DNA from Blood: Get the "Whole" Story

Automated 96-Well Purification of Genomic DNA from Whole Blood

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Abstract

Improvements in molecular analysis techniques have changed the "bottlenecks" in clinical and basic research. Until recently, analysis required the greatest amount of time and labor. Today however, the most time-consuming and laborious step is often DNA purification, especially when high-throughput processing is required. This article describes two new high-throughput, 96-well, walkaway automated systems for genomic DNA purification. The MagneSil® Blood Genomic, Max Yield System is designed for high-yield genomic DNA purification, purifying 4–9µg of DNA from 200µl of human whole blood and other samples. The MagneSil® ONE, Fixed Yield Blood Genomic System is designed to purify a "fixed DNA yield", 1.0µg ± 50%, from 60µl of whole blood. Both systems are designed to be implemented on a robotic liquid handler such as the Beckman Coulter Biomek® FX.

Both the MagneSil® Blood Genomic and the MagneSil® ONE Systems save time by automating traditionally time- and labor-intensive steps while providing a streamlined walkaway method that is an improvement over other automated systems.

Introduction

DNA analysis techniques are important for a wide variety of applications in genetic scoring and discovery. Whole blood is readily available and widely used for isolation of genomic DNA. However, traditional methods for purifying genomic DNA from small volumes of blood sacrifice quality or are too tedious to enable automated sample processing. Further, the dedicated equipment used in high-throughput genomic DNA isolation systems is typically highly specialized and inflexible. Promega has developed two genomic DNA purification systems that provide walkaway automated sample processing on the Biomek® FX liquid handler.

MagneSil® Blood Genomic, Max Yield System

For scientists who require purification of the maximum possible DNA yield from a blood sample we designed the MagneSil® Blood Genomic, Max Yield System[a] (Cat.# MD1360). This system will purify 4–9µg of DNA from 200µl of anti-coagulated whole blood, depending upon the white cell count of the donor sample. DNA purified with this system is suitable for PCR and multiplex PCR[b] (e.g., Y Chromosome Deletion Detection System[c]), fluorescent multiplex STR (e.g., PowerPlex® 16 System [d,e,f]) and the READIT® SNP Genotyping System[g] (Cat.# MD1290).

MagneSil® ONE, Fixed Yield Blood Genomic System

Access to complete genome sequences is redefining the types of questions investigated in many research fields. To this end, many novel genotyping methods have been developed in addition to single-locus amplification. These systems often rely on analysis of small amounts of DNA but may be limited to a defined range of input DNA for maximum reproducibility. Currently, the downstream application requirement for a narrow range of input DNA adds time and labor by requiring DNA quantitation and normalization.

The MagneSil® ONE, Fixed Yield Blood Genomic System[a] (Cat.# MD1370) was developed for applications, such as amplification-based genotype analysis, that require or benefit from a narrow range of purified DNA concentration. The MagneSil® ONE, Fixed Yield System is designed to purify a "fixed yield" of 1µg of DNA (±50%) from 60µl of anti-coagulated human whole blood. This saves time and labor by eliminating post-purification manipulation of samples. Like the MagneSil® Blood Genomic System, DNA purified with the MagneSil® ONE System is suitable for PCR and multiplex PCR (e.g., Y Chromosome Deletion Detection System), fluorescent multiplex STR (e.g., PowerPlex® 16 System) and the READIT® SNP Genotyping System.

Automated Methods

DNA purification with the MagneSil® Blood Genomic System starts with the setup of the liquid handler deck surface (Figure 1). Blood samples are pipetted into a 96-well, deep-well plate, the "working plate." Reagent reservoirs are filled with MagneSil® Paramagnetic Particles[a] (PMPs) and buffers. The robotic protocol transfers the appropriate amounts of each reagent to 96-well plates to act as "individual reservoirs" that prevent sample cross-contamination from using common reservoirs.

The DNA purification process begins with the addition of Lysis Buffer, Blood, and MagneSil® PMPs to the blood samples followed by thorough mixing. The combination of the proprietary Lysis Buffer and tip mixing lyses the blood cells and denatures the protein and heme, freeing the DNA so it can bind to the MagneSil® PMPs.

