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DNA extraction and PCR are among the most fundamental, essential, and inevitable experiments in plant genetics and biotechnology. Despite their long history, these protocols are still improved by researchers to perform more efficient and more successful experiments. In this review, we will try to introduce the key to successful DNA extraction and PCR experiments, especially for beginners of genetic analyses, to avoid making easy mistakes which often result in great waste of time and reagent. These keys are related to how to correctly measure DNA amount/purity, how to extract DNA from difficult plant species, and how to successfully amplify target DNA sequence from huge plant genomic DNA. Introduction For genetic analysis and molecular breeding of plants, we must extract DNA from the target plants at first, then perform PCR reactions. Model plants such as arabidopsis (Arabidopsis thaliana) and tobacco (Nicotiana tabacum) are relatively easy to extract DNA, but on the other hand DNA is not effectively extracted from many horticultural plants. Without preparation of high-density and high-quality genomic DNA, the quality of genetic studies is lowered, or even are inoperable. DNA extraction kits such as DNeasy (QIAGEN, Venlo, Netherlands) are recommended for extraction of relatively small amount of pure DNA from model plants, but you cannot use these kits for recalcitrant (difficult) plants. We have tried DNA extraction by ourselves from a variety of plants such as arabidopsis, rice, wasabi, locust tree, torenia, petunia, cyclamen, apple, and gentian. Genomic DNA is usually extracted from healthy leaves. People often extract DNA from soft tissues at shoot apices, but such samples are limited both in quality and in seasons of the year. This is why we usually extract DNA from expended leaves. The most recalcitrant plants in my life would be ginkgo, cyclamen, and rose. We especially needed to extract high-density and high-quality DNA from cyclamen in the research project of molecular breeding of flowers. After trials of novel methods, we discovered an effective method which we named Alkaline PVPP method. In the present report, we will introduce Alkaline PVPP and other DNA extraction methods, including rapid extraction methods specialized for preparation of PCR templates. We will also introduce easy but important tips/mistakes in general experiments of DNA extraction and PCR reaction. This knowledge will help successful performance of DNA extraction and PCR. Quantification of genomic DNA Before describing about DNA extraction, let us check how to quantify genomic DNA extracted from plants. Everyone who has extracted DNA should have measured UV absorbance. DNA concentration (c) in water solution is calculated by absorbance at 260 nm (A260) as: c = 50 A260 (ng L1) For example, a solution with the A260 value of 0.500 is estimated to contain 25 ng L1 of DNA in it. This measurement is correct for pure DNA samples. Measurement of UV absorbance used to be performed by UV/VIS spectrophotometer, but recently it is going to be replaced by NanoDrop (Thermo Scientific, Waltham, USA), which can measure absorbance with small amount (approx. 4 L) of DNA solution. It is often believed that the ratios of UV absorbances show quality of DNA: DNA is estimated to be pure if the ratios A260/A280 and A230/A280 are around 1.9. This is not true. Actually, extracted DNA cannot be quantified by UV absorbance. There are often great gaps between UV-estimated DNA concentrations and true DNA concentrations. DNA is not even contained in the solution, even if the UV absorbance values are good. This will be because there are impurities in plant-extracted DNA, which cause similar UV absorbance to that of DNA in many cases. The UV-estimated DNA concentrations are just estimations based on spectrometry (in spectrometry: is hereafter), then the value is expressed as follows: c = 50 A260 (ng-is L1) In order to measure the true amount of genomic DNA, you should perform agarose gel electrophoresis. We usually load 300, 100, and 30 ng of -DNA as standards in separate wells. These standards are electrophoresed with 300 ng-is of plant-extracted DNA. For example, the purity of extracted DNA is 33% if the band densities of 100-ng -DNA and plant extract are the same. Purities of plant DNA extract are usually less than 10% only with the popular treatments with PCI (later described), RNase, and ethanol precipitation. Intensities of DNA bands can be quantified with ImageJ. We should be careful so that band images are not saturated for correct calculations. Figure 1 shows CsCl-purified DNA samples extracted from different horticultural plants. Figure 1. Agarose gel electrophoresis of DNA samples extracted from horticultural plants. M, molecular weight marker. Modified from Kasajima et al. (2013) Meanwhile, RNA is also extracted together with DNA. RNA is visible in agarose gel as low molecular weight bands. Then, you should not confuse RNA with DNA. It is said that RNA affects enzymatic reactions, so RNA is degraded by RNase enzyme. It also often matters that sticky impurities in crude DNA extracts are lighter than water, and the samples will float out of the well before separation by electrophoresis. In this case, DNA is purified before electrophoresis, or additional loading buffer and electrophoresis buffer are added to samples. Also, rather than staining with ethidium bromide (EtBr) during electrophoresis, gel pictures are clearer when gels are stained with EtBr after electrophoresis. Recently appearing blue-light illuminators are not good. Gels should be illuminated by UV for clear gel images. GelRed (Biotium, Fremont, USA) enjoys good reputation in place of EtBr, although GelRed is expensive. DNA bands are also sharp when electrophoresed at 50 V, instead of 100 V. Polyacrylamide gel electrophoresis also gives sharp band. Close investigations of DNA purity and quantity will greatly help to judge if the plant-extracted DNA solutions are good enough for the experiments. DNA will be extracted by more competent method, if the quantity