Multipurpose Robot for Automated Cycle Sequencing

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ABSTRACT

Paramagnetic beads have the superior advantages of easy separation and resuspension by controlling the magnetic field. Previously, we have developed Magtration technology to automate paramagnetic bead handling and have built several automated instruments that handle 1–12 samples simultaneously. To achieve more high-throughput sample processing, two types of a 96-arrayed Integrated Magtration Unit (IMU) were developed, one installed with electromagnets and the other with thin rod-shaped magnets made of neodymium. A multipurpose robot (SX-96GC) equipped with the IMU was also developed for fully automatic processing of 96 samples in parallel. The cleanup of dye-terminator sequencing products was performed using the robot installed with the permanent magnet version of IMU. The results had quality comparable to those by the same protocol in manual handling or to those by the conventional protocols. The robot processed 96 samples in a microplate within 30 min. The protocol that can purify 384 samples within 1 h by processing two microplates concurrently was successfully designed.

INTRODUCTION

Magnetic beads are widely used for many purposes in molecular biology and diagnosis, such as DNA purification (1–3), immunoassay, or bacterial detection. Although they are thought to be suitable for automation, they have not been used in high-throughput analyses, including genome sequencing and medical diagnostics, because of a lack of high-throughput automated system.

Automation of the paramagnetic bead handling has been performed using conventional laboratory robots such as Biomek[®] (Beckman Coulter, Fullerton, CA, USA), BioRobot[™] (Qiagen, Valencia, CA, USA), or Genesis (Tecan, Männedorf, Switzerland) (4,5). In such systems, the supernatant is aspi-

rated by pipettor, while magnetic beads are packed at the bottom or at the side by the magnet under or by the side of the vessel, just as is done in manual handling.

Previously, we have developed a novel technology, named Magtration, for automatic and reliable magnetic bead handling. It requires only a conventional dispensing unit with a magnet that can be attached to and detached from the outer wall of the disposable pipetting tip. Magnetic beads in the suspension are captured during aspiration and/or dispensing at the inner wall of the tip by contacting the magnet with the outside wall of the tip. The captured magnetic beads can be quantitatively recovered by detaching the magnet and repeating the aspiration dispensing of liquid with the tip. This method has realized highly adaptive and fully automatic processing of a paramagnetic bead-based protocol. Magtration technology has been applied to various targets and fields including nucleic acid purification (6–8) and diagnostic immunoassays with chemiluminescence detection.

To achieve the effective capture and release of paramagnetic beads by this method, the magnetic force in the tube should be strong enough and be effectively reduced for quick resuspension. We have already succeeded in handling up to 12 samples by lined pipetting tools with Magtration technology. Each tip has to be equipped with a magnetic separator, and each magnet has to be fixed on the surface of the tip for gathering magnetic particles and moved away to reduce the magnetic force enough for release of the captured magnetic particles. However, it could not be applied to arrayed vessels in high density, such as the microplate format, because of the limit of the space for the magnet in the arrayed format.

Here we have developed arrayed magnetic separation units for the Magtration technology, which can process 96 samples in parallel, and have also developed a highly flexible robot equipped with the unit. To demonstrate the performance, the robot has been applied to the purification of the DNA se-

quencing reactants before loading to a DNA sequencer, which is one of the most tedious and not readily amenable steps to automation in DNA sequencing.

MATERIALS AND METHODS

Designing a 96-Channel Arrayed Unit for Paramagnetic Bead Handling

Magnetic bead handling by the Magtration technology is as follows. Liquid suspension of paramagnetic beads is aspirated in a disposable plastic tip. During aspiration and dispensing, a magnet is contacted with the outer side of the tip, and the beads are captured on the inner side of the tip. The beads can be resuspended by repeating aspiration and dispensing of the solution.

To perform magnetic bead separation by this method in 96- or 384-arrayed format, magnetic force should be controlled at each disposable tip, where only a limited space is available for each magnet. We have designed and built two types of a novel arrayed magnetic separation unit, named Integrated Magtration Units (IMU).