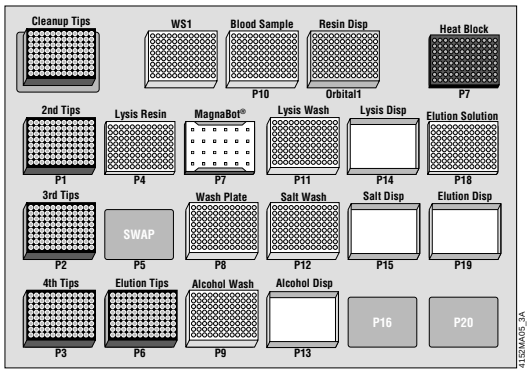

Figure 1. Example deck layout of the Biomek® FX instrument. Layout for the MagneSil® Blood Genomic, Max Yield System. Deck layouts may be different on different instruments and depend upon the individual instrument's configuration.

After the DNA has bound to the MagneSil® PMPs, they are separated magnetically by transferring the plate to the Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031). The supernatant is removed, and the MagneSil® PMP-bound DNA is serially washed in Lysis Buffer, Salt Wash and Alcohol Wash. For each step, the MagneSil® PMPs are resuspended in the washes, magnetically separated and the waste removed. Because of the need to perform several tip mixes during the method, the Biomek® FX liquid handler, with the capability of pipetting 96 wells at a time, was chosen as the optimal instrument for these applications.

After the final wash, the MagneSil® particles are dried at room temperature, and the plate is transferred to the Heater ALP (automated labware positioner) (Beckman Coulter Biomek® FX Heater ALP). The plate remains on the Heater ALP while Elution Buffer is added and a series of mixes and incubations are performed. The plate is then transferred once more to the Deep Well MagnaBot® 96 Magnetic Separation Device, and the MagneSil® PMPs are magnetically separated. The DNA-containing supernatant is transferred to a clean 96-well plate or similar storage receptacle, ready for PCR and other downstream applications.

The instrumentation method used for the MagneSil® ONE System is similar to the MagneSil® Blood Genomic, Max Yield method but uses MagneSil® ONE - Fixed Yield PMPs. Please refer to Technical Bulletins #TB312 (1) and #TB313 (2) for more information on the liquid handler requirements.

DNA Isolation from Blood

The procedures for purification of genomic DNA using the MagneSil® Blood Genomic, Max Yield or the MagneSil® ONE, Fixed Yield Systems allow genomic DNA to be isolated as either a maximum yield or fixed yield. Figure 2 shows representative yields from the MagneSil® ONE Fixed Yield System using replicate blood samples from several healthy donors. The system provided high-molecular weight DNA that was largely free of contaminating RNA. Purity was measured by $A_{260/280}$ ratios with results typically >1.7 (using A_{320} correction described in references 1 and 2). DNA yield is identical from blood collected with EDTA, ACD, citrate or heparin anticoagulants (data not shown).

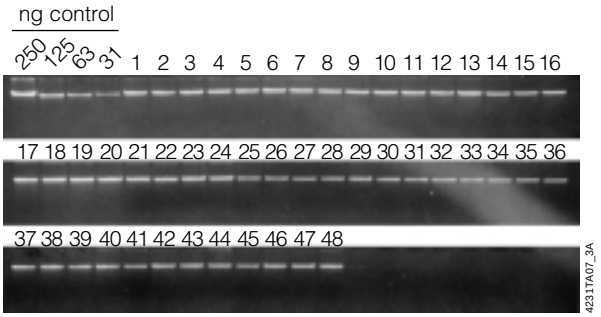

Figure 2. Consistent yield of genomic DNA purified with the MagneSil® ONE, Fixed Yield System. Genomic DNA standards were loaded onto the gel in the following amounts: 250, 125, 63 and 31ng. Forty-eight blood samples were purified with the MagneSil® ONE System, and 10µl of the purified DNA for each sample was loaded on the gel and visualized by ethidium bromide staining.

In some cases, blood samples that are shipped and handled at ambient or elevated temperatures contain white blood cells undergoing apoptosis. The MagneSil® Blood Genomic System purifies DNA across the broad size range seen in a typical apoptotic ladder (Figure 3).

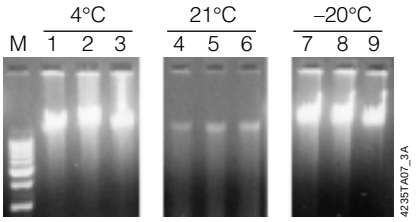

Figure 3. Apoptotic genomic DNA purification. Genomic DNA was purified with the MagneSil® Blood Genomic System following blood storage at the indicated temperatures for 8 days. Lane M, Promega Human Genomic DNA (Cat.# G3041).

The MagneSil® ONE, Fixed Yield System uses a novel mechanism for DNA purification where the MagneSil® ONE - Fixed Yield PMPs capture and release a consistent amount of DNA from the lysate. Table 1 illustrates the average DNA yield for the MagneSil® ONE System from 50µl of replicate blood samples from several healthy donors. This narrow range of yield greatly reduces the amount of time to go from a blood sample to genotype result by eliminating laborious normalizing and quantitation of each DNA sample.