is not enough, and DNA will be purified if the quality is not enough. DNA extraction with modified alkaline PVPP method Introduction We previously reported a DNA extraction method Alkaline PVPP method. This method can extract DNA from any horticultural plants, as far as we tested [1]. The NaCl concentration in the extraction buffer was modified after publication of this report [2,3]. This protocol would be the most competent method from most plant species. The protocol is as follows: Preparation of reagents Modified PVPP buffer (Tris-HCl, pH 9.5, 50 mM; EDTA, 10 mM; NaCl, 4 M; CTAB, 1%; PVPP, 0.5%). Also add 1% -mercaptoethanol immediately before use. PVPP is a white powder and insoluble in water, but PVPP becomes sticky in this solution. Then the buffer becomes white, half-transparent, and sticky. Store at root temperature. Tris represents tris(hydroxymethyl) aminomethane, EDTA represents ethylenediaminetetraacetic acid, CTAB represents cetyltrimethylammonium bromide, and PVPP represents polyvinylpolypyrrolidone. PCI (phenol-chloroform-isoamyl alcohol = 25:25:1). Phenol-chloroform without isoamyl alcohol is also often used in sample treatments, but isoamyl alcohol helps clear separation of the solid middle phase after centrifugation. Phenol is saturated with water or TE buffer (Tris-HCl, pH 8.0, 10 mM; EDTA, 1 mM) before preparation of PCI, and stored at 4C as a liquid form. Weighing plant samples You must be careful about the ratio between the weight of plant samples and the volume of extraction buffer, whichever protocol you follow. The most standard buffer ratio is 5 times more than leaf: 5 mL of buffer per 1 g of leaf. This ratio is increased to 10 when you extract DNA from difficult samples. The maximum leaf weight is 1 g when you extract with ordinary mortar and pestle, together with liquid nitrogen: larger amount of leaf causes insufficient grinding. The degree of grinding is in fact one of the most important factors for successful DNA extraction. Leaves should be ground to fine powder like Japanese Matcha. Alternatively, samples are frozen at 80C and crushed by using Micro Smash (Tomy, Tokyo, Japan), together with two metal balls in 2-mL plastic tubes. Micro Smash may cause slight degradation of DNA. There are many similar machines of this type, but crushing may not complete with many of the other machines. DNA extraction Cool mortar and pestle with liquid nitrogen, add frozen leaves, and crush immediately to fine powder. Hard leaves such as rice and camellia are cut to 1-mm width beforehand, and siliceous sand is added. Siliceous sand accelerates DNA fragmentation but should be added when grinding hard leaves. Add modified PVPP buffer and mix with leaf powder. Place at room temperature until the solution starts to thaw. Grind well again. Add samples to 15-mL or 50-mL plastic tubes, and heat in pre-heated heating block at 60C for 30 min. Longer heating will cause DNA degradation. Heating at 80C will slightly increase DNA yield. Invert tubes 2-3 times during heating. Cool samples to room temperature. Centrifuge and recover supernatant, when there are much solid substance in the solution. Add half volume (of the solution) of PCI, vortex well, and centrifuge at the maximum speed (such as 14000 rpm) for 5 min. Recover water (upper) phase to new tubes. Repeat the same step (PCI treatment) again. Dilute the sample with the same volume of distilled water. Add twice the volume of ethanol and mix well by inverting. Place at 80C for at least 15 min. Thaw samples and centrifuge at the maximum speed for 60 min at 4C. Discard supernatant and centrifuge briefly. Remove any solution by using pipette. Air-dry or more conveniently, dry by using hair dryer, to some extent (not completely dry). Dissolve in distilled water (typically 500 L of distilled water per 1 g of leaf sample). Add 2 g of RNase A, place at 37C or room temperature (25C) for 15 min. Store at 20C for several months. DNA samples will be more stably stored in ethanol for longer period. Other DNA extraction buffers Introduction The modified PVPP buffer needs relatively more experimental steps, then other DNA extractions buffers are usually used. CTAB buffer suffer from low capacity of DNA extraction, but it also extracts lower concentration of impurities. SDS buffer can extract DNA more efficiently that CTAB buffer. The protocol of DNA extraction with these buffers is the same as the protocol with modified PVPP buffer, except that PCI treatment do not need to be repeated, and the solution do not have to be diluted after PCI treatment. Reagents CTAB buffer (Tris-HCl, pH 8.0, 50 mM; EDTA, 10 mM; NaCl, 0.7 M; CTAB, 1%). Also add 1% of -mercaptoethanol immediately before use. SDS buffer (Tris-HCl, pH 7.5, 200 mM; EDTA, 25 mM; NaCl, 250 mM; SDS, 0.5%). Also add 1% of -mercaptoethanol immediately before use. SDS represents sodium dodecyl sulfate. When SDS precipitate at low temperature (e.g. in winter), warm the stock solution to completely dissolve SDS and mix well to homogeneity before experiments. DNA purification by isopropanol precipitation Introduction Isopropanol precipitation is a simple method for DNA purification. Here, a protocol which I adopted in my former experiments is introduced. Alternatively, DNA-containing solution can be mixed with the same volume of isopropanol, then treated just like the protocol for ethanol precipitation. We have also tried DNA purification by CTAB precipitation method, but this trial was far from successful [1]. Reagent High-Salt Solution for Precipitation (Takara, Kusatsu, Japan). Protocol Be careful so as not to directly touch isopropanol with your skin. Add half volume (of DNA solution) of High-Salt Solution for Precipitation and mix. Add the same volume (as High-Salt Solution for Precipitation) of isopropanol and mix. Place at room temperature for 10 min. Centrifuge at the maximum speed for 10 min at 4C. Discard supernatant. Add 1 mL of 70% ethanol. Place at 80C for 15 min. Thaw out sample and immediately proceed to centrifugation at the maximum speed for 5 min at 4C. Discard all supernatant and dry. DNA purification by CsCl ultracentrifugation