

Simple Method to Extract DNA from Mammalian Whole Blood

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Abstract: DNA isolation from cells is the 1st and the foremost step involved in molecular biology. The high quality and purity of extracted DNA and simplicity as well as low cost of the extraction method is an important consideration for a molecular biologist. Genomic mammalian DNA is very long and extraction must avoid high levels of centrifuge and severe shakes. There are several methods to extract DNA, including: Boiling, Salting out, Phenol-Chloroform, Isopropanol Precipitation methods. In this study, a method which seems to be superior to salting out and other methods has been developed. The extraction of DNA by this method is carried out from leukocytes. This modified method is simple, cheap and has a wider application than other methods.

Key words: DNA extraction, salting out and whole blood, isolation, molecular, mammalian

INTRODUCTION

Deoxyribonucleic Acid (DNA) is the genetic material of cells and a very long, thread like macromolecule; it is a double helix consisting of antiparallel strands. Each strand of polynucleotide chain consists of purine deoxyribonucleotides of adenine and guanine bases and the pyrimidine deoxyribonucleotides of cytosine and thymine bases. Two polynucleotide strands are joined together by hydrogen bonds between purine and pyrimidine bases (Vyas and Kohli, 2002).

In the eukaryotic cells, DNA is found in the nucleus as well as mitochondria and chloroplast. Chromosomal DNA exists in 2 forms heterochromatin and euchromatin. The DNA in heterochromatin is tightly coiled whereas euchromatin is more loosely organized and is believed to be the functional genetic material. Heterochromatin strains more intensely than euchromatin. However, the amount of DNA recovered is often limiting so DNA isolation from cells is 1st and foremost step involved in molecular biology (Vyas and Kohli, 2002).

In recent years, genetic techniques have come to be a powerful tool in several livestock applications. The DNA-based methods such as PCR are increasingly used in studies considering population genetic variability, QTL identification, marker-assisted selection and food traceability. These techniques require extraction methods that guarantee effective recovery of nucleic acid and removal of PCR inhibitors. Blood leukocytes are generally used as a source of DNA (D'Angelo *et al.*, 2007).

A rapid reliable method, producing a good yield of high molecular weight DNA from blood samples is essential to process the large number of samples required for linkage studies (Montgomery and Sise, 1990).

The high quality and purity of extracted DNA and simplicity as well as low cost of the extraction method is an important consideration for a molecular biologist.

Mammalian genomic DNA is very long and extraction must avoid high levels of centrifuge and severe shakes. There are several methods to extract DNA such as: boiling, salting out, phenol-chloroform, isopropanol precipitation methods (Kalmar *et al.*, 2000; Garg *et al.*, 1996).

Phenol is corrosive and toxic and the extraction steps are time-consuming. Methods recently published in the extraction of DNA have eliminated the phenol extraction steps (Montgomery and Sise, 1990). Salting out method is complicated and time-consuming too.

Extraction of DNA is usually carried out from leukocytes in most laboratories but extraction of DNA is carried out from milk, saliva, hair, nail and other tissues (Garg *et al.*, 1996).

One absorbance unit in wave length of 260 nm is $50 \mu\text{g mL}^{-1}$ of double strand DNA in 1 cm^3 quartz cuvette. Ratio of 260-280 nm reveals the purity of the DNA. If this ratio is between 1.8 and 2, the purity of DNA is suitable where this ratio is >2 this shows RNA contamination. The ratio of 260-280 nm <1.8 means the DNA is contaminated with protein or phenol (Eeles and Stamps, 1994).

The aim of this study was to develop a simple method to extract DNA from mammalian blood samples.

MATERIALS AND METHODS

Preparation of white blood cells: Venous blood was taken from mixed-age animals (20 cattle, 40 sheep, 20 goats) in fractions of 12 mL tubes, containing EDTA. White blood cells were collected from whole blood following lyses of the red blood cells within an equal volume of sterile ACT solution (7.47 g of ammonium chloride + 2.06 g of tris, added D.D.W to 1000 mL; pH = 7.2).

