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Electroelution Into a Salt Trap: Reviving an Old-School Approach to DNA Purification

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ABSTRACT

Recent advances in bioinstrumentation, such as the development of long-read sequencing, have reignited interest in methods for extracting long, intact nucleic acids from complex samples. One traditional method for this purpose is gel electrophoresis followed by electroelution from gel slices into a salt cushion. However, this method has become largely overlooked because its standard implementation is laborious, time-consuming, and incompatible with high-throughput workflows. In our recent work, we revisited this experimental approach and developed a simple, fast, and efficient method for purifying intact nucleic acids of varying lengths from complex samples. The method is available in both horizontal and vertical electrophoresis configurations, has the potential for automation and scalability, and is suitable for purifying high molecular weight (HMW) DNA for long-read sequencing. In this paper, we discuss the origins of the method, the stages of its development, and its advantages. The successful implementation of the method demonstrates how looking at a traditional technique from a new perspective can help meet the demands of next-generation technologies such as long-read sequencing. Our results highlight the importance of rethinking the applications of well-established yet underutilized methodological approaches in rapidly evolving fields such as biotechnology and bioinstrumentation.

1 | Introduction

Throughout the history of biomedical research, many concepts, approaches, and methods have made remarkable comebacks after being neglected and nearly forgotten. One example is phage therapy, which uses viruses to treat bacterial infections. Developed nearly a century ago, it was largely overlooked after the discovery of antibiotics. However, amid the current surge of

antibiotic resistance, there is renewed interest in using bacteriophages to treat bacterial infections, particularly those caused by antibiotic-resistant bacteria [1]. Another example is fecal microbiota transplantation (FMT), which was used in ancient China to treat severe diarrhea. Despite its long history, Western medicine largely ignored FMT until the mid-20th century, when its effectiveness in treating pseudomembranous colitis was proven. Since the FDA first classified FMT as an investigational

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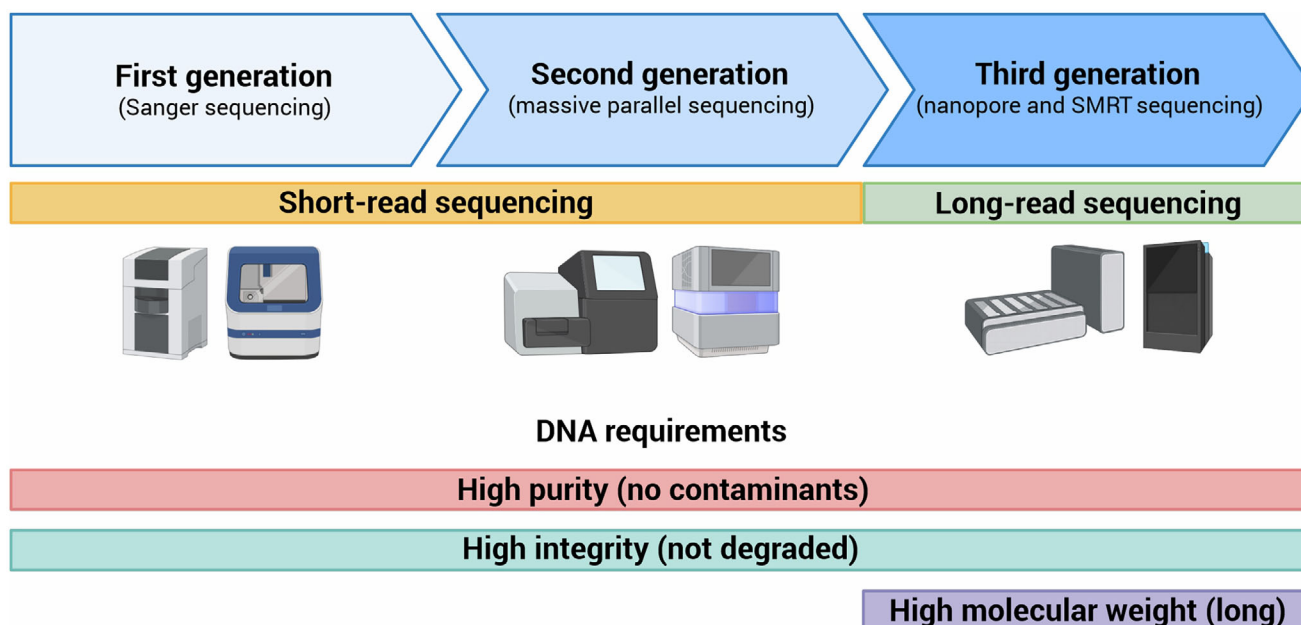


FIGURE 1 | The evolution of nucleotide sequencing methods has led to increased DNA requirements. SMRT: Single-Molecule Real-Time.

