Gelatin Substrate for DNA Extraction

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Received May 5, 2025
Revised June 23, 2025
Accepted June 23, 2025

The paper presents a method for creating a gel lifter using gelatin, glycerin, citric acid, and water. The efficiency of the developed gelatin gel lifters for collecting and subsequently extracting nucleic acids from various surfaces is studied. A comparison is made with traditional DNA collection methods, including cotton swabs and manual DNA extraction from *E.coli* samples. An assessment is made of the effect of the composition of the gel lifter substrates on their adsorption capacity.

Keywords: gel lifters, gelatin, DNA extraction, collection and sample preparation of nucleic acids.

DOI: 10.61011/TPL.2025.09.61862.8102

Collection and sample preparation of nucleic acids (NAs) are key stages in the analysis of samples for the presence of genetic material [1]. The quality and quantity of collected biological material have a direct effect on the results of subsequent molecular genetic studies. Traditional tools for DNA collection from surfaces, such as swabs, adhesive tapes, and gel lifters, have their own advantages and disadvantages and vary in efficiency, necessitating the search for new ways to improve it [2].

Gel lifters are flexible gelatin-based sheets that adsorb particles (including cells, hair, or nucleic acids) from surfaces brought into contact with them. They are elastic, biocompatible, and capable of releasing adsorbed material upon lysis. They also do not contain such PCR inhibitors as the bonding agent of adhesive tape [3]. Gelatin is a biopolymer that is used widely in medicine and pharmaceutics due to its availability and non-toxicity. The introduction of plasticizers (e.g., glycerol) improves the functional properties of gelatin films [4], and added citric acid stabilizes their structure [5]. It was demonstrated in [5] that a component ratio of 1:2.5:1.5:0.2 (gelatin : water : glycerol : citric acid) ensures optimum adhesion and thermal stability [5].

The aim of the present study is to compare the efficiency of traditional DNA collection with the developed technique utilizing a gel lifter based on gelatin, glycerol, citric acid, and water.

Powdered gelatin (type *A*, 220 bloom), glycerol as a plasticizer for flexibility, and citric acid were used to fabricate gel lifters for collecting DNA from surfaces. Two gel lifters with different ratios of components (gelatin (Ge), distilled water (H_2O), glycerol (Gl), and citric acid (CA)) were prepared:

- $Ge : H_2O : Gl : CA - 1 : 2.5 : 1.5 : 0.2$;
- $Ge : H_2O : Gl : CA - 1 : 6 : 3 : 0.8$.

Gelatin and distilled water were mixed in a given ratio, and the mixture was then heated to +55 °C for 5 min. Glycerol and citric acid were added successively to the homogenized solution under constant stirring. The resulting mixture was poured evenly into a Petri dish to form a 1 mm layer and dried at room temperature for 48 h. Disks with a diameter of 9.5 mm and a thickness of 1 mm were cut out from the obtained gel lifters for experiments (Fig. 1). They were stored in a refrigerator at a temperature of +4 °C.

The efficiency of genetic material collection by the studied method was compared with the efficiency of NA collection with a cotton swab.

The M-Sorb-OOM kit (Syntol LLC, Moscow) was used to extract *E.coli* DNA from the gel lifters. The measurement results were recorded using the real-time polymerase chain reaction (RT PCR) method and an ANK-48 analyzer (Institute for Analytical Instrumentation of the Russian Academy of Sciences, St. Petersburg).

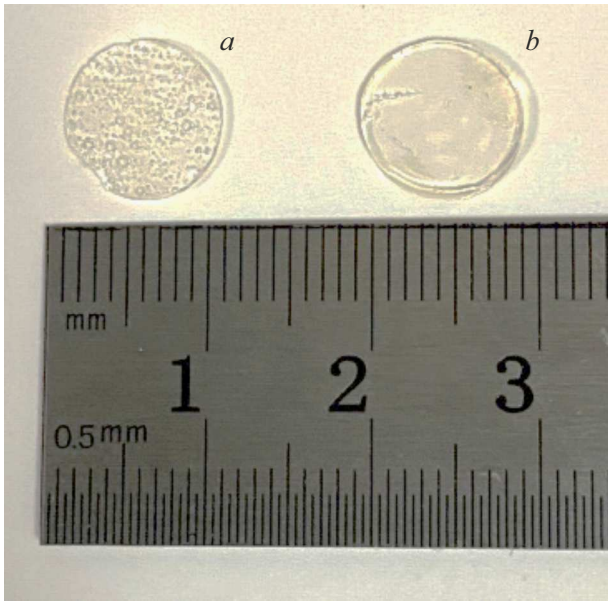

Figure 1. *a* — Gel lifter with a component ratio of 1:2.5:1.5:0.2; *b* — gel lifter with a component ratio of 1:6:3:0.8.

