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Standardization of DNA Extraction from Methanol Acetic Acid Fixed Cytogenetic Cells of Cattle and Buffalo

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Abstract: The aim of the study is to standardize the simple method for extracting DNA from cells fixed in fixative (3:1 ratio of methanol and acetic acid glacial) mostly used for chromosomal studies in cattle and buffaloes. These fixed cells were stored for more than 6 months at refrigerated temperature. The fixed cells were washed 2-3 times by the ice cold 1x Phosphate Buffer Saline (PBS) with pH 7.4, so that effect of fixative may be eliminated. The genomic DNA was extracted by adding cell lysis and nucleus lysis buffers. The quality and quantity of DNA were estimated. The readings of nano drop and agarose gel electrophoresis indicate good quality DNA isolated with a rapid and simple protocol routinely using in our laboratory. The method enables us to study the DNA of a cattle and buffaloes after completing cytogenetic investigation or in cases where DNA samples are otherwise not available. This protocol may be useful for molecular analysis of DNA from fixed cells palettes.

Key words: Genomic DNA, fixed cells, cytogenetics, fixative

INTRODUCTION

DNA extraction from various cells remains a challenge for the scientific communities for the studies at molecular levels. Efforts in past have developed various standard procedures to extract DNA from various sources and different species. Hence, DNA can successfully be extracted from fixed cytogenetic preparations and long-term refrigerated bone marrow specimens (MacKinnon *et al*., 2012), fixed cytogenetic cell suspensions (Barker *et al*., 1986; Nishigaki *et al*., 1988; Amorim *et al*., 2007), frozen and formalin fixed tissue section (Serth *et al*., 2000), Cladosporioid fungi (Moslem *et al*., 2010), cells fixed in Carnoy's fixative (Barrios *et al*., 1992), medicinal plants (Mohd-Hairul *et al*., 2011) etc. The efforts are being carried out to extract the DNA from different sources and species. The DNA extracted from various sources including fixed cytogenetic preparations have been utilized for molecular techniques like Polymerase Chain Reaction (PCR) yielded good PCR product to further analysis (Li *et al*., 1995; O'Leary *et al*., 1994; MacKinnon *et al*., 2012; Jonveaux, 1991). The aim of present studies is to standardize a procedure for DNA extraction from small volumes of fixed cell suspensions (3:1 Methanol:glacial Acetic acid) stored for 2-3 months duration at refrigerated temperature, previously prepared for conventional cytogenetic analysis in cattle and buffaloes.

MATERIALS AND METHODS

Collection of fixed cells: Peripheral blood cell pallets obtained from 5 normal cattle and 5 buffaloes were considered for the present study. They were previously submitted for Karyotyping. The cell pallets were simply stored at refrigerator for more than 6 months.

Washing of fixed cells: Cell suspensions were initially centrifuged at 5000 rpm for 3-5 min and discarded supernatant and again centrifuged at 5000-7000 rpm for 5 min at room temperature. The supernatant (methanol, acetic acid) was discarded and cells were resuspended in 400 μL of ice cold 1x Phosphate Buffer Saline (PBS) with pH 7.4. This washing step was repeated 2-3 times in order to remove methanol and acetic acid (Amorim *et al*., 2007).

DNA isolation: The cell lysis buffer containing 10 mM Tris, 10 mM KCl and 10 mM $MgCl_2$ with Triton X-100 in a ratio of 1000:30 μL was added to the cell palettes. It was mixed gently, incubated at room temperature for 5 min and finally centrifuged at 10000 rpm for 10 min. The supernatant was discarded and pellets were resuspended in 320 μL of nucleus lysis buffer containing 1 M Tris, 1 M KCl, 1 M $MgCl_2$, 5 M NaCl, 20% Sodium Lauryl Sulphate (SDS) and 0.5 M EDTA and mixed properly by pipette. The equal volume of phenol: chloroform: isoamyl alcohol (24:24:1) was added, mixed gently and centrifuged at

