J. Microbiol. Biotechnol. (2001), **11**(5), 890–894

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Rapid Isolation of Genomic DNA from Normal and Apoptotic Cells Using Magnetic Silica Resins

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Received: February 27, 2001
Accepted: May 25, 2001

Abstract The isolation of genomic DNA from mammalian cells is usually performed by cell lysis followed by protein digestion, extraction, and finally, ethanol precipitation of the chromosomal DNA. However, in the case of large sample numbers or when fast processing is required or when only small amounts of starting materials are available, such conventional methods are not efficient and are cumbersome to be applied. Some alternative methods have been described as well as having commercial DNA isolation kits to be available, nevertheless, there is room left for much improvement. In the present study, a novel method is introduced, where it simplifies conventional protocols by omitting some time-consuming steps such as protease incubation or DNA precipitation and its resuspension. Using paramagnetic silica resins, the genomic DNA was purified over a magnetic field, and the bound DNA was eluted with a low-salt buffer. The fidelity and effectiveness of this novel method was determined by using normal and apoptotic cells as a starting material and then compared to other protocols. The high speed and convenience along with its high efficiency in detecting apoptotic chromosomal DNA will prove this method to be an improved alternative in the isolation of genomic DNA from mammalian cells.

Key words: Genomic DNA, magnetic silica, apoptosis, dexamethasone, Jurkat cells

The extraction of chromosomal DNA from mammalian cells at present time has become an essential molecular biological tool in analyzing the genetic or physiological information of a given organ or organism. In the case of humans, isolated genomic DNA provides valuable information for predicting possible genetic diseases [9], or identifying individuals [16], determining the HLA haplotypes for bone marrow transfer [11], and solving paternity problems [18], etc. In the case of laboratory animals, the isolated genomic DNA also serves as an indicator for determining the integration or deletion of particular genes, which might have been transferred or removed by applying transgenic techniques [5] or gene knock-out systems [12], respectively.

The extracted chromosomal DNA further reflects the physiological status of the cell or tissue originated, since cells undergoing programmed cell death or apoptosis differ in the constitution of their genomic DNA from normal intact cells by generating a 200-bp ladder-like fragmentation pattern [2]. Actually, this 200-bp DNA ladder is a hallmark of apoptotic cells, and using this characteristic, the effects of various apoptotic or anti-apoptotic agents have been conveniently shown on several occasions [3, 14]. In this regards, an efficient and rapid method to isolate genomic DNA is pivotal for performing the above mentioned various assays, and in particular, methods for the simultaneous processing of large sample numbers without losing quality have attracted the interest of many laboratories. Currently, a variety of protocols are in use, all of which have their own advantages and disadvantages [1, 4, 13, 15]. However, most methods focus on the efficient recovery of the chromosomal DNA from intact cells, which on the other hand is not optimally suited for isolating fragmented chromosomal DNA, as it is in the case of the genomic DNA from apoptotic cells.

To provide a further alternative in the processing of chromosomal DNA with improved recovery rate of degraded DNA, in this study, a new method is introduced, which uses a solid matrix for binding genomic DNA and

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employs a magnetic field for collecting and concentrating the isolated material. It is expected that this novel protocol will shorten the processing time and facilitate the whole isolation process compared to the conventional methods since it requires no enzyme digestions step and it is safer in handling the material, by which it eliminates hazardous phenol extraction steps.

The current protocol is based on the use of a porous magnetic silica matrix, termed MagneSil™, which is a commercially available product (Promega, Madison, WI, U.S.A.). According to the supplier's information, these particles consist of 45% SiO_2 and 55% magnetite, and have an average diameter of 6.5 μm and pore sizes of >500 angstroms. Such physical characteristics made these particles suitable with high-capacity to bind to nucleic acids, and surface modification of this silica with anion exchange chemistry has led to a salt- and pH-dependent variation of DNA-binding characteristic that could be employed for the specific binding and elution of DNA molecules. In particular, under low pH conditions (pH 5.3), DNA binding capacities are nearly not affected, whereas in high pH environments

