Rapid isolation of animal mitochondrial DNA by alkaline extraction

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A simple technique for rapid isolation of mitochondrial DNA (mtDNA) from animal cells is described. The method is based on the selective alkaline denaturation procedure of Birnboim and Doly [(1979) Nucleic Acids Res. 7, 1513–1523] and avoids the use of CsCl gradient centrifugation. The yield of mtDNA is comparable to that obtained by standard techniques. This DNA is sufficiently pure for restriction analysis and cloning of mtDNA fragments.

mtDNA isolation *Rat mitochondria* *Restriction analysis* *Cloning*

1. INTRODUCTION

Structural analysis of mitochondrial DNA (mtDNA) by restriction endonuclease digestion and agarose gel electrophoresis has proven to be of wide importance for the assessment of genetic relatedness in systematic and population genetic studies [2,3]. Furthermore, this type of genomic analysis has wide applications in animal breeding and husbandry, e.g. for identification and monitoring of animal stocks. The standard methods for isolation of mtDNA are relatively tedious usually including a CsCl density centrifugation step [2] and thus impose limitations on the number of samples to be analyzed. Therefore simpler techniques for mtDNA isolation are a prerequisite for the utilization of restriction analysis of mtDNA as a routine diagnostic method.

All animal mtDNAs studied so far are strikingly uniform in size and structure [4,5]. They are covalently closed circular molecules of 15–19 kb and thus resemble many bacterial plasmids in structure. Because of this similarity we reasoned that rapid DNA extraction methods developed for the isolation of bacterial plasmids could also be applicable to mtDNA isolation. Here we describe the employment of the selective extraction procedure of Birnboim and Doly [1] for isolation of mtDNA from rat liver. The method yields mtDNA that is sufficiently pure for both restriction analysis and cloning of mtDNA fragments.

2. MATERIALS AND METHODS

2.1. *Animals*

Male rats (*Rattus norvegicus*) of the strain Wistar/Hannover were obtained from the National Laboratory Animal Center, University of Kuopio, Finland.

2.2. *Isolation of mitochondria*

Crude mitochondrial preparations were obtained from homogenized rat livers by differential centrifugation [3]. Nuclei and cell debris were removed by low-speed centrifugations (6 min at 700 × *g*). Mitochondria were thereafter pelleted (10 min at 10000 × *g*) and the pellet washed 3

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times. This crude mitochondrial pellet was directly subjected to DNA isolation, without any further treatment.

2.3. *Isolation of mtDNA*

The procedure for mtDNA isolation essentially follows that of Birnboim and Doly [1] developed for rapid isolation of plasmid DNA from bacteria. Mitochondria from 1–2 g tissue were resuspended in 200 μl buffer containing 50 mM glucose, 10 mM EDTA and 25 mM Tris-HCl, pH 8.0, on ice. To lyse the mitochondria and denature the contaminating nuclear DNA, 400 μl alkaline SDS solution (0.2 M NaOH, 1% SDS) was added and the suspension thoroughly mixed. After 5 min incubation on ice 300 μl of 3 M potassium acetate was added, and the contents of the tube gently mixed. The tube was maintained at $-70°C$ for 2 min and centrifuged for 10 min at $10000 \times g$ in a microcentrifuge, 750 μl of the clear supernatant was removed and the mtDNA precipitated by adding 450 μl isopropanol and holding the tube for 5 min at $-70°C$. The precipitate was collected by centrifugation, washed with 70% ethanol and dried under vacuum. This mtDNA pellet was then resuspended in 100 μl of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, treated with DNase-free RNase, and phenol extracted twice. The residual phenol was removed by ether and the DNA reprecipitated with ethanol in the presence of 150 mM NaCl. The precipitate was dried under vacuum and resuspended in 100 μl of 10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA.

2.4. *Restriction endonuclease analysis*

Restriction analysis was performed using 1–5 units enzyme/100 ng mtDNA, samples were incubated for 2–4 h at 37°C and restriction fragments separated by electrophoresis in 0.8 or 1.5% agarose slab gels. EtBr was included in the gels and running buffer for staining.

