

DNA-SIZING BY SURFACE SCANNING MICROSCOPY

S.Laib, M.Rankl, T.Ruckstuhl & S.Seeger

Institute for Physical Chemistry, University of Zürich, Zürich, Switzerland.

INTRODUCTION: The determination of DNA fragment sizes is of major importance in the investigation of genetic material. The presented work describes an approach to determine DNA-fragments on poly-l-lysine (PLL) coated glass coverslips [1]. Double stranded DNA-fragments were labeled with the intercalation dye TOTO-1-iodide stoichiometrically such that the amount of dye intercalated is proportional to the length of that fragment [2]. The fluorescence intensity of surface-bound DNA-TOTO-1 complex was measured by confocal scanning microscopy, revealing the brightness of every individual fragment.

METHODS: At a DNA concentration of 10 ng/ μ l fragments of different lengths were stained separately with TOTO-1-iodide in MES buffer (50 mM 2-Morpholino-ethansulfonic acid monohydrate, 50 mM NaCl, pH 6) yielding an average bp:dye ratio of 1:1. 250 μ l DNA solution diluted to a final concentration of 10^{-13} M was applied to PLL coated coverslips [3]. A surface area of $50 \times 50 \mu\text{m}^2$ was scanned by a confocal microscope with resolution of 640×640 pixels, which took less than 8 minutes.

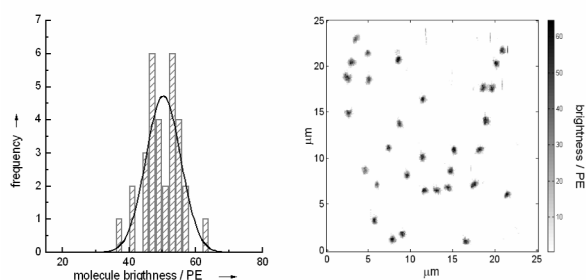


Fig. 1: Histogram and section of the scanning image of adsorbed pBR322 DNA fragments.

RESULTS: For calibration and examination of the proportionality of fragment length and brightness on single molecule level, DNA samples of defined sizes were measured.

Table 1: Calibration of the method with different DNA-fragment sizes

DNA-Fragment	Fragment size / bp	Centroid / PE	σ / PE	CV / %
PCR product	1 985	18.8 \pm 0.5	2.6	13.8
pUC 19	2 686	26.7 \pm 0.8	3.3	12.4
pBR 322	4 361	49.9 \pm 0.8	3.7	7.4
M13mp18 RF1	7 250	80.5 \pm 1.2	6.9	8.6
pHyR15AoriKncagGF	14 200	151.5 \pm 1.7	10.3	6.8

The obtained intensity distribution of the fragments was fitted with a Gaussian curve, resulting in average fragment intensity in photon electrons (PE) for each DNA-fragment (fig. 1). The results of the Gaussian fits are summarized in table 1. The

coefficient of variation (CV) is defined to be the standard deviation of a distribution divided by its means. For different sizes the CV varied from 7% to 13%.

Subsequently, the obtained calibration was used for DNA sizing of different fragments between 2 000 and 14 000 base pairs (bp). An intensity histogram of five different fragment sizes is shown in figure 2.

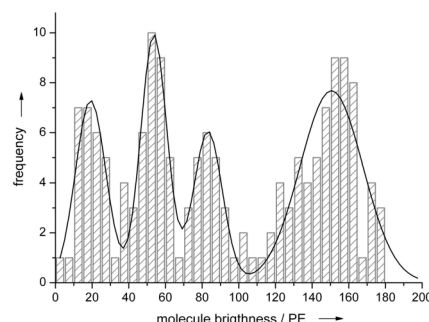


Fig. 2: Histogram of the molecule brightness from a mixture of different DNA-fragment sizes.

Fragments of 4.3, 7.2 and 14.2 kbp are clearly resolved with a CV of approximate 12%. Due to the CV's of table 1 we cannot expect to identify the fragments of 1.9 and 2.6 kbp in two separated peaks, although the broader peak (CV 25%) indicates the occurrence of more than one fragment.

DISCUSSION & CONCLUSIONS: The fluorescence based DNA-sizing technique is capable of determining fragments within an accuracy of typically 7-13%, which is comparable to data obtained by established methods and already sufficient for most applications. In comparison with gel electrophoresis, the new sizing method has high sensitivity concentration requiring several orders of magnitude lower amount of DNA. To ascertain the sizes of particular fragment, a quantity of 10^{-17} mol was sufficient. Additionally, the time exposure for the measurement is about ten times lower than for gel electrophoresis.

REFERENCES: ¹S. Laib, M. Rankl, T. Ruckstuhl, S. Seeger (2003), *submitted to Nucleic Acid Research*. ²P.M. Goodwin, et al. (2002) *NATO Science Series, 3. High Technology*, 87 (Structure and Dynamics of Confined Polymers), 351-370. ³M. Rankl, S. Laib, S. Seeger (2003) *Colloids and Surfaces B: Biointerfaces* 30, 177-186.