Research Paper

Volume : 2 | Issue : 11 | November 2013 • ISSN No 2277 - 8179

Optimized Protocols for Extraction of DNA in Plant and Blood Tissues

Biology

KEYWORDS : Molecular biology, methodology, nucleic acids, Spectrophotometer

Ednaldo da Silva Filho — Agrarian Science Institute, Federal Rural University of Amazon, Belém-Pará, Brazil. ZIPCODE: 66.077-901.

Taianara Tocantins Gomes Almeida — Hospital of Federal University of Pará, Belém-Pará, Brazil. ZIPCODE: 66.010-000.

ABSTRACT

There is a variety of works in the molecular biology of plants and animals using different methods of DNA extraction. Some procedures are performed using commercial kits or protocols using different buffered solutions and denaturing agents (protein and polysaccharide), which makes the method very laborious and polluting to environment with a high economic cost. This scientific note has objective to demonstrate two optimized simple methods, less polluting to the environment with little formation of waste, less expense in detected by UV spectrophotometry through of 230/260 and 260/280 rations demonstrating materials and a lot of quality. Samples of different kinds of vegetables and blood (human and bovine) were tested for protocols 3% CTAB and precipitation with salt methods, respectively. Results were that pure DNA is possible to be acquired with few contaminations.

Introduction

Some studies of molecular biology in plant and animal breeding use different protocols of DNA extraction in order to evaluate different molecular markers that may be associated with economic traits (Corva et al., 2009), studies of animal populations (Silva Filho, Schneider, and Silva, 2007), plant phylogeny (Renner, Schaefer, and Kocyan, 2007) and others. Therefore, the optimization of laboratory protocols contribute greatly reducing time and cost.

The extraction of DNA from plant tissue routinely in many studies is the CTAB (Cetyl trimethylammonium bromide) an agent that enables the precipitation of proteins and polysaccharides in the presence of a detergent, for the most used is SDS (sodium dodecyl sulfate) in highly saline solution. Several protocols are based on the methodology of Dellaporta, Wood and Hicks (1983), but with several modifications and adaptations.

The extractions of DNA from blood samples are routinely performed using commercial kits, as well as for methodologies based on the protocol of Sambrook, Fritsch and Maniatis (1989) using the phenol chloroform isoamyl alcohol as denaturing agent and precipitating protein. Another method of extracting DNA from blood samples is precipitation with salt, various protocols using this method are adapted from Olerup and Zetterquist (1992).

This scientific research has objective to demonstrate two methods for extracting of DNA from small changes to the procedures of the CTAB method of Dellaporta et al. (1983) for plant tissue tested in 12 plant species from Baixo Parnaíba of Maranhão State from Brazil and the method of precipitation with salt to blood tissue based Zetterquist and Olerup (1992) in samples from human and Nelore bovine breed.

Materials and Methods

Vegetable DNA extraction protocol

Samples of young sheet from twelve types of species (list in results) were collected in Maranhão State of Brazil and submitted to below protocol:

- To collect plant tissue of a vegetable leaf young and to stock in a paper envelope and to make the identification. To submit to drying at 60° C for 72 h. To macerate the plant tissue to obtain a powder. To weigh 150 mg of powder and to transfer to a polypropylene tube of 1.5 mL. To add 800 µL of 3% CTAB buffer (0.1 M Tris-HCl pH 8.0, 0.03 M EDTA pH 8.0, 1.2 M NaCl**,** 1% β-mercaptoethanol, 10 µg/µL proteinase K, 3% CTAB and ultra pure H2O as solvent) and to mix with a vortex to became a homogeneous mixture. To incubate at 65° C for 1 h.
- After incubation, to add 800 µL of chloroform: isoamyl alcohol (24:1) and to mix gently for 10 min. To centrifuge at 13,000 rpm for 10 min. After centrifugation, to transfer the supernatant to another 1.5 mL tube and to add 350 µL of absolute ethanol. To mix gently to form the cluster of DNA (pellet). To centrifuge at 13,000 rpm for 10 min. After centrifugation, to discard absolute ethanol by inverting the tube being careful not to lose the pellet of DNA. To add 500 µL of 70% ethanol and to centrifuge at 13,000 rpm for 10 min.
- After centrifugation, to discard 70% ethanol by inverting the tube being careful not to lose the pellet of DNA. To dry the pellet in order to remove all 70% ethanol at room temperature. After drying, to add 500 µL of TE buffer to dilute the pellet and to keep at low temperature (-20° C).