drug in 2012, it has become an increasingly common treatment for recurrent *Clostridioides difficile* infections. Importantly, modern FMT has evolved into a significantly different method that uses standardized oral capsules containing specific bacterial strains instead of crude stool suspensions administered via retention enema [2]. Yet another example, this time from molecular biology, is *in vivo* DNA assembly (IVA). This method uses bacteria's natural recombination machinery to assemble DNA plasmids directly inside the cell. Although first reported over 25 years ago, IVA failed to gain popularity due to the high cost of long primer synthesis and the lack of high-fidelity polymerases at that time. Once long oligonucleotides and commercial high-fidelity polymerases became available and affordable, the method gained prominence, offering a streamlined, cost-effective alternative to *in vitro* molecular cloning [3]. Thus, reevaluating established yet overlooked methods may align them with modern, real-world demands and breathe new life into them. In this paper, we describe how we revisited and revived an established yet underutilized nucleic acid purification method, electroelution into a salt trap, to meet the demands of modern technologies such as third-generation nucleotide sequencing.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two physiologically and therapeutically relevant nucleic acids, which are essential for numerous research and clinical applications. High-quality nucleic acids are necessary for various analytical and applied methods in fields such as molecular biology, genetics, molecular medicine, biotechnology, forensics, and food science. As these methods evolve, new and often more stringent requirements for analyzed nucleic acids may emerge. Nucleotide sequencing is a good example of this. Previous methods, such as Sanger sequencing and next-generation massive parallel sequencing (NGS), required relatively short stretches of pure, intact DNA. However, current third-generation sequencing methods require DNA that is not only pure and intact but also as long as possible (Figure 1). To ensure the success of third-generation sequencing, long DNA must first be separated from

impurities that could interfere with the sequencing process. The sample must also be free of stable chemical or mechanical complexes formed between impurities and DNA. However, complete removal of impurities and their complexes is difficult to achieve when the starting material is a complex mixture of chemically diverse molecules, including polysaccharides, polyphenols, proteins, peptides, oligonucleotides, lipids, pigments, humic substances, secondary metabolites, and various other low molecular weight compounds.

The literature describes a variety of experimental approaches to nucleic acid isolation and purification [4–20]. Their strengths and weaknesses are summarized in Table 1. With the exception of specialized methods such as cesium chloride gradient ultracentrifugation and electrophoresis, these approaches can be broadly divided into two categories: liquid phase extraction and solid phase extraction. A classic example of the former is extraction with phenol-chloroform [21]. In this approach, centrifugation separates the upper (aqueous) phase containing the nucleic acid of interest from the lower (organic) phase containing non-polar contaminants such as proteins and lipids. The DNA is then precipitated in the presence of a polar solvent (ethanol or isopropanol) and salt (sodium acetate) [22]. Although still in use, this approach often results in suboptimal DNA yields, requires the use of hazardous chemicals, and is not well suited for processing large numbers of samples. In addition, to obtain DNA of acceptable purity, organic extraction often needs to be repeated several times, reducing yields and prolonging exposure to hazardous chemicals. The idea behind solid-phase extraction is to bind DNA to a solid support so that unbound contaminants can be washed away [23–25]. Many types of solid supports have been successfully used for DNA purification, including silicon dioxide (silica) particles, borosilicate glass fiber, hydroxyapatite, diatomaceous earth, anion exchange resins, and coated magnetic beads [5, 6, 12, 26–28]. Solid-phase extraction on silica beads has become particularly popular in recent decades. It exploits the ability of nucleic acids to bind reversibly to silica in the

TABLE 1 | Comparison of commonly used nucleic acid purification methods and the proposed method.

Method	Characteristics of obtained nucleic acids				Technical, economic, and safety aspects			
	Achieved nucleic acid purity	Achieved nucleic acid yield	Suitable for purification of long DNA	Can reliably separate free nucleic acids from their covalent complexes and degradation fragments	Easy automation and scalability	Labor-intensive and/or time-consuming	Requires custom/consumables or equipment	Exposure to toxic chemicals
Buoyant density centrifugation (e.g., cesium chloride gradient ultracentrifugation)	High	Moderate (due to a sample desalting step)	Yes	Yes	No	Yes	Yes (ultracentrifuge)	Yes (ethidium bromide)
Liquid phase extraction (phenol-chloroform extraction)	Moderate for complex samples (may require a second extraction step)	Moderate or low (if two extraction steps are employed)	Yes	No	No	Yes	No	Yes (phenol-chloroform)
Solid phase extraction (silica membrane spin columns)	Moderate for complex samples	Moderate (low for HMW DNA)	No ^a	No	Yes (multiple spin columns)	No	No ^b	No
Solid phase extraction (magnetic silica-coated beads)	Moderate for complex samples	Moderate	Yes	No	Yes	No	No ^c	No
Electrophoresis followed by electroelution from gel slices (traditional electroeluters)	High	Moderate (due to a sample desalting step)	Yes ^d	Yes	No	Yes	Yes (electroeluter)	Yes (ethidium bromide)

(Continues)

TABLE 1 | (Continued)