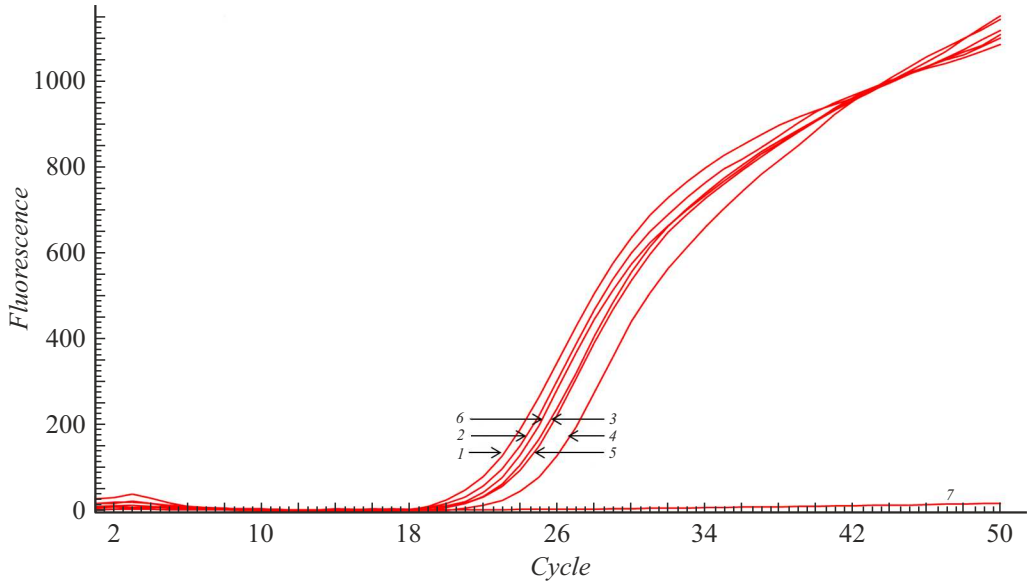

Figure 2. Plots of measurement amplification by RT PCR with an ANK-48 analyzer. *1* — Manual extraction in a test tube; *2* — collection with a cotton swab; *3* — gel lifter (1:2.5:1.5:0.2), dry spot collection; *4* — gel lifter (1:6:3:0.8), dry spot collection; *5* — gel lifter (1:2.5:1.5:0.2), wetting with lysis buffer; *6* — gel lifter (1:6:3:0.8), wetting with lysis buffer; and *7* — negative control sample.

Tests were conducted to determine the effect of a lysis solution and water on the gel lifter at different temperatures. A disk cut out from the gel lifter (9.5 mm in diameter and 1 mm in thickness) was introduced into an Eppendorf tube, which was filled with 500 μl of the lysis solution or distilled water and placed in a thermostat. It was found that gel lifters dissolve in the lysis solution without residue at a temperature above $+55\,^{\circ}$C in 3 min under stirring, but are insoluble in distilled water.

The efficiency of collection of nucleic acids from the surface was evaluated experimentally using a sample containing the genetic material of *E.coli*. A suspension of *E.coli* cells (100 μl) was pipetted onto a grooved polystyrene surface and dried at room temperature. DNA collection from the sample spot was carried out in several ways:

1) a gel lifter disk (compositions 1:2.5:1.5:0.2 and 1:6:3:0.8) was placed on a dry spot, leveled with a roller, and left at rest for 10 min;

2) a gel lifter disk (compositions 1:2.5:1.5:0.2 and 1:6:3:0.8) was wetted with lysis buffer, placed on a dry spot, leveled with a roller, and left at rest for 10 min; and

3) a cotton swab was wetted with lysis buffer, and a dry spot was wiped with it.

DNA collection was performed by applying the substrate to the surface with the sample, leveling the disk with a roller, and leaving it at rest for 10 min at room temperature. The gel lifters were then dissolved in the lysis solution at $+65\,^{\circ}$C. Further sample preparation was carried out in accordance with the standard procedure specified by the reagent manufacturer (Syntol LLC, Moscow). The results were detected by RT PCR using an ANK-48 analyzer (Institute for Analytical Instrumentation of the Russian Academy of Sciences, St. Petersburg).

Three measurements were performed for each method of collecting DNA from the surface. As an illustration, Fig. 2 presents a plot of amplification of one of three repetitions of the experiment; similar results were obtained in all repetitions. Numbers *1*–*6* denote the sample collection methods; *7* — negative control sample.

The table presents the results of *E.coli* DNA extraction from gel lifters in comparison with the genetic material collected from a cotton swab.

The smallest deviation from the control sample upon extraction in a test tube was 0.67 for the traditional method of material collection and 0.73 for collection with the (1:6:3:0.8) gel filter wetted with lysis buffer. It may also be noted that wetting with lysis buffer increases significantly the efficiency of genetic material collection. In addition, the gel lifter with the (1:6:3:0.8) component ratio turned out to be smoother and more sticky than the (1:2.5:1.5:0.2) gel lifter.

Thus, it was demonstrated that the developed gel lifter dissolves well in the lysis solution and does not dissolve in water at room temperature, which makes it potentially suitable for application as a tool for sample collection and transfer.

This method of DNA collection was found to be promising, since it allows for efficient collection and transfer of genetic material from the surface of a gelatin substrate to a sample.

Swabbing from surfaces is a common method of DNA collection. It is easy to use and cost-friendly. However, low efficiency of DNA collection from smooth surfaces and loss of material during transfer without specialized carriers are often noted. If genetic material is to be collected from ribbed or porous surfaces, it is advisable to use gel lifters.

Comparison of results obtained by RT PCR for the studied samples

Sample number	Description	Average threshold cycle (C_t) for FAM	Difference between the studied sample and extraction in a test tube (ΔC_t)
1	Manual extraction in a test tube	21.63 ± 0.16	–
2	Collection with a cotton swab	22.30 ± 0.17	0.67
3	Gel lifter (1:2.5:1.5:0.2), dry spot collection	23.04 ± 0.06	1.41
4	Gel lifter (1:6:3:0.8), dry spot collection	24.50 ± 0.15	2.87
5	Gel lifter (1:2.5:1.5:0.2), wetting with lysis buffer	23.32 ± 0.13	1.69
6	Gel lifter (1:6:3:0.8), wetting with lysis buffer	22.36 ± 0.14	0.73

NAs may be extracted by dissolving a gelatin gel lifter in a buffer solution.

The advantages of gel lifters are high efficiency of DNA collection, applicability to various surfaces, and stability during transfer. The cost is relatively low. The proposed gel lifter holds promise for application in various fields of medical and biological research.

Conflict of interest

The authors declare that they have no conflict of interest.

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Translated by D.Safin