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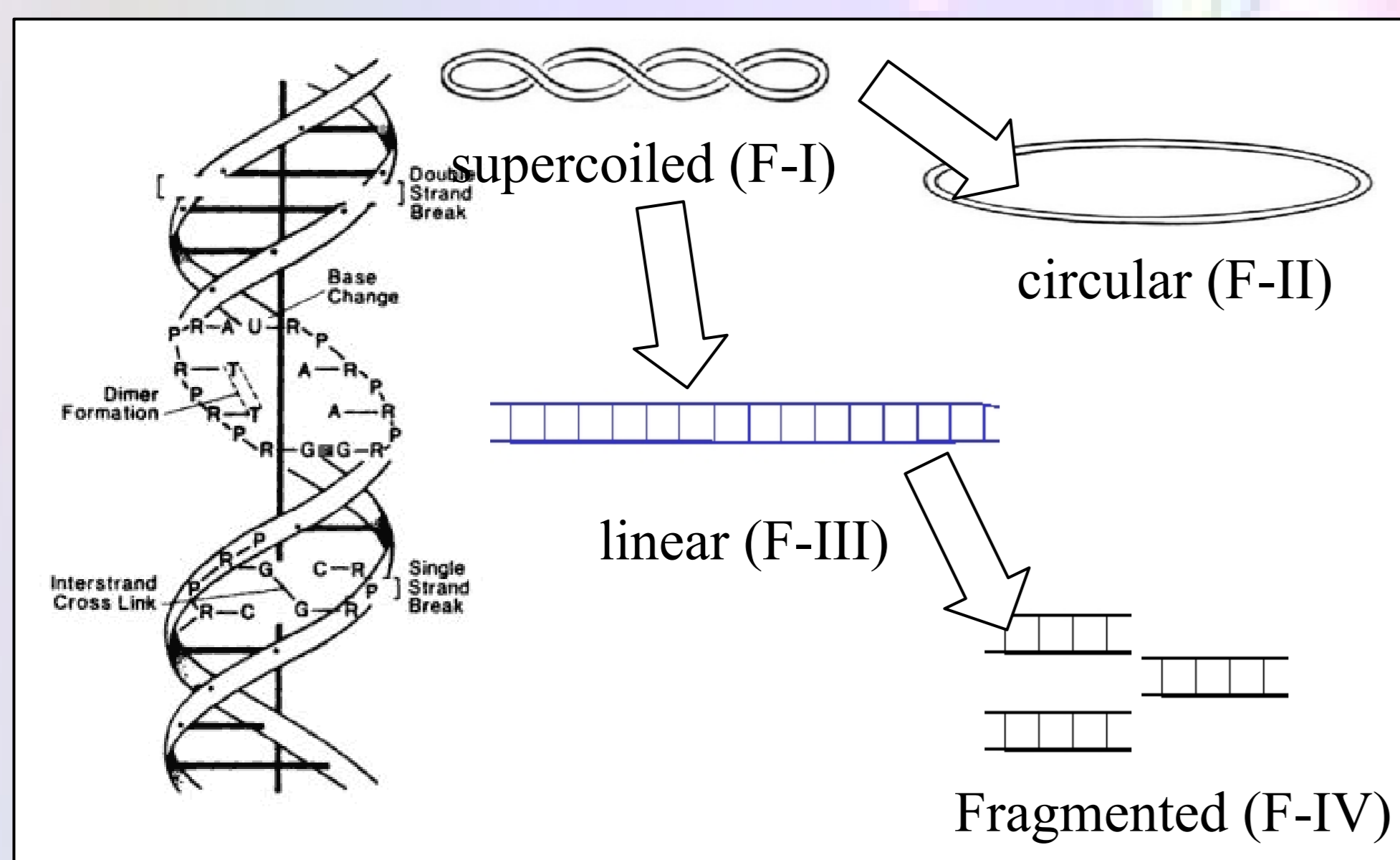
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Abstract

Plasmids have been used for studying how radiation can induce breakage of DNA molecules. Plasmids appear in three different forms, namely supercoiled, circular and linear, and that interaction with radiation can convert supercoiled DNA into circular form, if a single-strand break is produced, or into linear form, if a double-strand break is induced. It allows the use of simple electrophoresis technique for analyzing the number of breaks as a function of delivered dose. In this work we present a complete methodology for the quantification and study of the DNA damage by radiation. Also we present the results obtained for the full dose range between 0 and 200 Gy. We observe the fast decay of the fraction of supercoiled, which are transformed into the circular form. Also, at higher delivered doses, the fraction of circular molecules decreases while the fraction of linear plasmids increases. An analysis of these results will be presented in a forthcoming paper.