Method	Characteristics of obtained nucleic acids				Technical, economic, and safety aspects			
	Achieved nucleic acid purity	Achieved nucleic acid yield	Suitable for purification of long DNA	Can reliably separate free nucleic acids from their covalent complexes and degradation fragments	Easy automation and scalability	Labor-intensive and/or time-consuming	Requires custom/consumables or equipment	Exposure to toxic chemicals
Electrophoresis seamlessly followed by electroelution (Sage Science systems)	High	High	Yes ^d	Yes	Yes (PippinHT system)	No	Yes (proprietary precast gel cassette and computer-controlled instrument)	No ^e
Electrophoresis seamlessly followed by electroelution (proposed method)	High	High	Yes ^d	Yes	Yes (multiple electrophoretic columns)	No	No ^f	No ^e

^adedicated (more expensive) kits are available for the isolation of HMW DNA with moderate yield and purity.

^brequires proprietary spin columns.

^crequires proprietary magnetic beads.

^dthe separation of very long DNA molecules can be achieved using pulsed field gel electrophoresis (PFGE).

^edoes not require ethidium bromide or similar intercalating dyes if the elution timing is determined in advance (or selected based on marker migration).

^frequires electrophoretic columns.

presence of high concentrations of chaotropic salts such as guanidine hydrochloride. Impurities that remain unbound to the solid phase are removed by washing, after which the nucleic acid is selectively eluted with a low ionic strength aqueous buffer. Commercial kits based on this approach are available from several companies in a convenient spin column format. The advantages of these kits include shorter sample processing times, the absence of hazardous chemicals, and the ability to process large numbers of samples in parallel [5, 6, 24, 29, 30]. However, they still require multiple steps, proprietary chemicals and columns, and may result in suboptimal yields when isolating high molecular weight (HMW) DNA [31, 32]. The limitations of the above methods become even more apparent when the starting material is a complex biological mixture of chemically diverse molecules containing only small amounts of the target nucleic acid. Common examples include soil, food, clinical, forensic, agricultural, and environmental samples. In particular, current mainstream methods are unable to separate free nucleic acids from their covalent complexes and degradation fragments and have difficulty purifying nucleic acids from tightly bound high molecular weight compounds such as polysaccharides [10, 24, 26, 33]. This is a significant issue for downstream methods such as nucleotide sequencing, which require nucleic acids of high purity and integrity. Therefore, the search continues for a universal method that is fast and efficient, yet capable of purifying nucleic acids of any length from any biological sample, regardless of complexity.

2 | Origins of the Method

Our story began many years ago with a challenge. Despite our best efforts, we could not isolate sufficient amounts of pure genomic DNA from polysaccharide-rich plant samples using traditional methods. This experience made us realize the limitations of these methods when dealing with complex biological samples. The fact that DNA from our polysaccharide-rich samples could still be effectively separated by gel electrophoresis prompted us to evaluate the utility of this method for purifying HMW DNA from complex samples. Gel electrophoresis is well known as the preferred method for purifying long oligonucleotides because it provides one of the highest levels of purity available. Similarly, those familiar with molecular cloning know that DNA fragments can be consistently and reliably purified by gel extraction. The most common gel extraction method involves excising the DNA band of interest from the gel, dissolving the agarose slice in a chaotropic salt solution, and purifying the nucleic acid using solid-phase extraction (e.g., silica spin columns) or alcohol precipitation. Although this method produces sufficiently pure DNA, it is labor-intensive, requiring delicate gel handling, and time-consuming because it involves two purification steps: electrophoresis and agarose/chaotropic salt removal. These limitations largely eliminate the possibility of automation and high-throughput sample processing. Additionally, the two-step purification process reduces the overall yield, especially for HMW DNA. Another way to perform gel extraction is through the so-called “crush and soak” technique. In this technique, the gel slice is mechanically crushed and then soaked overnight in a buffer to extract the desired nucleic acid. In the final step, the extracted DNA is separated from the buffer constituents using conventional methods, such as alcohol precipitation. Unfortunately, the “crush

and soak” technique not only suffers from the same limitations as the previous method, but it also introduces a new problem: gel grinding-induced sample contamination with agarose particles and traces of polysaccharide sulfates, which can inhibit downstream enzymatic reactions [34, 35]. Other variations of the gel extraction method, such as those employing low-melting agarose or glass/cotton wool, are also not without issues [36]. The main limitation of all these methods is band excision, which is not only difficult to perform but also requires downstream agarose removal, adding more steps to the sample purification process. Therefore, we decided that our method should completely avoid nucleic acid band excision from a gel.

