Application of megahertz ultrasound to isolate nucleic acids

D G Petrov1, I E Antifeev1, R H Djenloda2, N N Germash1 and E D Makarova1

1Institute for Analytical Instrumentation Russian Academy of Science, Ivana Chernykh 33A, 198095, St. Petersburg, Russia
2Vernadsky Institute of Geochemistry and Analytical Chemistry RAS Kosygina 19, 119991 Moscow, Russia

E-mail: dimoon888@gmail.com

Abstract. The dependence of the yield of M. tuberculosis DNA from model solutions under the influence of megahertz ultrasound in the range from 1.2 to 3.0 W/cm² on magnetic particles was studied. The dependences of the DNA yield upon sounding for 1, 5, and 10 minutes are obtained. Under ultrasound exposure, the maximum yield is 98%, which is 39% higher than the DNA yield in the absence of a similar effect. It was shown that the efficiency of DNA extraction on magnetic particles using ultrasound is not linearly dependent on the intensity of scoring. The result of the electrophoresis of the sample after ultrasonic treatment of different intensities in megahertz for 1, 5 and 10 minutes is also shown.

1. Introduction
Isolation of nucleic acid (NK) is an important step in the preparation of samples in genetic analysis methods. Often the result is highly dependent on the presence of impurities in the samples, so the purity of the sample often determines the reliability of further analysis. [1]. The method of nucleic acid extraction on magnetic particles is the most popular; it is based on the adsorption capacity of nanocrystals on particles with a SiO2 surface [2, 3].

The principle of this method is quite simple: DNA is adsorbed on the surface of SiO2, no impurities are washed off by the wash solution stream, after which DNA is desorbed from the surface under the influence of an eluate buffer. This principle provides a quick, non-toxic method that can provide DNA isolation with an efficiency of about 50-75%. 100% efficiency cannot be achieved, since 5-10% of DNA can remain on the surface of SiO2 [4].

The binding between DNA and SiO2 is based on surface electrostatic interactions, and depends on the intensity of mass transfer. Obviously, the low rate of DNA adsorption on different surfaces is controlled by diffusion [5].

Studies have shown that we can use not only temperature, but also ultrasound to increase the efficiency of DNA extraction. Unique features of ultrasonic exposure are physical and chemical effects that can be used for various purposes. In particular, the ultrasonic effect shown on the diffusion process can increase the mass transfer intensity [6].

The aim of this work is to study the effect of ultrasonic (MHz) effects on the efficiency of DNA isolation. Tuberculosis on the surface of magnetic particles coated with SiO2 [6].

2. Materials and methods
To prepare model DNA solutions, Mycobacterium tuberculosis DNA was used at a concentration of 107 copies / ml, which is included in the Amplitude-Tub-RT commercial kit. To isolate
Mycobacterium tuberculosis DNA, the magnetic particles contained in the M-Sorb-Tub kit were used. A working sample of a concentration solution of $1 \times 10^5$ copies / ml was prepared by serial dilution using for this purpose the “Lysing Agent” (LR) from the “M-Sorb-Tub” kit. For the quantitative determination of DNA, the reagents supplied with the Amplitude-Tub-Rt qPCR kit were used: primers, buffer, probes, oligonucleotides, magnesium chloride. To conduct experiments to study the effect of ultrasound on the efficiency of DNA extraction, a setup based on the “Acoustic Filter” manufactured by the Institute of Atomic Energy, Russian Academy of Sciences, was used (RF patent No. 2393907). The installation diagram is shown in Figure 1 for contact with magnetic particles in the sample.

![Figure 1. Scheme installations for experiments with the magnetic particles](image_url)

The setup allows you to vary the intensity of ultrasound in a liquid medium in the range from 0.5 W / cm$^2$ to 3 W / cm$^2$, set the radiation frequency in the range from 2.5 to 3.0 MHz. A qPCR analysis was performed at ANA-32 manufactured by the IAI RAS. The device allows you to analyze 32 samples at a time. The operation mode (gain parameters) is selected in accordance with the instructions for the “Amplitude-Tub-Rt” set.

To prepare test samples, 800 μl of the working DNA solution was added to 800 μl of the Lysing Agent and mixed using a Cyclotemp-901 microfuge shaker. Thus, the DNA concentration in the solution was $2 \times 10^4$ copies / ml. Next, each sample for DNA extraction (sample for each ultrasound intensity), using magnetic particles, is subjected to the following manipulations:

1. DNA sorption.
   The sample was placed in a 2 ml glass cuvette, 20 μl of the solution and 500 μl of the precipitating solution were added. The contents of the glass cuvette were thoroughly mixed until the sorbent was evenly distributed in the device, and ultrasound was turned on as shown in Figure 1. After 1, 5 or 10 minutes, it was turned off in accordance with a series of experiments. Then, holding the magnetic particles, the supernatant was carefully removed.

