A new devise-mediated miniprep method

Mikhail BAryshev (Mihails.Barisevs@latnet.lv)

Research Article

Keywords: Plasmid DNA, Miniprep, Supercoiled plasmid, Plasmid DNA isolation

Posted Date: January 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1278978/v1

Abstract

Small-scale plasmid DNA preparation or miniprep is a fundamental technique in estimation cloning experiments and is widely used for DNA methylation analysis in epigenetic research. Current plasmid DNA minipreps use alkali - SDS based method in a three-solution format and require the spin column-based purification steps. This procedure demands the vortexing or pipetting of pelleted by centrifugation bacteria and mixing of solutions manually. Here we describe a centrifuge/mixer-based instrument having an ability to perform centrifugation, vibration, and rotor oscillation in order to perform all steps of plasmid DNA isolation by device only. We found that applying rotor oscillation-driving mixing of solutions added in the lysis and neutralization steps, the homogeneous mixing is achieved within 5 second at rotor oscillation amplitude 45 degrees and oscillation frequency 400±30 rpm, yielding maximal quantity and quality of plasmid DNA. No increase of host chromosome presence in purified by this approach occurs for high copy number plasmids as compared to manually performed miniprep, and indeed, there is a significant decrease in the presence of chromosomal fraction in low copy number plasmids. The supercoiled form of plasmid DNA purified at a rotor oscillation amplitude of 45 degrees does not turn into open circular (OC) isoform when the plasmid is stored for one year at plus four degrees, in contrast to the plasmid purified with rotor oscillation amplitude 270, 180 and 90 degrees. The programmed time-work-efficient protocol of plasmid miniprep installed in the device gives the extreme simplicity of plasmid minipreps speeding up and facilitating the isolation of plasmid DNAs.

Key Points

- New devise-mediated plasmid miniprep method (DM) performs all mixing steps without operator intervention.
- DM method produces plasmid DNAs free of the dCCC form and significantly reduces the contamination with genomic DNA in the low copy number plasmid.
- DM miniprep plasmids are reliable templates for bisulfite PCR sequencing analysis.

Introduction

Plasmid DNA minipreps are widely used procedures for recombinant plasmid purification to verify resultant constructs to be fit the appropriate downstream application. To perform multiple minipreps, the cost-effective and time-efficient systems that provide researchers with the appropriate quantity and quality plasmid DNA obtained with minimal manual handling, are a requirement of the laboratories to compete successfully in generating results. Most commonly used procedures include alkali with SDS (Birnboim and Doly, 1979), non-ionic detergents (Altschuler at al. 1994) zwitterionic detergents (Chowdhury and Akaike, 2005), using phenol, phenol/chloroform extraction with $MgCl_2$ addition (Cheng and Zhang, 2004; He at al. 1990; Kovalenko at al. 1994). Several approaches using instead of liquid bacterial culture the colony grown on plate agar as starting material for plasmid minipreps has been described (Sato 2012, 2014). The standard alkali SDS column-based method of minipreps rely on the irreversible denaturation of bacterial genomic DNA under high pH and the ability of plasmid DNA to re-associate upon subsequent neutralization. While chromosomal DNA along with some RNA, protein, and cell debris remains in the precipitate, the plasmids are in solution (Engebrecht at

al. 1991). Subsequently, other contaminants are removed by the addition of washing solution. The pure plasmid is then eluted in an elution buffer. In various modifications, instead of columns, the plasmid may be purified by organic extraction and ethanol precipitation or differential precipitation with polyethylene glycol/sodium chloride mixture. The final step all of these methods include air-drying for 10-15 minutes or using a vacuum desiccator.

The plasmids are known to exist in the enteric cells in different conformational states: supercoiled DNA (DNA I, or covalent closed circular DNA, i.e. cccDNA), relaxed circular DNA (DNA II, or open circular DNA, i.e. ocDNA) and, linear DNA (DNA III) forms (Vinograd and Lebowitz, 1966). In many cases, due to local pH extremes in solutions, the anchor base pairs may be lost. This treatment can then cause the plasmid to form incongruent complementary bases or cruciform loops after neutralization, resulting in an irreversibly denatured supercoiled plasmid form (DNA IV, i.e. dcccDNA) (Diogo at al. 1999), which is an undesirable impurity in the final plasmid preparation. Some studies have been done to remove this plasmid DNA form with arginine affinity chromatography and by combining denaturation, selective renaturation and aqueous two-phase extraction (Sousa at al. 2009; Frerix at al. 2007).

Despite column-based alkali procedure gives ready to use plasmid solution, the procedure demands the vortexing or pipetting of pelleted by centrifugation bacteria and the admixing of solutions manually. Here we describe the device with oscillation-driving mixing option for the steps of solutions admixing and ability for pellet re-suspension of 12 samples simultaneously. The time-work-efficient protocol of plasmid purification provides extreme simplicity of plasmid minipreps reducing the operating time by 40 - 60 percent compared to manually operating column-based approach. The plasmid DNAs obtained by using the device are free of dcccDNA plasmid form and presented mostly by cccDNA form suitable for basic molecular biology applications including sequencing, cell transfection and can form the complexes between amphipathic TAT peptide and plasmid DNAs that can be used for gene delivery in eukaryotic cells. In addition, a commercially available “classical” plasmid DNA miniprep column-based alkali kits are compatible with the developed device and can be successfully applied using the installed time- work-efficient protocol.

