Development of Nucleic Acid Extraction Technology and Its Product Strategy

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OVERVIEW: After sequencing the entire human genome, researchers are now turning their attention to clinical gene testing and analysis to explain gene function and differences among individuals. To research and to test genetic material, efficient and simplified techniques to extract nucleic acid from blood samples are essential. Hitachi has developed a new aspirate-discharge method, which incorporates silica, to extract nucleic acid from the whole blood. A nucleic acid sample under specified conditions is repeatedly aspirated and discharged inside a syringe using a silica carrier that is immobilized in a wet state. The byproduct of this method is a blood genome extraction kit that directly extracts nucleic acid from one to 10 mL of the whole blood without leukocyte separation. The kit achieves an average extraction rate of 91%. The extracted nucleic acid can then be used directly for subsequent gene analysis and testing. In addition, the kit does not have lower extraction efficiency when a frozen blood sample is used.

INTRODUCTION

AFTER sequencing the entire human genome, researchers are now turning their attention to clinical gene testing and analysis to explain gene function and differences among individuals. However, to research and to test genetic material, researchers require a pretreatment method to extract nucleic acid from a sample.

One long-established method to extract nucleic acid is to use an organic solvent like phenol. Recently, a method that uses silica is gaining popularity for its simplicity and speed.

Silica and nucleic acid binding has been known for a long time. In 1979, B. Vogelstein et al. developed a method to extract DNA (deoxyribonucleic acid) through gel electrophoresis[1)]. Improvements in both reagent and silica techniques have since led to the emergence of various kits and automatic devices[2)].

Below we describe the features of a blood genome extraction kit and its future development strategy. The kit incorporates a new aspirate-discharge method to extract nucleic acid using silica (see Fig. 1).

ASPIRATE-DISCHARGE METHOD

The new aspirate-discharge method immobilizes a silica carrier on a pipette tip or inside a syringe.

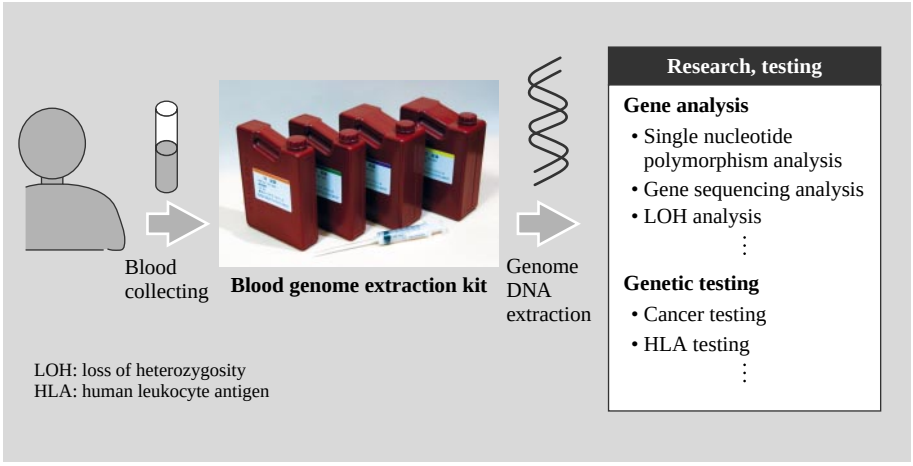

Fig. 1—Genomic DNA Extraction from Blood.
Blood contains genomic DNA that is the blueprint of life. Gene analysis and testing is used to extract genomic DNA found in blood.

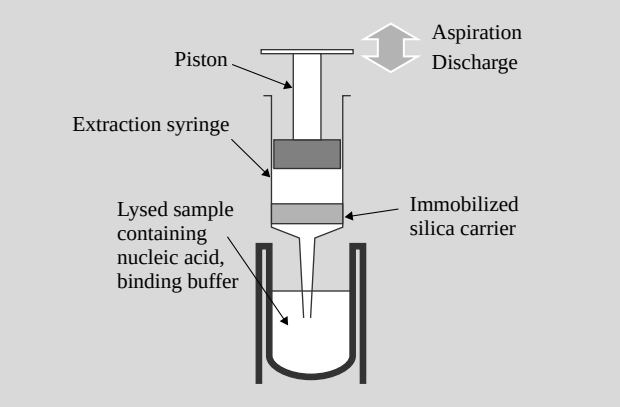

Fig. 2—Aspirate-discharge Method.
A silica carrier is immobilized inside a syringe. The nucleic acid binds to the silica efficiently and stably through repeated aspiration and discharge movements.

