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Capture of genomic DNA on glass microscope slides

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Abstract

It is well known that DNA strands bind to silica surfaces in the presence of high concentrations of chaotropic salts. We developed simple methods to evaluate binding and recovery of DNA on flat glass microscope slides and compared their properties to commercially available silica “spin columns”. Surprisingly, genomic DNA was recovered efficiently from untreated glass slides. Binding and elution times from glass slides were optimized in experiments with DNA samples of various sizes and defined buffers. Average DNA recovery from 500 ng of input genomic DNA varied from 25–53 % for the glass slide protocol. Yields were comparable to spin columns. Human serum albumin and plasma components decreased DNA binding to glass several-fold in a concentration dependent manner. These results support the concept of using flat glass slides as DNA purification surfaces in microfluidics devices for PCR sample preparation.

Keywords

DNA extraction; silica surfaces; PCR; microfluidics

The use of powdered glass or other high surface area silica materials has been widely adopted as a basic technique for purifying DNA from biological samples [1]. DNA strands bind efficiently to silica particles in the presence of high concentrations of chaotropic salts such as guanidinium thiocyanate or sodium perchlorate. After washing away contaminating substances with high salt buffers, the bound DNA is released from the silica surface in the presence of water or low salt buffer. The recovered DNA is suitable for use in PCR or other amplification methods. These methods are used extensively in medical diagnostics and forensics, in recombinant technology and for molecular genetics.

Silica-based DNA purification is compatible with microfluidic diagnostic devices. Such “lab on a chip” devices may help bring the benefits of PCR-based molecular diagnosis to users and point-of-care settings that lack the capacity for standard laboratory-based DNA purification [2] [3]. In such applications there are significant pressures to keep costs low. Therefore, DNA purification devices must be designed to use the least expensive materials possible, and without unnecessary engineering. Early researchers showed that the curved inner surfaces of glass test tubes could be used to purify DNA from agarose gels, but their binding capacity was deemed too small for most preparative experiments [4]. Currently, it is standard practice to maximize DNA binding surface area, for example by using silica glass bead matrices. However, such engineering measures may not be necessary to achieve satisfactory DNA purification yields.

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Flat glass surfaces such as cover slips or microscope slides have not previously been used for capturing and purifying DNA in the presence of chaotropic salts. We envision the use of larger (microscope-slide sized) flat glass materials as DNA capturing walls in microfluidics chambers [5]. Therefore, we developed simple methods to evaluate the DNA binding capacity and efficiency of flat glass surfaces and compared their properties to commercially available silica “spin columns”. Surprisingly, genomic DNA was recovered efficiently from untreated glass microscope slides. Interfering substances from human biological samples and the use of flat glass surfaces in microfluidics devices to purify genomic DNA for PCR sample preparation are discussed.

Materials and Methods

Precleaned glass microscope slides (soda lime glass, made in Switzerland, 25 × 75 mm) and glass cover slips (borosilicate glass, 60 × 24 mm) were purchased from VWR Scientific. Lambda DNA was obtained from Invitrogen as a component of the PicoGreen dsDNA quantitation kit (product no. P-11496). Salmon sperm DNA was purchased from Sigma and resuspended in TE buffer (10mM Tris-chloride pH 8.0, 1 mM EDTA) at a concentration of 10 mg/ml by stirring overnight at room temperature. The DNA solutions were diluted to 500 µg/ml in TE buffer and stored at −20°C.

In addition to the chaotropic salt containing buffers provided in the commercial DNA purification kit, we also prepared a set of Buffers that included Binding Buffer, Wash Buffer 1, Wash Buffer 2, and Elution Buffer. Binding Buffer contained 5M guanidine thiocyanate, 20 mM EDTA, and 1% Triton X-100 in 0.1M Tris-chloride pH 6.4. Wash Buffer 1 had the same composition except that it lacked the Triton X-100. Wash Buffer 2 had 10mM Tris-chloride pH 7.4, 2.5 mM EDTA, 50mM NaCl, and 50% ethanol. Elution Buffer contained 10mM Tris-chloride pH 8.0, 1 mM EDTA (TE).

