

A REPORT OF RAPID AND EFFICIENT METHOD FOR MITOCHONDRIAL DNA PURIFICATION FROM HUMAN PLATELETS

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ABSTRACT

Based on the similarity of prokaryotic DNA to that of mitochondrial DNA, a rapid and efficient method for DNA purification from mitochondrial extracts obtained from different sources, was carried out based on the modification method described by Marmur (1983) which resulted in a high degree of purity ($A_{260}/A_{280}=2$). In addition we have applied the same technique for the first time to purify the mitochondrial DNA of platelets.

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INTRODUCTION

Since human mitochondrial DNA (mt DNA) is the most known part of the genome in multicellular animals, the understanding of genetic complications through the use of restriction methods could result in a better understanding of the molecular basis of inherited diseases and their treatment. For instance mitochondrial myopathies are a group of complications that manifest themselves in skeletal muscles which in some cases result in CNS disorders. The disease is inherited maternally and caused by mitochondrial DNA mutations.¹ Many techniques are available for purification of mtDNA,^{2,3} some of them are long and time consuming.^{2,3} The present study portrays a simple and rapid method for the preparation of mtDNA with better or comparable results to other sources studied.

MATERIALS AND METHODS

Tris, succinic acid, SDS, EDTA and sodium perchlorate were purchased from Sigma (Germany), isoamylalcohol, ethanol, chloroform, sucrose, sodium citrate, potassium acetate glucose and isopropanol were purchased from Merck (Germany). High speed Beckman centrifuge Model J-21B and ultracentrifuge Beckman model L5-50 and Savant vertical disc elec-

trophoresis model DP-50 were used throughout the experiments. To obtain mtDNA of platelets, we first performed the Marmur technique (prokaryotic DNA purification) on the mitochondrial fraction of fresh calf heart. Briefly the above mitochondrial fraction was performed as follows: fresh calf heart was obtained from a local slaughter house and cut into pieces, grounded and homogenized in sucrose tris buffer pH=7.5. The homogenate was then centrifuged at X2000g for 20 minutes. The pellets consisted of cell debris and cell nuclei. After removal of lipid granules from the supernatant by filtration through a cheese-cloth, the supernatant was centrifuged for 15 minutes at X26000g. The precipitate contained mitochondria.⁵ It was further tested for purification by phase contrast microscope. The Marmur method for DNA extraction was applied with the exception that lysosome was not employed in the lysis stage and for removal of RNAs, RNase A was added in the first suspending medium. The mitochondrial fraction thus obtained, was suspended in a solution containing 0.15M, NaCl, 0.1M EDTA and 20 mg RNase A in a ratio of 2.5ml per 10 gr of the original tissue. 2ml of 25% SDS solution was then added and mixed gently. The mixture was incubated for 10 minutes at 60°C, and then one volume of chloroform/isoamyl alcohol in a ratio of 24:1 was added subsequently and mixed gently for 15 minutes on a rotating mixer. The resulting mixture was centrifuged at 10,000 g for 10 minutes. The supernatant (containing

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nucleic acids) was then mixed with two volumes of absolute ethanol. The resulting turbidity (ethanol precipitate) was complete when kept at -70°C for at least 1/2 hour. This mixture was then centrifuged at $\times 10,000g$ for 15 minutes. The DNA precipitate was then collected and resuspended in TE buffer (0.01 M Tris + 0.001 M EDTA), pH=8.0.

mtDNA Extraction From Platelets:

The isolation and collection of platelets from human blood were carried out according to the standard methods. The lysis of platelets was performed with alkaline EDTA solution according to the method of Shuster et al.⁷ The lysate was then homogenized in a suspension media recommended by Marmur.⁴ The remaining stages of purification were exactly as mentioned above. The DNA thus obtained was tested for purity using 1% agarose gel electrophoresis and the buffer used contained tris-phosphate 90mM, EDTA 2mM, pH=8 in constant voltage of 120 volts for one hour.⁸ The gels were then stained by 2 $\mu\text{g}/\text{ml}$ water solution of ethidium bromide and read at 336 nm.

RESULTS

The yield and the degree of DNA purification as determined by the spectrophotometric method for mitochondrial DNA from calf heart and human platelets was $A_{260}/A_{280}=2$. The electrophoretic results showed a distinct, sharp band for all the preparations. The electrophoretic movement of mtDNA, in an electric field was directly proportional to their molecular weights.

DISCUSSION

The main difficulty in DNA purification is deproteinization of the samples due to the lability of the DNA structure under the conditions in the study. In nuDNA, the best ratio obtained to date was $A_{260}/A_{280}=1.8$ (an index of DNA purification). In prokaryotic DNA the best ratio obtained was $A_{260}/A_{280}=2$. Due to the close similarity between bacterial and mitochondrial struc-

ture, we employed the method of prokaryotic DNA purification for mtDNA and obtained the highest degree of purity yet ($A_{260}/A_{280}=2$). We believe that by using the most moderate experimental conditions for the extraction of mt-DNA, i.e. pH, temperature and mechanical stress, the procedure guarantees to a great extent the intactness of DNA during its purification. In addition its preference to the previous methods could be assigned to its simplicity and the rapidity of the method. It is worthwhile to mention that in the purification steps of the mitochondrial genome, extreme care should be taken to avoid cross contamination of nuDNA with mt-DNA. This problem is usually overcome by separating the mitochondria from the source before the purification of mt-DNA. The main problem in mtDNA extraction from human sources is the small sample size obtained in biopsies and nuclear contamination. In addition the biopsy itself is tedious and time consuming therefore in some instances the samples are preferred to be obtained from placenta. However blood is the most convenient sample for mt DNA extraction. But there is still the fear of nuclear contamination. According to the above facts, the platelets derived from megakaryocytes were thought to be the most suitable source since they did not contain the nucleus. So far mt DNA extraction from platelets, which has been presented in this paper, has not yet been reported to the best of our knowledge. Most of the studies of human mt-DNA extractions were either on placenta, of biopsies derived from different organs, or total genomes (nuclear and mitochondrial) from whole blood. These mt-genomes could be identified by specific probes. We hope that in our future investigations we could use this rapid technique for restriction studies on abnormal mitochondrial DNA, and obtain a definite and correct map for the nucleotide sequences.

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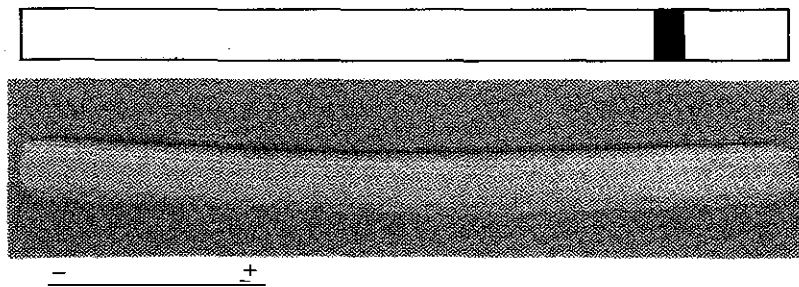


Fig. 1. 1% Agarose gel electrophoresis of mtDNA isolated according to our method.

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