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Optimization of the lysis process for flow-through isolation of nucleic acids

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The influence of the chemical lysis time and temperature on the results of *E. coli* DNA extraction was studied in order to reduce the time without losing quality of nucleic acid isolation. Values of threshold cycles (Ct) of the studied samples obtained by extracting DNA from *E. coli* bacteria were determined and analyzed at various lysis parameters: time (5, 7, 9, 11, 13 and 15min) and temperature (+45, +55, +65, and +75°). It has been shown that it is possible to reduce the lysis time by 67% (10 min) and cell lysis process temperature by 15% (10°C) without losing the efficiency as compared with the recommended conditions (15 min, 65°C).

Keywords: PCR, sample preparation, lysis, temperature, time.

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Molecular biology is currently one of the most topical areas of science. One of its main topics is studying nucleic acids (NA), which has of a great applied importance in such areas as medicine, agriculture, ecology, forensics, fundamental research, and many others. In view of the widespread implementation of molecular-biology methods in routine research, there arises the task of reducing the analysis time, as well as financial and labor costs.

The key stage of molecular diagnostics is sample preparation [1], in particular, NA extraction which quality largely determines the result of the analysis as a whole [2,3]. The initial and necessary process at the stage of NA isolation is the lysis of cells contained in the sample. Nowadays, there are many ways to destroy cell walls and membranes: mechanical methods (high-pressure homogenizer and bead mill) and non-mechanical/physical ones (heating, osmotic shock, cavitation), as well as chemical (exposure to alkalis and detergents) and biological (exposure to enzymes) methods [4]. Among the listed techniques, the chemical lysis methods may be highlighted; they provide high efficiency of the process at relatively low time and financial costs [5]. Notice that these methods are suitable for any type of cells, since they provide the release of target product through both the plasma membrane and outer membrane and bacterial cell wall; however, they may fail to ensure complete destruction of the cell. To overcome this problem, it is possible to use a combination of mechanical and non-mechanical methods for increasing the lysis efficiency [6,7].

The diagnostics speed is one of the most significant characteristics of diagnostics; thus, reducing the time without losing quality of isolation is a key task [8,9]. Optimal cell lysis conditions can allow not only achieving the maximum efficiency of the nucleic acid isolation, minimizing the material loss, and preventing the resulting NA contamination, but also significant reduction in the isolation time. Notice that today the task of reducing the duration of the NC isolation process is especially acute in the context of the necessity of acquiring mass diagnostics data. In addition, diagnostics needs universal optimal lysis parameters since those parameters are applicable to various objects requiring NA isolation. For instance, such universal lysis conditions can be realized in automated NA isolation systems used for diagnostics.

In this study, a widely used at present method of chemical lysis with detergent and heating was applied to destroy the cells. As a sample, such a conventional model object as bacterium *E. coli* was chosen. The bacterial cell wall complicates the lysis process; therefore, in selecting the optimal universal conditions it is important to take into account the possibility of its existence. The *E. coli* bacteria were cultured in the standard Lysogeny broth (LB) medium at +37°C for 18 h. The bacteria concentration was determined by measuring optical density OD600 with an Implen spectrophotometer. The LB medium was used as a blank sample. Concentration of the bacterial suspension used in the research was assumed to $(3.7 \pm 0.3) \cdot 10^8$ cells/ml.

The procedure of NA isolation was performed using a commercial kit (LLC „SYNTHOL") according to the protocol compliant with the kit. The sample was added to the lysis solution, and the mixture was incubated in a solid-state thermostat at the lysis temperature and time adopted in this study; the recommended values of these parameters for the kit were +65°C and 15 min, respectively. Upon thermostating, the sample was cooled, mixed with a sorbing solution of magnetic particles and precipitating solution, and incubated at room temperature for 5 min. After the stage of sorption, the magnetic particles were precipitated by centrifuging; then the sample was placed on a magnetic base, and the supernatant fluid was removed. The sediment was rinsed three times with washing solutions, after which the liquid was completely removed from the sample. Next, the sorbent was dried for 5 min at +65°C. To desorb NA, an eluting solution was added to the magnetic particles, and the mixture was incubated for 10 min at +65°C. After

Mean threshold cycle values (Ct) of the samples under study

Lysis temperature, °C	Lysis time, min					
	5	7	9	11	13	15
75	17.82	17.89	17.80	17.74	17.69	17.61
65	17.33	17.32	17.24	17.44	17.31	17.31
55	17.37	17.52	17.50	17.57	17.39	17.19
45	17.96	18.22	18.10	18.27	17.92	17.72