Introduction Ultracentrifugation with CsCl needs relatively long time of experiments, but DNA is highly purified by this treatment, and RNA is also removed. Ethidium bromide is often used to stain DNA in CsCl solution, but usage of GelRed instead of ethidium bromide halves the time of ultracentrifugation. GelRed is also only weakly carcinogenic, then you can keep safe environment. It seems that almost 100% of genomic DNA is recovered in CsCl ultracentrifugation [1,4]. In addition to CsCl ultracentrifugation, we have also recently noticed that silica monolith column (MonoFas, GL Sciences, Tokyo, Japan) could be used for high purification of genomic DNA within quite short time. Reagent TE buffer (Tris-HCl, pH 8.0, 10 mM; EDTA, 1 mM). Protocol Ethidium bromide is highly carcinogenic. Wear rubber gloves and perform experiments carefully. Dissolve DNA-containing sample in 3.0 mL of TE buffer. Add 3.0 g of CsCl and mix to dissolve. Add 120 L of ethidium bromide solution (10 mg mL1) or 20 L of GelRed solution (10000 concentration) and mix. Apply 3.8 mL of the solution to 5-mL centrifuge tubes. Ultracentrifuge at 50000 rpm for 48 h at room temperature. Orange DNA band will form near the middle layer of the tube (Figure 2). Figure 2. Cyclamen DNA separated by CsCl ultracentrifugation. Modified from Kasajima et al. (2013) Remove the above layers and recover the layer containing DNA (approx. 0.4 mL) to new tubes. Rinse with n-butanol for 5 times or more to remove ethidium bromide. Perform ethanol precipitation, after diluting DNA solution with water (see the protocol of DNA extraction). Note It will depend on experimental conditions, but the ultracentrifugation for long period (such as 48 h) was only successful with a swing rotor and was not successful with an angle rotor. We used Optima MAX Ultracentrifuge (Beckman-Coulter, Brea, USA), but other ultracentrifuge machine may not be successful. The reason for the failure of purification seems that the air inside the rotor leaks to the outside vacuum during ultracentrifugation, then selection of air-tight rotor will be the key to success. Rapid DNA extraction from arabidopsis Introduction DNA can be quite easily extracted from arabidopsis, then there exist very simple methods of DNA extraction from arabidopsis. DNA extract with these rapid methods contain impurity and the concentration of DNA is low, but this solution was enough for stable amplification of DNA by PCR [5]. Reagent SDS-D buffer (The SDS 2021 Copyright OAT. All rights reservh TE buffer by 10 times. -mercaptoethanol is not added). Protocol Arabidopsis leaf (around 5 mg) is sampled into a 1.5-mL plastic tube. Add 200 L of SDS-D buffer to the tube. Crush several times with plastic rod until the solution becomes pale green. Store sample at 20C until use. Apply 1 L of this DNA solution to a total of 20 L of PCR reaction. Rapid DNA extraction from torenia Introduction The rapid DNA extraction method from arabidopsis above was tested in transgenic (GM) torenia but failed to amplify T-DNA sequence. Then, a special protocol for torenia was developed [6]. Reagent SDS-R buffer (5 g mL1 of RNase A is added to SDS buffer, without addition of -mercaptoethanol). Protocol Leaf disc of torenia (approx. 5-mm square) is sampled into a 1.5-mL plastic tube. Add 200 L of SDS-R buffer. Crush with plastic rod for 5 times or more. Heat in heating block at 55C for 5 min. The solution becomes pale green. Store samples at 20C. When performing PCR analysis, dilute 10 times with sterilized distilled water. Apply 1 L of this diluted solution to a total of 20 L of PCR reaction. Summary of DNA extraction methods A DNA extraction method suitable to a plant species does not necessarily function in the other species. Such situations are often encountered and fluctuating as well. These problems are sometimes overcome by improving sample manipulations such as vigorously crushing frozen leaves to fine Matcha powder, and heating in heat block at suitable temperature and for suitable time. The protocol of DNA extraction is simple but consists of many important factors. In the present report we described all imaginable keys for successful DNA extraction from plants, based on our long experience. We would say that DNA can be more or less extracted from any plant species, if the protocols are faithfully followed. Alkaline PVPP method needs relatively many manipulations, then is not applicable to an analysis of hundreds of plants. The rapid methods for arabidopsis and torenia are the solutions to this problem. Finally, there is close relationship between the protocol of DNA extraction and the protocol of PCR analysis. However clean DNA be prepared, incorrect PCR conditions will fail to amplify target DNA. On the contrary, the target DNA can be amplified even from low-concentration template DNA, when the PCR condition is correct. Thus, following sentences will explain how to successfully perform PCR amplification by using plant genomic DNA as the template. Tips of PCR reactions Amount of DNA samples in PCR reaction Of course, certain amount of template DNA must be added to the reaction mix, but excessive amount of DNA will inhibit PCR amplification. Typically, 5 ng-is of template DNA is quite enough for amplification. The concentrations of DNA stocks are often quite high. Except for low-density DNA such as the solution prepared by rapid extraction method of arabidopsis, DNA stocks are usually diluted by 100 to 1000 times with sterilized distilled water before added to the reaction. It is also important to compare a series of DNA dilutions (e.g. 1/1, 1/10, 1/100, 1/1000, and 1/10000) to determine the best dilution scale. In the case of cDNA, which are prepared by reverse transcription of RNA, samples should be diluted by 2, 5, or 10 times before PCR reaction. This is because the components of reverse transcription reaction partly inhibit PCR reaction. Utilization of high-fidelity PCR polymerase High-fidelity type DNA polymerase would be more successful (on average) than the ordinary type, when amplifying target DNA sequence from the genome of horticultural plants. PCR