2.5. *End labelling of restriction fragments*

The products of restriction digestion were alternatively end labelled with [α-^{35}S]dATP according to Drouin [6]. In this case staining with EtBr was omitted. The dried gels were exposed to X-ray film.

2.6. *Cloning of mtDNA fragments*

Restriction endonuclease *Bam*HI-digested mtDNA was mixed with similarly treated pBR322. This mixture was ligated with T_4 ligase and transformed to *Escherichia coli* HB101 cells made competent with $CaCl_2$ [7]. Transformants were selected for ampicillin (Ap) resistance and screened for the presence of mtDNA inserts by their sensitivity to tetracycline (Tc); inserts into the *Bam*HI restriction site of pBR322 inactivate the tetracycline resistance marker. Plasmid DNA was isolated as described [1] and restriction analysis performed as above.

3. RESULTS AND DISCUSSION

3.1. *Isolation and restriction analysis of rat mtDNA*

To test the utility of the alkaline extraction procedure for isolation of animal mtDNA, we attempted to extract mtDNA from crude mitochondrial preparations from rat liver. Rat was chosen as a model animal since the restriction patterns of its mtDNA are well established [8–12]. Mitochondria prepared from fresh livers of Wistar rats were directly subjected to the DNA extraction procedure. The purity of this DNA was tested by restriction endonuclease digestion and agarose gel electrophoresis. Fragment patterns obtained with the following enzymes: *Eco*RI, *Bam*HI, *Xba*I, *Hpa*II, *Hae*III and *Hin*dIII are shown in fig.1. The restriction fragment patterns are identical to those published previously from rat liver mtDNA of type B [8,9]. Furthermore, no contaminating chromosomal DNA was detected in this analysis (fig.1) and no problems were encountered in obtaining total digests of the mtDNA with the enzymes used. We obtained a reproducible yield of about 1–2 μg mtDNA/g wet tissue, isolated from crude mitochondrial preparations. This is comparable to yields obtained previously by CsCl gradient centrifugation [3].

We also attempted to extract mtDNA with phenol or phenol-chloroform directly from the crude mitochondrial preparations. Although mtDNA was obtained, problems were encountered in trying to achieve complete digestion of this DNA and furthermore the preparations were contaminated with chromosomal DNA (not shown).

The sensitivity of restriction analysis can be in-

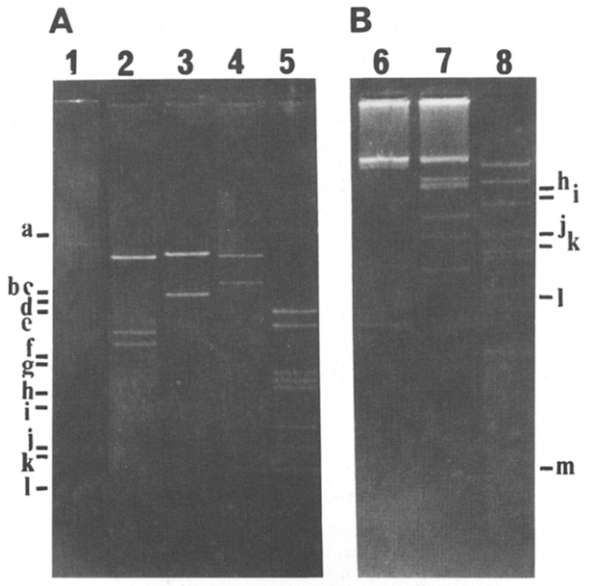

Fig.1. Restriction endonuclease fragment patterns of rat liver mtDNA. The digests were analyzed by electrophoresis in 0.8% (A) and 1.5% (B) horizontal agarose slab gels. The mtDNA was isolated and digested as described in section 2. The digests were as follows (lanes): 1, undigested; 2, digested with *Eco*RI; 3, *Bam*HI; 4, *Xba*I; 5, *Hpa*II; 6, *Eco*RI; 7, *Hpa*II; 8, *Hae*III. Phage λ DNA digested *Eco*RI and *Hin*dIII was used as a size marker. The DNA fragments were visualized by staining with EtBr. The letters a–m denote the migration of λ DNA fragments of the folllwing molecular masses in kb: 21.23 (a), 5.14 (b), 4.98 (c), 4.27 (d), 3.52 (e), 2.02 (f), 1.91 (h), 1.37 (i), 0.95 (j), 0.83 (k), 0.56 (l), 0.13 (m).