Materials And Methods

Bacterial strain and DNA plasmids

Invitrogen (Carlsbad, Ca) supplied XL1-blue *Escherichia coli* strain used for plasmid propagation

in LB medium. Antibiotics were purchased from Sigma-Aldrich. High copy number plasmids used were: pcDNA 3.1/His/LacZ 8.6 kb from Invitrogen (Carlsbad, Ca); pEGFP vector Clontech, (Palo Alto, USA); Low copy number pR322 plasmid was from our lab. pCR4-TOPO Invitrogen (Carlsbad, Ca) vector was used to clone 585 and 750 bps in size PCR generated fragments of *PPARg2* and *Oct4* gene promoter regions that has been used in DNA methylation analysis by PCR bisulphite sequencing. ZymoPURE™ II Plasmid Midiprep Kit was from Zymo Research Corp. The QIAGEN Plasmid Midi Kit was from QIAGEN Company.

Standard column-based plasmid miniprep

Standard column-based plasmid minipreps were performed according to GeneJET Plasmid Miniprep Kit, ThermoFisher Scientific (https://tools.thermofisher.com/content/sfs/manuals/MAN0012655_GeneJET_Plasmid_Miniprep_UG.pdf).

DNA quantification

Average concentrations of the plasmid DNA was quantified using the NanoDrop microvolume sample retention system, Thermo Fisher Scientific, (Waltham, MA).

Restriction analysis and DNA sequencing

Restriction endonucleases and T4 DNA ligase were obtained from Thermo Fisher Scientific and used with the buffer stocks recommended and provided by the company.

DNA sequencing was performed using the ABI BigDyeTerminator Cycle Sequencing Kit v3.1 according to the manufacturer's instructions on a Gene Amp 9700 PCR machine and the sequences were detected on an ABI 3130XLGenetic Analyzer.

Assessment of host genomic DNA in minipreps by SQ-PCR

To obtain semi-quantitative data on the presence of host genomic DNA in plasmid preparation, a four 5 x fold serial dilutions of host chromosome (purified with the Wizard gDNA purification kit; Promega) in the range 0.008-1 ng were used as the reference of host DNA amount in PCR reactions and it has been compared with amplification of 100 ng plasmid DNAs. Forward, 5'- TTCCCACGGACATGAAGACTACA-3' and reverse, 5'-ATCCTGCGCACCAATCAACAA-3' *E coli* K-12 strain-specific primers were used to amplify a 1.687 bp fragment (Kuhnert at al. 1995). The relative intensity of the band of the genomic DNA amplified from the plasmid DNA minipreps was quantified in relation to the bands of reference diluted samples by the image analysis software (Fujifilm Image Gauge Ver. 4).

Time efficiency

Time efficiency of the device-mediated minipreps (DM) relative to the standard column-based minipreps (SM) was calculated as the ratio of the total SM and DM procedure completion times amplified by 100. T (tot) SM/T (tot) DM x 100 = % time efficiency. Subtracting 100% we will find how much the DM minipreps more time-efficient, in percentage, compared to SM. The T (tot) were considered as the sum of the handling times, a time needed for sample/solution manipulation by the operator, and technological times which are mediated by working equipment's and incubation time according to appropriate protocols.

The data presented in Table 1 illustrate the time efficiency of the DM minipreps.

PC3 cell line transfection

Prostate cancer-derived PC3 cells were cultured in RPMI-1640 culture medium supplemented with 10% fetal bovine serum (Invitrogen Corp.), penicillin (100 U/mL), and streptomycin (100 µg/mL) (Invitrogen Corp.) at 37 ◦C in a 5% CO_2 atmosphere incubator. The cells were seeded in 24-well plates at a density of 1.8×10^5 per well

one day before transfection to obtain 80% confluence. Before transfection, the growth medium was removed and 350 µL of Opti-MEM reduced serum medium (Invitrogen Corp.) was added. K4® Transfection System consisting of the K4® Transfection Reagent and the K4® Multiplier were obtained from (Biontex Laboratories GmbH) and used according to company provided protocol. The transfection efficiency was determined after 24 h of transfection as the percentage of green cells compared to the total cell number.

Gel retardation assay

One micrograms of pcDNA 3.1/His/LacZ plasmid (Clontech, Mountain View, CA, USA) was pre-mixed with various amounts of the GFP-TAT fusion protein (0, 2.5, 5, 7.5, 10, and 20 µg) in phosphate-buffered saline (PBS) to a final volume of 20 µl and incubated at 37 ^{o}C for 30 min in to form peptide/DNA complexes. (*GFP-TAT* fusion was created by fusion PCR and cloned into IPTG-inducible vector. The fusion protein was expressed in *E. coli* and pure protein subsequently was purified by Ni-NTA chromatography). These complexes were analyzed by electrophoresis on a 1% agarose gel in TAE buffer, followed by staining with ethidium bromide.