DNA binding to glass microscope slides

Unless otherwise noted, samples were prepared by adding purified salmon sperm DNA to a final volume of 0.3 ml in Binding Buffer. DNA containing solutions were layered onto each slide, spread with a pipette tip, and incubated for 20–30 minutes at room temperature unless otherwise noted (covered with Petri dish lid). After incubation, the slides with pooled DNA solutions were transferred to a vertical position in a 50 ml conical polypropylene centrifuge tube. Residual liquid containing unbound DNA was removed from slides by centrifugation. All centrifugations were carried out in a Beckman Coulter Allegra 6R centrifuge equipped with a 16 cm radius rotor with swinging buckets that can hold 50 ml conical centrifuge tubes. Slides in 50 ml conical centrifuge tubes were centrifuged at 1000 RPM (150 × g) for 5 minutes. The DNA treated surfaces were then rinsed with 0.5 ml Wash Buffer 1 and centrifuged as described above, transferred to a fresh 50 ml tube, rinsed twice with 0.5 ml of Wash Buffer 2 and centrifuged as described above. Wash time incubations were as fast as permitted by benchtop manipulations and generally <1 min. The slides were then layered with 0.2 ml of Elution Buffer, covered with 60 × 22 mm glass coverslips to prevent evaporation and transferred to a fresh 50 ml tube, capped and incubated in a vertical position for 15–20 minutes unless otherwise noted. A final centrifugation of the slides as described above allowed recovery of ~0.2 ml of the eluant for DNA quantitation.

DNA purification using spin columns

DNA spin columns containing porous silica matrices were purchased as part of a kit from Qiagen that also contained the required chaotropic salt and wash solutions (QIAMP Blood Mini Kit Cat. No.51104). Samples of either lambda DNA or salmon sperm DNA (500 ng DNA) were mixed in 300 µl of TE buffer and purified as per the manufacturer’s instructions except

that protease treatment was not performed. The DNA was recovered using 200 μ l of Elution buffer and quantitated with the PicoGreen assay.

DNA quantitation

Quantitation of DNA in 100 μ l samples was performed using the PicoGreen Assay (Invitrogen) as described by the manufacturer. The PicoGreen reagent was diluted 1:200 into TE buffer and 100 μ l of the reagent was added to each 100 μ l DNA sample into an opaque black 96 well plate (Corning). After 5 min, samples were read on a Bio-Tek Synergy HT micro well plate reader in fluorescence mode. The excitation wavelength was set at 485 nm and emission was set at 528 nm using optical filters. A standard curve of DNA dilutions was concurrently prepared for each sample set and used to quantitate the amount of DNA recovered in each experiment.

Kinetic experiments

Samples of salmon sperm DNA (500 ng) were diluted into Binding Buffer (Buffer Set I) and spread onto glass slides. At selected time points, the binding reaction was stopped by removing the bulk solution with a pipettor and rinsing the slide with Wash Buffer 1 followed by immediate centrifugation. Slides were then further processed as described above. For the elution time course, glass slides were pre-bound with DNA. Elution Buffer was added together with a 60 $\times$ 24 mm glass coverslip and at selected time points, the elution was stopped by centrifugation. DNA was quantitated using the PicoGreen assay as described above.

Effect of human serum albumin (HSA) and plasma on DNA binding

Samples of recombinant HSA solution (200 mg/mL in a proprietary aqueous buffer: Recombumun product Novozymes Delta Limited, Nottingham England) or human plasma (Puget Sound Blood Center, Seattle WA) were diluted directly into Binding Buffer (Buffer Set I) at a ratio of 1 part sample to 2 parts buffer. The final sample volume was 0.3 ml. 1 μ g of salmon sperm DNA was added to the diluted sample. DNA purification was then carried out as described above.