PCR Amplification of Purified DNA

To illustrate the quality of DNA purified with the MagneSil® Blood Genomic and MagneSil® ONE Systems, we used multiplex PCR to analyze DNA purified from blood. Carryover of excessive salts, metal ions, heme and protein often presents a challenge to robust and

Table 1. Average Yield of Genomic DNA Purified with the MagneSil® ONE System.

Sample Number	n	Yield (ng)
18	4	935
19	4	799
21	4	1,030
22	4	916
23	4	1,077
24	4	829
25	4	871
28	4	864
29	4	817
32	4	871
34	4	950
36	4	1,079
Average	**48**	**920 (±93)**

consistent amplification of all products within a multiplex. Figure 4 shows multiplex amplification from the human Y chromosome (Y Chromosome Deletion Detection Kit, Version 1.1, Cat.# MD1101). DNA purified from blood by the MagneSil® Blood Genomic and MagneSil® ONE Systems amplified well across the broad size range of products.

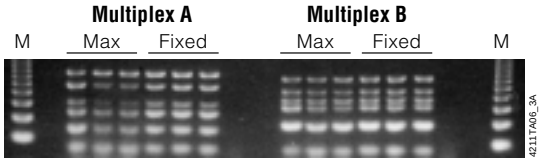

Figure 4. Multiplex PCR analysis. Genomic DNA purified with either the MagneSil® Blood Genomic System (Max) or the MagneSil® ONE System (Fixed) was amplified using the Y Chromosome Deletion Detection System, Version 1.1 (Cat.# MD1101). Ten microliters of the amplification reactions for Multiplex A and B were run on a gel and visualized by ethidium bromide staining.

Blood Card Punches

The use of blood cards (e.g., FTA® or S&S903 cards) allows archiving of samples, ease of transport and safety in handling potentially infectious liquid samples. For some scientists, blood card punches have become a significant percentage of samples processed. We developed a method to purify DNA from blood cards using the MagneSil® Blood Genomic System. Briefly, blood card punches are first incubated in a 96-well, deep-well plate in TE buffer/DTT solution at 65°C for 30 minutes. Following this incubation, Lysis Buffer, Blood, is added, mixed by vortexing and incubated for 2 additional hours at 90°C. The plate is cooled and the supernatant is collected, placed on the deck of the Biomek® FX, and processed in the method as liquid blood samples. Approximately 120–140ng of total DNA can be purified from two 2.5mm blood card punches using this method. Total DNA yield is quantitated using an amplification of the human THO1 allele and an external set of standards (Table 2). This is done because

Table 2. Average Yield of Genomic DNA Isolated from 2 × 2.5mm Blood Card Punches.

Sample #	Double-Stranded DNA (ng)	THO-1 Total DNA (ng)
1	19	65
2	25	158
3	57	146
4	60	167
5	26	153
6	27	90
7	20	72
8	16	152
Mean (n=8)	**31 (±17)**	**125 (±43)**

the majority of purified DNA is single-stranded due to the extended incubations at elevated temperatures. The purified DNA is suitable for direct PCR amplification. For a complete protocol, please contact Promega.

Cross-Contamination Testing

For routine testing of cross-contamination between wells of a plate, we developed an assay to amplify the DYS214 sequence found on the human male Y chromosome (3), which is present in multiple copies in wildtype male genomic DNA and absent in most females. The X chromosome has a sequence similar to the DYS214 probe sequence (4), and in the absence of Y chromosome-specific DNA, the X chromosome-specific amplicons (of 260 and 600 bases) will be generated. These X Chromosome-specific products are readily distinguishable from the 230-base Y chromosomme-specific amplicon. The ratio of Y chromosome-specific template versus X chromosome-specific template, as determined by the TaqMan® real-time PCR assay, indicates the amount of each PCR product that is generated. However, some female donors, particularly those that have been pregnant with male children, carry some male cells in their blood. These male cells may persist in the woman's blood for years (5), and such female donors will generate Y chromosome-specific DYS214 amplification products in our test.

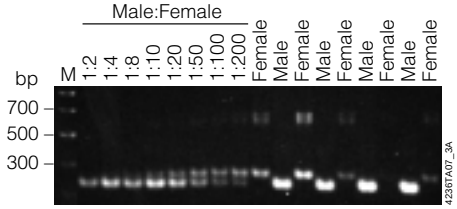

Figure 5. Cross-contamination assay results. Samples of blood from one female who never had a male child and one male subject were purified in alternating wells using the MagneSil® Blood Genomic, Max Yield System. The purified DNA was then amplified with DYS214-specific primers. Dilutions of male DNA into female DNA are shown, followed by alternating male and female DNA that was purified and amplified in adjacent wells. None of the female samples showed Y chromosome-specific products.