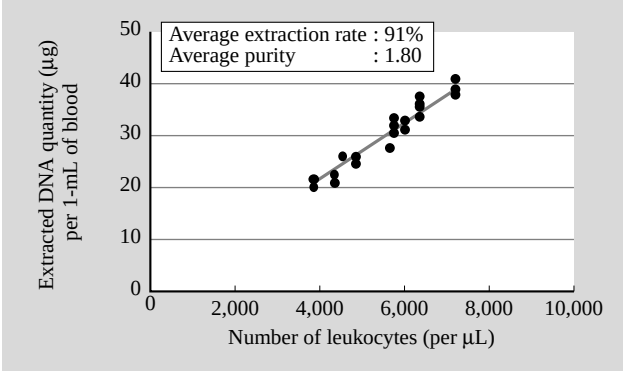

Fig. 3—Result for DNA Extraction from Whole Blood.
The figure shows results for nucleic acid extraction (n = 40) using blood samples with different leukocyte levels. After extraction, PicoGreen quantification is used to determine DNA concentration.*
* PicoGreen is a registered trademark of Molecular Probes, Inc.

After repeated aspiration and discharge, nucleic acid binds efficiently to the silica. The nucleic acid is then washed and eluted. Researchers can then directly use the eluted solution to perform gene analysis and testing. Fig. 2 shows an overview of the aspirate-discharge method.

In the aspirate-discharge method, first a sample containing nucleic acid is lysed. A solution is added to help bind the nucleic acid to a silica carrier after which the whole solution is mixed. An extraction syringe containing a silica carrier is then used to draw in the mixed solution containing nucleic acid through the upward movement of the syringe piston. At this time, the mixture and the carrier make contact, which causes the nucleic acid to bind specifically to the carrier. After drawing in, the mixture inside the syringe is discharged through the downward movement of the syringe piston. At this time as well, the mixture and the carrier make contact, which causes the nucleic acid to bind specifically to the carrier. Through repeated upward and downward piston movement, the mixture is drawn in and discharged many times, which ensures stable binding reproducibility between carrier and nucleic acid. After binding is complete, it is possible to dispose of the mixture by using the discharge movement only.

After binding, washing and elution are performed. Once again the upward and the downward movement of the piston is used to ensure stable yields for purification and elution.

BLOOD GENOME EXTRACTION KIT

Kit Features

The blood genome extraction kit is a trial product that incorporates an aspirate-discharge method. The kit can directly extract nucleic acid from one to 10 mL of blood without leukocyte separation. The kit has a special reagent and an extraction syringe used for binding, washing, and elution.

Silica carrier inside the extraction syringe plays an important role in the stability of aspiration and discharge. Pore size is crucial to both a porous carrier and a fibrous carrier. In this kit the pore size has been optimized to extract genomic DNA from blood. In addition, the reagent and the protocol used with this kit provide a simple and efficient way to extract DNA from blood.

Extraction Result for Nucleic Acid from Blood

Fig. 3 shows nucleic acid results for multiple samples using the blood genome extraction kit. A 7-mL vacuum blood-collection tube with EDTA-2Na as an anticoagulant and a 2-mL vacuum blood-collection tube with EDTA-2K as an anticoagulant are used to take blood from the same person. Blood from the 7-mL tube is used to extract nucleic acid and blood from the 2-mL tube is used to measure leukocytes. The kit has a standard protocol in which nucleic acid is extracted from the blood without leukocyte separation.

After extraction, the nucleic acid solution is evaluated for concentration using PicoGreen DNA quantification and for purity using absorbance measurement with a spectrophotometer (absorbance ratio at wavelength of 260 and 280 nm).

The amount of DNA extracted per 1 mL of blood is related to the number of leukocytes in the sample

blood. This proportion is present because leukocytes contain most of the nucleic acid in blood. As shown in Fig. 3, the kit can extract a very high average of 91% of the genomic DNA contained in leukocytes.

In addition, the absorbance ratio index shows a very high average purity of 1.80. With this purity after extraction, the nucleic acid solution is ready for use in a PCR (polymerase chain reaction) or other experiment.

Blood Retention State

A blood extraction sample may be preserved in a frozen or unfrozen state. During development of the kit, the reagent and the protocol were optimized to handle nucleic acid extraction from either frozen or unfrozen samples.

When preserving in an unfrozen state, nuclease action in the blood tends to break down genomic DNA. When preserving in a frozen state, nucleic acid may not be extractable, or the rate may be very low, due to extraction principles or kit characteristics. So researchers urgently desire a kit that stably extracts nucleic acid from frozen blood and overcomes the problems of sample deterioration and preservation limits.

First, multiple blood samples are collected from the same person and preserved at various temperatures using 7-mL vacuum blood-collection tubes with EDTA-2Na as an anticoagulant. After that, DNA is extracted using the standard protocol for the blood genome extraction kit. Once the nucleic acid is extracted, PicoGreen quantification is used to evaluate the DNA concentration, with the yield immediately after blood collecting on retention day zero taken as 100%. The results are shown in Fig. 4.