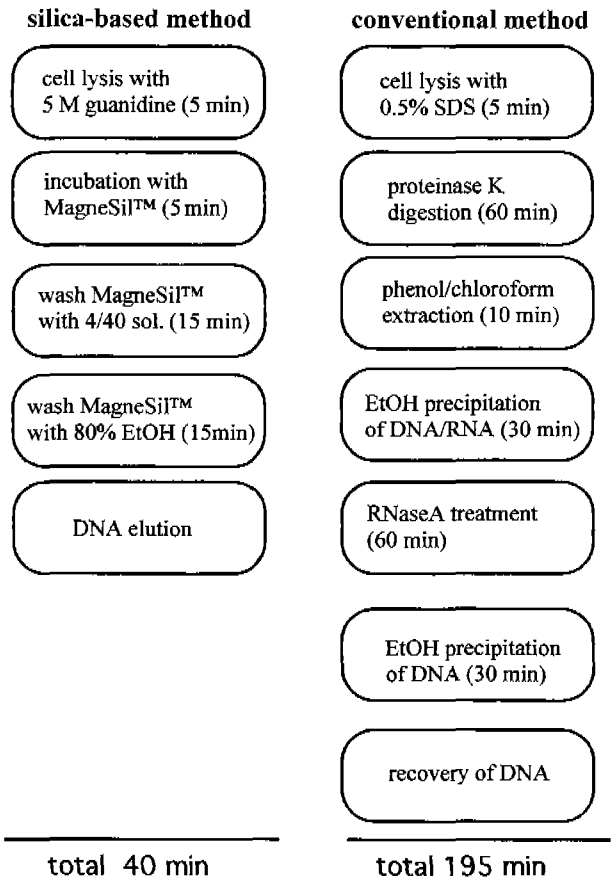

Fig. 1. Schematic presentation of the magnetic silica-based genomic DNA extraction method.
The whole procedure for extracting genomic DNA from normal or apoptotic cells using the MagneSil™ resins is depicted in this figure.

(pH 8.5), almost all the bound DNA could be eluted with 0.2 M NaCl. Therefore, in the present study, by adjusting the loading and elution conditions with different ionic strength and pH conditions, a specific protocol could be prepared to allow for rapid and quantitative isolation of genomic DNA from mammalian cells. In particular, the efficiency in the recovery of small DNA fragments, which are usually generated in apoptotic cells, was of a much higher order, so that there is a possibility for this new protocol to be suited for detecting apoptotic cells. A complete procedure for this method is schematically shown in Fig. 1. Here, it can be seen that both the incubation time and processing steps have been largely reduced in comparison to the conventional manual methods.

In practice, 1×10^7 cells were harvested from tissue cultures [8] or isolated from tissues and the cell pellet was resuspended in 500 μl of a 5 M guanidine thiocyanate solution (Sigma, St. Louis, MO, U.S.A.). While cells were completely lyzed by incubating for 2 min at room temperature (RT) in this buffer in a micro-reaction tube, the MagneSil™ particles were prepared for the next step. To prepare for this, the stock solution (Promega) was vigorously shaken to ensure a homogeneous suspension from which 100 μl was added to the cell lysate. The lysate/bead mixture was then incubated for an additional 5 min under occasional shaking. Magnetic particles were then harvested by placing the micro-reaction tube in a strong magnetic field for 30 sec, and the solution was carefully removed while beads were still attached on the magnet. Beads were then washed twice by the same procedure in 500 μl of the "4/40 wash solution" (4.2 M guanidine-HCl, 40% isopropanol). After the last wash, the tube was then placed on the magnetic unit for 30 sec to allow the solution to be cleared. Residual solution was then completely removed while leaving the tube in the magnetic field, and beads were washed three times with 80% EtOH. After the last wash, beads were dried in air for 5 min while still being attached on the magnet but the cap of the reaction tube was opened. MagneSi™ particles were then resuspended in 500 μl of TE buffer to elute the bound DNA, and after incubation for 3 min at RT, the beads were removed from the suspension by placing the tube on the magnet. The clear solution, which contains genomic DNA, could be either directly used for further assays or concentrated by precipitation with 2 vol of absolute EtOH.

The actual application of this protocol was first shown by using Jurkat human T-cells as a material source (Fig. 2), which were induced to apoptosis with camptothecin [10]. The cells from a stationary culture were transferred into fresh media containing 10 μM of campothecin and incubated for 24 h in a CO_2 cell incubator under normal cell culture conditions. Genomic DNA was then isolated by using the MagneSil™-based method from both intact and apoptotic cells and analyzed in a TAE-buffered agarose gel [7]. The

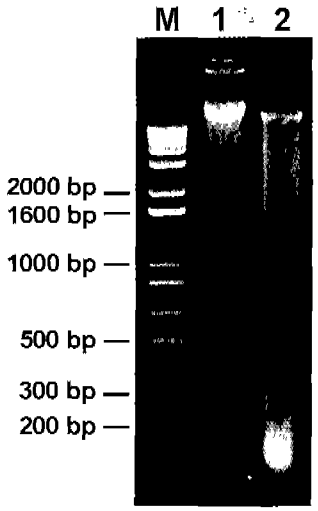

Fig. 2. Isolation of genomic DNA from normal and apoptotic cells using MagneSil™ particles.
Using the newly developed procedure, genomic DNA was isolated from both normal and apoptosis-induced Jurkat cells. Apoptosis was induced by treating with campothecin. Successful induction of apoptosis was previously confirmed by the flow cytometric analysis, using FITC-conjugated annexin V. Analysis of each 10 μg of the isolated genomic DNA in a 1.8% TAE-buffered agarose gel. M, molecular weight marker; 1. Normal Jurkat cells; 2. Apoptotic Jurkat cells.