Results

Assays for evaluating DNA binding properties of flat glass slides

Microscope glass slides are inconvenient vessels for holding liquids, and may lead to the observed variability in the DNA binding assay results. For example, pooled liquids can spill and the large surface area promotes rapid evaporation. Complete recovery of liquids from the surfaces is tricky, but spinning the slide in a 50 ml centrifuge tube was effective. The point was to evaluate the large glass surfaces for use as a purification matrix in inexpensive laminated DNA purification devices. In such a device, the glass surface would serve as a wall in a container for nucleic acid containing samples and buffers and wash solutions could be conveniently channeled through the space between the glass slides.

Purified genomic DNA was mixed with the Binding Buffer reagent (see Materials and Methods). The key component in the Binding Buffer is the chaotropic salt (5M guanidinium thiocyanate). Glass slides were then placed on the lab benchtop and solutions of 500ng DNA in 0.3ml Binding Buffer were simply spread on the surfaces using a pipettor and retained by surface tension. After allowing DNA binding to occur, the pooled liquid was removed by low speed centrifugation. The surface was washed using a similar process. Bound DNA was eluted and quantitated using the PicoGreen dsDNA quantitation method. This fluorescent assay for measuring dsDNA is very sensitive, but (as per the manufacturer's instructions) requires calibration curves with DNA standards each time an assay is run. The slopes of the standard

curves varied from day to day even though the same calibration standards were used consistently. Residual chaotropic salts in the eluted DNA can also affect the PicoGreen assay.

Comparison of DNA recovery from glass microscope slides and silica spin columns

Recovery of purified DNA using the glass slide method described above was compared to a standard DNA purification spin column kit. Table 1 shows the percent recovery of DNA following the methods in the spin column kit and from an experiment using the glass slide method. The efficiency of DNA recovery for high molecular weight DNA from salmon sperm was compared with the recovery of DNA from bacteriophage lambda (~50,000 MW) using both methods under normal conditions (see Materials and Methods) or using human plasma as the diluent instead of TE buffer for the DNA sample. The DNA binding/recovery of the DNA was slightly better for the spin column method with salmon sperm DNA in TE buffer but otherwise equivalent for both DNA types in both types of conditions tested (buffers vs. plasma). The comparable recovery of genomic DNA for the glass slides and the spin columns indicated remarkable performance for an inexpensive, off the shelf product. We further optimized the glass slide method as described below.

Optimization of the DNA binding and wash buffers

Since composition of the commercial assay buffers is not described in technical manuals, we developed a defined set of assay buffers for optimization of the glass slide method. Chaotropic salt containing buffers have been described elsewhere, for example by Glenn, Bavis, and Bollback: www.uga.edu/srel/DNA_Lab/TSilicaDNA.rtf. The critical issue with the buffers is to ensure that DNA remains bound to the glass surface until elution with the low salt (TE) buffer. DNA binding is not pH sensitive in the range of 6.4–8.5. DNA binding is equally efficient in a range of guanidine thiocyanate concentrations of 1–5 M, and falls off below 1M. Since DNA is not soluble in ethanol, ethanol can be added as a co-solvent to perhaps help dissolve cellular membrane materials, proteins or other biological contaminants. The commercial formulation for Lysis Buffer (AL) specifies addition of ethanol to enhance protease digestion perhaps by facilitating the dissolution of proteins, thereby improving the ability of the protease to hydrolyze them. We did not deem ethanol dilution to be required in our assays since the DNA was already deproteinized. In the assays described here DNA quantities recovered by including or excluding ethanol from the Binding Buffer did not vary significantly from those obtained with the commercial buffers. This indicated that our defined buffers can effectively substitute for the commercial buffer set for the DNA samples tested.

