A DNA Extraction Procedure Practicable Under Field Work Conditions

J. SCHMIDTKE[1]

Institut für Humangenetik und Anthropologie, Albertstrasse 11, D–78 Freiburg i. Br. (German Federal Republic, BRD), 3 October 1975.

Summary. A DNA extraction procedure is described which is designed to be used in field work. A crude initial cell precipitate, which is easily obtained, may endure storage of months.

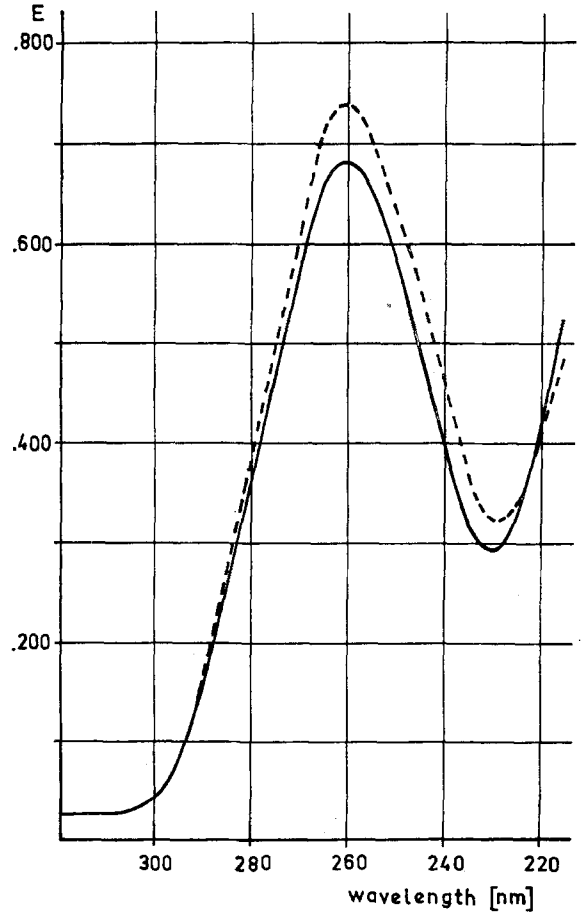
A procedure is described which allows one to prepare DNA from blood of vertebrates with nucleated erythrocytes, and which, in the initial stages, requires only the simplest technical equipment. The method has been developed for field work purposes, and has thus far been applied to a large number both of freshwater and seawater teleostean fish, and to a variety of amphibians. The procedure is explained here with blood of the rainbow trout (*Salmo irideus*) as an example. As a control, a conventional DNA extraction from nuclei isolated from erythrocytes derived from the same animal was performed.

Methods. 10 drops of blood from the caudal vessels were collected in 10 ml 0.1% heparin – 0.65% NaCl aqueous solution (about 10^5 cells/mm^3). This suspension was halved, and one half processed into nuclei. For this purpose the erythrocytes were isolated by centrifugation (10 min, 200 $\times g$), suspended in 20 ml of Ringer's Amphibian solution containing 0.5% glucose, and then lysed with Triton-X-100 (Serva; final concentration 0.15%); another 20 ml of Ringer's amphibian-glucose solution was immediately added to the hemolysate, from which nuclei were spun down at 3000 $\times g$ in a Sorvall centrifuge.

The second half of the original blood suspension was poured into a 100 ml Erlenmeyer flask and treated as follows: A 4% Triton-X solution was added under moderate shaking until a gelatineous substance was obtained. Then 2-propanol was added slowly, while initially shaking very gently and more vigorously at the end, until a dark reddish-brown network was obtained. This substance was removed from the solution by means of forceps with bent arms, transferred into a petridish with 75% ethanol – 0.1 *M* NaCl, kept there for several minutes and moved in the solution from time to time. The substance was finally air-dried on filter paper overnight, and kept in a sealed vessel at room temperature for 1 week. (The time span of storage is not critical for several months.) Immediately prior to the final DNA extraction the substance was allowed to swell in water for approximately 20 min and was dispersed in a Dounce homogenizer.

Both the isolated nuclei as well as the sample just described were subjected to 1 h treatment with pronase (Merck), $CaCl_2$, and LiCl (final concentrations 0.25 mg/ml, 5 m*M*, and 1 *M*, respectively; in a final volume of 10 ml). After addition of 0.5 ml of a 10% sodium dodecyl sulfate solution, another hour of incubation at 37 °C was permitted. DNA was extracted essentially as described by KIRBY[2], applying 3 successive extractions with equal volumes of phenol-cresol-hydroxyquinoline (100 g phenol + 14 ml m-cresol + 0.1 g 8-hydroxyquinoline + 25 ml H_2O) and chloroform. From the aqueous phase, DNA was precipitated with 2-propanol and redissolved in 15 m*M* NaCl – 1.5 m*M* Na-citrate pH 7.0.

Both DNA preparations were treated subsequently with ribonuclease (RNase A, Boehringer, final concentrations 7.5 Kunitz U/ml; RNase T 1, Boehringer, 23 Egami U/ml) for 2 h. Pronase-SDS-, and phenol treatments were performed as described above. The final DNA yields were 0.17 mg (from isolated nuclei) and 0.4 mg (from the direct precipitate). Both DNA samples were scanned in a Beckman DB spectrophotometer with recording attachment. As can be inferred from the Figure, both preparations have almost identical absorption curves. The 260/280 wavelength ratios were 1.85 and 1.86 respectively.



Absorption curves of DNA extracted from isolated nuclei (solid line) and from a propanol precipitate of Triton-lysed erythrocytes (broken line) derived from a specimen of *Salmo irideus*. The DNA was solved in 15 m*M* NaCl – 1.5 m*M* Na-citrate.

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[2] K. S. KIRBY, in *Methods of Enzymology* (Academic Press, New York and London 1968), vol. 12, p. 87.