2. DNA flushing.
   500 μl of the washing solution were introduced into the glass cuvette, the contents of the tube were mixed in a microcentrifuge, until the sorbent was evenly distributed. Expected 1 minute. Then, holding the magnet, the supernatant was removed. Similarly, the procedure was carried out with a precipitating solution of 2.3.

3. DNA desorption.
200 μl of the elution solution was added to the cuvette, the contents were mixed and incubated for 10-15 minutes. at 75 °C and periodically mixed in a vortex. Holding a magnet, the solution was taken for PCR-RV analysis.

To determine the DNA concentration, 10 μl of the solution obtained in the solution was collected twice and analyzed by qPCR using an ANA-32 sample calibration instrument (n = 2).

3. Results

Under the influence of ultrasound on a sample with magnetic particles, the movement of magnetic particles throughout the volume of the solution was visually observed. The nature of the movement varies depending on the intensity of the ultrasound, and the speed of the visually observed mass (group) movement of particles in the cell increases with increasing intensity of the ultrasound.

Figure 2 is a graph of the average values of the DNA yield during sorption on magnetic particles under the action of ultrasound of varying intensity when irradiated for 1 minute.

![Figure 2](image2.png)

**Figure 2.** Dependence of DNA output values of the ultrasonic impact at the sorption on magnetic particles.

Figure 3 is a graph of the average values of DNA yield during sorption on magnetic particles under the action of ultrasound of varying intensity when irradiated for 1 minute 5 minutes and 10 minutes.

![Figure 3](image3.png)

**Figure 3.** Dependence of DNA output values of the temperature at the sorption on magnetic particles. Solid line - 1 min; dotted line - 5 min; broken line - 10 min.
The result of the electrophoresis (comet assay) show the plasmid under the influence of ultrasound on his different intensity and different exposure time is shown in Figure 4.

According to the results of the analysis, we can conclude that the destruction of the plasmid into fragments occurs at intensities close to 3.0 W/cm² and a time of more than 5 minutes. Thus, the possibility of detection using qPCR can be saved. Also, the fact of plasmid fragmentation at high intensities and durations of sound can explain the appearance of “super-efficient” DNA extraction (efficiency of more than 100%), in which individual DNA fragments can randomly become targets for primers, in gain, thus artificially increasing with gain efficiency. However, this process has a low value, comparable with the accuracy of the entire procedure for DNA extraction from sample preparation in order to close specific indications.

4. Conclusion
A study of the effect of megahertz ultrasound on the output of M. tuberculosis DNA using model solutions and commercial magnetic particles in the intensity range from 1.2 to 3.0 W / cm² showed that this approach can serve as an alternative to temperature exposure. With effective ultrasonic treatment, the maximum yield is 98%.

There is every reason to believe that these results are mainly related to the occurrence of hydrodynamic flows, which affect the speed and efficiency of mass transfer. Thus, the use of megahertz ultrasound can significantly increase the efficiency of DNA extraction, which creates the prerequisites for the use of ultrasound to create high-performance systems for the separation, purification and concentration of DNA.
Acknowledgments
Kits of reagents for nucleic acid isolation and qPCR were provided JSC Syntol (Moscow, Russian Federation).

References
[1] Antonova O S, Korneva Yu V, Belov Yu V, Kurochkin V E 2010 Effective methods of release of nucleic acids for carrying out analyses in molecular biology Nauchnoe Priborostroenie 20 (1) 3-9
[2] Boom R, Sol C J A, Salimans M M et al. 2009 Rapid and simple method for purification of nucleic acids J. Clinical Microbiology 28(3) 495–503
[3] Herzer S, Ed. A S Gerstein Willey-Liss 2005 DNA purification. Molecular biology problem solver: a laboratory guide 1 167–193
[4] Esser K, MarxW H, Lisowsky T 1998 MaxXbond: first regeneration system for DNA binding silica matrices. Nature Methods 1(2) 1–2
[5] Kurochkin V E 2006 Methods and tools for the express immunoassay A new approach to solving the problem PACS 176 994–999
[6] Dzhenloda R K, Petrov D G, Shkinev V M, Spivakov B Y 2017 DNA recovery from environmental samples on suspension columns under a combined action of ultrasound and magnetic fields followed by polymerase chain reaction detection Journal of Mendeleev Communications 27(3) 302-303