successful induction of programmed cell death was previously confirmed by using the fluorescence-activated flow cytometry [6], in which the outer-membrane-exposed phosphatidyl serine residues were detected with fluorescein-isothiocyanate (FITC)-conjugated Annexin V (Roche Biochemicals, Inc.). The positive staining with this apoptotic cell-specific reagent (data not shown) confirmed the induction of cell death in these camptothecin-treated Jurkat cells, and subsequently, the current protocol was successfully applied in isolating the genomic DNA from both intact and apoptotic cells (Fig. 2).

To compare the efficiency of this novel protocol to other conventional methods, a couple of representative genomic DNA isolation methods were selected. One is based on the use of a series of solutions, which proceeds by the ethanol precipitation of chromosomal DNA from cell extracts which has been predigested with proteinase K and pretreated by phenol extraction. A standard protocol has been recently described by Woehrl and Haecker [19], where the isolation of genomic DNA was performed by the following procedure: cells were harvested by centrifugation and resuspended in cell lysis buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, 0.5% SDS, 500 μg/ml proteinase K) followed by incubation for 1 h at 56°C. Protein fraction was then extracted with phenol/chloroform/isoamylalcohol, and nucleic acids were ethanol-precipitated. The pellet was then dissolved in Tris-EDTA buffer containing RNase A (10 μg/ml) and incubated for 1 h at 37°C before analysis. Most of the current manual genomic DNA isolation procedures are based on this concept, however, some of the commercial kits which have optimized these procedures are available and they include the Wizard® genomic DNA isolation kit from Promega along with the Puregene® DNA isolation kit from Gentra (Minneapolis, MN, U.S.A.).

The other method is based on the use of solid matrices, such as membrane filters and resins, to immobilize the genomic DNA from the cell lysate on a solid matrix. After washing out the contaminating proteins and RNA, the matrix-bound DNA is usually eluted by using special buffer solutions. Most of the current commercial DNA isolation products are based on this concept, and these are available as the "A.S.A.P. genomic DNA isolation kit" from Roche Biochemicals (Mannheim, Germany), the "GeneEluter" from Supelco, Inc. (via Sigma, St. Louis, MO, U.S.A.), or the "Elu-Quik kit" from Schleicher and Schuell (Keene, NH, U.S.A.) as well as the filter system from Qiagen (Hilden, Germany), etc.

To compare the effectiveness of the three DNA extraction methods as described above, genomic DNA from normal and apoptotic cells were isolated by each of the respective protocols. For this, thymuses were dissected from young adult SD rats and processed to a single cell suspension. Genomic DNA was then isolated from these thymocytes after overnight culture in RPMI-1640 media (Gibco, Grand Island, NY, U.S.A.) in a cell incubator. Genomic DNA from apoptotic thymocytes was isolated after a one-day-treatment with dexamethasone under the same conditions. Dexamethasone, which is a synthetic glucocorticoid [20], is known as a classical apoptosis-inducer in thymocytes [17], and the *in vitro* treatment of isolated thymocytes resulted in generating of a profound apoptotic DNA ladder. Figure 3 shows the result from this experiment, where the same amounts of genomic DNA isolated using the three different methods are compared. It is obvious that the isolation method with MagneSil™ is as good as the others in isolating intact chromosomal DNA, but even better when applied in extracting DNA from apoptotic cells. In particular the MagneSil™-based method appears to show a much higher efficiency in the recovery of short DNA fragments with two possible reasons: First, this effect could have resulted due to the high DNA binding capacity of the silica resins, so that even degraded DNA is efficiently recovered from the cell lysate, which are otherwise missed in the other two methods. The other factor would be the relative inefficiency of the other two methods in the selective isolation of genomic DNA, since in these conventional methods, recurring RNA contamination is observed. In particular, in the case of the membrane filter-based method, the spin-filtration of the cell lysate through the nitrocellulose membrane not only easily led to the loss of small DNA fragments, but also sometimes resulted in the retaining of large molecules, such as 18S and 28S RNA, so that there were usually RNA contamination in genomic

