Original Article

A New Method for DNA Isolation from Bone Marrow & Sperms of White Mice

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Summary:

Background: A quick and easy method was developed for extraction of DNA of eukaryotes from different samples, which are bone marrow and sperms in white mice Mus musculus strain (Balb/c).

Patients and Methods: this method using high salt buffer, Ethylene diemine tetracetec acid (EDTA), Trypsine,Sodium Dodecyl Sulfate(SDS), and urea without using Proteinase-K digestion or ultracentrifugation.

Results: This method was successful in extracting DNA from different samples in eukaryotic and this DNA is suitable for Hind III digestion.

Conclusion: Without further clean-up, the extracted DNA can be used for restriction endonuclease digestion or for numerous applications.

Key words: DNA, Eukaryotes, extraction, Mice, bone marrow, Sperm, Hind III digestion.

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Introduction:

DNA isolation involves extracting the DNA molecule from the cell in clean form. In prokaryotes, the DNA is a naked (protein-free) molecule that lies in a tightly super coiled mass in the cytoplasm and is not surrounded by a membrane. Extraction can be effected easily by bursting the cell and eliminating all non-DNA matter from the lysed cell. Prokaryotes possess a special carbohydrate –cum-protein cell wall around each cell. This has to be first degraded by an enzyme. Lysozyme has used for this purpose (1). DNA (or genomic) is in chromosomes within a nucleus in eukaryotes. Several different procedures have been developed over the past years for the isolation of DNA free from, such as used cesium chloride equilibrium density centrifugation , lithium chloride precipitation followed by a chromatography on glass powder column ,chromatography through Sepharose columns CL-4B and Sephacryl S-300 or S-100 or Ultrogel A2(2). Other methods involve chromatography or hydroxyapatite columns or precipitation with calcium chloride (3). Here we describe a new method for isolation of DNA from eukaryotes, different samples such as bone marrow & sperms. This method is a simple and quick without using Protinase-K or use Ultracentrifuge. The DNA which extraction by this method can be used for genetic analysis and cloning studies, the extracted DNA is suitable for restriction endonuclease digestion and for laboratories routine applications.

Materials and Methods:

Animals: The experimental animals were Balb/c albino Swiss mice strain *Mus musculus* (from the colony of the medical college, Baghdad university) with age (8-12) weeks and weighing (25±2**)** weeks and weighing (25±2) gm. They had sacrificed by cervical dislocation

And taking:

A) The DNA from femur according to (4) and the bone marrow was extraction by syringe by injection 5ml of PBS buffer with 37c^{o}. The suspensions had centrifuged at 2000 rpm for 10 min; this process has Done two times.

B) The DNA from sperms according to(4),the sperms were extraction from the epididymis with a scissor to small pieces and put it in Petri dish with normal saline, the suspensions were centrifuged at 2000 rpm for 10 min.

Reagents: General laboratory chemicals were from Sigma Chemical Co., St. Louis, M.O. and BDH, Darmstadt, F.R.C. Restriction endonucleases were from BRL. Life Technologies, Inc. Gaithersburg, MD 20877 USA. All reagents were prepared as buffers solutions and sterilized by autoclaving or filtration.

I. Buffer A: Dissolve (8) gm of NACL, (0.2)gm of KCL and (3)gm of Tris-base in(800)ml of distilled water and then added (0.15)gm of phenol red at PH(7.4) (equilibrated by(1)mol/L HCL), Finally the volume became(1)Liter by distilled water.

II. Buffer B: Containing (0.01) mol/L of EDTA (PH=8) and (1%) of Trypsine.

III. Buffer C: Containing (10) m mol/L of Tris-HCL (PH=7.5), (0.01) mol/L of EDTA (PH=8), (300) m mol/L of Sucrose and (10) m mol/L of Triton-X100 (in Liter).

IV. Buffer D: Containing (0.1) m mol/L of Tris –HCL (PH 7.5), (0.01) mol/L of EDTA (PH=8), (8) mol of Urea and (10) gm of SDS (Sodium dodecyl Sulphate) in Liter.

V. TE (PH=8): Containing (10) m mol/L of Tris-HCL (PH=8) and (1) m mol/L of EDTA (PH=8).

DNA extraction: Mixed (2) ml of buffer (A) to the sediment of Samples (bone marrow and sperms) in

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centrifuge tube, the mixture was incubated on ice for (15) min and then was centrifuged at (4000) r pm for (10) min; the solution was resuspended in (5-10) volume of cold buffer (A), this step has been done two time.The sediment were re suspended in (5) ml of TE buffer and mixed the sediment with (1) ml of (B) buffer vortexing and incubating for (1) hr in (37) c °.
Add (2) volume of (c) buffer,then the solution was mixed by inversion and centrifuged at (14000) rpm for (15) min. Resuspended the sediment in (2) ml of (D) buffer and add (1) volume of phenol (equilibrated by (0.01) mol/L of Tris-HCL (PH=7.6) then centrifuged at (10000) rpm for (15) min, the aqueous phase was collected and extracted again with an equal volume of phenol / chloroform / isoamyl alcohol\ (25/24/1 by vol).

