

Low- to Moderate-Throughput Automation

Automation of Genomic DNA Purification Using the MagneSil® KF, Genomic System

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Abstract

DNA analysis techniques have become central in many aspects of modern basic and clinical research. These methods require high-quality DNA from diverse sample types that might include blood, tissue, buccal swabs, body fluids and feces. Traditionally, smaller laboratories have used time- and labor-intensive manual genomic DNA purification methods, as automated instruments have been too large, too expensive and not suited for their lower throughput. This article describes a new system for 1- to 15-sample, low- to moderate-throughput automated genomic DNA purification. The MagneSil® KF, Genomic System is designed for use on the Thermo Electron KingFisher® mL. Using this instrument, DNA purification takes under 25 minutes. The purified DNA is suitable for PCR, fluorescent STR, as well as more stringent applications such as multiplex PCR (e.g., Promega Y Chromosome Deletion Detection System), and SNP detection systems such as Promega READIT® SNP Genotyping System.

Promega developed the MagneSil® KF, Genomic System to provide a “middle ground” between the traditional extremes of low-throughput manual and high-throughput automated DNA purification.

Introduction

Genomic DNA purification is central to many aspects of modern molecular genetics. Several established methods exist for purifying genomic DNA from blood and other samples including detergent lysis/precipitation or lysis/binding of DNA to a filter or paramagnetic particle. Although these methods present several genomic DNA purification options, they are generally designed for either time- and labor-intensive manual purification or high-throughput automated purification.

Promega developed the MagneSil® KF, Genomic System to provide a “middle ground” between the traditional extremes of low-throughput manual and high-throughput automated DNA purification. The Thermo Electron KingFisher® mL instrument presents the unique ability to use paramagnetic particle-based methods in a convenient and flexible 1- to 15-sample/batch scheme.

Convenience and flexibility come from the fact that several commonly used sample types can be processed using the same instrument method. Although this system was initially tested for genomic DNA purification from 200µl of anticoagulated whole blood, customer feedback suggests that other sample types can be rendered into a liquid or semi-liquid form to be processed using the MagneSil® KF System on the KingFisher® mL. This article presents examples of genomic DNA purification using several sample types and downstream applications using the purified DNA.

The MagneSil® KF System uses optimized chemistries and a free downloadable method for the KingFisher® mL instrument (www.promega.com/automethods/). Figure 1 shows the layout of the KingFisher® mL 1 × 5 tube strip. One sample can be processed per 5-well tube strip. To begin the protocol, the user must dispense the reagents into each well of the tube strip. As shown in Figure 1, the first well contains 200µl of MagneSil® KF Particles and 800µl of Lysis Buffer KF. Up to 200µl of the sample material is added to the first well prior to the start of the protocol. The second well contains 1,000µl of Salt Wash. The third and fourth wells each contain 1,000µl of Alcohol Wash. The DNA is eluted in 200µl of Nuclease-Free Water in the fifth well of the tube strip.

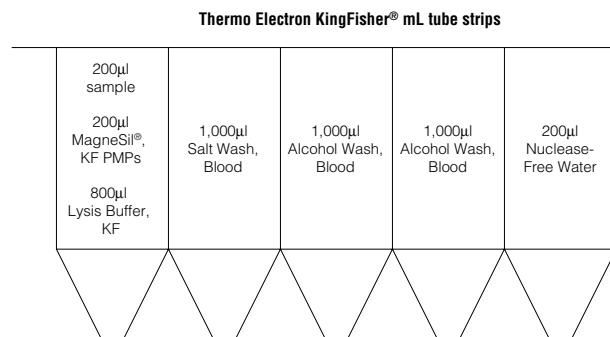


Figure 1. Contents of the KingFisher® mL 1 × 5 tube strip for genomic DNA purification with the MagneSil® KF, Genomic System.

Automation of Genomic DNA Purification... continued

Yield and Purity

The MagneSil® KF System can purify DNA from 200µl of whole blood drawn into blood tubes containing anticoagulants. Common anticoagulants such as EDTA, acid citrate dextrose (ACD), citrate and heparin all work equally well with this purification system. The DNA yield will vary with the white cell content of the blood sample. Generally, the MagneSil® KF, Genomic System will purify 2–6µg of genomic DNA from 200µl of normal human whole blood. Whole blood with anticoagulants can be stored for up to 3 weeks at 4°C with little loss in DNA yield or purity. Frozen blood can also be used with this system, and long-term storage at –20 to –70°C does not grossly affect DNA yield or quality.

