DNA fragment size determination: Are electrophoresis-based methods capable of detecting chromatin?

Chromatin pre-treatment alters DNA migration and apparent fragment size

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INTRODUCTION

Host cell-derived DNA is a critical impurity in bionanoparticle-based products, including viruses, virus-like particles and extracellular vesicles. When DNA is associated with histone proteins as chromatin, it may evade detection by standard analytical methods. In electrophoresis-based assays, chromatin can prevent DNA from entering the gel, leading to an underrepresentation of larger DNA fragments.

In this study, DNA migration patterns were compared using polyacrylamide- and agarose-based gels. Samples were analyzed untreated, after Proteinase K treatment, or following nuclease treatment by a salt-active nuclease capable of digesting both DNA and chromatin, as previously reported[1–3].

LARGE DNA AND CHROMATIN FRAGMENTS MIGHT BE OVERLOOKED

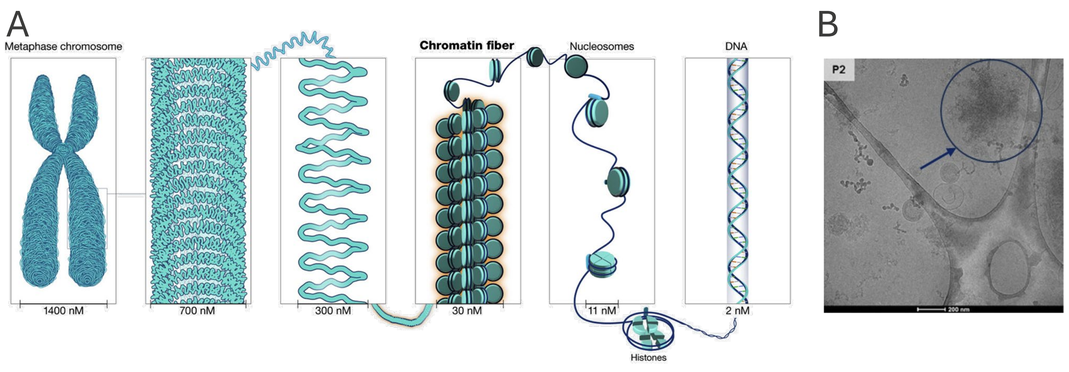
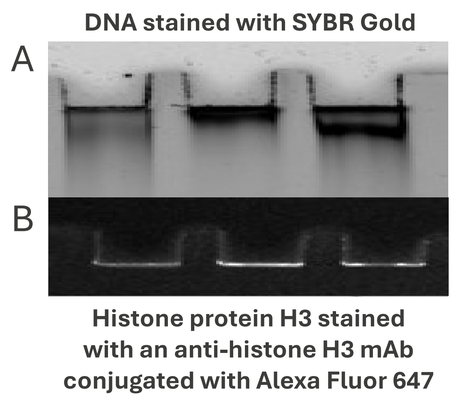


Figure 1: *(A) DNA packaging levels from metaphase chromosome to double-stranded DNA (courtesy of the National Human Genome Research Institute); (B) Cryo-EM picture of chromatin (Aguilar et al [1]).*

FLUORESCENT LABELLING ON SAME GEL ENABLES CHROMATIN IDENTIFICATION



- DNA labelling confirms large fragments in gel wells
- Histone protein labelling confirms chromatin in gel wells

Figure 2: *Fluorescent labelling of DNA (A), followed by fluorescent labelling of histone protein H3 (B), both on the same gel.*

DNA MIGRATION PATTERNS ARE AFFECTED BY SAMPLE PRE-TREATMENT

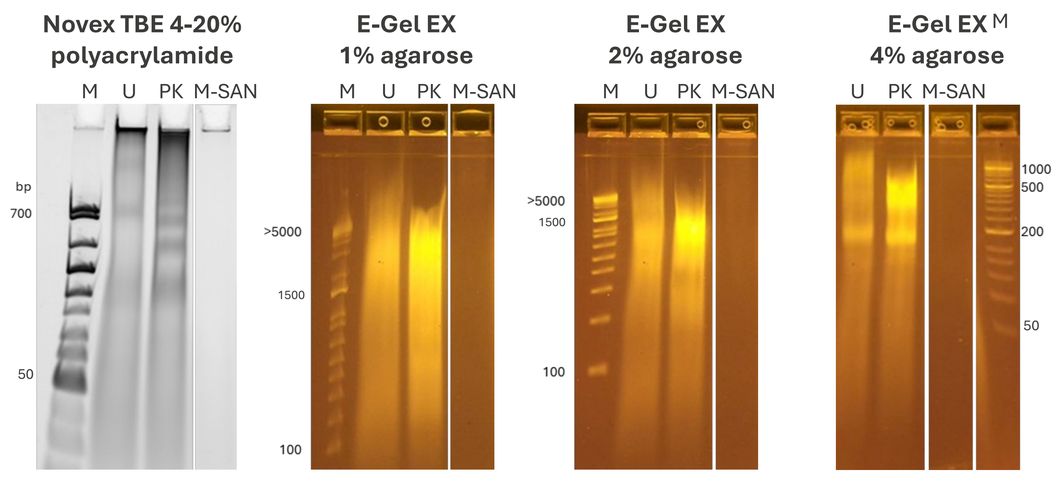


Figure 3: *Migration patterns of DNA fragments in different gels. M: marker, U: untreated sample, PK: Proteinase K treated sample, M-SAN: salt-active nuclease treated sample.*

Untreated sample:
Limited or no migration of large chromatin-associated DNA into the gel; signal remains in the well

Proteinase K treated sample:
Digestion of DNA-binding proteins (e.g. histones) leads to reduced DNA-protein interactions, resulting in smaller apparent fragments and increased migration

M-SAN treated sample:
Digestion of DNA and chromatin; residual signal in the well my indicate the presence of encapsulated DNA

CONCLUSION

Electrophoresis does not reliably represent chromatin-associated DNA in crude samples.

While smaller DNA fragments may migrate, larger chromatin-associated DNA is retained. Disruption of chromatin (e.g. by proteinase treatment) enables migration but alters native fragment size.

This should be considered when assessing host cell DNA in bioprocessing.

[1] Aguilar, PP. et al. J Chrom A 1627, 461378 (2020)
[2] Mayer V. et al. Biotechnol Prog 39(4):e3342 (2023)
[3] Mayer, V. et al. J Chrom A 1738, 465470 (2024)

Webinar: "False Confidence, Real Cost - How Chromatin Escapes Standard DNA Analytics and Undermines Your Downstream Process"

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