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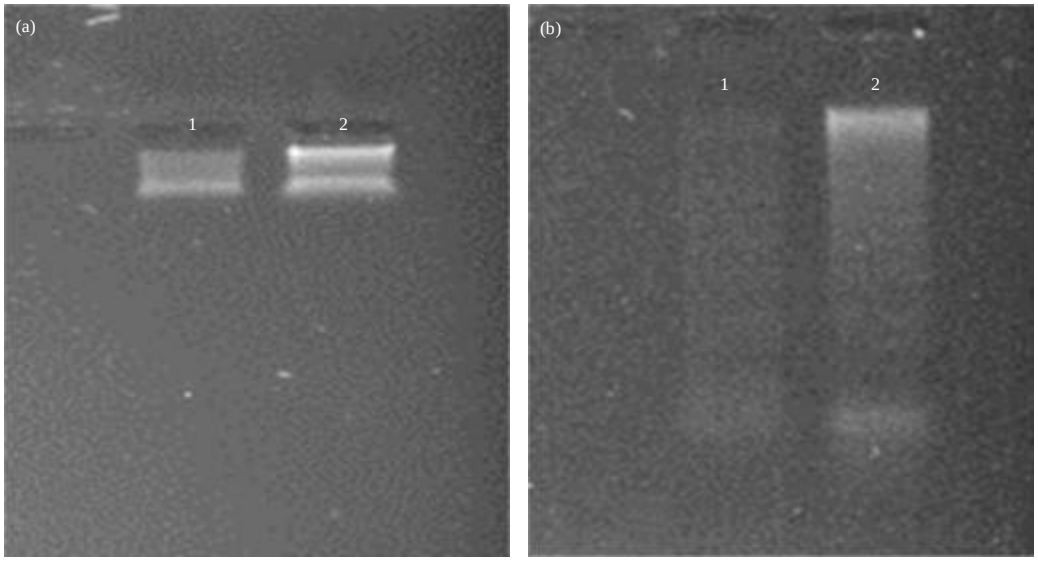

Fig. 1(a-b): Genomic DNA on 0.8% agarose gel extracted from fixed cells (a) Gel run time 15 min and (b) Gel run time 50 min. Line 1: Cattle DNA and Line 2: Buffalo DNA, respectively

11000 rpm for 10 min. After centrifugation, an aqueous phase was collected and transferred in a clean Eppendorf tube. A double volume of chilled absolute alcohol was added in eppendrof tube which was mixed gently and kept at -80°C for 10 min, followed by centrifuge at 11000 rpm for 10 min. Removed supernatant and washed DNA pellet with 70% alcohol by centrifugation at 11000 rpm for 10 min 2-3 times to remove alcohol and residuals. DNA pellet was dried at room temperature, suspended in 1xTE buffer (pH 8), mixed it till DNA completely dissolved, which can be kept at 4 or -20°C for storage.

Assessment of DNA: The quality and quantity of DNA was evaluated by nano drop 2000 (Thermo Scientific) and an electrophoresis gel stained with ethidium bromide.

RESULTS AND DISCUSSION

The nano drop readings indicate the quantity of DNA extracted from fixed cell palettes was 16.16 and 43.5 μg μL^{-1} in cattle and buffalo respectively. The quality of DNA at 260/280 OD was estimated to be 1.8 and 1.9 in cattle and buffalo respectively. The higher molecular weight DNA was obtained in case of buffalo as compare to cattle. The quality and quantity was also visualized on 0.8% agarose gel (Fig. 1). The DNA quality could have been much better if the fixed cell pallets are stored at -20°C instead of refrigerated temperature as fixed cell's DNA is not degraded at low temperature. Because of quality and quantity, the isolated genomic DNA may be used for genetic diseases diagnosis and genotyping any trait in cattle and buffalo in the absence of DNA extracted from blood, semen, body tissue etc.

MacKinnon *et al.* (2012) extracted DNA from the fixed cytogenetic preparations and long-term refrigerated bone marrow specimens and used for SNP array. Once the genomic DNA is isolated from any source of material, the same may be utilized for PCR (O'Leary *et al.*, 1994; Li *et al.*, 1995). Although, blood is the traditional source of genomic DNA, however, cell suspensions can also be used as an alternate source of DNA for genetic studies. The method enables us to study the DNA of cattle and buffaloes after completing cytogenetic investigation or in cases where DNA samples are otherwise not available.

The method was successfully developed to isolate genomic DNA from acetic acid and methanol fixed cells which are otherwise used for chromosomal studies in cattle and buffaloes.

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