Blood DNA extraction protocol

Blood samples from human and bovine (Nelore breed) were collected and submitted to below protocol:

- To collect blood and to procedure for extraction of leukocytes with 1.5 mL of blood and to add in polypropylene tubes of 1.5 mL, to make the identification of the sample and to centrifuge at 4,000 rpm for 10 min. After centrifugation, to discard the blood serum being careful not to remove the cell layers. To add the solution of hemolysis (0.014 M ammonium chloride) until to complete 1.5 mL. To mix with a vortex to homogenize and to centrifuge at 4,000 rpm for 10 min. After centrifugation, to discard supernatant, being careful not to remove the sediment of white cells and to repeat this procedure until the layer of leukocytes cells to be clear.
- To resuspend the leukocytes cells with 500 µL of extraction solution (10 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 mM EDTA pH 8.0, 0.5% SDS, 10 µg/µL proteinase K and ultra pure H2O as solvent) and to mix with a vortex until the sediment be dissolved, to incubate at 55° C for 4 h or overnight for activation of the detergent and proteinase K (Emphasizing that the tube should be shaken at least twice during heating). After incubation, to add 210 µL of TE buffer (10 mM Tris-HCl pH 7.4, 7.5 or 8.0 and 1 mM EDTA pH 8.0) and 250 µL of solution of 5 M sodium chloride. To mix gently (about 10 X) and to freeze (-5° C) for 15 min.
- After cooling, to centrifuge at 13,000 rpm for 10 min. To transfer the supernatant to another 1.5 mL tube with care not to remove the sediment. To add 1 mL of absolute ethanol frozen (-5° C). To mix gently until to form the cluster of DNA (pellet). To centrifuge at 13,000 rpm for 10 min. After centrifugation, to discard absolute ethanol by inverting the tube being careful not to lose the pellet of DNA. To add 500

μL of 70% ethanol and to centrifuge at 13,000 rpm for 10 min;

- After centrifugation, to discard 70% ethanol by inverting the tube being careful not to lose the pellet of DNA. To dry the pellet in order to remove all 70% ethanol at room temperature. After drying, to add 300 μL of TE buffer to dilute the pellet and to keep at low temperature (-20° C).
- DNA samples were analyzed by UV Spectrophotometry with wavelengths at 230, 260 and 280 nm. The dilution factor was 100 and spectrophotometer curve was 100 for 100 μL. The rations among wavelengths were 230/260 and 260/280 rations. The rations were compared to methodology of Clark and Christopher (2000).

Results and Discussion

DNA extractions from vegetable issue for about 150 mg to concentrations and quantifies of reagents showed pure concentrations of DNA, only Sucupira roxa (*Diplotropis purpurea*) showed possible protein and/or phenol contamination detected by UV spectrophotometry for 230, 260 and 280 nm of wavelengths through 230/260 and 260/280 rations (Table 1).

Table 1. Results of qualities for vegetables by UV spectrophotometer after DNA extraction.

Popular names *Specie name*	230/260 ration	260/280 ration
Sucupira branca *Pterodon emarginatus*	1.68	1.79*
Sucupira roxa *Diplotropis purpurea*	1.10	1.51**
Mamona *Ricinus communis*	1.50	1.80*
Murici *Byrsonima verbascifolia*	1.68	1.77*
Manga *Mangifera indica*	1.59	1.74*
Caju *Anacardium occidentale*	1.70	1.81*
Parkia *Parkia platycephala*	1.69	1.80*
Mandioca *Manihot esculenta*	1.73	1.83*
Pião-manso *Jatropha curcas*	1.60	1.73*
Urucum *Bixa orellana*	1.55	1.70*
Bacuri *Platonia insignis*	1.72	1.85*
Janaúba *Plumeria bracteata*	1.66	1.79*
*260/280 ration of ~ 1.80 suggests pure DNA **260/280 and 230/260 ration <1.80 and >0.50, respectivally suggest protein and/or phenol contamination (Claker and Christopher, 2000).		