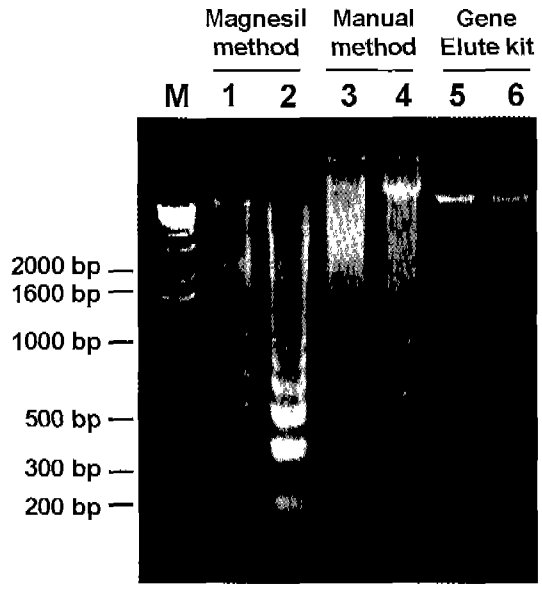

Fig. 3. Comparison of genomic DNA extraction methods.
To show the efficiency of the MagneSil™-based method, genomic DNA was isolated from apoptotic thymocytes by using several other conventional methods, which were compared to each other. Into each lane, 10 μg of DNA as determined spectrophotometrically at $OD_{260\,nm}$ was loaded. M, molecular weight marker; 1, 3, 5: Genomic DNA from thymocytes cultured overnight in normal cell culture media. 2, 4, 6: Genomic DNA from thymocytes, which were induced to apoptosis by campothecin treatment.

DNA preparations. Most certainly, it is also because of this fact that the amount of the agarose gel-loaded DNA of the filter-based method seems to differ for the other two preparations (Fig. 3, lanes 5 and 6). While in all six lanes (Fig. 3), the same amount of DNA (10 μg) as determined by spectrophotometry at OD_{260} has been loaded, lanes 5 and 6 appears to have much less DNA. RNA contamination in this DNA preparation is regarded to have attributed to an apparently higher DNA concentration than it actually is, which could also be confirmed in another RNA agarose gel (data not shown). On the contrary, the silica-based method did not show RNA contamination, which together with the short processing time and simple handling procedure, makes it obvious that this novel method will be a convenient alternative in genomic DNA isolation, especially when handling apoptotic cells or tissues.

To determine the sensitivity of the current protocol, in a further step, the MagneSil™ method was used to isolate the genomic DNA from decreasing numbers of cells of the same apoptosis-induced rat thymocytes (Fig. 3). The result from this assay is shown in Fig. 4 where the sensitivity of this method was estimated to range above 5×10^5 cells. Therefore, in the isolation of genomic DNA from smaller numbers of cells, an assumption can be made that the current method would not be suitable. On the other hand, the upper limit in cell numbers for one DNA isolation assay has not been determined. However, the use of

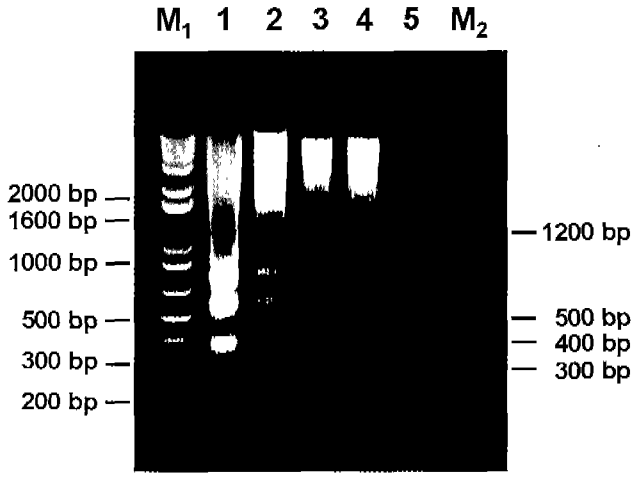

Fig. 4. Sensitivity of the MagneSil™-based genomic DNA isolation method.
Different numbers of thymocytes were used as the source for isolating the genomic DNA by the current method. M1, M2: molecular weight markers. 1, 1×10^7 cells; 2, 5×10^6 cells; 3, 1×10^6 cells; 4, 5×10^5 cells; 5, 1×10^5 cells.

1×10^8 cells for one tube was still manageable. In conclusion, 5×10^6–1×10^7 cells gave the best results with the described protocol.

In viewing the currently rising interest in various experimental procedures involving the isolation of genomic DNA, the current method is expected to give an improved alternative over the conventional methods in terms of processing time, convenience, and costs. Further applications such as the isolation of chromosomal DNA from bacterial or yeast cells as well as the isolation of genomic DNA directly from tissues with the current method need to be tested, and their successful application will indeed prove the versatility of this novel method.

Acknowledgments

This study was supported by the One Bio Co., and the TIC fund of the Chungnam National University, Korea.

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