Instead of chemically or mechanically extracting the desired nucleic acid from a gel, it can be eluted using the same electric field that pulls it through the gel during electrophoresis. This process, known as electroelution, can be performed immediately after electrophoresis in a seamless manner, eliminating the need for gel cutting and subsequent nucleic acid extraction from the gel slice. Historically, however, electroelution has primarily been used to recover purified nucleic acids from gel slices [37–41]. This approach led to the development of a generation of devices called electroeluters in the late twentieth century. Many of these devices worked as follows: First, the target nucleic acid was purified by gel electrophoresis. Next, the band of interest was excised from the gel and transferred to an electroeluter. Finally, the nucleic acid was electroeluted from the gel slice (Figure 2). It is clear that this workflow is not much simpler than those involving gel dissolution or grinding. It also requires an electroeluter, an additional piece of equipment. This is paradoxical because electrophoresis and electroelution work on the same principle and should not require separate instruments. The inability to seamlessly integrate electrophoresis and electroelution was probably the main reason why dedicated electroeluters were eventually phased out. In light of the above, we realized that we needed to rethink the nucleic acid elution strategy and figure out how to use the same instrumentation for both electrophoresis and electroelution.

An example of how electrophoresis and electroelution can be seamlessly integrated into a single instrument is the SageELF system (Sage Science Inc., USA). Based loosely on the method of Stabile and Wurtzel [42], the system employs a precast agarose gel cassette with two sets of perpendicularly oriented electrode ports for electrophoresis and subsequent electroelution [42]. First, the starting sample is loaded onto the cassette, and the gel is electrophoresed using the first set of electrodes. Then, the purified nucleic acids are electroeluted from the gel into 12 separate sample collection wells using the second set of laterally positioned electrodes. The SageELF system allows for both nucleic acid purification and fractionation, with the resulting fractions being pure enough to be used in demanding applications. It is not only an elegant engineering solution, but also a powerful tool with many practical applications. Nevertheless, SageELF has its drawbacks. For example, it is not well suited for processing large numbers of samples simultaneously. The PippinHT system, which works on a similar principle, was specifically designed to address this limitation. Most importantly, however, all such systems require expensive equipment and consumables, including computer-controlled instruments and proprietary gel cassettes with built-in sample collection wells. We decided that our method

