A NUCLEIC ACID UNIVERSAL PURIFICATION, ISOLATION METHOD AND DEVICE

Ruslan Kalendar

National Laboratory Astana, Astana,010000, Kazakhstan

ruslan.kalendar@nu.edu.kz

Background: DNA isolation is the first step for many molecular workflows, and success depends on removing contaminants such as polysaccharides, polyphenols, lipids, pigments, humic substances, and other inhibitors. Sequencing is especially sensitive to DNA purity. With third-generation long-read platforms, purity alone is not enough high molecular weight and intact DNA are essential to realize reads spanning tens to hundreds of kilobases.

Materials and methods: We present a DNA purification method combining agarose gel electrophoresis with electroelution. It exploits the reduced electrophoretic mobility of DNA in high-salt conditions. After DNA separation in a standard gel, a high-salt gel block is placed ahead of the DNA path, leaving a gap (sample collection reservoir). Reapplying current causes DNA to migrate into the gap, where it slows and accumulates. DNA is then easily collected by pipetting and used directly or after desalting. This cost-effective method requires no specialized equipment and is ideal for challenging samples with complex biomolecular mixtures.

Results: Isolating high molecular weight (HMW) DNA from complex samples like plants and soil is typically difficult, yielding low purity and quantity. Plant cells contain polysaccharides and polyphenols that interfere with extraction, while soil samples pose greater challenges due to fragmented DNA and humic substances. Despite these obstacles, our method consistently produced HMW DNA with high yield and purity. Conventional methods often recover less than 10% of starting DNA due to poor retention or partitioning losses. In contrast, our approach based on a distinct principle achieved yields up to 50% for plant samples and 30% for soil.

Conclusion: This on-gel electroelution method yields HMW DNA for long-read sequencing and other demanding applications from eukaryotes, prokaryotes, organelles (mitochondria, chloroplasts), large DNA viruses, and more. It is particularly useful when other approaches are impractical enabling single step recovery from complex, inhibitor-rich samples with low target abundance. The workflow is low-cost, scalable, and readily automatable.

Acknowledgement: This study was funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (grant AP23483529).

Key words: nucleic acid, DNA purification and extraction, electroelution, DNA sequencing, long-read sequencing

References:

1. Kalendar, R., Ivanov, K.I., Akhmetollayev, I., Kairov, U., Samuilova, O., Burster, T., Zamyatnin, A. An improved method and device for nucleic acid isolation using a high-salt gel electroelution trap. Analytical Chemistry 96(39), 15526-15530 (2024).

2. Kalendar, R., Ivanov, K.I., Samuilova, O., Kairov, U., Zamyatnin, A. Isolation of high- molecular-weight DNA for long-read sequencing using a high-salt gel electroelution trap. Analytical Chemistry 95(48), 17818-17825 (2023).