Blood samples that have been shipped at ambient temperature in anticoagulant tubes will often undergo apoptosis and other forms of DNA degradation during shipment (or storage). The MagneSil® KF, Genomic System is designed to purify genomic DNA from degraded samples (Figure 2). While much of the DNA may be present as apoptotic fragments, purified DNA still performs in many amplification-based analysis methods.

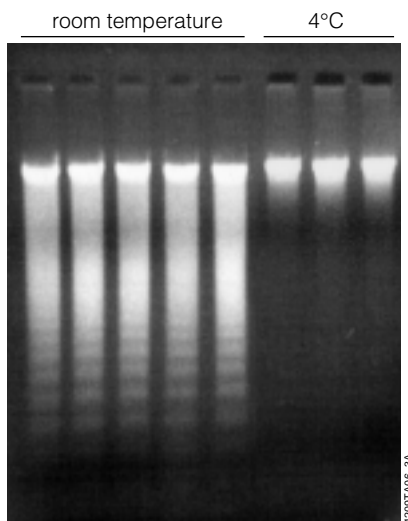


Figure 2. DNA isolated from blood stored at room temperature. Blood was collected with EDTA as an anticoagulant and stored for 5 days at either room temperature or 4°C. DNA was isolated using the MagneSil® KF, Genomic System.

To illustrate the quality of DNA purified with the MagneSil® KF System, we amplified^(a) a single-locus target from human genomic DNA. Figure 3 shows robust amplification of the expected 1.8kb product from the apolipoprotein E allele (ApoE). To more stringently test DNA quality, we analyzed DNA purified from blood using the Promega Y Chromosome Deletion Detection System (Cat.# MD1101). The tests consist of four multiplex PCR amplifications with amplicons ranging in size from 109bp to 370bp. Robust amplification of each product within and between multiplex amplifications indicates the high purity of DNA free of heme, protein, excessive salt and other potential PCR inhibitors (Figure 4).

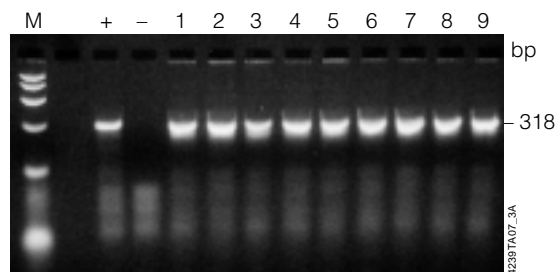


Figure 3. Single-locus amplification from blood genomic DNA isolated using the MagneSil® KF System. Single-locus amplification of the APO-E allele was performed using 5µl (~50ng) of DNA isolated using the MagneSil® KF System. Lane M, PCR Markers (Cat.# G7531). Lane (+), Genomic DNA (Cat.# G3041) positive control. Lane (-), water negative control. Lanes 1–9, DNA samples.

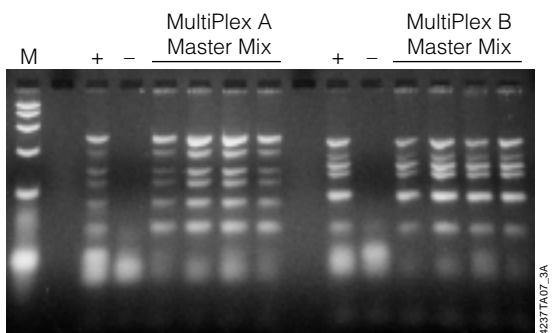


Figure 4. Multiplex PCR analysis using blood genomic DNA isolated using the MagneSil® KF System. MultiPlex A and B Master Mixes were used to amplify genomic DNA using the Y Chromosome Deletion Detection System^(b). Lane M, PCR Markers (Cat.# G7531). Lane (-), negative control. Lane (+), positive control.

Applications

The pace of discovery in the research and service laboratory has been advanced by access to new sample types as well as new molecular analysis methods. In both cases, it is critical to have genomic DNA purification methods with the flexibility to work with multiple sample types at a suitable throughput. Although originally designed for genomic DNA purification from blood, the MagneSil® KF, Genomic System has been successfully used with a broad variety of sample types and downstream applications.

Detection of *Helicobacter*

The pathogenic organism *Helicobacter pylori* was first isolated from humans in 1983, and numerous papers have identified *H. pylori* as the causative bacterial agent for certain types of recurrent gastric ulcers in humans. Several *Helicobacter* species have been found to colonize and infect rodents such as mice and rats. Traditional methods of detection, including histologic observation and microbial culture, have proven difficult to use. Many animal facilities have turned to PCR analysis as part of routine health screening.