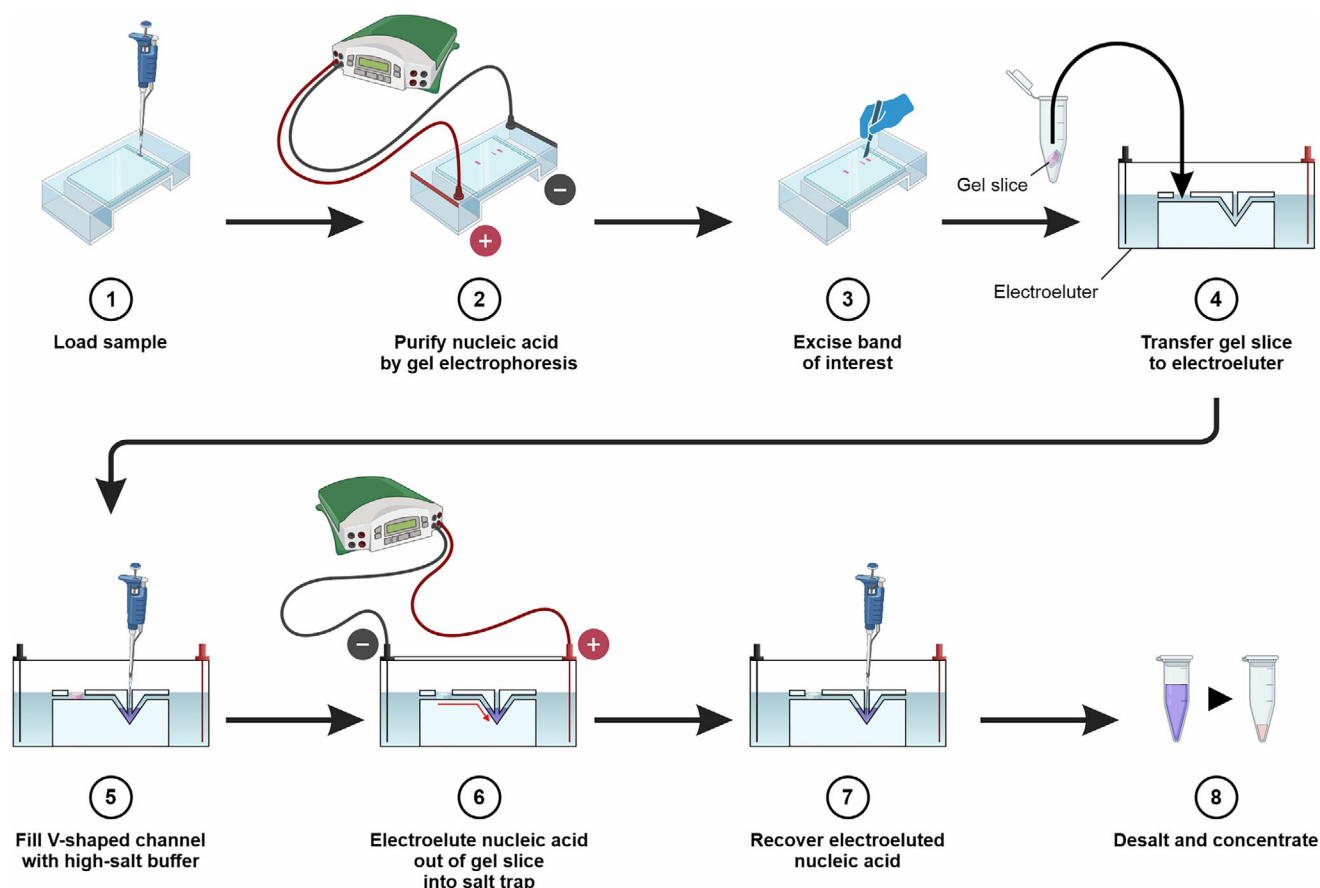


FIGURE 2 | Workflow of the traditional nucleic acid purification method that involves trapping electroeluted nucleic acids in solutions of high ionic strength. The diagram illustrates the process of electroeluting the desired nucleic acid from an agarose gel slice and capturing it in a salt cushion. Note that the method is labor-intensive and requires delicate gel handling. It also requires a separate instrument, the electroeluter.

should use less sophisticated hardware wherever possible to make it suitable for both high-end and budget-conscious laboratories alike.

Several simple, low-tech methods integrating electrophoresis and electroelution have been described in the literature. The central question addressed by these methods is how to collect the nucleic acid after it has been electroeluted from the gel. Historically, one of the first solutions was to slice the gel with a razor blade immediately ahead of the band of interest and insert a nucleic acid-binding membrane. A variant of this procedure is described by Kormanec et al. [43]. To remove unwanted longer nucleic acid fragments, another membrane can be inserted upstream of the band of interest. Although the membrane efficiently captures the electroeluted nucleic acid, its extraction back into solution requires a series of incubations followed by alcohol precipitation. Since this protocol involves many steps and requires manual gel handling, it is clearly not suitable for automated high-throughput workflows. Furthermore, yields can be insufficient, especially for high molecular weight DNA. An alternative approach is to electroelute the nucleic acid into a well excised from the gel ahead of the band of interest. While this solution is simple and logical, it is difficult to implement without trapping the electroeluted nucleic acid in the well. Without such trapping, the free-floating, fast-moving nucleic acid would almost certainly escape the well. Several trapping solutions have been proposed, including filling

the well with hydroxyapatite [44] or polyethylene glycol [45], but they all had limitations and never gained popularity. Therefore, we focused on finding a simple, efficient, and versatile trapping solution that would not require additional sample purification at the end of the workflow.

One promising solution is to trap the electroeluted nucleic acid against a high-salt barrier. This approach has previously been used successfully in traditional electroeluters [37–39]. The idea behind this approach is as follows: Positively charged counterions (cations) and negatively charged co-ions (anions) surround the negatively charged nucleic acid in solution, forming the so-called ion atmosphere [46]. The ion atmosphere contains more attracted cations (counterion accumulation) and fewer repelled anions (co-ion depletion). In solutions of high ionic strength, counterions electrostatically shield the negative charge of the nucleic acid, reducing its electrophoretic mobility [47, 48]. This allows the electroeluted nucleic acid to be trapped in a sample collection reservoir filled with a high-salt buffer, often called a salt cushion (Figure 2). However, there are two problems with this trapping approach. The first is that filling the reservoir with a high-salt buffer is simple to implement in standalone electroeluters, but not in instruments that seamlessly integrate electrophoresis and electroelution. The second is that trapping the nucleic acid in a high-salt buffer would require an additional sample purification (desalting) step. This, in turn, would reduce the

yield and make the method more laborious and time-consuming. Therefore, we decided to look at this trapping approach from a different perspective. We thought, what if we added salt to a gel instead of a buffer? This would allow for the gradual release of counterions into the sample collection reservoir, slowing the migration of the electroeluted nucleic acid and causing it to accumulate. Such a trapping approach is suitable for nucleic acids of all types and sizes and, if performed correctly, should not require an additional sample desalting step. From a technical point of view, it is possible to simply insert a high-salt gel after the sample collection reservoir, achieving compatibility with instruments that seamlessly integrate electrophoresis and electroelution. Thus, we proposed the high-salt gel electroelution trap as a simple and potentially effective solution for capturing electrophoretically purified nucleic acids.

3 | Method Development: From Horizontal to Vertical Electrophoresis

We performed proof-of-concept validation and initial testing of the high-salt gel electroelution trap with horizontal agarose gels, which is the standard configuration for nucleic acid separation. For more information on these experiments and the trapping system we developed, we refer the reader to our previous work [49]. Here, we will only briefly describe the proposed workflow. The first step of the workflow is to purify complex biological samples by electrophoresis in a horizontal channel filled with agarose gel, which we call the separating gel. After the nucleic acid of interest has been separated from impurities and has reached the end of the separating gel, the run is paused, and a tray containing a precast high-salt gel is inserted into the channel (Figure 3, top panel). A buffer-filled gap, which we call the sample collection reservoir, is left between the separating gel and the high-salt gel. When the current is reapplied, excess counterions from the high-salt gel gradually reduce the electrophoretic mobility of the nucleic acid, causing it to accumulate in the sample collection reservoir. The final step is to pipette the purified nucleic acid of interest from the reservoir. In most cases, the salt concentration in the collected sample is compatible with the downstream enzymatic reactions, eliminating the need for an additional sample desalting step. The proposed method is simple, inexpensive, and yields high-quality nucleic acids from even the most complex biological samples. However, there is still room for improvement. For example, the method requires precise sample loading into loading wells, which makes the process difficult to automate. In addition, some current passes through the electrophoresis buffer around the immersed high-salt gel, resulting in sample loss during electroelution.

