PROTOCOL FOR ISOLATION OF GENOMIC DNA FROM LIVESTOCK BLOOD FOR MICROSATELLITE ANALYSIS*


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ABSTRACT

Isolation of pure high molecular weight genomic DNA is a pre-requisite in microsatellite studies. The phenol-chloroform method of DNA extraction from whole blood usually involves several steps which are time consuming. Further the phenol is corrosive and toxic. A simple procedure called High salt method was further optimized for isolation of high molecular weight genomic DNA from much lesser quantity of buffalo blood i.e. only from 200 µl, which helped in avoiding wastage of blood sample and also saving costly chemicals. The optimized High salt method produced good yield of pure high molecular weight genomic DNA.

INTRODUCTION

Satellite DNA is a broad term to describe genetic material which includes the telomeres, minisatellites and the microsatellites. Shorter repetitive DNA sequence having repeats of 15 or more bases were termed as minisatellites (Jeffreys et al., 1985). In the late 1980s, even shorter repeats of satellites of two to six bases were isolated and named microsatellites (Litt and Luty, 1989).

The microsatellite is a sequence of varying number of short tandemly repeated segments of 1-5 base pairs at a unique physical location in the genome, which varies among individuals sufficiently to have its pattern of inheritance tracked through families. Microsatellite markers have been successfully utilized to determine the genetic variation between various cattle breeds (Moazami Goudarzi et al., 1997; Martin-Barriel et al., 1999). Microsatellite loci have been successfully also used to assess the phylogenetic relationships in the tribe bovini (Ritz et al., 2000) and clarifying the evolutionary relationship of closely related populations (Takezaki and Nei, 1996).

The phenol-chloroform extraction method for DNA extraction from whole blood involves several steps. (Blin and Stafford, 1976; Andersson et al., 1986; Viaman et al., 1986). Further Phenol is corrosive and toxic. These methods required larger quantities of blood sample and costly chemicals with modern facilities. Montgomery and Sise (1990) tested a simple method called high salt method for the extraction of DNA from large volume of (15 ml) sheep blood, which eliminated phenol-chloroform extraction steps. Hence, a simple and faster method, by Senthil et al. (1996) was further standardized using extremely lower quantity of blood (200 µl only) to enable saving of costly chemicals. The method is feasible for ordinary laboratory.

MATERIAL AND METHODS

Optimized method : Blood (200 µl ) Collected initially containing anticoagulant sodium heparine, was taken in a 1.5 ml capacity microcentrifuge tube, mixed thoroughly and kept in frozen condition till isolation of genomic DNA. The steps detailed here were to be carried out at room temperature.

1. Heparinized venous blood sample (200 µl) was centrifuged at 6000 rpm for 10 min.
2. Discarded the plasma by leaving RBCs and WBCs.
3. For lysis of RBCs, added 500 µl of ice-cold RBC lysis buffer (0.17 M NH4Cl) and kept it in ice for 5 to 10 min.
4. Spin down the leucocytes at 4000 rpm for 15 min.

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Discarded the supernatant containing lysed RBCs.

Again added 300 µl of ice-cold RBC lysis buffer in the microcentrifuge tube, containing leucocytes and keep it in ice for 5 min for complete lysis of RBCs.

Spin down the leucocytes at 4000 rpm for 15 min and discarded the supernatant containing lysed RBCs.

Later on added 200 µl of TE buffer (10 mM Tris HCl, 0.1 mM EDTA, pH 8.0) to the tube containing spined down leucocytes.

Resuspended the pellet by vigorous vortexing.

Added 2 µl of proteinase K (10 mg/ml), 7 µl of 0.5 M EDTA (pH 8.0) and 7µl of 20 % SDS mix it well by vigorous vortexing.

Incubated the vortexed sample at 50°C in a water bath for 3 hours with occasional shaking, which helped in the digestion of sample.

To the digested sample, added 65 µl of saturated NaCl solution, mixed it well by vortexing and spin it at 5000 rpm for 15 min at room temperature.

Transferred the supernatant to a sterile microcentrifuge tube and added 25 to 30 µl of 3 M sodium acetate (pH 5.5).

Added 300 to 400 µl of 95 % ice cold ethanol and spin down the pellet by centrifuging it at 5000 rpm for 15 min at room temperature. Discarded the ethanol by leaving DNA pellet attached to tube wall.

Finally dissolved the DNA pellet in 50 µl of TE buffer (pH 8.0) or sterile water and store it at 4°C (for long life storage it should be stored at -20°C).

RESULTS AND DISCUSSION

The purity and quantity of DNA was checked by Agarose gel electrophoresis (Fig. 1). Also the DNA samples exhibited the ratios of optical density between 1.70 to 1.90 at 260 and 280 nm indicating good deprotenization (Senthil et al., 1996 and Yadav et al., 2003). The procedure yields 150 ± 25 µg of DNA from 200 µl of blood, which was sufficient for microsatellite studies. This rapid procedure was tried to extract DNA in cattle, buffaloes, goats, and sheep also.
The DNA obtained was of good quality and suitability of it for microsatellite studies was confirmed by resolving the optimized PCR product (Fig. 2) on urea PAGE-gel (Fig. 3). All the steps were carried out using microcentrifuge machine and required 6 to 6.5 hours of time. In conclusion, this procedure appears to be simple and inexpensive for microsatellite studies in Indian laboratories.

ACKNOWLEDGEMENT

The authors are grateful to Dr. B.R. Yadav, (NDRI, Karnal) and Dr. D.K. Sadana (NBAGR, Karnal) for their technical guidance, to Dr. Rathod, Dr. Kashid and Dr. Deepak Kale (NDRI, Karnal), and Mr. Ulhas Kadam (National Grape Research Station, Pune) for their skillful help.

REFERENCES