seems to be equally successful with the ordinary polymerase in arabidopsis with relatively small genome, but PCR was more successful with high-fidelity polymerase in rice in our experience. The relative frequency (concentration) of the target sequence is very low in the large genomes of horticultural plants, thus higher selectivity of PCR amplification will be needed in many horticultural plants. We usually use KOD Plus Neo (Toyobo, Osaka, Japan) in crop plants. Many other polymerases are also released from many companies. Setting the best condition of the reaction Usage of high-fidelity polymerase is not enough for success of PCR. After designing suitable primers, PCR is tested with the gradient annealing temperature from 46C to 68C, with 2C or 4C intervals. If the target is not amplified at any temperature, primers should be re-designed. Primer3 software is nice for primer design. Adenine residues are not added to DNA ends when amplified with high-fidelity polymerases. Sequences are TA-cloned after A attachment by using an enzyme kit. High-throughput manipulations Manipulations for PCR reaction also need a lot of time, when large number of samples are analyzed. For the sake of shortening the time of manipulation, PCR reactions are not needed to be prepared on ice. A key of PCR reaction is to homogenize PCR mixes well before thermal cycles. Vortexing all samples on a tube rack greatly shortens the time of mixing, instead of mixing one by one by using pipette. The number of PCR cycles are not enough for 30 cycles in many cases, then PCR is usually performed for 40 cycles. Agarose gel is prepared in 200-mL PYREX medium bottle, by heating with microwave at 400W. Heating is repeated for several times: the first heating is 1 min, then the following heating are 30 s. After completely melting the agarose powder, bottle is cooled by tap water and agarose solution is applied to gel tray. Keeping moisture with aluminum sheet, gel is completely solidified at room temperature for 1 h. Conclusion The essential keys to the success of DNA extraction and PCR reaction described in the present report will help all beginners and all researchers of plant genes. However highly genetic technologies are developed by researchers, the most fundamental experiments, that are DNA extraction and PCR reaction, are inevitable. Thus, knowledge on successful protocols will benefit any kind of genetic analyses. The present report is an English version of a review written in Japanese [2], with modifications in part. Japanese readers are also recommended to read the Japanese version. Conflicts of interest The author declares no conflict of interests. References Kasajima I, Sasaki K, Tanaka Y, Terakawa T, Ohtsubo N (2013) Large-scale extraction of pure DNA from mature leaves of Cyclamen persicum Mill. and other recalcitrant plants with alkaline polyvinylpolypyrrolidone (PVPP). 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 Dont understand how DNA extraction kits work? We cover the basics in this article so you can perfect your nucleic acid isolation and get high-quality DNA. We give a lot of troubleshooting help on RNA and DNAextraction here at Bitesize Bio because almost everything we do in molecular biology requires DNA or RNA at the very first step whether it is qPCR, molecular cloning, next-generation sequencing, or something else. These days, most labs use commercial DNA extraction kits that use the silica spin filter method to get high-quality DNA. These allow rapid and efficient purification of DNA (or RNA). But it does mean many people just follow the instructions and dont understand how DNA extraction kits work. The spin columns contain a silica resin that selectively binds DNA and RNA, depending on the salt conditions and other factors influenced by the extraction method. These DNA extraction kits make the whole process much easier and faster than the DNA isolation methods of old when things are going well. However, the downside of using a kit is that if you dont understand what is in the black box of the kit, it makes troubleshooting much more difficult. So in this article, Ill explain in some detailhow DNA extraction kits work and what is going on at each step. Ill also go over some common problems specific to using silica columns in DNA extraction that can be overcome or avoided with just a little extra understanding. The lysis formulas mayvary based on whetheryou want to extract DNA or RNA, but the common denominator is a lysis buffer containing a high concentration of chaotropic salt. Chaotropes destabilize hydrogen bonds, van der Waals forces, and hydrophobic interactions. Proteins are destabilized, including nucleases, and the association of nucleic acids with water is disrupted setting up the conditions for the transfer to silica. Chaotropic salts include guanidine HCL, guanidine thiocyanate, urea, and lithium perchlorate. Besides the chaotropes, there are usually some detergents in the lysis buffer to help with protein solubilization and lysis. There can also be enzymes used for lysis depending on the samples type. Proteinase K is one of these, and actually works very well in these denaturing buffers; the more denatured the protein, the better Proteinase K works. Lysozyme, however, does not work in the denaturing and so lysozyme treatment is usually done before adding the denaturing salts. One comment about isolating plasmid DNA: the lysis is very different than extraction forRNA or genomic DNA extraction because the plasmid has to be separated from the genomic DNA first. If you throw in chaotropes, youll release everything at once and wont be able to differentially separate the small circular DNA from thehigh molecular weightchromosome. So, in plasmid preps the chaotropes are not added until after lysis, and the salts are used for binding. An excellent in-depth article onalkaline lysisis here and also another article on the difference between genomic DNA and plasmid DNA is available for further reading. The chaotropic salts are critical for lysis, but also for binding the DNA (or RNA) to the column. Additionally, to enhance and