4- DNA Purification: The tubes were incubated on ice for (15) min, then ammonium acetate (3mol/L) added to the crude DNA sample to give a final concentration of (2.5) mol/L and mixed the solution well then added two and half volume of cold absolute ethanol and incubated in (4) c° for (24) hr. After centrifugation (14000) rpm for (15) min,
The pellet was resuspended in (1) ml of 70% ethanol, recentrifuged, and the pellet briefly vacuum dried. The pellet was re suspended in (50) µL of TE buffer.

5-Agarose gel electrophoresis:

DNA samples were loaded in electrophoresis on a 0.8% agarose (5) gel prepared in TBE (50 m mol/L of Tris –OH, 50 m mol/L boric acid, 1m mol/L EDTA). The gels were examined under UV light (302 nm) and photographs were taken.

6-Restriction endonuclease digestion:

Ten microliters of DNA extract (from different samples) was digested with Hind III (4U/µL) for 1hr at (37) c°, this enzyme was used because was on hand. The DNA digest had analyzed by agarose gel electrophoresis.

Results:

The result of DNA extraction had shown in Fig-1-.As had seen, the procedure was able to extract DNA from bone marrow and sperms in mammalian. The amount of RNA contamination was minimal as judged by the ethidium bromide-staining pattern observed on the agrose gels.
The restriction endonuclease digestion of DNA extracted with Hind III in bone marrow and sperms are shown in Fig-2-.The digestion were done in both samples with DNA obtained from a single preparation. The figure shows that the enzyme Hind III was able to Digest the DNA when compared to the other lanes

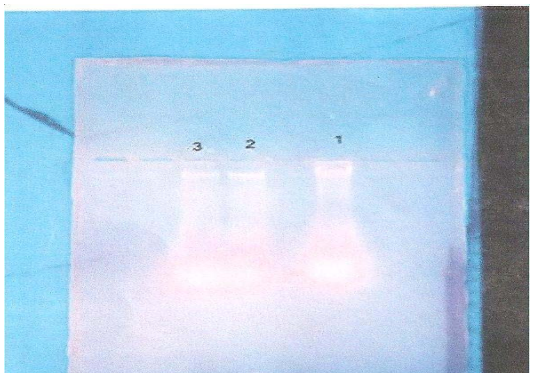

Fig-1- Agarose gel electrophoresis of DNA extracted from bone marrow and sperms in mice.
Lane-1- the DNA extracted with using Protinase-K in bone marrow in mice.
Lane-2- the DNA extracted by this procedure in bone marrow.
Lane-3- the DNA extracted by this procedure in sperms.

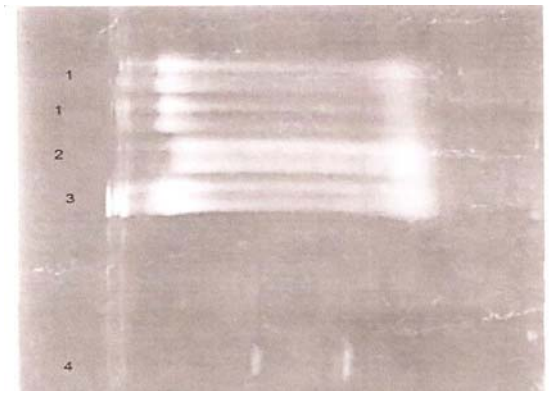

Fig-2- Agarose gel electrophoresis of Hind III digested DNA from
Bone marrow and sperms.
Lane-1- the DNA extracted by this procedure in bone marrow in mice.
Lane-2- the DNA extracted by this procedure in sperms.
Lane-3- the Hind III digested DNA from bone marrow.
Lane-4- the Hind III digested DNA from sperms.

Discussion:

The procedure presented has based on using different materials to isolated DNA from bone marrow and sperms in mice without using Proteinase-K digestion or ultracentrifugation, in this procedure had used EDTA (6) which chelating agent can draw the (Mg+2) ions from the cellular membrane when using Trypsine with EDTA lyses the cells and destroy components. Sodium dodecyl sulphate (SDS) and urea (7) which is denature the proteins in other hand using of phenol and

chlorophorm to discard the destroyed proteins. This method was successful in extracting DNA from different samples in eukaryotic and this DNA are suitable for Hind III digestion. The amount of RNA contamination, as judged by the location and intensity of the fluorescence observed in ethidium bromide stained agarose gel, varied from extracted to extracted, But appeared low. In summary, this procedure provides a rapid and safe method to extract DNA from a variety of samples. The extracted DNA is suitable for restriction endonucleasa digestion or for numerous applications.

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