Dr. Ken Henderson and Paulo Silva of Charles River Labs (Wilmington, MA) developed a method to purify DNA from mouse and rat feces using the MagneSil® KF System. They have compared the performance of DNA isolated using the MagneSil® KF System to DNA isolated using their own “homebrew” rapid lysis method in PCR analysis (Figure 5).

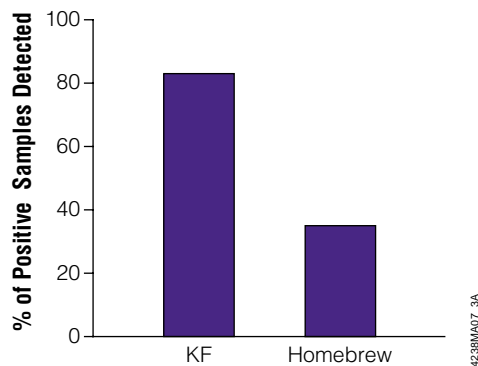


Figure 5. Comparison of performance in PCR analysis of DNA isolated from rat fecal pellets using the MagneSil® KF System or a “homebrew” method. DNA from 28 different samples was analyzed by fluorogenic PCR using a conserved PCR primer set that detects all *Helicobacter* species in the 50- to 100-copy range. Data is presented as the percent of samples that tested positive for *Helicobacter* by the production of a 375bp amplification product.

Five to seven milligrams of fecal pellet were placed in Lysis Buffer KF in a Qiagen Mixer Mill (2 minutes at 20Hz). The homogenate was incubated for 10 minutes at 75°C and centrifuged at 13,000rpm for 1 minute. Supernatant (minus 50µl near fecal debris) was removed and placed in a KingFisher® mL 1 × 5 tube strip analogous to a liquid blood sample. DNA purification proceeded with no modification.

All the rat fecal pellets used in this experiment came from rooms that tested positive for *Helicobacter*. The homebrew protocol works well for mouse fecal pellets, but not for rats, guinea pigs, hamsters, gerbils, or rabbits due to fecal-associated PCR inhibitors. DNA isolated using the MagneSil® KF System performed significantly better in PCR analysis than DNA from the homebrew purification most likely because of the removal of these inhibitors.

Routine Purification of a Range of Sample Types

Small- to medium-sized clinical labs are routinely presented with a broad variety of sample types. To illustrate the flexibility of the MagneSil® KF System, Dr. Greg Tsongalis’s lab at the Hartford Hospital (Hartford, CT) developed methods to purify genomic DNA from tissue and other samples using the MagneSil® KF, Genomic System. Table 1 shows the modifications to the standard protocol for each sample type and the downstream applications that have been performed using the purified DNA.

Table 1. Purification of genomic DNA from different sample types.

Sample	Protocol Modifications	Applications
Buffy coat	Centrifuge up to 200µl of blood. Remove buffy coat and bring the volume to 200µl. Add to tube 1 of the 1 × 5 tube strip and process the same as blood.	BRAC2 allele 11.2 SNP
Bone marrow	Bone marrow lavage is treated like blood, except 200µl of a liquid sample containing chunks of marrow is added to tube 1 of the 1 × 5 tube strip.	Factor V (Leiden) SNP Factor II (prothrombin) SNP
Virus in blood	None	Qualitative PCR for HBV, Parvo B19, CMV gpB, EBV <i>Bam</i> H I-W, HHV8 KSS330
Dacron® swab	Swirl swabs in 200–500µl of Lysis Buffer KF for 1 minute with occasional gentle shaking. This is basically enough liquid to wet the entire swab from top to bottom. “Squeeze” the tips of the swab to remove as much absorbed liquid as possible. Process 200µl of liquid using the blood protocol.	Multiplex PCR for HSV1 and HSV2
Nasopharyngeal swab	Same as Dacron® swab.	PCR for <i>Bordetella pertussis</i>

Automation of Genomic DNA Purification... continued

Conclusion

The MagneSil® KF, Genomic System, designed for use on the KingFisher® mL instrument, provides consistent high-quality DNA from blood and other samples. Together, this system and instrument provide a unique level of performance and automated throughput for the small- to medium-size laboratory. In this article, we have described methods to purify genomic DNA from blood and other samples for use in PCR and specialized amplification-based analysis. Please contact Promega for the latest information on these and other sample types.

Protocols

- ◆ *MagneSil® KF, Genomic System Technical Bulletin #TB322*, Promega Corporation.
(www.promega.com/tbs/tb322/tb322.html)



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Ordering Information

Product	Size	Cat.#
MagneSil® KF, Genomic System	200 preps	MD1460

For Laboratory Use.

Products may be covered by pending or issued patents. Please visit our Web site for more information.

^(a)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.

^(b)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.

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