Introduction

The radiation may induce in DNA different damages, single strand break (SSB) or double strand break (DSB), they are very related with the cell survival. After the irradiation they may appear in four different shapes. Initially most of the sample is in supercoiled shape. Using the methodology below we may quantify the radiation action over the DNA for subsequent analysis

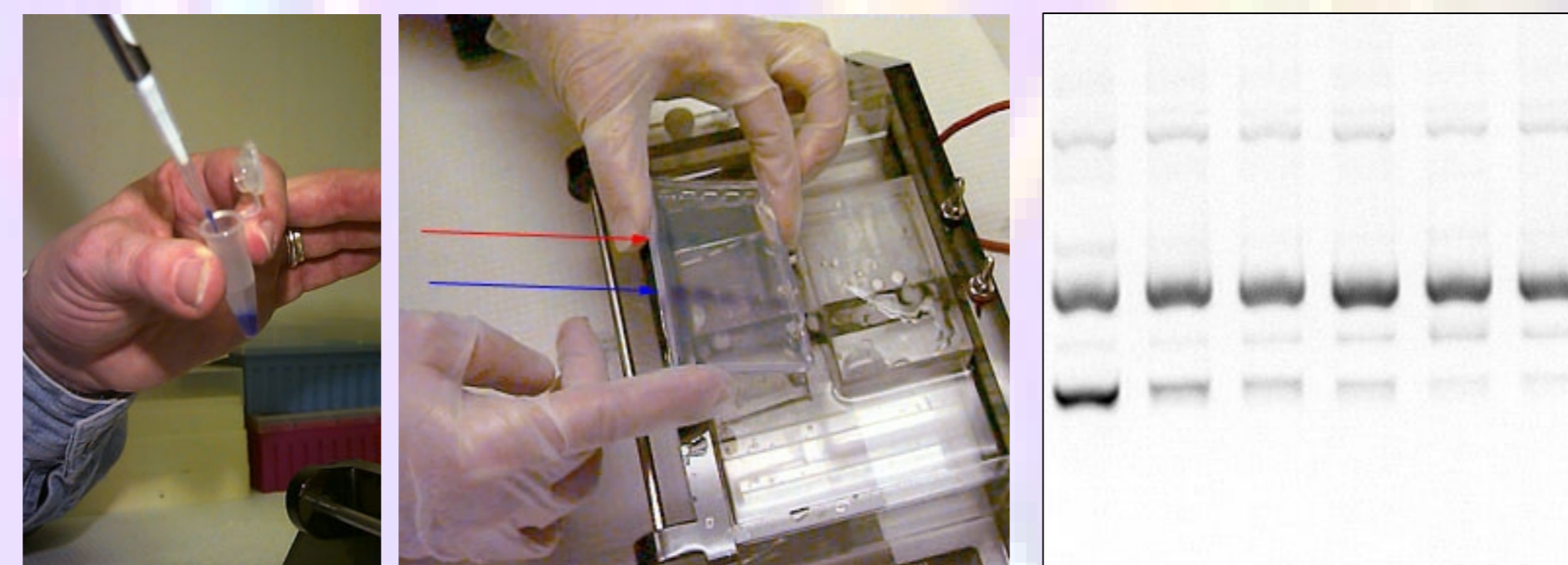


CTR-IPEN, at ambient temperature. The doses were from 0 to 200 Gy with a step of 5Gy, 40 samples in totality. The doses rate was 1.28Gy/s.



