Using of «bubble sensors» to control the quality of sequencing by the Illumina / Solexa method

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Abstract. In the modern world, sequencing is an integral part of medicine, biology and other scientific fields. The Illumina / Solexa method is a new generation method and relates to methods of mass parallel sequencing. One of the features of using this method is the sequential pumping of various chemicals through the flow cell in which the reaction occurs. For uniformity and high quality of DNA sequencing, it is necessary that the amount of gas in liquids be minimized. Because many it can adversely affect both during chemical reactions and at the stage of recording reaction results. This article will examine the sequencing system using the Illumina/Solexa method using bubble sensors. An algorithm was developed that periodically receives information from bubble sensors in a microfluidic tube. The information received is processed and allows at certain stages to report deviations from the normal conditions for sequencing. The experimental results are presented.

1. Introduction
Deterioration of the ecological situation \cite{1-5}, man-made disasters that lead to mutations \cite{6-9}, required the development of various methods for research. Currently, sequencing of the sequence of nucleotides that enter DNA and RNA has become a familiar and necessary phenomenon in various fields \cite{10-14}.

This method has several advantages in terms of informativeness of research in comparison with other methods, for example, optical spectroscopy \cite{15-20} or nuclear magnetic spectroscopy \cite{21-27}. The nuclear magnetic spectroscopy has a higher measurement accuracy compared to other methods \cite{26-33}. But the information contained in the NMR spectrum is not enough for a genetic analysis with a unambiguous result. The using of the various methods for optical signal processing techniques \cite{15-20, 34-37} in the optical spectroscopy does not allow to obtain a complete DNA chain. Therefore, DNA sequencing methods remain the most reliable.

One of the widely used methods for carrying out this process is the use of the Illumina / Solexa method. The result of sequencing is data on the sequence of nucleotides that make up the sample. One of the important features of the implementation of this sequencing method is the use of an optical system during detection.

Illumina / Solexa sequencing uses a large number of different chemicals. For ease of
implementation, these reagents are placed in test tubes, and then through the flow channels enter the reaction cell.

In this connection, the problem of getting into the system of bubble bubbles of gas dissolved in reagents arises. In addition, air can enter the system at the junction of the flow channel with the reaction cell.

The ingress of gas bubbles into the reaction cell at the stage of carrying out amplification can lead to a decrease in the number of clusters, which in turn will negatively affect the quality of further analysis.

On the other hand, the ingress of gas bubbles at the detection stage leads to a decrease in the total number of clusters in the field of view of the detection system. The used of other industrial devices for determining bubbles is excluded [33-35].

To solve this problem, it was proposed to use the so-called “bubble sensor”, which allows counting the number of gas bubbles passing through the reaction cell at all stages of sequencing.

2. Working process of bubble counting system

To count the number of bubbles, it is advisable to use an optical sensor as a sensitive element. This fact is justified primarily by the fact that flow tubes, due to their transparency, transmit light. The radiation intensity will be highly dependent on the medium. Accordingly, if a gas bubble passes through the flow channels, the value of the radiation intensity will change its value.

Therefore, it is necessary to use a combination of a radiation source and a photodetector. The obvious solution in this case will be the use of optocouplers.

However, this measuring circuit will be sufficiently effective only if the gas bubble enters the field of view of the optocoupler. In the event that the bubble is smaller than the diameter of the tube or moves along the walls of the tube, the value of the radiation intensity recorded by the photodetector may not be high enough to determine the passage of the bubble.

To solve this problem, it was decided to use two optocouplers located perpendicular to each other. The block diagram of the total operation of the bubble detection system is shown in Figure 1.

![Figure 1. Block-diagram of signals in using of «bubble sensors».

A feature of the work is the use of a comparator. After the bubble passes through one of the optocouplers, the level of radiation received by the photodetector changes. The radiation level of the photodetector is converted into an electrical signal.

The converted electrical signal is fed to the input of the comparator, the second input of which receives the reference signal. The level of this signal corresponds to the minimum level of radiation arising from the passage of a bubble. This signal is generated at the output of the digital-to-analog microcontroller converter. As a result of this, the level of this signal can be adjusted to different types of reagents and bubble sizes and is usually determined experimentally.
If the signal level from the optocoupler exceeds the value of the reference signal, a voltage is generated at the output of the comparator, which corresponds to the level of a logical unit, which in turn is interpreted by the microcontroller as the presence of a bubble.

Similarly, the processing of signals from the second optocoupler occurs. It is also important to note that optocouplers are located behind the reaction cell, since this allows one to take into account the possible ingress of gas bubbles at the junction of the reaction cell with the flow channels.

The converted electrical signal is fed to the input of the comparator, the second input of which receives the reference signal. The level of this signal corresponds to the minimum level of radiation arising from the passage of a bubble. This signal is generated at the output of the digital-to-analog microcontroller converter. As a result of this, the level of this signal can be adjusted to different types of reagents and bubble sizes and is usually determined experimentally.

If the signal level from the optocoupler exceeds the value of the reference signal, a voltage is generated at the output of the comparator, which corresponds to the level of a logical unit, which in turn is interpreted by the microcontroller as the presence of a bubble. Similarly, the processing of signals from the second optocoupler occurs.

3. Analysis data from «bubble sensors»

During sequencing, the control program sends requests to the microcontroller, to which the sensors are connected, requesting the number of registered gas bubbles every 5 seconds (see Figure 2). Further analysis is carried out in two ways:

- The number of bubbles for a period of 5 seconds should not exceed 500
- The number of bubbles for the entire duration of the sequencing should not exceed 25,000

If the number of bubbles is exceeded in one of the two above parameters, the general sequencing algorithm proceeds to interrupt the system.

These threshold values of the number of bubbles were obtained experimentally and are justified by a significant deterioration in the sequencing results if these values are exceeded.

![Block diagram of the main algorithm with using «bubble sensors»](image)

**Figure 2.** Block diagram of the main algorithm with using «bubble sensors».

4. Conclusion

Thus, the developed system for analyzing the number of gas bubbles passing through the reaction cell during mass parallel sequencing using the Illumina / Solexa method can significantly improve the quality of the output data of the analysis.
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