influence the binding of nucleic acids to silica, alcohol is also added. Most of the time this is ethanol but sometimes it may be isopropanol. The percent ethanol and thevolume have big effects.Too much and youll bring in a lot ofdegraded nucleic acids and small species that will influence A260 readings and throw off some ofyour yields.Too little, and it may become difficult to wash away all of the salt from the membrane. The important point here is that the ethanolinfluencesbindingand the amount added is optimized for whatever kit you are using. Modifying that step can help change what you recover so if you are having problems with RNA or DNA recovery and want to troubleshoot it, that can be a step to evaluate further. Another way to diagnose problems is to save the flow-through after binding and precipitate it to see if you can find the nucleic acids you are searching for. If you used an SDS-containing detergent in lysis, try using NaCl as a precipitant to avoid contamination of the DNA or RNA with detergent. Your lysate was centrifuged through the silica membrane and now your extracted DNA or RNA should be bound to the column and the impurities, cellular proteins, and polysaccharides should havepassed through. But, the membrane is still dirty withresidual cellular proteins and salt. If the sample was from plants, there will still be polysaccharides, maybe some pigments left on the membrane, or if the sample was blood, the membrane might be tinted brown or yellow. The wash steps serve to remove these impurities. There are typically two washes, although this can vary depending on the sample type. The first wash will often have a low amount of chaotropic salt to remove the protein and colored contaminants. This is always followed by an ethanol wash to remove the salts. If the prep is something that didnt have a lot of protein to start, such as plasmid preps or PCR clean up, then only an ethanol wash is needed. Removal of the chaotropic salts is crucial to getting high yields and high purity DNA or RNA. Some kits will even wash the column with ethanol twice. If saltremains behind, the elution of nucleic acid is going to be poor, and the A230 reading will be high, resulting in low 260/230 ratios. After the ethanol wash, most protocols have a centrifugation step to dry the column. This is to remove the ethanol and is essential for a clean eluant. When 10 mM Tris buffer or water is applied to the membrane for elution, the nucleic acids can become hydrated and will release from the membrane. If the column still has ethanol on it, then the nucleic acids cannot be fully rehydrated. Skipping the drying step results in ethanol contamination and low yields. I do not see ethanol absorbance on the Nanodrop, so it wont show up in your readings. The main indicators of a problem are that when you try to load the sample onto an agarose gel, the DNA will not sink. Even in the presence of loading dye.Another indicator is thatif you put the sample in the -20C, it doesnt freeze. The final step in the DNA extraction protocol is the release of pure DNA or RNA from the silica. For DNA preps, 10 mM Tris at a pH between 8-9 is typically used. DNA is more stable at a slightly basic pH and will dissolve faster in a buffer. This is true even for DNA pellets. Water tends to have a low pH, as low as 4-5 and high molecular weight DNA may not completely rehydrate in the short time used for elution. Elution of DNA can be maximized by allowing the buffer to sit in the membrane for a few minutes before centrifugation. RNA, on the other hand, is fine at a slightly acidic pH and so water is the preferred diluent. RNA dissolves readily in water. If you experience DNA/RNA yields lower than you expected for a sample, there are many factors to think about. Usually, it is a lysis problem. Incomplete lysis is a major cause of low yields. It could also be caused by incorrect binding conditions.Make sure to use fresh high-quality ethanol (100% 200 proof) to dilute buffers or for adding to the binding step. Low-quality ethanol or old stocks may have taken on water and not be the correct concentration. If the wash buffer is not made correctly, you may be washing off your extracted DNA or RNA. If the extracted DNA is contaminated with protein (low 260/280) then maybe you started with too much sample and the protein was not completely removed or dissolved. If the DNA has a poor 260/230 ratio the issue is usually salt from the bind or the wash buffer. Make sure that the highest quality ethanol was used to prepare wash buffers and if the problem continues, give the column an additional wash. Some samples have a lot more inhibitors compared to others. Environmental samples are especially prone to purity issues because humic substances are solubilized during extraction. Humics behave similarly to DNA and are difficult to remove from the silica column. For this type of sample,specialized techniques exist toremove the protein and humics prior to the column step. This is more of a concern for RNA preps and an article that gives specific advice on RNA Isolation is here. Mainly with RNA extractions, degradation occurs from improper storage of the sample or inefficient lysis, assuming of course that you eluted with RNase-free water. For DNA extractions, degradation is not a huge problem because for PCR, the DNA can be sheared and it works fine. But if you were hoping to not have so much sheared DNA, then you may have used too strong a lysis method. PCR cleanup obviously isnt a DNA extraction technique per se, but it is a nice and easy technique because it is simply adding a high concentration of binding salts (typically between 3-5 volumes of salt per volume of PCR reaction) and centrifugation through the column. So when PCR Clean-up kits fail, it can be particularly frustrating.The first question I ask people is did you check the results of the PCR on a gel? because you cannot UV check a PCR reaction and get an accurate DNA quantitation. There is way too much in a PCR reaction absorbing UV at 260: nucleotides, detergents, salts, and primers. In my experience, a failure of a PCR clean-up kit