



Simplicity in Large-Volume DNA Isolation

MagneSil® Genomic, Large Volume System, for Large-Sample Genomic DNA Isolation

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Abstract

The MagneSil® Genomic, Large Volume System, is a scalable, automated genomic DNA isolation system capable of processing sample volumes of 1–10ml. Unlike traditional salting-out, organic or differential extraction methods, the MagneSil® Genomic, Large Volume System, provides a simple, robust method of direct lysis, binding, washing and elution that can purify genomic DNA from whole blood samples including those that have been mishandled. The purified DNA is of sufficient quality and yield to be used in applications like multiplex or real-time PCR.

The MagneSil® Genomic, Large Volume System, and the Tecan Freedom EVO™ instrument provide an integrated solution for automating genomic DNA purification from large-volume samples.

Introduction

Genomic DNA analysis is used increasingly for a wide variety of applications in genetic scoring, discovery and identification methodologies for pharmaceutical and clinical research. Oftentimes, the samples that are collected for purification of genomic DNA and subsequent analysis are large sample volumes, such as whole blood drawn into Vacutainer® tubes. Moreover, these samples are often not collected at the same location where they are processed. Transportation conditions to the DNA isolation laboratory can vary widely and impact sample integrity.

Furthermore, increasing demand for large-scale genomic DNA isolation drives researchers to look for automated purification options. Automation of traditional, large-volume genomic DNA isolation requires dedicated equipment, which is typically expensive, highly specialized and inflexible.

We developed the MagneSil® Genomic, Large Volume System^(a), to address the varied challenges for a robust, automatable genomic DNA purification method for large sample volumes. Here we describe a new system that integrates a robust chemistry with novel hardware and automation implementation to provide a total integrated solution for automated large-scale genomic DNA isolation from various sample types including whole blood, tissue, bacteria and tissue culture cells.

Automation for Large-Volume Purification

The MagneSil® Genomic, Large Volume System, is designed for scalable, automated genomic DNA isolation from large-volume samples. The MagneSil® Genomic, Large volume System, eliminates the need for laborious centrifugation steps and the use of organic solvents. The simple procedure (direct lysis, binding, washing, elution; Figure 1) purifies genomic DNA directly from a wide variety of sample types, including whole blood, with similar yield and quality regardless of sample storage or shipping conditions. The MagneSil® Genomic, Large Volume System, has been automated on the Tecan Freedom EVO™ liquid-handling instrument, providing walkaway, large-scale purification of genomic DNA from 1–10ml samples (Figure 2).

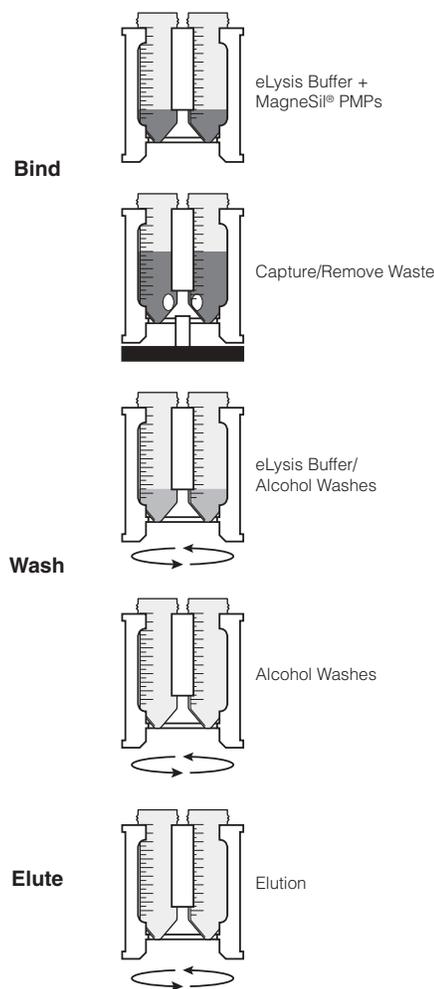


Figure 1. The simple, scalable protocol for the automated MagneSil® Genomic, Large Volume System.