The one has four coils connected electrically in parallel between the upper and the lower plates (Figure 1a). The magnetic force grown by an electronic current is applied to the individual dispensing tip from a 1-mm gap between the projections

of the two plates. The magnetic force was completely erased by the demagnetizer equipped with the power-supplying unit (KR-M102A-24; Kanetec, Nagano, Japan) immediately after the electric current to the IMU was turned off.

The other unit, the paramagnetic IMU, has specially designed thin rod-shaped magnets ($2 \times 5 \times 15$ mm) made of neodymium, which is known as one of the strongest magnetic materials. Forty-eight pieces of the magnet were mounted on the metal plate (Figure 1b). The 96 dispensing tips were placed through the magnet plate so that one permanent magnet plate can give magnetic force on two tips. The magnet plate moves down when the magnetic force is applied to the tips by a ball-and-screw mechanism driven by a pulse-motor. While the magnet plate moves up or down, the magnets are detached from or attached to the dispensing tips, respectively, by adjusting the horizontal relative position between the magnets and the tips by a cum-and-pin mechanism.

High-Throughput Multipurpose Robot with the 96-Channel Magnetic Bead-Handling Unit

Figure 1c shows the overview of the robotics. The IMU was set accessible to a turntable on which eight microplates (Thermo-Fast 96 Skirted Plate; ABgene House, Surrey, UK) could be mounted. The robotic arm was placed at the center of the turntable to transfer the microplates. A 12-nozzle reagent dispensing unit, three heat/cool blocks (two of them are on the turntable), a sample plate storage box, the disposables-supplying units (one for microplates and the other for tips), and the incubation box were equipped, surrounding the turntable for quick access by the robotic arm. The position of each unit was carefully designed so that multiple plates could be concurrently processed at the different steps, thereby greatly reducing the process time. Paramagnetic particles for the 96 samples can be separated in parallel using the IMU.

Purification of Sequencing Products by an Automated Protocol Using Magnetic Beads

Sequencing reaction was done using ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA polymerase, FS (Applied Biosystems, Foster City, CA, USA), following the manufacturer's recommendations. The plasmid vector, pGEM®-3Zf(+) (Promega, Madison, WI, USA) was prepared by the alkaline lysis method followed by CsCl purification. Using 3.2 pmol of a biotinylated M13 universal sequencing primer (-21 M13 primer: biotin-5'-TGTAACGACGGCCAGT-3'), 0.3 µg of the plasmid were sequenced.

Paramagnetic bead purification of sequencing products was performed by using MagExtractor®-Sequencing Clean up-Dye Terminator Removal Kit (TOYOBO, Osaka, Japan) and DYNAPURE™ Dye Terminator Removal version 2 (Dyna AS, Oslo, Norway). These kits use streptavidin-coated paramagnetic beads and are designed to clean up the sequencing reaction mixture by adsorbing the products that contain the biotinylated primer. The purification was performed according to the manufacturer's protocol, with slight modifications when necessary for automatic processing as indicated.

Figure 1. Arrayed units for automated handling of paramagnetic beads and a platform of a high-throughput automated system. (a and b) The mechanisms and photos of the IMUs with the electromagnet and the permanent magnets, respectively. (c) A platform of the automated system (SX-96GC). A turntable holds eight 96-well microplates and moves them to access the IMU. A robotic arm at the center of the turntable can access the peripherals to carry microplates between the turntable and the peripherals. 1, robotic arm; 2, IMU; 3, reagent dispenser with 12 nozzles; 4–6, heat/cool blocks; 7, incubation box; 8, microplate-supplying unit; 9, tip rack-supplying unit; 10, sample plate storage box; and 11, trash box.

Product Application Focus

After the removal of the beads, the eluent was applied to the capillary gel sequencer (ABI PRISM 3700 DNA Analyzer) (injection time, 50 s; voltage, 2500 V; cuvette temperature, 46°C). The manual purification of the sequencing products was performed by ethanol precipitation or by Centri-Sep™ spin columns (Princeton Separations) according to the Applied Biosystems' recommendations.

RESULTS AND DISCUSSION

Magnetic Bead Handling by IMU

Performance of the electromagnetic IMU was evaluated using Dynabeads M-280. The magnetic force by an electronic current of 2 A at 90 V was 0.15 T, which was strong enough for complete capture of the beads from 50 µL of 10 mg/mL suspension within 180 s during five repeats of aspiration and dispensing. However, the temperature of the unit increased and reached 74°C after 180 s. An interval of 270 s was necessary to cool the unit until the next use to reduce electric resistance and to generate strong magnetic force.

