# QUANTITATIVE DNA DETERMINATION IN GELS

N. A. Khodarev, I. A. Sokolova, and I. I. Votrin

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Electrophoresis of DNA in agarose and polyacrylamide gels has proved to be the best of the modern methods for separating DNA fragments of different molecular weights. Nevertheless, its use for determining absolute or relative amounts of DNA in different fractions is sufficiently laborious, it requires consideration of many factors and, it can be said that in general no methods yet described have earned universal approval.

In the investigation described below a number of the factors affecting quantitative determination of DNA in gels with the aid of photography and densitometry of the negatives are analyzed and conditions enabling quantitative determination of DNA in gels are suggested.

## EXPERIMENTAL METHOD

Methods of isolation and purification of DNA were described previously [1]. Electrophoresis of DNA in blocks of 2% agarose (from Chemapol, Czechoslovakia) was carried out as described previously [2]. Samples were applied to the block through the same recess. The empty channels on both sides of each sample were used to determine the baseline. After electrophoresis the blocks were exposed in a solution of ethidium bromide (2.5 μg/ml) for 15 min. The solution of ethidium bromide was prepared from a concentrate immediately before the experiment. Gels to be photographed were mounted on a Plexiglas frame against a black background, under the "Neva-2M" enlarger. The gels were illuminated by two chemiscopes (from Camag, Switzerland), located 17 cm from the gel surface and 25 cm apart, and tilted to an angle of 60° to the surface of the gel. The wavelength of excitation was 254 nm. Photographs were taken on "Tasma" film with a sensitivity of 130 GOST (State Standard) units, through a red filter. Negatives were developed by means of a single-use developer to which the name ABC was given. It was made up from concentrates containing the following components: solution A: 250 g crystalline sodium sulfite, 250 ml water, 5 g boric acid; solution B: 870 ml water, 35 ml glycerol, 35 g boric acid, 5 g anhydrous sodium sulfite, 40 g metol, 50 g hydroquinone; solution C: 855 ml water, 50 g potassium bromide, 410 g potash. The original solutions were kept in darkness at room temperature. The working solution of developer was made up by mixing 250 ml water, 12.5 ml of solution A, 2.5 ml of solution B, and 5 ml of solution C. It was found that with developing at 20°C for 5 min the coefficient of contrast reached 0.8, which is optimal for the type of photographic film used [3]. Negatives and gels were scanned on a Chromoscan-200 — Scan-201 system (Joyce-Loebl, England).

## EXPERIMENTAL RESULTS

The optical density of negatives (D) is known to be related to exposure (B) by the equation $D = K \cdot \log B$. The graph of D as a function of log B, or the characteristic curve of photographic negatives, is S-shaped with a linear area over a certain range of values of log B [3]. Calculations of the quantities of DNA in gels can be considerably simplified if the measurements are made entirely within the linear region of the characteristic curve [4]. Since exposure is the product $A \cdot t$, where A is the brightness of the object and t the length of exposure, the effect of each of these factors on optical density of the negative was studied. For visualization of DNA by means of ethidium bromide the value of A is the intensity of fluorescence. With saturating quantities of ethidium bromide, such as were used in these experiments, the value of A is determined by the DNA content. Dependence of fluorescence

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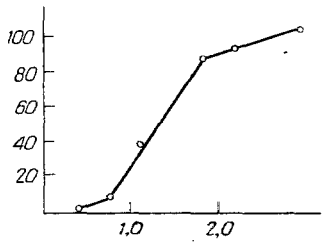

Fig. 1

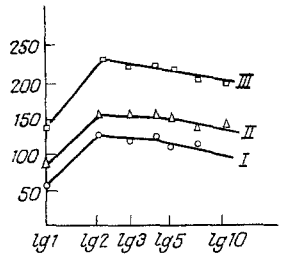

Fig. 2

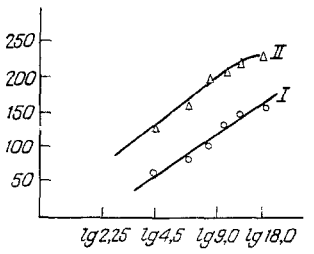

Fig. 3

Fig. 1. Dependence of intensity of fluorescence on DNA content in 2% agarose gels. Abscissa, DNA content of mononucleosomes (in μg); ordinate, peak amplitude (in mm).

Fig. 2. Dependence of weight of densitograms and exposure time. I) 4.5 μg, II) 6.75 μg, III) 13.5 μg, total DNA of rat liver chromatin hydrolysate. Abscissa, logarithm of exposure time (in min); ordinate, weight of densitograms (in mg).

Fig. 3. Dependence of weight of densitograms on quantity of DNA applied to gel. Expsure: I) 1 min, II) 2 min. Abscissa, logarithm of DNA content (in μg); ordinate, weight of densitograms (in mg).

on the DNA content in the fraction of mononucleosomes from autolysate of rat liver chromatin is illustrated in Fig. 1 (results were obtained on the Scan-201 scanning fluorometer). Clearly this relationshiop is of a complex, triphasic character, although there is a region in which DNA content is proportional to transmission of fluorescence (from 0.67 to 1.8 μg DNA of mononucleosomes). Dependence of total optical density of the negatives of 2% agarose gels with fractionated mononucleosomes and their oligomers on the duration of exposure is illustrated in Fig. 2. These graphs show that the differences between optical density of densitograms and base lines increase only within an interval of 2 min. This is followed by a phase of decline, due to the fact that the optical density of the region of the negative corresponding to zones of DNA has reached maximal values whereas the optical density of regions of the negative corresponding to control (empty) channels continues to rise with an increase in the duration of exposure. As a result of this, the maximal exposure time was limited to 2 min. Dependence of optical density of the negatives on the logarithm of the quantity of DNA of chromatin autolysates applied to the gel is shown in Fig. 3. Clearly this dependence is linear over the range from 4.5 to 18 μg for an exposure of 1 min and from 4.5 to 12 μg over an exposure of 2 min. To assess the error of these measurements, known quantities of DNA from chromatin hydrolysates obtained by degradation of nucleases to different depths were applied to the gel and the quantities found after densitometry were compared with the true amounts. To compare the results they were expressed as a ratio of quantities of a standard obtained in parallel determination (a 40-min autolysate of rat liver chromatin, obtained as described previously [2], was used as the standard). The accuracy of the measurements was found to depend largely in the distribution of DNA in the gel and the similarity of this distribution with those for the standard. Meanwhile, within the range of hydrolysis of chromatin from 5 to 20% of acid-soluble DNA the error of the measurements did not exceed 10%.

The conditions chosen thus ensure a linear dependence between the optical density of the negatives and logarithm of the total DNA in the gels, provided that certain parameters of exposure and development are observed and the DNA content lies within a certain range. It must also be pointed out that if special methods are used to process the densitograms [4, 5], these conditions will allow not only total DNA to be determined, but also DNA in individual fractions of gel.

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