We addressed these limitations by switching from horizontal slab electrophoresis to vertical column electrophoresis [50]. The improved system utilizes two nested, partially overlapping vertical electrophoretic columns (Figure 3, bottom panel). The upper column, which is smaller in diameter, contains a thin layer of separating agarose gel at the bottom that is overlaid with electrophoresis buffer (see the detailed protocol in the [Supporting Information](#)). This column, which we call the separating column, serves to purify the target nucleic acid. In the first step of the workflow, a complex sample containing the nucleic acid of interest is loaded onto the gel surface in the separating column. It is important to note that sample loading onto the gel surface is

much easier than loading into a well of a slab gel. The procedure requires less precise control and is therefore easier to automate for high-throughput applications. In the next step, the loaded sample is electrophoresed until the desired nucleic acid reaches the end of the gel. The power is temporarily switched off, and a second column of larger diameter is inserted from below. This column, which we call the trapping column, contains a layer of precast high-salt gel overlaid with electrophoresis buffer. As its name implies, the column acts as a nucleic acid trap and is inserted outside of the separating column, causing the two columns to partially overlap. When the power is restored, the desired nucleic acid is electroeluted from the separating column into the buffer-filled gap between the separating gel and the high-salt gel. Excess counterions from the high-salt gel slow its movement and cause it to accumulate. Importantly, there is a tight seal between the high-salt gel and the column wall, making it impossible for the current to bypass the trapping column. This results in faster sample processing and better recovery compared to the horizontal trapping system. In the final step of the workflow, the power is switched off, the separating column is lifted up and set aside, and the nucleic acid is collected by pipetting from the top of the trapping column.

The proposed method is a simple, fast, and efficient way to purify nucleic acids, including HMW DNA for long-read sequencing, from complex biological samples. While the method is available in both horizontal and vertical configurations, the vertical configuration outperforms the horizontal in several ways. First, it is more user-friendly, increasing the method's potential for automation and scalability. Second, the vertical implementation eliminates the problem of electric current bypass, resulting in faster purification and better sample recovery. Third, the vertical column configuration allows for the purification of several nucleic acid species from the same sample. This is achieved by inserting interchangeable trapping columns one at a time as the desired nucleic acids reach the end of the separating gel in the upper column.

4 | Value of the Method

The key advantage of the proposed method is that it provides a straightforward and efficient solution for purifying nucleic acids from difficult samples. Such samples are complex mixtures of chemically diverse biomolecules, such as peptides, proteins, oligonucleotides, polysaccharides, polyphenols, humic substances, proteins, lipids, pigments, and secondary metabolites. Separating target nucleic acids from all these chemically unrelated molecules is a difficult task, often requiring multiple steps in traditional purification methods. The proposed method achieves such separation in a single step through the use of gel electrophoresis. The unique feature of gel electrophoresis is its ability to separate molecules based on differences in charge and size. This allows for the simultaneous purification of nucleic acids from a variety of chemically related and unrelated molecules, making the method ideal for processing complex samples. Furthermore, gel electrophoresis typically requires only minimal preliminary sample preparation to achieve a good separation. We have successfully prepared crude samples for gel loading using simple and inexpensive SDS/Proteinase K or CTAB nucleic acid extraction methods. The SDS/Proteinase K method has

