucleic acids, are happier in the aqueous phase (with a few exceptions listed below) and remain there. Now for the nitty-gritty. Phenol versus Phenol/Chloroform versus Chloroform One of the most frequently asked questions I am asked when training someone is what are the differences between the different organic phases used in extraction. Here's the breakdown, the best I understand. Phenol – What we're really talking about here is the buffer saturated phenol, which consists of a solution that is actually about 72% phenol, 28% water. Since phenol is a weak acid, the solutions we use have been balanced with buffer to bring pH to a particular target, whether acidic for RNA purification or slightly alkaline for DNA purification. In addition to a certain amount of water dissusing in phenol, there is a certain amount of phenol that dissolves in water – to balance the aqueous phase will contain about 7% phenol. This is thought to aid in extraction, as this dissolved phenol helps to denatur proteins while they are still in the aqueous solution. The buffer saturated phenol has a density that is only slightly higher than that of water. Phenol/Chloroform – This is a mixture of buffer-saturated phenol and chloroform, usually about 1:1 for DNA purification with other proportions sometimes used for RNA purification. Isoamile alcohol is sometimes included as an anti-foaming agent, but is generally believed to be an inert and optional addition. This solution is commonly used instead of buffer saturated phenol for a couple of reasons. As I mentioned earlier, the density of the phenol saturated buffer is only slightly higher than water. So if your aqueous phase contains enough salt or any other solute that would increase your density, then you could end up with the phase reversal during extraction, where your aqueous phase is below the phenol, rather than above it. Chloroform is significantly denser than water, so adding it to the organic phase increases the overall density of that phase, helping to prevent phase reversal. In addition, chloroform (and some say isoamile alcohol) help reduce the interface – the diffuse edge between the two phases populated by molecules that cannot decide where they want to go. These can be partially denatured proteins, DNA (depending on pH) and/or partially denatured DNA binding proteins that still cling to DNA, and it's a real pain in the butt. If you pipette some of this material when removing the aqueous phase, then the purity of the sample decreases. If you are too shy while pipetting, then you hurt your performance. If you're lucky, then whatever you wanted to keep is sitting on it. The addition of chloroform to mixing helps reduce this. (But I have an even better solution to this problem that I'll tell you below.) Chloroform – This is normally used after phenol or phenol/chloroform extractions. While pure chloroform does not as well as the organic solutions mentioned above for protein extraction, it works well to extract phenol from aqueous solutions. Remember when I said the aqueous phase contained 7% phenol after balance? Remember also when I said phenol likes to denature proteins? If you don't get rid of (or at least severely reduce) phenol in your now protein-free nucleic acid solution, it could re-chase you by partially or completely inhibiting the enzymes with which you treat DNA or RNA at the end of the line. Presented with a good chloroform home, however, phenol will separate from its nucleic acids. The chloroform itself is approximately 10 times less soluble in water than phenol (0.8%) and it's less unnaturally for proteins. I was also told a long time ago that phenol with DNA is less likely during ethanol precipitation once, but I can't find a reference to back that point up. Ether – This can also be used to extract phenol back out of the aqueous phase. However, due to the explosive potential of ether and the tendency of biology types to have Bunsen burners and strikers in their laboratories, it has been largely replaced by chloroform. Not so pretty pink A note of caution: do not use your phenol or phenol/chloroform if the solution is turning pink. Phenol oxidation produces a pink/brown compound, and this compound will cause your DNA to be skidded and your RNA degraded. Most commercial phenol solutions contain an

antioxidant to inhibit this oxidation, and phenol dampened to an acid pH appears to be resistant to oxidation, but it is not a bad idea to move a part of the buffer saturated phenol (from the brown bottle it probably entered) to a clear bottle or tube to inspect it before starting its extraction. PH matters – a lot Occasionally someone does a phenol extraction and does not retrieve anything from the DNA in the sample. If this happens to you, or someone in your lab, your first question should be What phenol did you use? Labs that do DNA and RNA work will likely have hermetic phenol solutions and basic cushioned phenol, or someone will buy a new bottle of phenol without paying attention to pH. Dna extraction containing acid phenol samples results in DNA denaturation, and once denatured, DNA partitions to the organic phase. This is a key feature