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Protocols for Extraction DNA of Sample for Genetic Analysis

S. Treebonmuang , W. Nualchuen and K. Srisakwattana

Research and Development Centre for Livestock Production Technology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

*Corresponding author: chetasing@yahoo.com

Introduction

Currently biotechnology has a role in the development of various fields. To obtain new knowledge on biological science, a molecular study is necessary. The extraction of the DNA is the technique for isolate DNA in biological samples .There are details vary in each creature. In general, the basic principles of methods are the same; release of nucleic acid from cells, stabilization of nucleic acid against degradation and separation of nucleic acid from other components. This provides the purified DNA ready for use in different application such as identity testing, forensic science, sequencing genomes and detecting for the paternity or parentage (6). The appropriate preparation procedures for each type of samples are necessary. Those different types of DNA require different methods of isolation and the method used is dependent upon the final application. Although the advantage of using commercial kit are reported. The objective of this study is to show the steps of commercials kits related to conventional steps in the DNA extraction of various sources: : blood, tissue, cell culture or plant. The understanding of conventional steps will support and increase the efficiency of using commercial extraction kit.

Materials and Methods

Preparation of sample for DNA extraction

The extracted genomic DNA of different sample relies on the following main principles. **Step 1**: Breaking cells : the cells are broken open to expose its DNA by physical methods such as disruption by grinding , vortexing or using chemicals to destroy the cells (lysozyme for prokaryotes and SDS for eukaryotes), depending on the type of samples. In our lab, breaking cell by physical method will be used for plant and rumen fluid and chemical methods are used for lysis cell from blood and cell culture**_Step 2**: Separated of DNA : DNA from protein and other impurities with an organic solvent. The commonly used are phenol and chloroform, often a proteinase is add to degrade DNA associated proteins. The nucleic acid is dissolved in the water's upper layers. **Step 3:** DNA precipitation with alcohol: Nucleic acid dissolved in alcohol. It is visible to the naked eye.

Step4: DNA hydration: DNA solubilization and DNA should be stored in water or TE buffer (10mM Tris,0.1mM EDTA pH 8.0). then frozen at -20 degrees Celsius. At present, using DNA commercial kit is more convenient. For example, Genomic DNA mini kit (plant, blood, culture cell) is available. There are also 4 steps of DNA extraction protocol. It's column purification system as showed in figure 1. (9,3). The entire procedure is not required the phenol-chloroform extraction and can be finished within 60 min.

Confirming the presence and quality of the DNA

The purity of the DNA are important for further process, the concentration and quality of DNA in the sample can be determined by a spectrophotometer. The purity of DNA will be calculated from absorbance of DNA (A260) and protein (A280). The ratio absorbance should not be less than 1.8 to 1.9, if the ratio is lower than this suggests that a high-protein contamination. Absorbance at 260 is converted into DNA concentration by following method:

A260/OD of 1.0 = a concentration of 50 ug/ml of double-stranded DNA (dsDNA)

DNA conc. (ug/ml)= measured OD(260mm) x 50ug/ml x dilution factor…..(5)

Alternatively, we can check the amount and the size of the extracted DNA by using 2 % agarose gel electrophoresis. The electrophoresis is a technique used to separate molecules on a charge of using electricity. The DNA will show that the presence of band and quality of DNA sample.