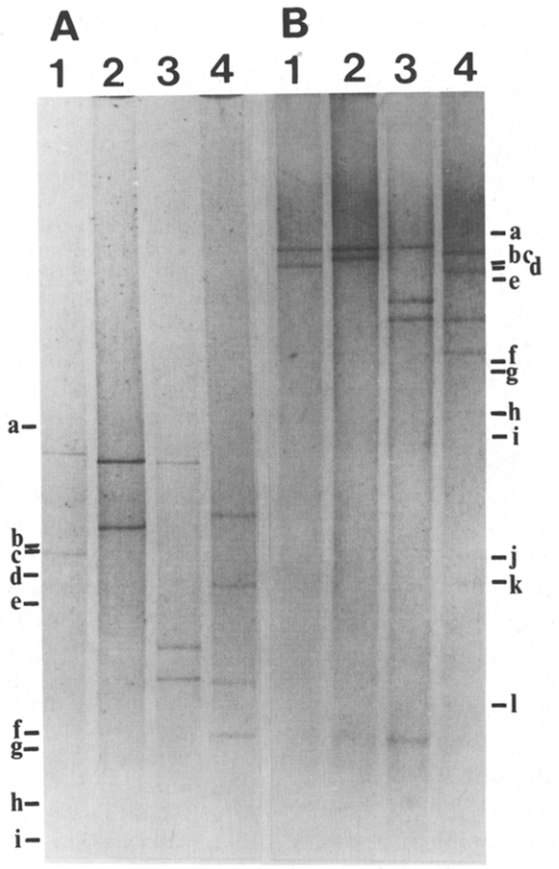

Fig.2. End labelling of mtDNA restriction fragments. Isolated mtDNA from rat liver was digested with restriction endonucleases, the fragments were end labelled with [α-^{35}S]dATP as described in section 2 and the digests analyzed by electrophoresis in 0.8% (A) and 1.5% (B) agarose gels. The isolated mtDNA was digested as follows (lanes): 1, *Bam*HI; 2, *Xba*I; 3, *Eco*RI; 4, *Hin*dIII. Phage λ DNA cleaved with *Eco*RI and *Hin*dIII and labelled with [α-^{35}S]dATP was used as molecular size marker (a–l as in fig.1). The fragments were visualized by autoradiography of dried gels.

creased considerably by radioactive labelling of the restriction fragments, thus less material can be used [3]. To test whether the mtDNA isolated by the alkaline extraction procedure was sufficiently pure for this type of analysis, the fragments were end labelled [6] prior to electrophoresis. The results (fig.2) clearly show that end labelling is applicable to these mtDNA preparations, and that CsCl purification of the mtDNA is not necessary. The amount of mtDNA used for one digest was about 10 ng. Thus an mtDNA preparation from 1 g liver provides enough material for about 100 restriction digests.

3.2. *Cloning of mtDNA fragments*

To test further the utility of this simple mtDNA isolation procedure, we attempted to clone the 2 *Bam*HI fragments of rat mtDNA into pBR322. The isolated mtDNA was digested with *Bam*HI and ligated with similarly cleaved pBR322 and then transformed to *E. coli* HB101 selecting for Ap resistance. The transformants containing inserts were screened for by their Tc sensitivity. Recombinant plasmids from several Tc-sensitive transformants were isolated and subjected to restriction analysis (fig.3). We could easily clone both of the