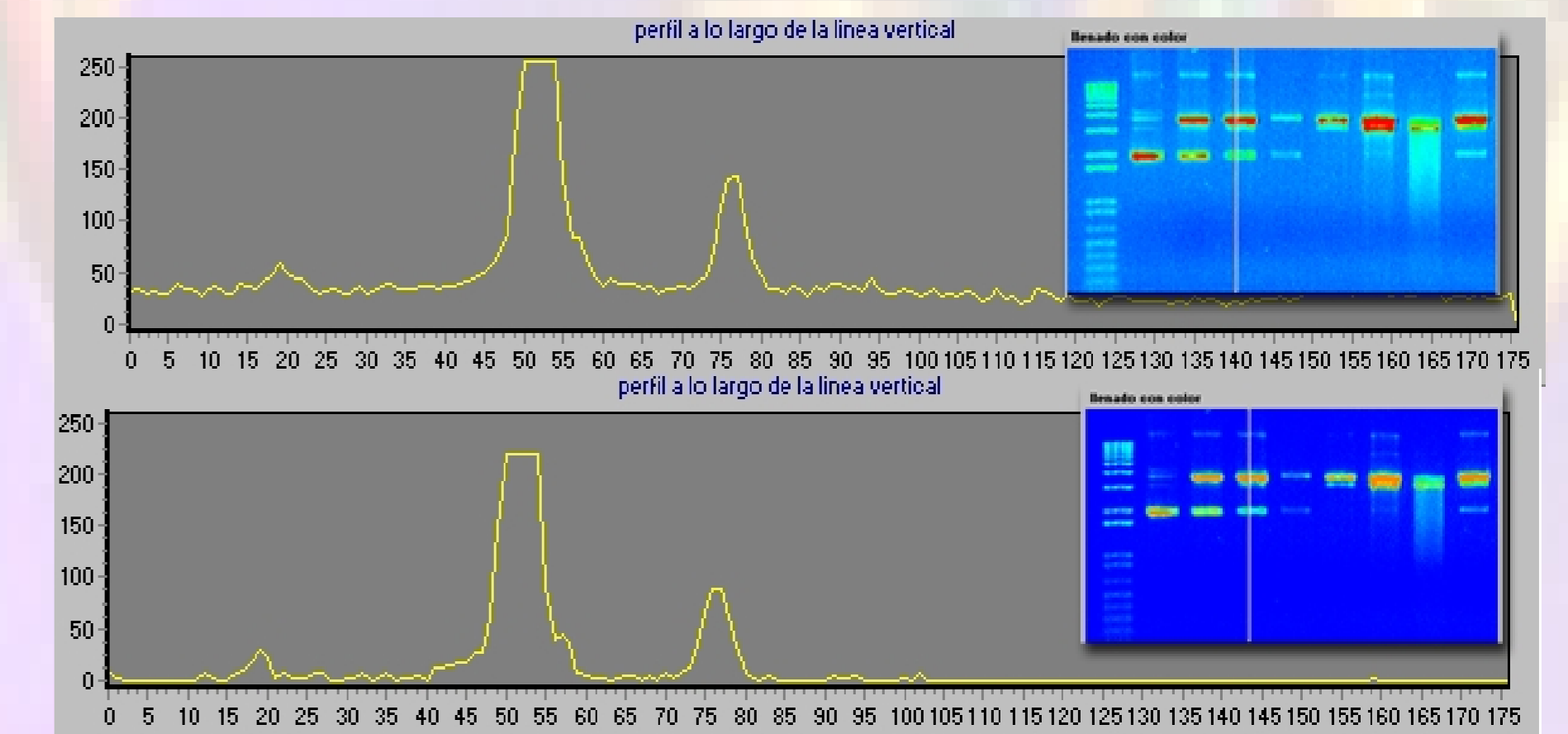