of many RNA purification protocols, which is one of the reasons why buffer saturated acid phenol is used. Now, sometimes DNA phenol extractions from a lab begin to fail (without DNA recovery afterwards) and phenol pH is questioned. If you are in this place, you cannot simply dip your pH meter into it, and you cannot use paper because the pH indicator on paper was characterized in aqueous solutions. The method I have used is to dilute 1 ml of the buffer saturated phenol with 9 mls of methanol at 45%, mix and then measure the pH with a standard pH meter. The safest way to adjust the pH is to the aqueous phase at the top of the phenol solution with a fresh aliquot of buffered water of 100 mM (usually Tris pH 7.9 for DNA work), mix the phases well, and then let the bottle settle until the phases are well separated again. Then pH again. Mixing your phases Phenol/chloroform extractions are incredibly efficient: less than 1% of the average protein remains in the aqueous phase after the first extraction has reached balance. The trick is to bring the extraction to balance, of course. The more surface there is between the two phases, the faster this happens, and that surface is larger, the higher the finer emulsion you have created. This can be achieved by vortexing phases for a couple of minutes, as many protocols require, but not all samples can be vortex. If you are purifying very large DNA, such as genomic DNA, then you may have to mix your sample much more smoothly, and therefore perform each extraction for much longer. So at this point, follow your protocol and be very careful about trying to shave time out of this step. Effects of denaturation and digestion Some protocols require protein denaturation and possibly digestion with Protinase K prior to extraction. Both steps are attempts to reduce the amount of material that is trapped in the interface and thus improve the performance of the recovered DNA or RNA. I have never seen any negative effects of denaturing proteins with SDS before extraction. On the other hand, digestion of the protein could reduce the purity of the nucleic acid that is recovered. While whole proteins are almost guaranteed to be divided into the organic phase, once the protein is digested into small peptides, not all of those peptides will have the same chemical character as the entire protein, and each will have its own partition number. It may not matter much if you have some peptides in your nucleic acid, depending on your downstream application, but it is formally possible that these contaminants could affect your future sample amount. However, I discovered a better way to eliminate the dreaded interface... Phase Lock gel® This is one of those things that seems to be in 50% of labs, but less than 10% of the people I've talked to know what it is or how it works. I discovered this at a critical point in my research, and saved my thesis. In short, Phase-lock gel is a gooey gel, like Vasoline that has a slightly higher density than water. If you add its extraction on top of it in a centrifugal tube, and then the centrifuge, the Phase Lock gel accumulates between the aqueous and organic phases, separating the two and preventing the formation of the DNA/RNA hungry interface. An internet search didn't appear any pictures of this process that I thought was good enough, so I took some of mine. In this small demonstration, the red dye is taking the place of our precious nucleic acid, and the blue dye is replacing the protein. A) Phase Lock gel® pelletize at the bottom of a 1.5 1.5 Eppendorf tube. B) After adding phenol/chloroform and aqueous phase, complete with false DNA (red) and fake protein (blue) in the aqueous phase. C) After gently stirring for 5 minutes. D) After centrifugation. Note that the gel now separates the organic phase from the aqueous phase. E) After a second addition of phenol/chloroform and gentle agitation for 5 minutes. F) After the second centrifugation. Fake DNA could now be extracted with chloroform (in the same tube, if space allows) to remove residual phenol. As you can see, the gel forms a stable partition between the two phases, and if you want to extract the sample a second time and there is still space in the tube, then it can be done, using the same tube two or more times without compromising the purity of the sample. You cannot vortex the two phases of a tube containing this reagent, but you can mix votex into a separate tube, then add the sample to the tube with the gel and centrifuge. They have this gel in two different flavors: one for regular samples (light) and one for high density samples, as solutions with high concentrations of salt or proteins (heavy). Using SDS to denature the proteins in my sample before extraction and then using Phase Lock gel® to separate the phases has constantly given me DNA samples with 260/280 ratios of 1.8 and more than 98% recovery. Very good things. When preparing this article I came across this website, which has a lot of useful information. Visit if you want to learn more about phenol. Now to hear from you: what are your tricks and tips for extracting perfect proteins? Facebook Twitter LinkedIn More