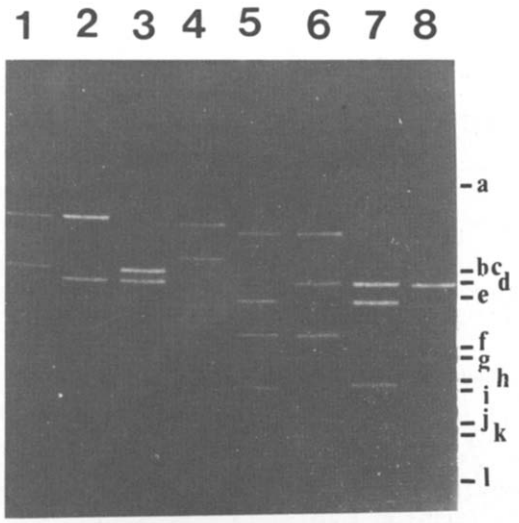

Fig.3. Cloning of the mtDNA from rat. The 2 *Bam*HI fragments of the mtDNA were cloned to the *Bam*HI site of pBR322. The pBR322-mtDNA plasmids (pTK301 contains the rat mtDNA *Bam*HI fragment A and pTK302 the *Bam*HI fragment B) were isolated from *E. coli* HB101 and subjected to restriction analysis in 0.8% agarose gels. Lanes: 1, rat mtDNA digested with *Bam*HI; 2, pTK301 digested with *Bam*HI; 3, pTK302 digested with *Bam*HI; 4, mtDNA cleaved with *Xba*I; 5, mtDNA cleaved with *Bam*HI and *Xba*I; 6, pTK301 cleaved with *Bam*HI and *Xba*I; 7, pTK302 cleaved with *Bam*HI and *Xba*I; 8, pBR322 cleaved with *Bam*HI (pBR322 does not contain *Xba*I restriction sites). Size markers as in fig.1.

*Bam*HI mtDNA fragments using mtDNA isolated by the alkaline extraction procedure (fig.3).

In conclusion, we have demonstrated that the simple alkaline extraction procedure [1] used routinely for isolation of bacterial plasmids can be readily applied to the isolation of animal mtDNA from crude mitochondrial preparations. The mtDNA obtained is sufficiently pure for standard restriction enzyme analysis and also for cloning of mtDNA fragments. Consequently, the method should have wide applicability for both population genetic studies and as a diagnostic tool in animal breeding and husbandry. This technique avoids the necessity of highly purified mitochondrial preparations and the tedious CsCl density gradient purification of mtDNA. The whole isolation procedure can be carried out during 1 day and does not require heavy equipment. To increase the sensitivity of this method, which would allow the use of tissue sampling methods that avoid the killing of the animals, radioactive labelling of the fragments can be employed. Furthermore, this procedure should provide a simple alternative for the cloning of mtDNA fragments.

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REFERENCES

[1] Birnboim, H.C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513–1523.
[2] Avise, J.C. and Lansman, R. (1983) in: Evolution of Genes and Proteins (Nei, M. and Koehn, R.K. eds) pp.147–164, Sinauer Associates Inc., Sunderland, MA.
[3] Lansman, R.A., Shade, R.O., Shapiro, J.F. and Avise, J.C. (1981) J. Mol. Evol. 17, 214–226.
[4] Wallace, D.C. (1982) Microbiol. Rev. 46, 208–240.
[5] Brown, W.M. (1983) in: Evolution of Genes and Proteins (Nei, M. and Koehn, R.K. eds) pp.62–88, Sinauer Associates Inc., Sunderland, MA.
[6] Drouin, J. (1980) J. Mol. Biol. 140, 15–34.
[7] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[8] Hayashi, J.-I., Tagashira, Y., Moriwaki, K. and Yosida, T.H. (1981) Mol. Gen. Genet. 184, 337–341.
[9] Hayashi, J.-I., Gotoh, O. and Tagashira, Y. (1981) Biochem. Biophys. Res. Commun. 98, 936–941.
[10] Brown, G.G. and Simpson, M.V. (1981) Genetics 97, 125–143.
[11] De Vos, W.M., Bakker, H., Saccone, C. and Kroon, A.M. (1980) Biochim. Biophys. Acta 607, 1–9.
[12] Feldman, H. and Grosskopf, R. (1979) Curr. Genet. 1, 13–19.