While the development of the IMU with the electromagnet was in progress, the use of a permanent magnet was also ex-

Figure 2. Process diagrams of the purification of sequencing products. The process diagram of purifications by the permanent magnet (for four microplates), electromagnet, and the manual method are indicated. Five microliters (50 µg) of the paramagnetic beads and 15 µL binding buffer were dispensed into the 96-well microplate by the 12-channel automatic dispensing unit. Then, 20 µL sequencing reaction were added and mixed by the 96-channel IMU without using the magnet. The reaction was incubated for 5 min at room temperature for the biotinylated sequencing products to bind to the streptavidin-coated paramagnetic beads. The beads in the suspension were captured while dispensing the suspension at approximately 20 µL/s. After washing the DNA-bound beads in 50 µL wash buffer twice, the beads were resuspended in 20 µL loading buffer. The DNA was eluted off the beads by heating at 95°C for 2 min. I, mixing the sample and the bead suspension; II, washing the beads twice; and III, removal of the magnetic beads from the purified products. Hatched and black boxes designate binding of DNA onto the beads and heat denaturation, respectively.

amined for the IMU. The magnetic flux density was approximately 0.4 T, nearly three times as strong as that of the electromagnet unit. The magnet was set and moved as shown in Figure 1b. Although it is difficult to apply the use of permanent magnets to the 384-well format because of the space problem, the IMU with the permanent magnets exhibited better performance and reliability for capturing the magnetic beads.

Two types of paramagnetic beads were used to evaluate the performance of bead handling by the IMUs. The two kits used in this work, from Dynal and TOYOBO, had the paramagnetic beads with different sizes of 2.8 and 0.75 μm , respectively. Although the Dynal beads were completely captured by both IMUs, the TOYOBO beads sometimes dropped off the tip with the electromagnet IMU. The larger beads with the 3- μm diameter prepared in the same process as the TOYOBO beads could be perfectly captured by both IMUs, the same as Dynal beads (data not shown). These results indicate that the magnetic beads with a larger size have increased the reliability in automatic processing and will decrease the process time for magnetic capturing.

Application to the Purification of Sequencing Products

The robot with the permanent magnet IMU was applied to the purification of the sequencing reaction products, which is an important process in large-scale sequencing. After the cycle sequencing reaction, the 96-well microplate stored in the cooling box equipped with the robot was automatically transferred onto the turntable by the robotic arm. We designed the protocol to purify 384 samples (96 samples $\times$ 4 plates) within 1 h completely automatically, as shown in Figure 2, in which two microplates were concurrently processed in the manner of an assembly line. By using the first block of the process diagram, 96 samples were purified within 30 min.

No differences in the quality of the sequencing results were observed between the manual and the automated purification by using the Dynal or TOYOBO kit (data not shown). The number of bases called as "N" in an average of more than 10 samples was 0.40, 0.33, and 0.31 in the region between 50 and 600 bases for ethanol precipitation, the column method, and the automated method using the TOYOBO kit, respectively. Unlike ethanol precipitation, our automated protocol dramatically reduced labor and the need for technical skills, which allows quick initiation of a sequencing project without finding skillful labor and technical training.

The conventional liquid-handling robot with magnets under the microplate should capture paramagnetic beads at the side but not the bottom of the well for the supernatant to be removed. Thus, it is difficult to resuspend the beads in a small volume of the eluent. Our arrayed Magtration unit has enabled reliable and quantitative handling of paramagnetic beads, including resuspension of the beads, and, thus, has made it possible to integrate any paramagnetic bead-based protocols. Although the electromagnet IMU is hard to generate magnetic force as powerful as the permanent magnet IMU, mainly because of the heating problem in the present work, the use of an electromagnet is ideal for preparing an arrayed unit of higher density, such as for 384- and 1536-well formats. Improvement of the electromagnet IMU is under

progress. The high throughput and highly adaptable features of the IMU and the automation robot open up the application of biomagnetic beads to high-throughput screening and commercial inspections.

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