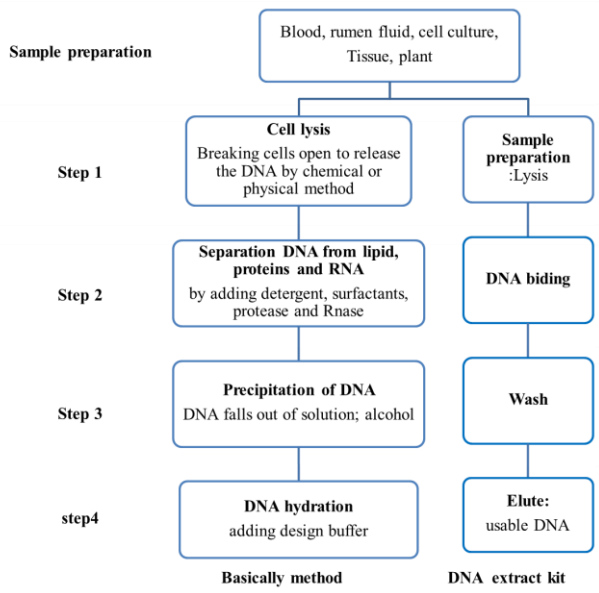
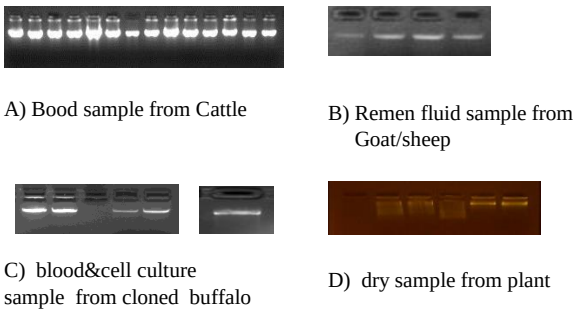


Figure 1: Flowchart of four steps of DNA extraction method, corresponding step between basically method and DNA extraction kit .It takes

Results and Discussion

In this study, The DNA sample were extracted from blood samples various species, (cattle , buffalo), remen fluid sample (goat) and cell culture (cloned buffalo).Type of samples are important for optimal process of appropriated method at early stage of preparation. In figure 2, the quantity of DNA was checked by 2%agarose gel electrophoresis , this step will showed the quantity of DNA and a sufficient amount to be studied in the future. The DNA samples are exhibited the ratios of optical density between 1.70 to 1.90 at 260 and 280 mm indication good deprotenization. It mean that the DNA has a purity and good quality. The important points between conventional and commercial extraction kit are compared in table 1



A) Bood sample from Cattle

B) Remen fluid sample from Goat/sheep

C) blood&cell culture sample from cloned buffalo

D) dry sample from plant

Figure2. Representation of quality and quantity of DNA different source extraction from commercial kit by the 2% agarose gel shows A) Bood sample from Cattle, B) Remen fluid sample from Goat/sheep, C) blood&cell culture sample from cloned buffalo and D) dry sample from plant

Table 1 The important points between conventional and commercial extraction kit are compared

Step	Basically Methods	Conventional extraction kit
step1	Efficiency of grinding or vortexing are developed on time, round per minute, the size of granules.	Nature of biological material and preparation method from conventional procedure will support the awareness of early stage preparation, such as varying time of lysis.
step 2	Extraction using phenol/ chloroform the yield of DNA depended on amount of starting materials and perhaps, the skill of extraction used those solvent.	DNA binding
step 3	The yield of extracted DNA depended on starting materials and the skill of pipetting precipitated DNA using cutting edge tip.	DNA washing
step 4	The longer disserving time will ensure the efficiency of solubilizing	DNA Elusion : Usable DNA
Disadvantage	*Phenol/chloroform are corrosive and toxic *Higher amount of starting material *More chance of losing DNA at each step *Laborious work and time consuming * The purity of DNA instability	*Expensive *Must have an understanding of all stages. *The device has an expiration date
Advantage	*not expensive	*more convenient and rapid * high yield DNA and high purity * fewer amount of starting material

The phenol chloroform extraction method for DNA extraction from whole blood involves several steps (1). Further phenol is corrosive and toxic. These methods required large quantities of blood samples and costly chemicals with modern facilities (10). However, the use of DNA extraction kits, although it is a convenient way and rapid more the conventional methods. If we do not understand the use of the kit in each step, it makes troubleshooting much more difficult (5).

In conclusion, information from conventional methods will increase awareness of using commercial kit and protecting from loss of valuable materials and equipped with the research

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