to work frequently is caused by a PCR reaction that has failed and so there was nothing to clean up. But if you know you had a strong PCR product,the best approach is to just save your flow-through fraction after binding. If the DNA doesnt bind, thats where it is. You can always rescue it and then clean it up again. And then call tech support and ask for a replacement kit. As scientists, of course, we want to know exactly what is going on with our experiments and be able to troubleshoot without having to call technical service first. I hope that this article helps clarify some of the science around the silica spin filter method for RNA and DNA extractions so you can make your own diagnosis and fixes. So, when you do call technical service, youll have double-checked a few of the most likely causes of problems first and instead of going through a lot of rigmarole, you can get to a resolution much faster. Even if that is a free replacement DNA extraction kit! Hopefully, you now understand more about how DNA extraction kits work. Any other problems with silica spin filter preps that you dont understand? Let us know or ask a question in the comments below and well discuss! Originally published on June 28, 2010. Updated and revised October 2021. This document describes DNA extraction protocols for fungi, yeast, and bacteria. The fungal protocol involves grinding fungal material, adding CTAB extraction buffer and chloroform/isoamyl aAI-enhanced title and descriptionThis document describes DNA extraction protocols for fungi, yeast, and bacteria. The fungal protocol involves grinding fungal material, adding CTAB extraction buffer and chloroform/isoamyl aAI-enhanced title and descriptionProcess of isolating DNA from cellsFor the various methods, see Nucleic acid methods.This article needs additional citations for verification. Please help improve this article by adding citations to reliable sources. Unsourced material may be challenged and removed.Find sources:"DNA extraction"news newspapers books scholar JSTOR (May 2014) (Learn how and when to remove this message)The first isolation of deoxyribonucleic acid (DNA) was done in 1869 by Friedrich Miescher.[1] DNA extraction is the process of isolating DNA from the cells of an organism isolated from a sample, typically a biological sample such as blood, saliva, or tissue. It involves breaking open the cells, removing proteins and other contaminants, and purifying the DNA so that it is free of other cellular components. The purified DNA can then be used for downstream applications such as PCR,[2] sequencing, or cloning. Currently, it is a routine procedure in molecular biology or forensic analyses.This process can be done in several ways, depending on the type of the sample and the downstream application,[3] the most common methods are: mechanical, chemical and enzymatic lysis, precipitation, purification, and concentration. The specific method used to extract the DNA, such as phenol-chloroform extraction, alcohol precipitation, or silica-based purification.[4]For the chemical method, many different kits are used for extraction, and selecting the correct one will save time on kit optimization and extraction procedures. PCR sensitivity detection is considered to show the variation between the commercial kits.[5]There are many different methods for extracting DNA, but some common steps include:Lysis: This step involves breaking open the cells to release the DNA. For example, in the case of bacterial cells, a solution of detergent and salt (such as SDS) can be used to disrupt the cell membrane and release the DNA. For plant and animal cells, mechanical or enzymatic methods are often used.Precipitation: Once the DNA is released, proteins and other contaminants must be removed. This is typically done by adding a precipitating agent, such as alcohol (such as ethanol or isopropanol), or a salt (such as ammonium acetate). The DNA will form a pellet at the bottom of the solution, while the contaminants will remain in the liquid.Purification: After the DNA is precipitated, it is usually further purified by using column-based methods. For example, silica-based spin columns can be used to bind the DNA, while contaminants are washed away. Alternatively, a centrifugation step can be used to purify the DNA by spinning it down to the bottom of a tube.Concentration: Finally, the amount of DNA present is usually increased by removing any remaining liquid. This is typically done by using a vacuum centrifugation or a lyophilization (freeze-drying) step.Some variations on these steps may be used depending on the specific DNA extraction protocol. Additionally, some kits are commercially available that include reagents and protocols specifically tailored to a specific type of sample.[6]DNA extraction is frequently a preliminary step in many diagnostic procedures used to identify environmental viruses and bacteria and diagnose illnesses and hereditary diseases. These methods consist of, but are not limited to:Fluorescence In Situ Hybridization (FISH) technique was developed in the 1980s. The basic idea is to use a nucleic acid probe to hybridize nuclear DNA from either interphase cells or metaphase chromosomes attached to a microscopic slide. It is a molecular method used, among other things, to recognize and count particular bacterial groupings.[1]To recognize, define, and quantify the geographical and temporal patterns in marine bacterioplankton communities, researchers employ a technique called terminal restriction fragment length polymorphism (T-RFLP).Sequencing: Whole or partial genomes and other chromosomal components, ended for comparison with previously published sequences.