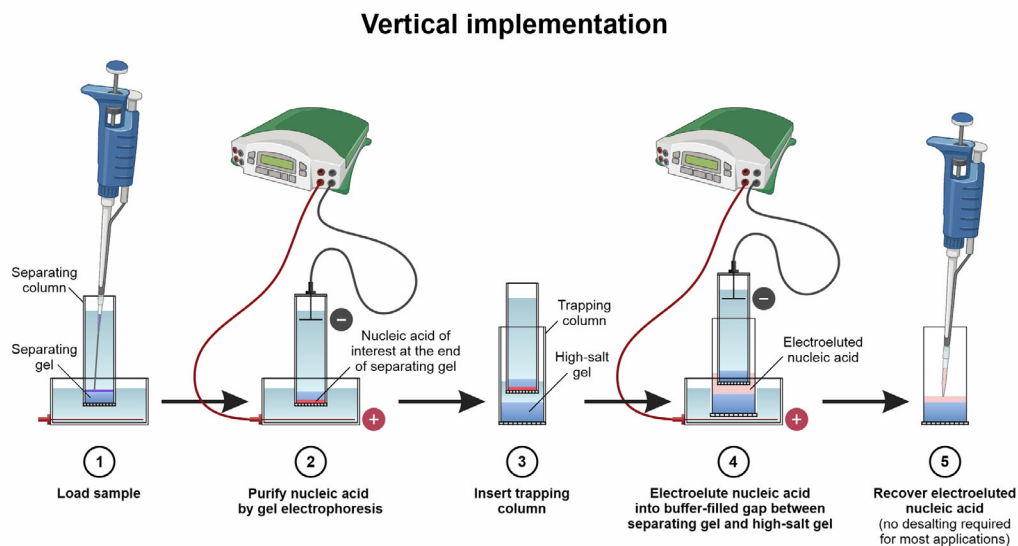
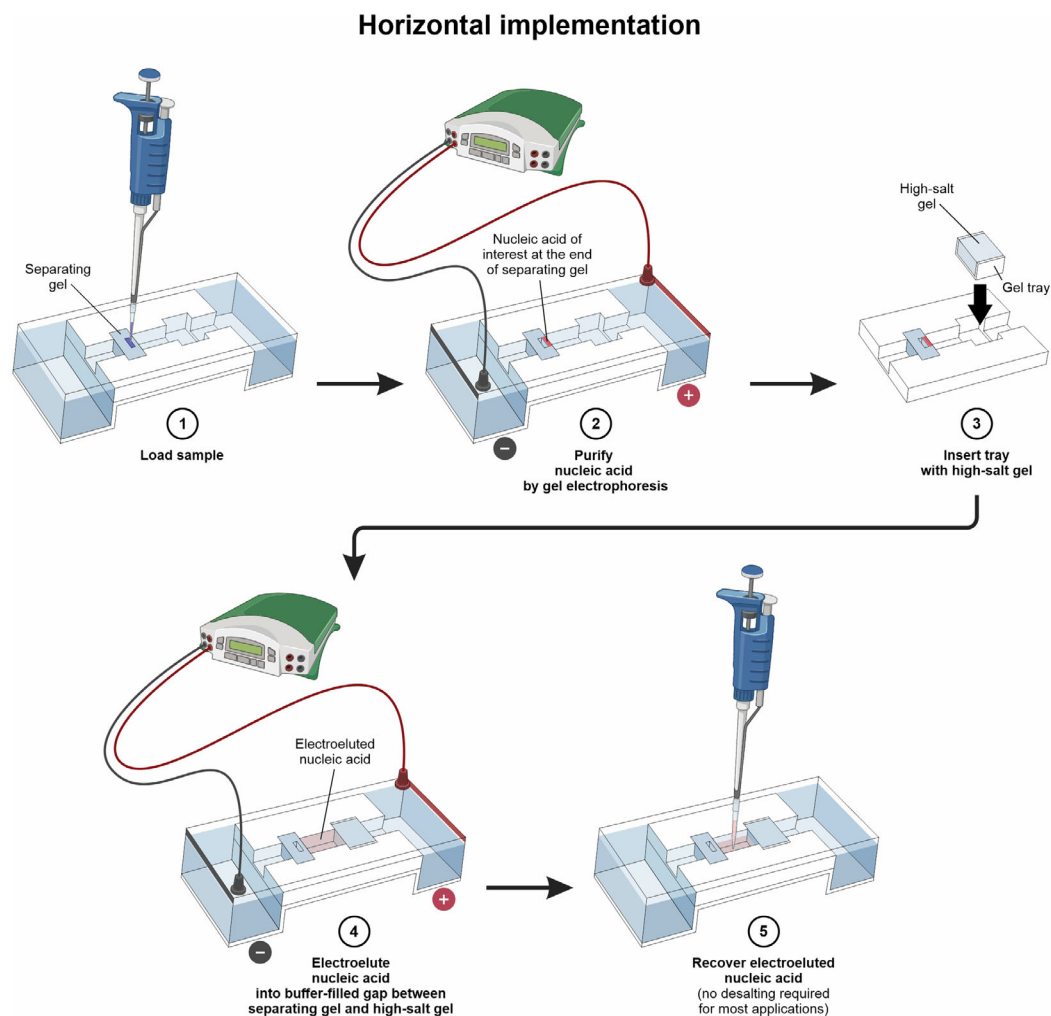


FIGURE 3 | Horizontal and vertical implementations of the purification method that uses a high-salt gel to trap electroeluted nucleic acids. This method is faster and less labor-intensive than the traditional method because it involves fewer steps and does not require careful gel handling. It also seamlessly integrates electrophoresis and electroelution, eliminating the need for the separate instruments used in the legacy method. Additionally, most downstream applications do not require a desalting step at the end of the workflow. The vertical implementation improves upon the horizontal implementation by solving the problem of an imperfect seal around the high-salt gel, which allows some electric current to bypass it. This results in faster processing and better sample recovery and purity. The vertical implementation also does not require precise sample loading into loading wells, which hinders process automation.

the added advantage of inactivating nucleases in cell lysates. Notably, nucleic acid purification by gel electrophoresis requires less starting DNA or RNA than other purification methods. This is particularly useful for processing complex biological samples containing small amounts of target nucleic acid but large amounts of other undesired molecules.