The solution was gently shaken and the red blood cells lyses showed a change in the color of the solution from blood red to a dark clear red. Five min after the addition of ACT solution white cells were collected by spinning at an rpm of 4000 for 5 min. Supernatant solution was discarded and pelleted white blood cells were resuspended by adding of 5 mL of PBS solution (8 g of NaCl + 0.2 g of KCL + 1.44 g of Na₂HPO₄ + 0.24 g of KH₂PO₄ added D.D.W to 1000 mL; pH = 7.4) and then spun 2 or 3 times at an rpm of 4000 for additional 5 min.

Extraction of DNA: Lyses buffer (5 mL) containing of 100 mM of trisHCl, pH = 8.5 + 0.5 M of EDTA + 10% SDS + 5 M of NaCl 40 mL added D.D.W. to 1000 mL is added to the white cell solution. Proteinase K (200 µg) is added to the solution. The tubes were incubated at 50°C in a shaker incubator for overnight for digestion. Adding more proteinase K to the solution can reduced the duration of digestion to several hours).

After digestion, one volume of ethanol (-20°C) is added to the lysate and the samples are mixed or swirled until precipitation is completed (20-30 min, until it become completely transparent).

The DNA is recovered by lifting the aggregated precipitate from the solution, using a disposable tip. Excess liquid is dabbed off and the DNA is dispersed in a pre-labeled eppendorf tube, (depending on the size of the precipitate containing 500 µL of TE (10 mM of tris HCl + 0.1 mM of EDTA, pH 7.5).

The DNA was washed in TE solution and 500 µL of TE is added, complete dissolution of the DNA requires several hours of agitation at 37°C or preferably overnight. It is important that the DNA is completely dissolved to ensure reproducible removal of aliquots for analysis.

Analysis of DNA: Concentration of the DNA measured in a scanning spectrophotometer. The DNA was diluted with TE solution by a 1:1000 dilution. The ratio of 260/280 was calculated. Extracted DNA samples were amplified for PCR amplification. Each reaction mixture (25 µL) contained 100 ng of DNA template, 3 mM MgCl₂, 0.4 µM each primer (forward: 5'-CCAGAGGACAATAGCAAAGCAA-3',

reverse: 5'CAAGATGTTTTTCATGCCTCATCAACAGGTC-3', according to Davis *et al.* (2002), 400 M of each dNTP, 2.5 U of *Taq* DNA polymerase, 50 mM KCl and 20 mM tris-HCl, pH 8.4. The PCR reactions were carried out in a thermocycler (eppendorf Germany) under the following conditions: a denaturation step at 94°C for 3 min followed by 30 cycles of 94°C for 45 sec, 55°C for 45 sec and 72°C for 2 min. A final extension step at 72°C for 10 min was performed.

The PCR products were electrophoresed in a 2% agarose gel, stained with ethidium bromide. The resulting DNA fragments were visualized by UV transillumination.

RESULTS

The extraction of DNA following SDS-proteinase K digestion of animal blood cells by the new modified method, yielded a high molecular weight DNA as determined by agarose gel electrophoresis (Fig. 1). The mean yield of DNA from 5 mL of whole blood was 358/58±52.

This method has been routinely used in the laboratory and the DNA extracted by this method can be amplified with wide variety of primers. The ratios of optical density at 260 and 280 nm are between 1.8 and 2.0 indicating deproteinisation. The optical density of 76.25% of the samples (61 out of 80) was 1.8-2.0, while 12.5% (10 out of 80) had OD of <1.8 and 11.25% (9 out of 80) of samples indicated OD of >2 (Table 1).