DNA binding and release times from glass slides

The optimum binding and release times were determined for the glass slide purification method for high MW (genomic) DNA. An experiment to measure the time needed for DNA adsorption utilized 500 ng of salmon sperm DNA which was suspended in 300 µl of Binding Buffer and applied to a glass slide for the time periods indicated in Fig. 1. Binding was halted by centrifugation of the DNA binding mix from the glass slide. The glass slides were then rinsed sequentially using the two wash buffers and the bound DNA was eluted using 200µl of TE buffer. Binding increased over time during this experiment from 5 minutes to 30 minutes (Fig. 1) but started to plateau at about 20–30 minutes. Over 85% of the recovered DNA bound to the glass surface in the first 20 minutes of the 30 minute experiment. For subsequent experiments, an adsorption time period of at least 20 minutes was used based on these results.

The kinetics of elution of high molecular weight DNA was followed in a similar way. Elution reached maximal concentration at the first time point (5 min) and did not increase significantly after 30 min. We therefore conclude that elution is essentially complete within minutes. It is not clear that the recovery of DNA from the glass surface is quantitative, but the DNA concentration reaches equilibrium rapidly.

Reproducibility of DNA binding to glass slides

Experiments were performed to explore the slide-to-slide variation between several independent preparations. Sixteen slides were prepared in which 500 ng of salmon sperm DNA was dissolved in 0.3 ml of Binding Buffer and each slide was treated for 30 minutes. The slides were subsequently rinsed in Wash 1 and Wash 2, and bound DNA was eluted with 200 μ l of TE buffer. One slide gave low yield due to human error and was eliminated from the data set. As shown in Table 2, for the 15 slides, the average yield was 56% $\pm$ 10%. As mentioned earlier, there is day to day variability in the absolute quantitation of recovered DNA. For example, the % DNA recovery shown in Table 1 was 25% $\pm$ 5%. It is not clear whether the wide range of DNA recovery shown in Table 2 (101–384 ng) reflects the variability of the glass slides, the flat surface liquid handling techniques, or the PicoGreen measurement assay.

Effect of Human serum albumin and plasma on DNA binding to glass slides

We next studied the binding and recovery of DNA in complex solutions containing other biological materials. As shown in Fig. 2 A, the DNA recovery on glass slides was reduced ~30% when the DNA was adsorbed in the presence of a physiological amount of human serum albumin (40 mg/mL HSA). We have not seen similar studies of substances that affect DNA binding to glass, but a common method for “passivating” surfaces to prevent DNA binding is to coat them with albumin. Thus it was not surprising that a decrease in DNA binding was observed. Similarly, when 33% plasma was added to the binding buffer, recovery was reduced ~70% as depicted in Fig. 2B. The interfering effects of plasma components on DNA recovery from both the glass slide and spin column purification methods is also depicted in the series of 10 preparations depicted in Table 1 where the DNA was dissolved directly into 100% plasma before being put through the purification protocols. It is not clear what components in plasma (other than HSA) contribute to decreased DNA binding, but other proteins can compete for glass binding sites under the chaotropic salt conditions.

Discussion

We report here that flat glass microscope slides (untreated, soda lime glass) can be used in a simple procedure to capture DNA from dilute solutions. Capture of long DNA fragments (genomic DNA) on flat glass surfaces is surprisingly rapid and efficient in the presence of chaotropic salts. The DNA binding properties of glass slides are similar to glass tubes [4] but DNA recovery in small volumes is facilitated by the flat slides compared to the curved glass surfaces. The capacity of DNA binding on the interior walls of 10 $\times$ 75 mm glass tubes was reported to be ~1 μ g [4] but the capacity of the glass slide surfaces was not studied here. DNA recovery using the glass slide method is somewhat variable, but may improve when the glass slides are incorporated in microfluidic devices. The various binding/washing and elution buffers are changed at each step by carefully placing the glass slide in a 50 mL centrifuge tube and spin drying. Using an optimized protocol we have been able to achieve nearly comparable recovery of genomic DNA versus silica spin columns (56% recovery, Table 2). Human serum albumin and other plasma components decrease efficiency of DNA binding to the silica surface and this is the case for with the standard silica spin columns as well (3–4 % recovery from plasma, Table 1).