[7]Cells that are to be studied need to be collected.Breaking the cell membranes open exposes the DNA along with the cytoplasm within (cell lysis).Lipids from the cell membrane and the nucleus are broken down with detergents and surfactants.Breaking down proteins by adding a protease (optional).Breaking down RNA by adding an RNase (optional).The solution is treated with a concentrated salt solution (saline) to make debris such as broken proteins, lipids, and RNA clump together.Centrifugation of the solution, which separates the clumped cellular debris from the DNA.DNA purification from detergents, proteins, salts, and reagents is used during the cell lysis step. The most commonly used procedures are:Ethanol precipitation usually by ice-cold ethanol or isopropanol. Since DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation. Precipitation of DNA is improved by increasing ionic strength, usually by adding sodium acetate.Phenolchloroform extraction in which phenol denatures proteins in the sample. After centrifugation of the sample, denatured proteins stay in the organic phase while the aqueous phase containing nucleic acid is mixed with chloroform to remove phenol residues from the solution.Minicolumn purification relies on the fact that the nucleic acids may bind (adsorption) to the solid phase (silica or other) depending on the pH and the salt concentration of the buffer.Cellular and histone proteins bound to the DNA can be removed either by adding a protease or having precipitated the proteins with sodium or ammonium acetate or extracted them with a phenol-chloroform mixture before the DNA precipitation.After isolation, the DNA is dissolved in a slightly alkaline buffer, usually in a TE buffer, or in ultra-pure water.The most common chemicals used for DNA extraction include:Detergents, such as SDS or Tween-20, which are used to break open cells and release the DNA.Protease enzymes, such as Proteinase K, which are used to digest proteins that may be binding to the DNA.Phenol and chloroform, which are used to separate the DNA from other cellular components.Ethanol or isopropanol, which are used to precipitate the DNA.Salt, such as NaCl, which is often used to help dissolve the DNA and maintain its stability.EDTA, which is used to chelate the metals ions that can damage the DNA.Tris-HCL, which is used to maintain the pH at the optimal condition for DNA extraction.Some of the most common DNA extraction methods include organic extraction, Chelex extraction, and solid phase extraction.[8][9] These methods consistently yield isolated DNA, but they differ in both the quality and the quantity of DNA yielded. When selecting a DNA extraction method, there are multiple factors to consider, including cost, time, safety, and risk of contamination.Organic extraction involves the addition of incubation in multiple different chemical solutions;[9] including a lysis step, a phenol-chloroform extraction, an ethanol precipitation, and washing steps. Organic extraction is often used in laboratories because it is cheap, and it yields large quantities of pure DNA. Though it is easy, there are many steps involved, and it takes longer than other methods. It also involves the unfavorable use of the toxic chemicals phenol and chloroform, and there is an increased risk of contamination due to transferring the DNA between multiple tubes.[10] Several protocols based on organic extraction of DNA were effectively developed decades ago,[11] though improved and more practical versions of these protocols have also been developed and published in the last years.[12]The chelex extraction method involves adding the Chelex resin to the sample, boiling the solution, then vortexing and centrifuging it. The cellular materials bind to the Chelex beads, while the DNA is available in the supernatant.[10] The Chelex method is much faster and simpler than organic extraction, and it only requires one tube, which decreases the risk of DNA contamination. Unfortunately, Chelex extraction does not yield as much quantity and the DNA yielded is single-stranded, which means it can only be used for PCR-based analyses and not for RFLP.[10]Solid phase extraction such as using a spin-column-based extraction method takes advantage of the fact that DNA binds to silica. The sample containing DNA is added to a column containing a silica gel or silica beads and chaotropic salts. The chaotropic salts disrupt the hydrogen bonding between strands and facilitate the binding of the DNA to silica by causing the nucleic acids to become hydrophobic. This exposes the phosphate residues so they are available for adsorption.[13] The DNA binds to the silica, while the rest of the solution is washed out using ethanol to remove chaotropic salts and other unnecessary constituents.[9] The DNA can then be rehydrated with aqueous low-salt solutions allowing for elution of the DNA from the beads.This method yields high-quality, largely double-stranded DNA which can be used for both PCR and RFLP analysis. This procedure can be automated[10] and has a high throughput, although lower than the phenol-chloroform method. This is a one-step method i.e. the entire procedure is completed in one tube. This lowers the risk of contamination making it very useful for the forensic extraction of DNA. Multiple solid-phase extraction commercial kits are manufactured and marketed by different companies; the only problem is that they are more expensive than organic extraction or Chelex extraction.Specific techniques must be chosen for the isolation of DNA from some samples. Typical samples with complicated DNA isolation are:archaeological samples containing partially degraded DNA, see ancient DNA[14]samples containing inhibitors of subsequent analysis procedures, most notably inhibitors of PCR, such as humic acid from the soil, indigo and other fabric dyes or haemoglobin in bloodsamples from microorganisms with thick cellular walls, for example, yeastsamples containing mixed DNA from multiple sourcesExtrachromosomal DNA is generally easy to isolate, especially plasmids may be easily isolated by cell lysis followed by precipitation of proteins, which traps chromosomal DNA in insoluble fraction and after centrifugation, plasmid DNA can be purified from soluble fraction.A Hirt DNA Extraction is an isolation of all extrachromosomal DNA in a mammalian cell. The Hirt extraction process gets rid of the high molecular weight nuclear DNA, leaving only low molecular weight mitochondrial DNA and any viral episomes present in the cell.Main article: Quantification of