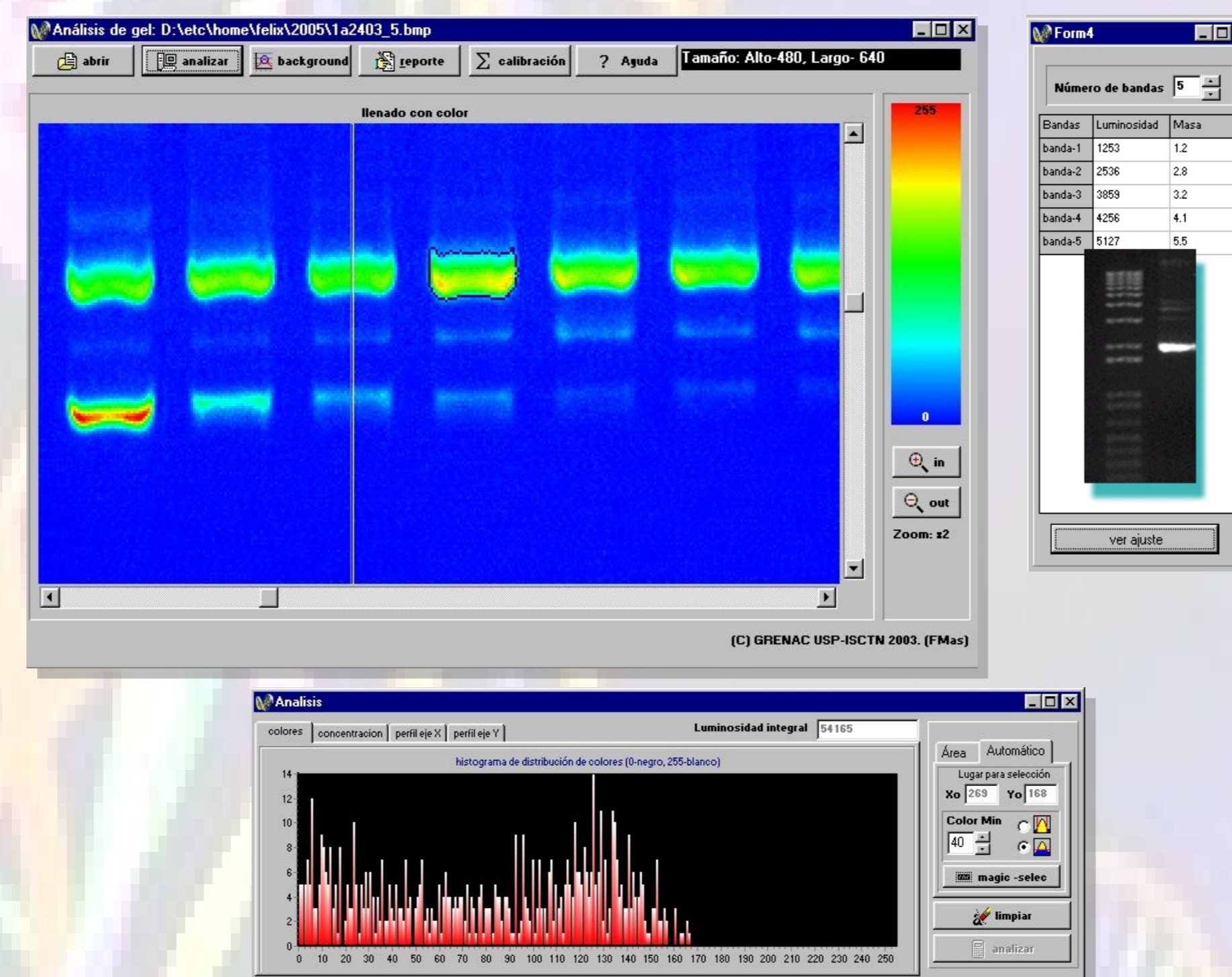
Electrophoresis

