

# DNA obtained by *ab initio* synthesis forms hyperbranched net-like structure

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## INTRODUCTION

*Ab initio* DNA synthesis is unusual synthesis of dsDNA from tens bp to kbp long by thermophilic DNA polymerases (Tth, Taq, Bst etc.) from free dNTPs in the complete absence of added DNAs. This synthesis is extremely enhanced if a thermostable restriction endonuclease (TspRI, etc.) or nicking endonuclease (Nt.BstNBI, Nt.AlwI, etc.) is added to the reaction with thermophilic DNA polymerase.

The structure of the DNA synthesized *ab initio* has not been sufficiently investigated. As believed, the reaction product is a linear double-stranded DNA in the B form. However, some preliminary evidence suggests more complex structure.

## OBJECTIVE

To study the structural specificity of high-molecular-weight DNA products synthesized *ab initio* by Bst DNA polymerase LF itself and in the presence of the nicking endonuclease Nt.BstNBI.

## METHODS

*Ab initio* DNA synthesis of high-molecular-weight DNA, pulsed field gel electrophoresis, purification.

Identification of specific structural features of the DNA:

- analysis by nucleases (DNaseI, mung bean nuclease, restriction endonucleases HinfI and DraI),
- atomic force microscopy (NTEGRA spectra) including analysis of AFM images (Gwyddion 2.49, FiberApp) and statistical analysis (SPSS Statistic 19).

## CONCLUSIONS

High-molecular-weight DNA is double-stranded and a significant amount of DNA branches and forms net-like structures. The data may be very useful to develop techniques requiring fast and inexpensive preparation of highly structured DNA and DNA nanomaterials.

## RESULTS

### ANALYSIS BY NUCLEASES

Based on the known sequence data, restriction endonucleases were chosen. DNA synthesized by Bst polymerase was hydrolyzed by DraI (5'-TTTAAA-3') only partially. The DNA synthesized in the presence of Nt.BstNBI were extremely difficult to digest with HinfI (5'-GANTC-3') (fig. 1a).

A single-strand specific nuclease mung bean and nonspecific endonuclease DNaseI easily cleaved this DNA (fig. 1b).

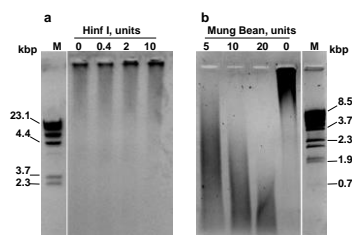


Figure 1.

### AFM

DNA strands branched and formed net-like structures (fig. 2a). The most of *ab initio* synthesized DNA molecules consisted of linear ds strands. The DNA contained single-stranded and triple-stranded segments. The net-like structures may be assumed to be three-dimensional (3D). The DNA molecules obtained in the synthesis with the nicking endonuclease Nt.BstNBI were structurally similar to those obtained without adding the enzyme.

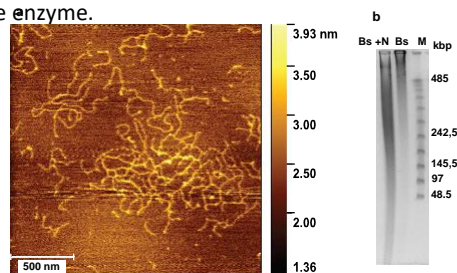


Figure 2.

### THE DNA LENGTH

The maximum length of the DNA was more than 500 kbp as determined by PFGE (fig. 2b). On the other hand, maximum of measured contour lengths for individual DNA molecules was about 100-150 kbp. The reaction products with net-like structures got stuck in agarose gels that can account for the disparity of the data on the DNA length determined by pulse-fielded gels and AFM

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