Complex plant and soil samples are among the most difficult for nucleic acid extraction. Plant samples are characterized by an abundance of polysaccharides that are difficult to separate from nucleic acids. In traditional purification methods, polysaccharides are often co-extracted with nucleic acids, forming viscous solutions. Contamination of nucleic acids with polysaccharides is detrimental to many downstream applications, including those that rely on template-dependent synthesis (e.g., enzymatic nucleotide sequencing, PCR amplification, and DNA profiling) [51–53]. Soil samples, on the other hand, contain humic substances that also inhibit polymerases and nucleic acid-modifying enzymes [33, 54]. As with polysaccharides, humic substances are difficult to remove from nucleic acid extracts using traditional methods. Unlike traditional methods, gel electrophoresis can separate nucleic acids from polysaccharides or humic substances in a single step. Therefore, we selected challenging plant and soil samples for experimental testing to evaluate the method's performance. We found that our method could produce high-quality nucleic acid preparations from these samples [49, 50] (Figure S1), which are notoriously difficult to obtain by other methods. In addition, gel electrophoresis followed by electroelution is a very gentle purification strategy that largely eliminates the risk of mechanical shearing of nucleic acids. Thus, the proposed method can extract nucleic acids from complex biological samples in a single step with high yield and purity while preserving their integrity. The quality of nucleic acids produced by the proposed method was sufficient for a number of demanding applications, including long-read nucleotide sequencing.

Another advantage of the proposed method is that it can separate target nucleic acids from their degradation fragments. This is where the method really shines, as most other methods simply cannot accomplish this task. Fragmented DNA is often present in soil and stool samples and, if not removed, it will compete with full-length DNA for pore occupancy during long-read sequencing. Therefore, removing nucleic acid fragments is essential for any DNA extraction method intended for long-read sequencing. Because our method utilizes gel electrophoresis, it can successfully separate full-length DNA from its degradation fragments, thereby increasing the fraction of long sequencing reads. Due to its versatility, gel electrophoresis can also separate free nucleic acids from their natural covalent complexes. Two common examples of such complexes are those formed between nucleic acids and proteins [55] and nucleic acids and oxidized polyphenols [56]. Regardless of their composition, covalent nucleic acid complexes must be removed from purified samples because they can inhibit downstream applications. We have experimentally confirmed that the proposed method effectively separates free nucleic acids migrating in a gel from large covalent complexes that can hardly enter the gel matrix [49]. Thus, the proposed method has the additional advantage of removing nucleic acid complexes and degradation fragments from complex biological samples.

5 | Conclusion

The aim of this paper was to describe how we brought a new perspective to the concept of trapping electroeluted nucleic acids in solutions of high ionic strength. We took the idea of a salt trap from traditional electroeluters, improved it by using a salt-containing gel as the ion source, and turned it into a method capable of producing nucleic acids for cutting-edge, demanding applications such as long-read sequencing. The resulting method is simple, fast, and inexpensive, and can produce nucleic acids with high yield and purity from even the most complex biological samples. Because gel electrophoresis is at the heart of the method, it is universally applicable to the separation of nucleic acids from a wide range of chemically unrelated and related molecules, including difficult-to-separate nucleic acid complexes and degradation fragments. The use of a high-salt gel as an electroelution trap eliminates the need for sample desalting for most downstream enzymatic applications. The method is easy to implement and seamlessly integrates electrophoresis and electroelution, offering the potential for automation and scalability. With further research and optimization, the method can become a reliable mainstream tool for isolating nucleic acids with high purity and integrity from complex biological samples for various demanding applications.

In a more general sense, the method can serve as an example of how traditional, well-established techniques can be repurposed to meet the requirements of modern, next-generation technologies. As we phase out such “old” techniques and replace them with new approaches, we often neglect or forget the underlying concepts. As a result, stereotypes can emerge that restrict these concepts to certain obsolete instruments, such as electroeluters. Rather than accepting such stereotypes, we encourage readers to examine the physicochemical principles behind these “old” techniques and evaluate their potential applications in conjunction with modern technology. Looking at traditional, old-school methods from a different perspective can produce innovative and often unexpected results that may reshape the future of emerging technologies.

Author Contributions

R.K. conceived the idea, developed the method, performed the experiments, and wrote the manuscript. K.I., T.B., O.S. analyzed the results and wrote the manuscript. A.Z. and T.B. provided scientific guidance and resources, as well as contributed to the study's design. All authors reviewed the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

The original gel electrophoresis data of this study are publicly available in Figshare at <https://doi.org/10.6084/m9.figshare.30927881.v1>.

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