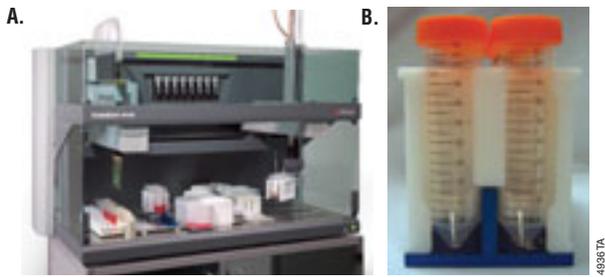


Figure 2. Integrated solution implementation. Combination of novel hardware integrated on the Tecan Freedom EVO™ workstation to use common, inexpensive 50ml tubes for automated, large-scale genomic DNA purification. **Panel A.** Shows the Tecan Freedom EVO™ workstation running the automated MagneSil® Genomic, Large Volume System, method. **Panel B.** Shows the eight-place MagneBot® Magnet used to capture the MagneSil® Paramagnetic Particles.

Solution to Traditional Isolation Method Concerns

The MagneSil® Genomic, Large Volume System, purification method addresses many of the challenges of traditional centrifugation-based, organic, salting-out or differential extraction methods (Table 1) while maintaining both yield and concentration (Figure 3). The purification procedure incorporates lysis of the entire sample followed by direct capture of genomic DNA from the lysate. Differential centrifugation, which can be problematic for mishandled samples, is not required to separate the white and red blood cells. After washing to remove contaminants such as heme or cellular proteins, the genomic DNA is eluted directly from the MagneSil® Paramagnetic Particles (PMPs) into an aqueous solution and is ready for use in downstream applications. There is no need for tedious overnight DNA pellet rehydration, or risk of losing the DNA pellet during post-purification handling.

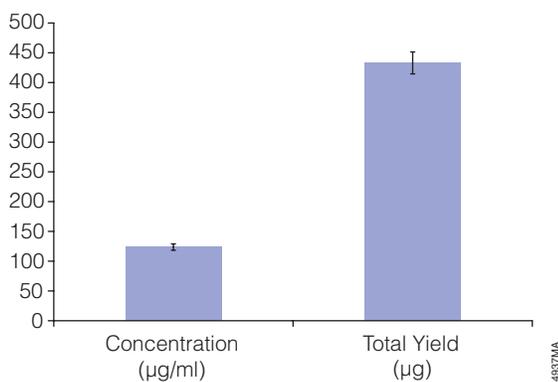


Figure 3. Average concentration and yield of purified genomic DNA. DNA was isolated from 10ml freshly drawn human whole blood (white cell count: 1×10^7 cells/ml). Concentration and yield were quantitated by measuring absorbance (A_{260}) of purified genomic DNA from four isolations. Yield may vary depending on white cell count per milliliter of whole blood.

The genomic DNA can be purified regardless of sample storage or shipment conditions and is suitable for a variety of downstream applications including single and multiplex PCR, restriction digestion and real-time PCR. Figure 4 shows a real-time PCR amplification curve with purified genomic DNA. The success of the real-time PCR amplification demonstrates the purified genomic DNA is of high quality. The fluorescent dyes present in the amplification reaction are sensitive to contamination resulting in a poor amplification curve and compromised linearity.

In Figure 5, we examined the quality of DNA isolated from EDTA- citrate- or heparin-treated human blood. The DNA was isolated with the MagneSil® Genomic, large Volume System, and amplified using a multiplex PCR kit, the PowerPlex® 16 System. This system amplifies 16 short tandem repeats (STR) loci in a single reaction and is sensitive to contaminants is the purified DNA. Equivalent STR profiles were obtained for all samples regardless of the anticoagulant, indicating that the genomic DNA was of high quality.

Robust Chemistry for Stored or Frozen Samples

Methods of sample transport are often uncontrolled, with samples exposed to a wide variety of temperature and pressure fluctuations. In addition, sample freezing can affect the integrity of the sample, especially whole blood samples, causing white blood cell lysis and release of genomic DNA from the cells.