nucleic acidsA diphenylamine (DPA) indicator will confirm the presence of DNA. This procedure involves chemical hydrolysis of DNA: when heated (e.g. 95C) in acid, the reaction requires a deoxyribose sugar and therefore is specific for DNA. Under these conditions, the 2-deoxyribose is converted to w-hydroxylevulinyl aldehyde, which reacts with the compound, diphenylamine, to produce a blue-colored compound. DNA concentration can be determined by measuring the intensity of absorbance of the solution at the 600nm with a spectrophotometer and comparing to a standard curve of known DNA concentrations.Measuring the intensity of absorbance of the DNA solution at wavelengths 260 nm and 280 nm is used as a measure of DNA purity. DNA can be quantified by cutting the DNA with a restriction enzyme, running it on an agarose gel, staining with ethidium bromide (EtBr) or a different stain and comparing the intensity of the DNA with a DNA marker of known concentration.Using the Southern blot technique, this quantified DNA can be isolated and examined further using PCR and RFLP analysis. These procedures allow differentiation of the repeated sequences within the genome. It is these techniques which forensic scientists use for comparison, identification, and analysis.In this method, plant nuclei are isolated by physically grinding tissues and reconstituting the intact nuclei in a unique Nuclear Isolation Buffer (NIB). The plastid DNAs are released from organelles and eliminated with an osmotic buffer by washing and centrifugation. The purified nuclei are then lysed and further cleaned by organic extraction, and the genomic DNA is precipitated with a high concentration of CTAB. The highly pure, high molecular weight gDNA is extracted from the nuclei, dissolved in a high pH buffer, allowing for stable long-term storage.[15]DNA storage is an important aspect of DNA extraction projects as it ensures the integrity and stability of the extracted DNA for downstream applications.[16]One common method of DNA storage is ethanol precipitation, which involves adding ethanol and a salt, such as sodium chloride or potassium acetate, to the extracted DNA to precipitate it out of solution. The DNA is then pelleted by centrifugation and washed with 70% ethanol to remove any remaining contaminants. The DNA pellet is then air-dried and resuspended in a buffer, such as Tris-EDTA (TE) buffer, for storage.Another method is freezing the DNA in a buffer such as TE buffer, or in a cryoprotectant such as glycerol or DMSO, at -20 or -80 degrees Celsius. This method preserves the integrity of the DNA and slows down the activity of any enzymes that may degrade it.It's important to note that the choice of storage buffer and conditions will depend on the downstream application for which the DNA is intended. For example, if the DNA is to be used for PCR, it may be stored in TE buffer at 4 degrees Celsius, while if it is to be used for long-term storage or shipping, it may be stored in ethanol at -20 degrees Celsius. The extracted DNA should be regularly checked for its quality and integrity, such as by running a gel electrophoresis or spectrophotometry. The storage conditions should be also noted and controlled, such as the temperature and humidity.It's also important to consider the long-term stability of the DNA and the potential for degradation over time. The extracted DNA should be stored for as short a time as possible, and the conditions for storage should be chosen to minimize the risk of degradation.In general, the extracted DNA should be stored under the best possible conditions to ensure its stability and integrity for downstream applications.There are several quality control techniques used to ensure the quality of extracted DNA, including:[17]Spectrophotometry: This is a widely used method for measuring the concentration and purity of a DNA sample. Spectrophotometry measures the absorbance of a sample at different wavelengths, typically at 260nm and 280nm. The ratio of absorbance at 260nm and 280nm is used to determine the purity of the DNA sample.[18]Gel electrophoresis: This technique is used to visualize and compare the size and integrity of DNA samples. The DNA is loaded onto an agarose gel and then subjected to an electric field, which causes the DNA to migrate through the gel. The migration of the DNA can be visualized using ethidium bromide, which intercalates into the DNA and fluoresces under UV light.[19]Fluorometry: Fluorometry is a method to determine the concentration of nucleic acids by measuring the fluorescence of the sample when excited by a specific wavelength of light. Fluorometry uses dyes that specifically bind to nucleic acids and have a high fluorescence intensity.PCR: Polymerase Chain Reaction (PCR) is a technique that amplifies a specific region of DNA, it is also used as a QC method by amplifying a small fragment of the DNA, if the amplification is successful, it means the extracted DNA is of good quality and it's not degraded.Qubit Fluorometer: The Qubit Fluorometer is an instrument that uses fluorescent dyes to measure the concentration of DNA and RNA in a sample. It is a quick and sensitive method that can be used to determine the concentration of DNA samples.[17]Bioanalyzer: The bioanalyzer is an instrument that uses electrophoresis to separate and analyze DNA, RNA, and protein samples. It can provide detailed information about the size, integrity, and purity of a DNA sample.Biology portalBoom methodDNA fingerprintingDNA sequencingDNA structureEthanol precipitationPlasmid preparationPolymerase chain reactionSCODA DNA purification^ a b "Fluorescence In Situ Hybridization (FISH)". Genome.gov. Retrieved 2022-10-23.^ Gupta, Nalini (2019). "DNA extraction and polymerase chain reaction". Journal of Cytology. 36 (2): 116117. doi:10.4103/JOC.JOC_110_18. ISSN0970-9371. PMC6425773. PMID30992648.^ Srivastava, Akhileshwar Kumar; Kannaujiya, Vinod Kumar; Singh, Rajesh Kumar; Singh, Divya (5 October 2020). DNA Extraction - an overview | ScienceDirect Topics. Elsevier Science. ISBN978-0-12-821710-8. 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