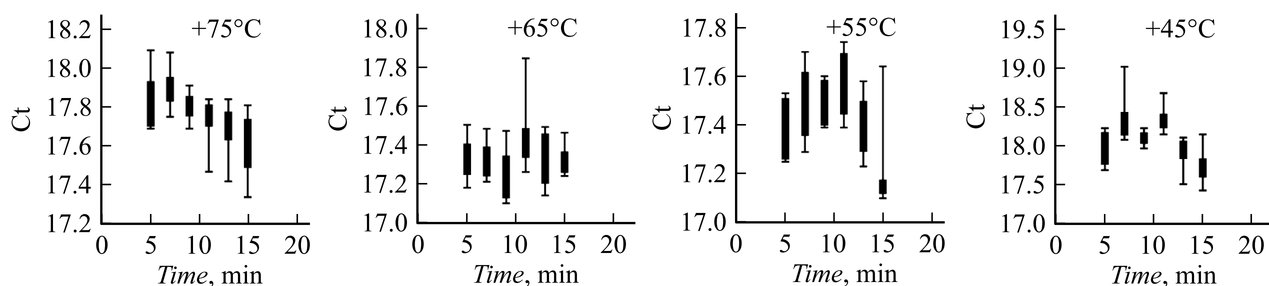

Figure 1. Block diagrams containing the obtained data on threshold cycles (Ct) for temperature groups (+75, +65, +55, +45°C).

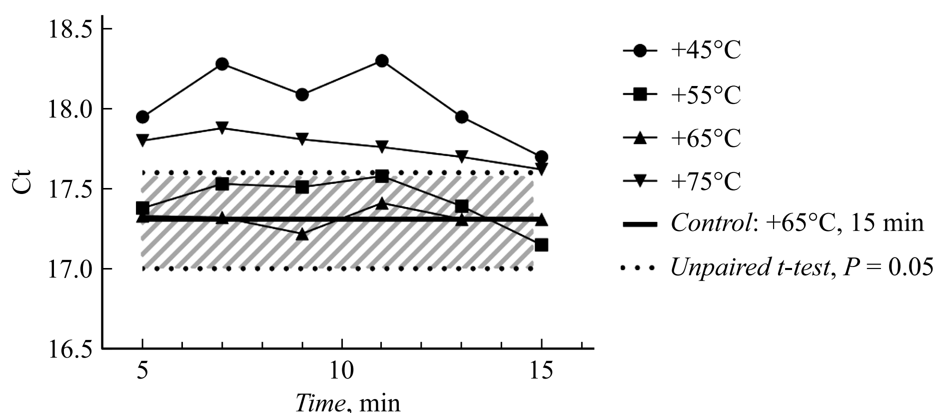

Figure 2. Average threshold cycles (Ct) for the temperature groups versus the lysis time. In the plot, the value of the control threshold cycle for lysis during 15 min at +65°C is designated. The range of threshold cycles at $P \leqslant 0.05$ is indicated by dotted lines and hatching.

thermostating, the magnetic particles were precipitated by centrifuging, the sample was placed on the magnetic base, and the supernatant fluid containing NA was collected and transferred to a clean test tube. The procedure of NA isolation was repeated ten times for each value of time and temperature; as a negative isolation control, distilled water was used.

The efficiency of lysis during NA isolation was assessed by real-time polymerase chain reaction (PCR-RT) with the obtained samples. PCR-RT was conducted using a commercial kit („SYNTHOL“ LLC) according to the protocol compliant with the kit at device ANK-48 („SYNTHOL“ LLC. The threshold cycles (Ct) of the samples under study were determined. For each time and temperature, the Ct parameter was averaged over ten measurements, and block diagrams were constructed for the temperature groups. Average threshold cycles Ct of the studied samples are listed in the Table.

Fig. 1 presents block diagrams for the temperature groups (+75, +65, +55 and +45°C); error limiters allow assessing the data dispersion.

The groups (ten measurements) for each temperature and time were compared with a reference group of measurements corresponding to the recommended parameters of the commercial kit for isolation (+65°C, 15 min). The comparison was performed via the unpaired t-test; groups significantly different from the reference group were revealed, as well as the Ct value corresponding to the significance threshold $P = 0.05$. The data obtained are plotted in Fig. 2.

The obtained data were processed with the GraphPad Prism 8.0 package.

Literary sources have shown the possibility of varying the lysis temperature; however, long-term heating can damage the NA structure, and, therefore, the temperature of material release for further analysis should be selected based on the research objectives. The results of the study showed that it is possible to reduce the cell lysis temperature by 15% (10°C) without loss of efficiency as compared with the recommended conditions (65°C). Thus temperature increase led to a decrease of efficiency due to NA damage, which fully corresponds with literature data.

The main issue in diagnostics in the case of large-scale sample preparation seems to be the speed of performing the standardized isolation process with the use of the diagnostic kit. As a result of the study, there was shown the possibility of reducing the time of lysis with the commercial kit by 67% (10 min) without loss of efficiency in comparison with the recommended conditions (15 min).

Thus, in the framework of this work there were found optimal conditions for the NA isolation using the commercial kit, which allow reduction in the lysis temperature and significant decrease in the lysis time without loss of efficiency.

In future, we plan to continue studying the lysis efficiency, for instance, to extend the ranges of the studied parameters and include in the study other model objects.

Conflict of interests

The authors declare that they have no conflict of interests.

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