Chem Rep, 2025, 5(1): 298-307
DOI: 10.25082/CR.2024.01.005

RESEARCH ARTICLE

An Easy Method to Non-Destructively Separate the Strands of a Circular Duplex Plasmid Chromosome: A Preliminary Report

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Received: May 30, 2025;
Accepted: July 15, 2025;
Published: July 25, 2025.

Citation: Biegeleisen K. An Easy Method to Non-Destructively Separate the Strands of a Circular Duplex Plasmid Chromosome: A Preliminary Report. *Chem Rep*, 2025, **5**(1): 298-307.
https://doi.org/10.25082/CR.2024.01.005

Abstract: A straightforward and cost-effective method has been developed for the non-destructive separation of the two strands of a circular duplex plasmid chromosome. This method was adapted from the original protocol reported by Wang and Wu (1996), who achieved strand separation using prolonged low-current electrophoresis. In contrast, the method described here utilizes a two-step denaturation process: initial alkali denaturation to pH 13 to generate "Form IV" DNA, followed by denaturation in 90% formamide at 90°C under low-salt conditions. The resulting single strands were observed to migrate as two discrete bands on agarose gel electrophoresis, indicating successful strand separation without degradation. This approach offers a reproducible, rapid, and inexpensive alternative for investigating the topology and helicity of circular DNA molecules.

Keywords: DNA topology, plasmid, Form IV, strand separation, non-helical DNA, circular DNA

1 Introduction

A biological theory posits that the structure of DNA within living chromosomes has never been definitively determined, despite existing substantial support for this notion. This theory, however, has been overlooked for many decades. The Watson-Crick double-helix structure of DNA was determined using X-ray crystallography. However, this method cannot be applied directly to chromosomes. To perform X-ray crystallography on DNA, several preparatory steps are necessary.

The chromosome must be removed from the cell nucleus, which results in its fragmentation into numerous pieces, thereby destroying any aspect of DNA structure that requires chromosomal integrity. Histones or protamines, which provide numerous positive charges that interact with the negative charges on DNA phosphate groups, must be removed. The proposition that the removal of these proteins does not affect the DNA structure is implausible. Most of the water must be removed from the DNA, as crystals cannot form under the high humidity conditions within the cell nucleus. Given these limitations, it is presumed that fully intact, unperturbed chromosomal DNA adopts the double-helix structure. However, since the publication of the Watson and Crick Nature article in 1953, only two studies have specifically addressed the question of helicity in native, unperturbed chromosomes: Stettler et al. (1979) [1] and Crick et al. (1979) [2]. The Stettler study reported an uncontrolled experiment and drew conclusions that were not supported by the presented data. The Crick publication was a review paper that argued for the Watson-Crick helical structure in topoisomerase experiment bands but did not discuss the topology of the native structure prior to topoisomerase treatment, which was presumed a priori to be helical without justification.

The only reliable evidence for topological net helicity in unperturbed living systems is the difficulty in non-destructively separating the strands of small circular plasmid or viral chromosomes. Notably, both non-destructive separation and reannealing of small circular DNA have been reported [3,4], yet these findings have been largely ignored by the molecular biology community.

Further evidence against helicity in unperturbed chromosomal DNA is the inability to solve the structure of the complex between protamine and DNA in sperm cells if DNA is presumed helical. To date, only one published atomic model for the protamine-DNA complex exists,