Glass slides or cover slips seem ideal materials for construction of microfluidic devices to purify DNA. The inexpensive and clean optical glass parts can be laminated into devices with various chamber and fluid transport features. Despite growing interest in developing “lab-on-a-chip” devices for inexpensive DNA isolation[2,3] [6] the utility of readily available, unmodified glass surfaces in DNA capture chambers has not been described. Several devices have been designed to miniaturize the sample sizes for micro-PCR analysis [7], and silica based DNA capture surfaces have been engineered with higher binding capacity. For example, silica

resins have been optimized for DNA capture in microchambers in the presence of chaotropic salts [8,9]. Another approach has been to use microfabricated silica structures etched into a surface to increase surface area [10]. DNA binding molecules such as amino silanes have been coated onto glass slides to improve capacity in extraction devices and can release the DNA at high pH [11]. Other polyamine coatings such as polylysine have been used to coat microscope slides for making DNA microarrays, but these are not designed to release the DNA. Porous polymers with embedded silica particles have also been reported [12].

Flat glass surfaces may have advantages over finely divided silica particles for efficient capture of genomic DNA in biological samples in microfluidic devices. After Boom et al. [1] published their “1 step” method for DNA isolation using silica particles, the use of monolithic glass surfaces [4] was not further examined by the scientific community. The glass slides are not convenient for routine purification of nucleic acids from complex biological samples compared to the easy-to-use commercial spin columns. We intend to improve convenience of the slides by using them as walls in microfluidic containers and we will report performance of such devices in subsequent publications. The gentle DNA binding and recovery process described here may have further advantages for assays requiring long, unbroken strands of genomic DNA.

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Fig. 1.

Kinetics of DNA binding to glass slides. 500 ng of DNA in 0.3 ml Binding Buffer was adsorbed onto each glass slide for the time period indicated by each time point. Binding was halted by centrifugation of the DNA binding mix from the glass slide. The glass slides were then rinsed sequentially using the two Wash Buffers and the bound DNA was eluted using 200 μ l of TE buffer. One glass slide was used for each time point.

A.

B.

Fig. 2.

A. Effect of human serum albumin (HSA) on DNA recovery from glass slides. Increasing amount of HSA were added to 0.3 ml of Binding Buffer containing 1000 ng of salmon sperm DNA. 40 mg HSA/ml is the physiological concentration of HSA in blood.

B. Effect of plasma on DNA binding to glass slides. Increasing volumes of human plasma were added to 0.3 ml of Binding Buffer containing 1000 ng of salmon sperm DNA up to a maximum concentration of 0.1 ml of plasma/0.3 ml of Binding Buffer.

Table 1

Comparison of spin column and glass slide DNA isolation methods

	% DNA Recovered (n=5 for each data point)		
	Lambda DNA	Salmon Sperm DNA*	Salmon Sperm DNA in Plasma
Spin Column	28±8	61±16	3±2
Glass Slide	16±8	25±5	4±1

p value 0.01 by paired t test comparing spin column to glass slide method

Recovered quantities of high molecular weight DNA were compared with bacteriophage lambda DNA using either the glass slide method or the spin column method in the presence or absence of plasma. Each data point represents the average and standard deviation for 5 experiments. For the slide preparations, 500 ng DNA samples were either diluted directly into 0.3 ml Lysis buffer or into 0.15 ml plasma to which 0.15 ml of the Lysis buffer were then added, washed with buffers 1 and 2, and eluted with 0.2 ml TE. For the spin column preparations, DNA samples were prepared in 0.3 ml TE buffer or plasma, purified as per the manufacturer's instructions (without protease treatment) and eluted with 0.2 ml of TE as well. Samples were quantitated using the PicoGreen assay.

Table 2

Reproducibility of salmon sperm DNA recovery from glass slides

Elution Temp.	Glass slide variability from 500 ng input DNA			
	% recovery	DNA recovery (number of slides)	Standard deviation (%CV)	Range
Room temp.	Avg = 53%	Avg = 267 ng (n=15)	± 67 ng (25%)	102 – 384 ng