Development of a hydraulic system for bridge amplification

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Abstract. In this paper, we consider the features of creating a hydraulic system with the goal of conducting bridge amplification, which is part of the Illumina/Solexa method. The result of the work is an automated system with all the necessary functional qualities.

1. Introduction
Deteriorating global environmental conditions, the production increased with using of the different technology, the emergence of new viruses and diseases and much more, required the development of various methods for control and research of condensed media [1-10]. For solved these difficult tasks are necessary the different methods [8-23]. These methods should be applicable in various conditions. Comparing the results obtained with their help can get the reliable information of the medium parameters [6, 11-15, 23-39]. These methods differ to complexity of each other in dependence from solved tasks.

One of the most difficult tasks is the research of the genome, including the human genome. This direction is now actively developing in medicine. The research of the genome is inextricably linked with the study of the sequence of nitrogenous bases that make up deoxyribonucleic acids (DNA). One such technique is to “read” this sequence or otherwise a sequencing process [40, 41].

Sequencing methods are constantly being improved and currently the next generation sequencing methods (NGS) are the most relevant. One such method is the Illumina / Solexa method, which is a mass parallel sequencing method.

The advantages of this method are: the ability to obtain a large amount of data in a relatively short period of time, i.e. high sequencing speed, as well as high accuracy achieved by mass. In addition, due to the high speed, the cost per unit of information received is significantly reduced.

One of the components of this method is bridge amplification, an important process which results in DNA segments ready for sequencing, called clusters, fixed on a special surface of the substrate with immobilized oligonucleotides, which act as primers during the reaction. The process of bridge amplification is based on a sequential increase in the number of segments of the test sample. After passing a special sample preparation, the sample goes through the stage of presynthesis [42].

2. Hydraulics features
Next, it is necessary to perform several amplification cycles, which consist in a sequential process of linearization of the chain, for which a special linearization mixture is used. After this, it is necessary that the linearized library interact with the mixture for amplification during the experimentally set time. Before carrying out the next cycle, it is necessary to conduct denaturation by feeding formamide. Several cycles are necessary to increase the number of clusters [42].

Based on the characteristics of the reactions, it became necessary to create an automated system for its implementation. The main requirements that are presented to the system include: the ability to
pass reagents through the space in which the substrate with the primers is located, as well as their removal from this space, the ability to automatically select reagents for pumping, the ability to control the pumping and removal process, and the ability to change the temperature at which the reaction takes place on the substrate. In addition, it is extremely important to be able to verify that the clusters are successfully grown on the surface of the substrate.

The block diagram of the developed hydraulic system is shown in figure 1.

![Figure 1. Block diagram of the hydraulic system.](image)

The necessary reagents are placed in a tripod, which is connected by flow channels to a hydraulic switch. This switch allows you to connect the flow channels that go to the tanks with reagents, with the flow channel that goes to the reaction cell, in which there is a substrate with primers.

The hydraulic switch has 24 possible positions, which allows the use of up to 24 different reagents for the reaction. The reaction cell itself is a C-shaped channel, the surface of which is covered with immobilized oligonucleotides. Segments of DNA clinging to these primers undergo an amplification process.

A precision syringe pump is used to pump and remove reagents. In the event of a discharge of the syringe, the reagent fills the flow channels, and then the cell itself together with the pump syringe. When the syringe is empty, the reagents contained in it are sent to the drain tank. To implement the temperature regime, a thermal cycling system is used.

3. Operation of the hydraulic system

Based on the tasks set, it is necessary to implement the algorithm of the hydraulic system. The block diagram of this algorithm is presented in figure 2.

![Figure 2. Block diagram of hydraulic operation algorithm.](image)
In the course of work, a problem arose of controlling the fluid flow rate in the flow channels of the hydraulic system. In addition, for carrying out the reaction, it is necessary that the system in a state of hydrostatic equilibrium in which there is no fluid flow. Due to this problem, it was decided to use the LPG10-1000 calorimetric flow sensor. The choice of this sensor was justified by the parameters of permissible flow rates, as well as the coincidence of the diameter of the flow part of the sensor with the flow channels of the hydraulic system. A decrease of the fluid flow rate below a certain threshold signals a transition of the system to the state of equilibrium.

The transition to the state of hydrostatic equilibrium is necessary before performing the following fluid pumping procedure, since premature switching of the position of the hydraulic switcher will lead to a decrease in the volume of reagent entering the reaction cell.

Before the first pumping of each of the using reagents, it is also necessary to fill them with flow channels leading from test tubes to a hydraulic switcher, which will subsequently allow repulsion solely from the volume of the reaction cell, as well as the flow channel connecting the hydraulic switcher to the reaction cell.

One of the problems that arose during the development of the hydraulic system was the control of the airing into the reaction cell at the junction of the cell inlet with flow channels coming from the hydraulic switcher. Air can also enter the reaction cell if there is gas dissolved in the liquids contained in the test tubes, which in turn can lead to low quality amplification of a sample.

To reduce the concentration of air dissolved in the reagents, they must be degassed before use.

The developed system uses the so-called bubble sensors, the operation of which is based on receiving signals received from two optical couplers.

This tracking system allows you to draw preliminary conclusions about the quality of the performed bridge amplification without photo registration of the formed clusters.

During the experiment conducted on the developed system, it was possible to successfully carry out bridge amplification, the result of which was the presence of amplified clusters on the surface of the substrate of the reaction cell.

In order to confirm the presence of clusters, an optical detection system was used, the results of which are presented in figure 3.
Each cluster is a sequence of nucleotides. To detect each of the nucleotides, they are joined by dyes that have the ability to fluorescence. To detect clusters, you need to make a laser illumination, and then get an image that captures the fluorescence of dyes.

4. Conclusion
Thus, the developed automated system allows bridge amplification, which is extremely necessary for sequencing using the Illumina/Solexa method. This improves measurement accuracy.

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