Bloods samples tested demonstrated high quality with pure DNA without contamination, but a stage after heating must be made with carefully so that sediment is not transferred along with light phase, therefore, the light phase must be alone because the sediment has so much contaminations (Table 2). Sambrook et al. (1989) commented that the care of the transfer of the supernatant should be of paramount importance to avoid contamination with protein and/or reagents such as phenol and chloroform denaturing and alcohol after precipitation of DNA. Not only DNA extraction methodology is important to have a DNA sample with quality, therefore, the chemical from plastic tubes to conservation could to demonstrate possible levels of contaminations (Lewis et al., 2010).

Table 2. Results of qualities for blood by UV spectrophotometer after DNA extraction.

Samples *Specie name*	230/260 ration	260/280 ration
Human *Homo sapiens*	1.64	1.84*
Bovine *Bos tauros indicus*	1.69	1.91*
Human*** *Homo sapiens*	0.82	1.10**
Bovine*** *Bos tauros indicus*	0.84	1.19**
*260/280 ration of ~ 1.80 suggests pure DNA **260/280 and 230/260 ration <1.80 and >0.50, respectively suggest protein and/or phenol contamination (Claker and Christopher, 2000). ***Samples that were not transferred only light phase (contend sediment).		

Conclusions

DNA extraction vegetable and blood methodologies demonstrated reliable quality of purity of DNA with few costs and contaminants to environment. However, care during the execution of protocols to avoid contamination to purity of DNA, principally protein.

Acknowledgements

This work was supported by the Center for Agricultural and Environmental Sciences, Federal University of Maranhão, Campus IV, Chapadinha, MA. Brazil.

REFERENCE

Clark, W., & Christopher, K. (2000). An introduction to DNA: Spectrophotometry, degradation, and the "Frankengel' experiment. In S. J. Karcher (Ed.), Tested studies for laboratory teaching (pp. 81-99), v.22. Proceedings of the 22nd Workshop/Conference of the Association for Biology Laboratory Education (ABLE). | Corva, P. M., Fernández Macedo, G. V., Soria, L. A., Papaleo Mazzucco, J., Motter, M., Villarreal, E. L., Schor, A., Mezzadra, C. A., Melucci, L. M., & Miquel, M. C. (2009). Effect of leptin gene polymorphisms on growth, slaughter and meat quality traits of grazing Brangus steers. Genetics and Molecular Research, 8(1), 105-116. doi: 10.4238/vol8-1gmr556 | Dellaporta, S. L., Wood, J., & Hicks, J. B. (1983). A plant DNA minipreparation: version II. Plant Molecular Biology Reporter, 1(4), 19-21. doi: 10.1007%2FBF02712670 | Lewis, L. K., Robson, M. H., Vecherkina, Y., Ji,C., & Beall, G. W. (2010). Interference with spectrophotometric analysis of nucleic acids and proteins by leaching of chemicals from plastic tubes. BioTechniques, 48(4), 297-302. doi: 10.2144/000113387 | Olerup, O., & Zetterquist, H. (1992). HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSCP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens, 39(5), 225-235. doi: 10.1111/j.1399-0039.1992.tb01940.x | Renner, S. S., Schaefer, H., & Kocyan, A. (2007). Phylogenetics of Cucumis (Cucurbitaceae): cucumber (C. sativus) belongs in an Asian/Australian clade far from melon (C. melo). BMC Evololuciory Biology, 10. 7-58. doi: 10.1186/1471-2148-7-58 | Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular cloning: a laboratory manual. 2nd Edition. Cold Spring Harbor Laboratory Press, New York. | Silva Filho, E., Schneider, M. P. C., & Silva, A. L. C. (2007). Variabilidade Genética de Cavalos baseada em DNA Microssatélites. Revista Trópica – Ciências Agrárias e Biológicas, 1(1), 76-87. http://www.ccaa.ufma.br/revistatropica/Artigos_nr1/zootecnia/VariabilidadeGeneticaDeCavalos_zoot_art.prf |