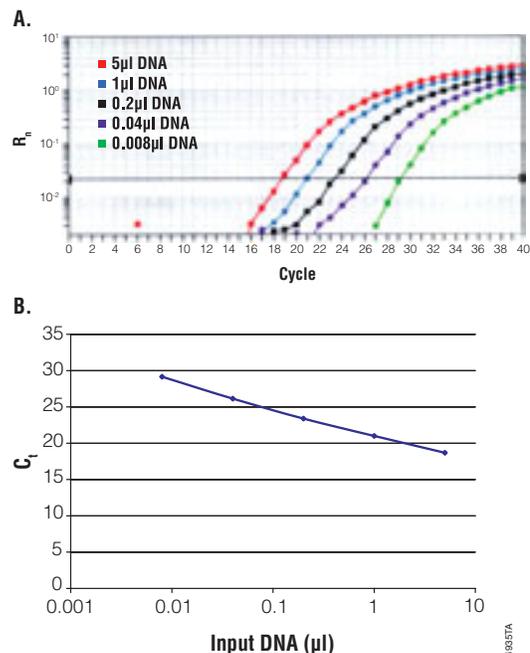


Figure 4. Assay for quality of purified genomic DNA by real-time PCR. Decreasing volumes of isolated human DNA were analyzed using β -actin real-time PCR control reagents from Applied Biosystems. **Panel A.** Amplification curve for the DNA volume range tested. **Panel B.** Linear detection for the amplification.

MagneSil® Genomic, Large Volume System...continued

Once the integrity of a sample has been compromised, the performance of traditional genomic DNA purification methodologies that rely on differential centrifugation of intact cells from the total sample volume can be questionable and unreliable (Table 1). The MagneSil® Genomic, Large Volume System, does not require intact cells for successful purification of genomic DNA from the sample. The MagneSil® Genomic, Large Volume System, isolates genomic DNA from whole blood and other sample types regardless of storage temperature (Figure 6).

Table 1. MagneSil® Genomic, Large Volume System, Solutions to Traditional Precipitation-Based Purification Challenges.

Centrifugation-Based Method Challenge	MagneSil® Genomic Purification Solution
Differential centrifugation requires intact white blood cells. Shipment and storage of whole blood samples may result in white blood cell lysis.	Genomic DNA can be purified from any whole blood sample regardless of storage or shipment conditions. Special sample collection and storage tubes (e.g., PAXgene® tubes) are not required.
Complete resuspension of cell pellet is required for good lysis. Loss of white blood cells during decanting and cell resuspension as well as inefficient cell resuspension results in low yields.	No centrifugation is required. Whole sample volumes are lysed, and genomic DNA is directly captured from the sample by the MagneSil® PMPs.
Genomic DNA precipitation by centrifugation can result in the loss of DNA pellet during the multistep process for DNA precipitation and washes.	No DNA precipitation or centrifugation is required. DNA is bound to easily visible MagneSil® PMPs.
Resuspension of genomic DNA pellet in aqueous solution is not automatable. Lengthy incubation time required for rehydration of precipitated, dried DNA pellet.	Easily automated. Genomic DNA is eluted directly from the MagneSil® PMPs into an aqueous solution.

Integrated and Flexible Automated Solution

We have automated the MagneSil® Genomic, Large Volume System, on a Tecan Freedom EVO™ workstation to provide a fully integrated chemistry and instrumentation solution. This integrated solution was designed to meet various throughput needs while maintaining broad functionality. The automated purification procedure provides scalability in two dimensions to easily adapt to daily needs. First, the volume of each sample is detected by the instrument, and the automated purification method then scales the reagent volumes per sample purification without user intervention. This feature allows processing of variable sample volumes within a single run as well as making the most efficient use of both time and reagent consumption. Second, the same method can be used for batch sizes from 1 to 24 samples per run. An alternative method can be used for batch sizes up to 96 samples per run. This allows sample batch size flexibility based on your work requirements rather than having to batch sample runs according to instrumentation requirements. Finally, genomic DNA can be purified directly from common sample collection tubes such as Vacutainer® tubes.