Table 1: The ratio of 260/280 OD of DNA samples from sheep, goat and cattle

Ratio of 260/280 OD	Sample	(%)
1/8-2	61	76.25
>2	9	11.25
<1.8	10	12.50
Total	80	-

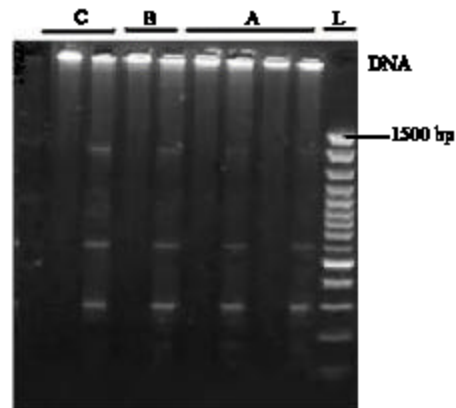


Fig. 1: Agarose gel of 8 undigested samples, a): Sheep, b): Goat and c): Cattle

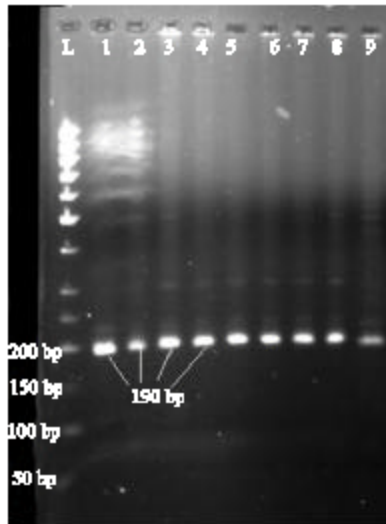


Fig. 2: Agarose gel of the FecB gene in sheep

The DNA extracted from sheep samples were amplified in a PCR reaction using primers from FecB gene after which sharp bands produced after electrophoresis (Fig. 2).

DISCUSSION

DNA must be extracted from large numbers of samples for genetic linkage and diagnostic tests for desirable genes or parentage determination (Montgomery and Sise, 1990). Whole blood provides a convenient source of nucleated cells for extraction of genomic DNA (Montgomery and Sise, 1990).

Simple procedure with fewer steps are superior to commercial kits (Schiffner *et al.*, 2005). Extraction of digested sample with phenol produces a good yield of high molecular weight DNA, suitable for analysis of genomic DNA (Montgomery and Sise, 1990; Adell and Ogborna, 1990). However, the phenol extraction steps are time-consuming and limit the number of samples that can be processed in a day. Furthermore, phenol is toxic and corrosive. The high salt method is a suitable alternative to phenol extraction (Montgomery and Sise, 1990). The method that involved the fewest manipulations and thus, the least potential for sample loss was a simple detergent lysis buffer containing SDS with proteinase K, which produced superior results than procedures requiring multiple washing steps, tube transfers, or adherence to matrices. For example, some DNA may irreversibly bind to a matrix and although this loss is inconsequential for high copy number DNA samples, this loss, albeit minute, is crucial. The SDS does

not inhibit amplification and increases the efficacy of proteinase K (Schiffner *et al.*, 2005). In the salt method a large centrifuge is required repeatedly to spin the volume of lyses buffer. This modified method is simple, quick, reliable and provides a yield of DNA that is at least equivalent to the salt and phenol extraction method. Commercial kits were not suited for the isolation of DNA from samples containing only a few cells. The other non-commercial methods often involved many handling steps such as transferring to new tubes or adsorption of DNA to matrices, which may have lead to a loss in DNA (Schiffner *et al.*, 2005). Analysis of the samples has demonstrated that the DNA is of high molecular weight. Amplifying of the sheep DNA samples has shown that the samples are suitable for routine PCR.

CONCLUSION

The advantages of this modified method are as follows:

- This method is fast and requires neither hazardous chemicals nor special devices
- The result demonstrates that extracting DNA using this method increases the success rate of PCR amplification
- This method does not involve numerous enzymes or multiple steps, which increases the possibility of contamination and DNA degeneration

This method is suitable for PCR-RFLP, single nucleotide polymorphism and most applications without any need for sample enrichment.

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