Figure 5 shows DYS214 amplifications of female blood samples alternated in the 96-well plate with male blood samples (so that the female samples are surrounded by male samples on four sides but not diagonally in the 96-well plate during sample processing). This test shows that cross-contamination during automated purification of genomic DNA is less than 0.05% by the semi-quantitative measurement the assay provides.

Conclusion

The MagneSil® Blood Genomic, Max Yield System provides high-yield, high-quality DNA from blood in a walkaway, automated 96-well format. The MagneSil® ONE System allows the user to add a specific amount of DNA to a reaction by obtaining a "fixed yield" of DNA. Both of these systems help save time by automating traditionally time- and labor-intensive steps. Automation provides streamlined walkaway methods that are an improvement over other automated systems. In this article, we have described methods to achieve a maximum yield or fixed yield of genomic DNA from blood and blood card punches. These systems and the associated automated methods provide new tools to improve the productivity of scientists in clinical and basic research.

References

1. *MagneSil® Blood Genomic, Max Yield System Technical Bulletin* #TB312, Promega Corporation.
2. *MagneSil® ONE, Fixed Yield Blood Genomic System Technical Bulletin* #TB313, Promega Corporation.
3. Skaletsky, H. *et al.* (2003) *Nature* **423**, 825–837.
4. Whitehead Institute/MIT Center for Genome Research: www.genome.wi.mit.edu/cgi-bin/contig/sts_info?sts=DYS214, (accessed 25 February 2003).
5. Banchi, D.W. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93(2)**, 705–708.

Protocol

- *MagneSil® Blood Genomic, Max Yield System Technical Bulletin* #TB312, Promega Corporation. (www.promega.com/tbs/tb312/tb312.html)
- *MagneSil® ONE, Fixed Yield Blood Genomic System Technical Bulletin* #TB313, Promega Corporation. (www.promega.com/tbs/tb313/tb313.html)

Ordering information

Product	Size	Cat.#
MagneSil® Blood Genomic, Max Yield System*[a]	1 × 96 preps	MD1360
MagneSil® ONE, Fixed Yield Blood Genomic System*[a]	1 × 96 preps	MD1370
Y Chromosome Deletion Detection System, Version 1.1**[c]	25 reactions	MD1101
READIT® SNP Genotyping System**[g]	100 reactions	MD1290

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*For Laboratory Use.

**For Research Use Only. Not for use in diagnostic procedures.

[a] U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756 and other patents and patents pending.

[b] The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

[c] Use of the Y Chromosome Deletion Detection System, Version 1.1, requires performance of the polymerase chain reaction (PCR), which is the subject of European Pat. Nos. 201,184 and 200,362, and U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-La Roche. Purchase of the Y Chromosome Deletion Detection System, Version 1.1, does not include or provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of the Y Chromosome Deletion Detection System, Version 1.1, may, therefore, be required to obtain a patent license, depending on the country in which the system is used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.

These products are designed and sold for use in the multiplex PCR process covered by U.S. Pat. No. 5,582,989. A limited license has been granted under the patent to use only this amount of the product to practice the multiplex PCR process and is conveyed to the purchaser by the purchase of this product.

[d] Use of Promega's STR Systems requires performance of the polymerase chain reaction (PCR), which is a patented process. Purchase of Promega's STR Systems does not include or provide a license with respect to PCR. Users of Promega's STR Systems may, therefore, be required to obtain a license with respect to the use of PCR. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.

[e] This product may be covered by one or more of the following patents issued to Promega Corporation for multiplex amplification of STR loci: U.S. Pat. Nos. 5,843,660, 6,479,235 and 6,221,598, Australian Pat. No. 724531 and Canadian Pat.No. 2,118,048. Other patents are pending.

[f] U.S. Pat. No. 6,238,863 and other patents pending.

[g] READIT® products may be covered by one or more of the following patents: U.S. Pat. Nos. 6,335,162, 6,159,693, 6,235,480, 6,312,902, 6,270,974, 6,277,578, 6,270,973, 6,268,146, 6,379,898 and 6,391,551. Other patents are pending.

Use of this method may or may not require performance of the polymerase chain reaction (PCR), which is covered by patents issued and applicable in certain countries. Accordingly, users of this product may be required to obtain a patent license from the appropriate patent owner.