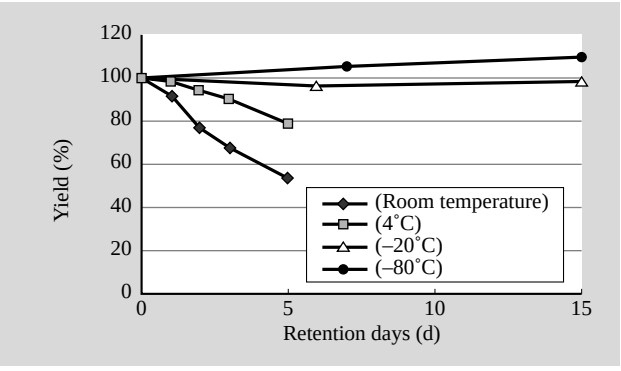

Fig. 4—Retention Effect.
Multiple blood samples are collected from the same person and preserved a specified number of days at 4, –20, and –80˚C. The figure shows extraction results using the standard protocol (n = 2). After extraction, PicoGreen quantification is used to determine DNA concentrations. The yield is calculated using blood at retention day zero as 100%.

For unfrozen preservation at room temperature and at 4˚C, the yield drops the longer a solution has been preserved. By contrast, no drop in yield is seen over time for solutions preserved at -20˚C and at -80˚C. The yields show how freeze preservation prevents DNA deterioration in blood. It also shows how the kit makes frozen blood suitable for nucleic acid extraction.

FUTURE DEVELOPMENT

Diversification

In addition to DNA extraction from whole blood as described above, customers require nucleic acid extraction from other types of samples. In particular, extracting RNA (ribonucleic acid) from various types of cells and tissues is essential to the study of gene expression. However, when genomic DNA is contained in the same sample as extraction RNA, gene expression analysis becomes difficult.

Through developing the aspirate-discharge method, Hitachi has discovered reagent conditions that enable RNA-only binding. In the future, we will introduce a kit that can selectively extract only the RNA from a blood or a cell sample.

Hitachi is also planning to optimize extraction syringe shape based on the volume of a sample. For example, a pipette tip is most effective if the sample volume is very small.

Automation

The syringe's piston control incorporates a simple, automated mechanism to extract nucleic acid through an aspirate-discharge method developed by Hitachi. In the future, Hitachi plans to automate the aspirate-discharge method on a pipette tip through a pressure mechanism as used in dispenser devices.

Miniaturization

Micromachining applications in biotechnology are starting to have an impact on integrated functionality and higher throughput. Hitachi is developing a number of devices that aim to integrate and to simplify the nucleic acid extraction process. Fig. 5 shows a prototype device that incorporates micromachining technology. This micro device, the size of a business card, integrates various processes from separating serum from blood to extracting nucleic acid from serum.

This device combines centrifugal and capillary forces that can perform all processing for one sample

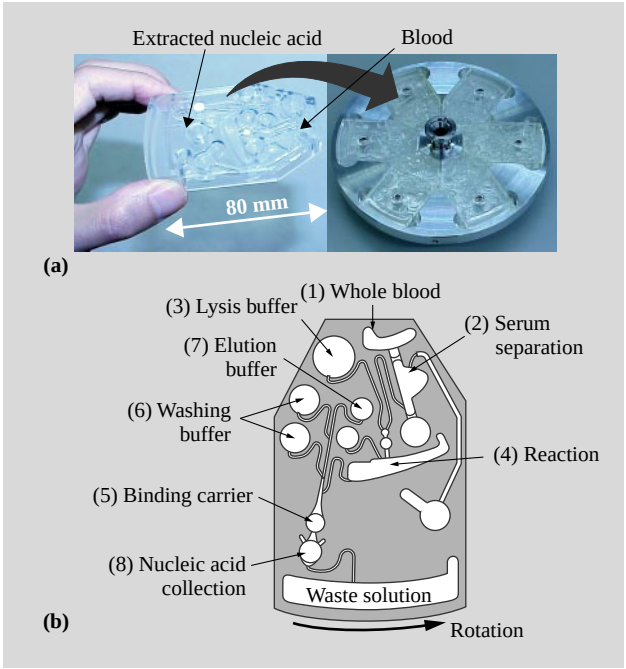

Fig. 5—Micro Nucleic Acid Extraction Device and RNA Extraction Process.
The micro device is a prototype (a) that uses micromachining technology. Processes (1) to (8) proceed in order based on centrifugal and micro flow controls. Blood that drops into the device is used for RNA extraction.

using independently sealed channels. As a result, a contamination-free extraction environment will become a reality.

Systematization

Nucleic acid extraction is a gateway to gene analysis and testing. Combining the three processes would achieve a highly efficient system for analysis and testing. For example, such a system would be able to extract genomic DNA from blood or cells of the same person then test for cancer genes through DNA comparisons.

CONCLUSIONS

This paper has discussed nucleic acid extraction through a new aspirate-discharge method, a blood genome extraction kit based on the new method, and the future development strategy for the kit.

The blood genome extraction kit based on Hitachi's aspirate-discharge method provides a safe and an efficient way to extract genomic DNA from the whole blood. Researchers in laboratories can use the kit as an excellent pretreatment system for gene analysis.

In the future, Hitachi will automate the kit, increase the types of samples that can be used, and expand the range of application.

REFERENCES

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