Gamma irradiated pBKS in water was subjected to agarose gel electrophoresis. After electrophoresis the gel was illuminated with UV light and an analog image in gray scale of the ethidium fluorescence was digitalized using the Eagle Eye® II system.



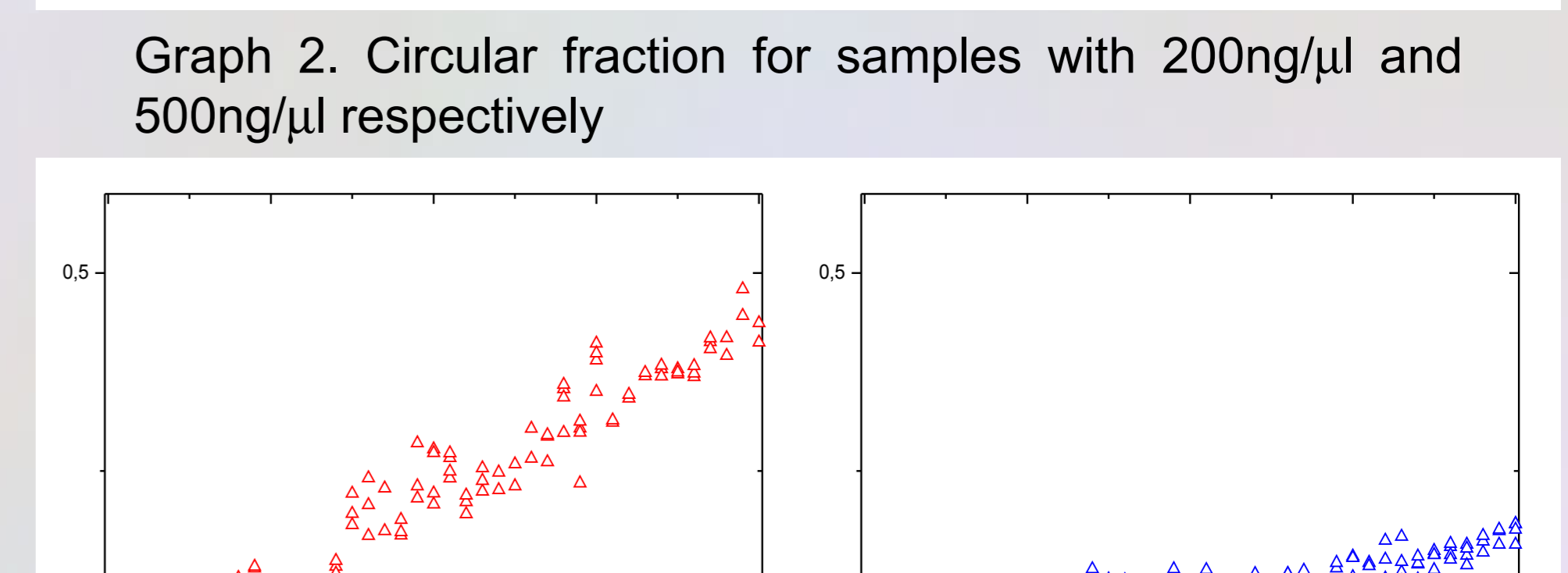
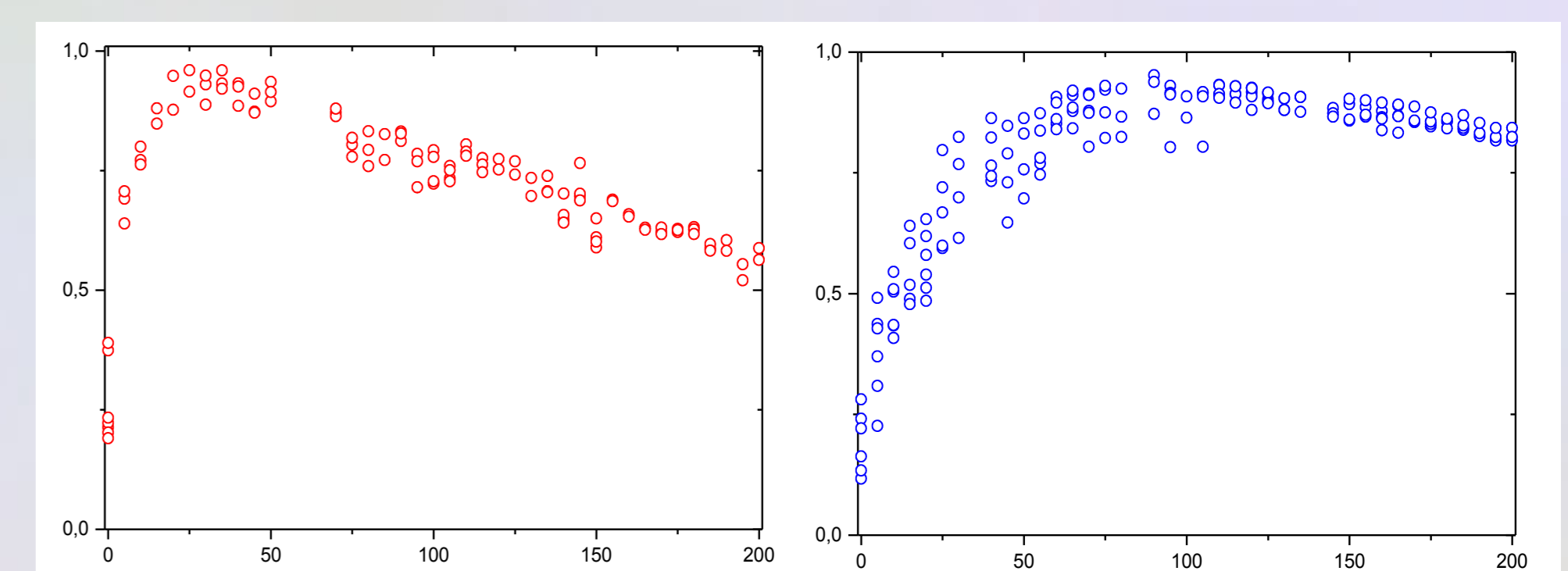
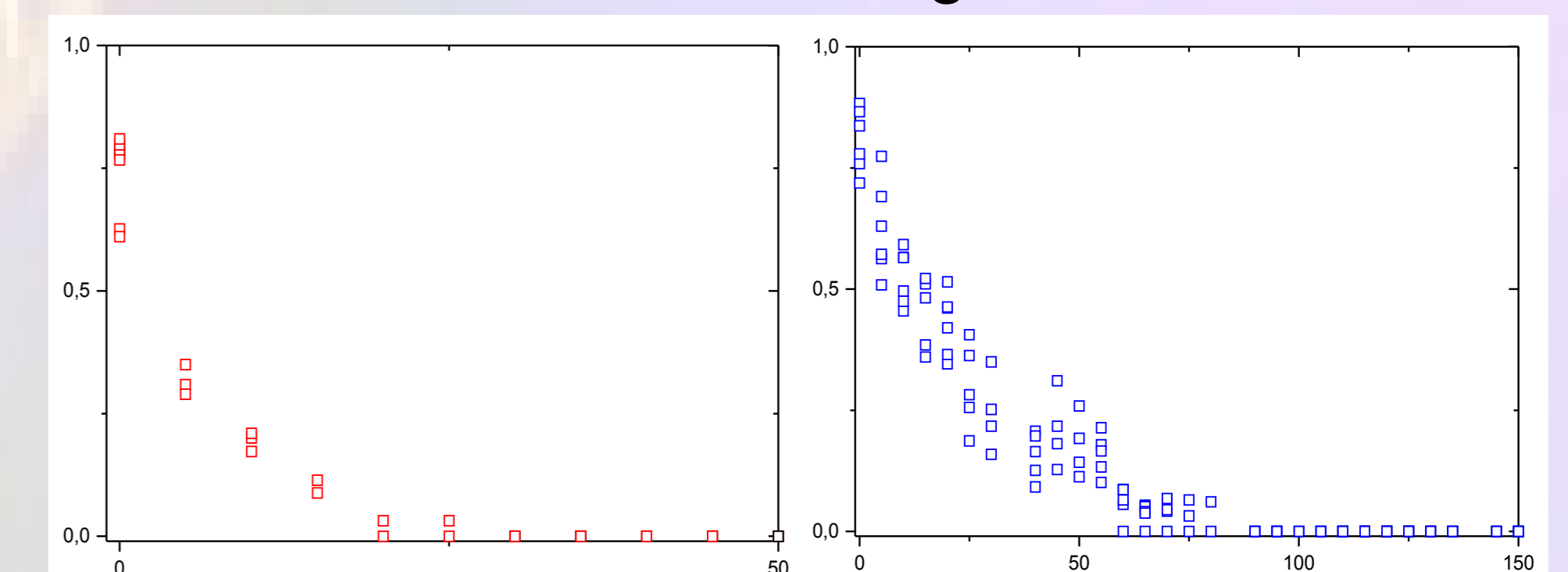
Electrophoresis analysis

The relative amount of DNA in each of the three plasmid conformational forms was estimated using the software GelAnalis developed by our group.



Results

With the integrated luminosity in each canal, was estimated the fraction of each DNA form. Were used a correction factor 1.4 for the supercoiled luminosity (Milligan,1992). The method demonstrate to be reproducible and we get the expected results. The fraction of supercoiled decrease exponentially. The Circular fraction increase until a value where start to decrease, converting in lineal DNA.



Graph 3. Circular fraction for samples with 200ng/μl and 500ng/μl respectively

An analysis of this data is in another poster in this meeting.

Acknowledgements

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The Method

Preparation of plasmid DNA

Plasmid DNA pBluescript® (pBKS, 2961 pb) was isolated from E. coli and after it was purified. Where prepared samples in two different concentration in aqueous solution, once with 200ng/ul and the other with 500 ng/ul. The samples ready for irradiation were kept in plastic test tubes from the Eppendorf®.



Samples irradiation

Samples with 10 uL in the two different concentration were irradiated with 60Co γ-gamma rays in a Gamma Cell of Cobalt in the

References

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