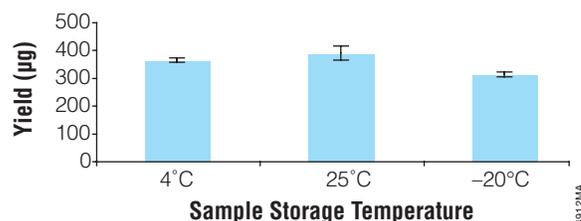


Figure 6. Effect of storage temperature on yield of purified genomic DNA. Genomic DNA purified with the MagneSil® Genomic, Large Volume System, from 10ml whole blood samples (white cell count: 9×10^6 cells/ml) stored at 4°C, room temperature (25°C), or -20°C for seven days. Yield was measured by absorbance (A_{260}) and is an average of three 10ml whole blood genomic DNA purifications per storage condition.

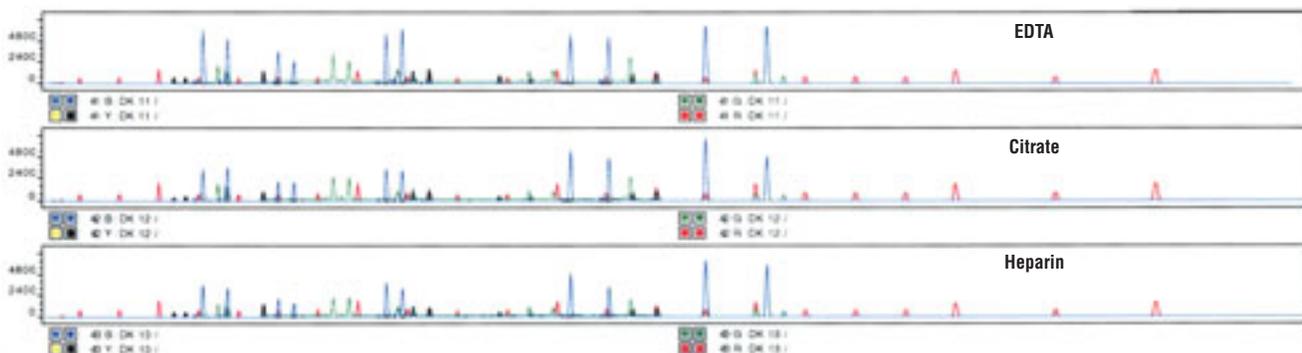


Figure 5. Assay for quality of purified genomic DNA by STR analysis of isolated DNA. PowerPlex® 16 STR analysis (multiplex amplification of 16 loci in a single reaction) were performed on 1ng of DNA isolated from human blood collected in EDTA-, citrate- or heparin-treated tubes and the fluorescently-labeled amplified samples were analyzed on an ABI 310 instrument.

Conclusion

The MagneSil® Genomic, Large Volume System, is designed for automated, scalable purification from a variety of large-volume samples between 1 and 10ml, including whole blood, regardless of sample storage and handling conditions. The system uses only the amount of reagents required to process each sample, maximizing efficiency and value on a per-preparation basis.

Without the need for centrifugation, the MagneSil® Genomic, Large Volume System, provides genomic DNA of sufficient yield and purity even from mishandled samples. The DNA can be used in a wide variety of downstream applications including monoplex and multiplex PCR, restriction enzyme digestion and real-time PCR.

Protocol

- ◆ *MagneSil® Genomic, Large Volume System Technical Bulletin #TB549*, Promega Corporation.
(www.promega.com/tbs/tb549/tb549.html)

Not Pictured

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Ordering Information

Product	Size	Cat.#
MagneSil® Genomic, Large Volume System	8 isolations	A4080
	48 isolations	A4082
	96 isolations	A4085

For Laboratory Use

Related Products

Product	Size	Cat.#
MagnaBot® Large Volume Magnetic Separation Device	1 each	V3471
Tube Holder, 50ml Tubes	1 each	Z3631
Heat Block Insert	1 each	Z3651
Heat Block Adapter, 50ml Tubes	1 each	Z3661
Shaker Top Adaptor	1 each	Z3671

For Laboratory Use

^(a) U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756 and other patents and patents pending.

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