Results

Instrument for devise-mediated miniprep

The instrument for device-mediated minipreps was created on basis of ELMI centrifuge/mixer and has a capacity of up to 12 samples (Fig. 1A). It has a special two-parts rotor (Fig. 1B). The upper part of the rotor comprises the angle disk with openings while the lower part is the disk with specially shaped openings whose axes lie in one plane with the openings of the upper part of the rotor. This feature of the rotor drives concussion of tubes in the openings of the lower part rotor under rotor vibration, which, in turn, results in quick resuspension of bacterial pellets. The introduced option of back-and-forth rotor movement performs the oscillation-driving mixing of solutions added without tube inversion.

Plasmid yield by DM miniprep depends on oscillation-driving mixing parameters and exceeds that by SM method

The mixing of miscible fluids of different densities is needed to be done within the miniprep procedure. The mixing as a degree of homogeneity of two or more liquids plays a pivotal role in the quality and quantity of the final product. In miniprep, it is conventionally carried out by tube inversion. This step is operator-dependent and some variability in the homogeneity of mixing might be happening, which may have an effect on yield and quality of plasmid DNA. Unlike commonly used 1,5 ml tubes, a 2 ml tube have a cylinder-like geometry and suite to be used in rotor oscillation-driving mixing and will promote the plasmid yield increasing.

We have introduced the oscillation-driving mixing to avoid the manual operation and to make a process of plasmid DNA isolation as ‘semi-automated’. The oscillating motion of the rotor provides for oppositely vectored acceleration forces, which are automatically imposed on the constituent of the tube to mix the solutions. To ensure proper mixing, the tube is periodically oscillated in opposite directions so that the various formed components will gravitate first toward one end of the tube wall and then toward the opposite end of

the tube wall. We have compared the effect of oscillation-driving mixing at varies amplitudes and frequencies on plasmid DNA yield (Fig. 2A, B, C, D) and found the process of mixing is time, oscillation amplitude and oscillation frequency-dependent and affects the plasmid quality and yield. Having evaluated parameters affected plasmid yield by this approach, we found that combination of oscillation amplitude 45 degrees, oscillation frequency 400 rpm ensures the homogenous mixing of the admixed solutions during minipreps in a 5-10 second, and, yielding the amount of plasmid DNA exceeding that of the manually isolated plasmid DNA (Fig. 2A, C, D). Besides higher plasmid yield, an improvement the quality of the plasmid is also observed: pEGFP plasmid is free of dcccDNA plasmid form (Fig. 3A, C). It is interesting to note, that plasmid yield at rotor oscillation amplitude 90 degree is less than that obtained at 270 and 180 degrees. Furthermore, plasmid yield at these three amplitudes do not reach that obtained by SM method even at prolonged to 20-second time mixing (Fig. 2A, B), reflecting, thereby, incomplete/improper mixing. In general, the mixing efficiency is increased upon decreasing of amplitude oscillation but not in the case of 90 degree. However, the mixing performance at amplitude 45 degree, which is half of 90 degrees, and oscillation frequency 400 rpm, provides a homogenously mixed solution in 5-10 second, resulting in exceeding plasmid yield compared to SM method (Fig. 2.A, D). Moreover, plasmids purified under these conditions retained supercoiling after storage for one years at +4 degrees in contrast to the plasmids isolated with rotor oscillation amplitude 270, 180 and 90 degrees (Fig. 3B).

Of note, the low copy number plasmid prepared demonstrated the absence of visible chromosomal impurity on the gel, in contrast to manually prepared plasmid (Fig. 3A, B). Having determined the mixing parameters giving improved miniprep, we have established the DM miniprep and used that in the plasmid purification.

Devise-mediated miniprep

Miniprep consists of 7 pre-programmed successive steps by using GeneJET column-based plasmid miniprep kit. The pre-set optimized parameters for each step of miniprep are installed in the device and represent the DM miniprep protocol as shown in Fig. 1C. Of what you need to perform miniprep, is to add/remove solutions and press the start button of the following step to obtain the ready for use plasmid DNA in the final step.

F1. Spin 2 ml overnight grown bacterial culture. Remove supernatant.

F2. Add 250 µl of resuspension solution. Press Start. All pellets will be re-suspended.

F3. Add 250 µl of lysis solution and then add 350 µl of neutralization solution. Press Start. All solutions will be mixed and automatically centrifuged removing the main impurity.

F4. Pour supernatant in column/tube ensemble. Press Start. Discard the flow throw.

F5. Add the washing solution. Press Start. Discard the flow throw.

F6. Place tubes into the device. Press Start. The remaining of washing solution will be removed.

F7. Add 50 µl of elution buffer and put tubes into the device for 1 minute. Having increased during operation the temperature within the device will promote the plasmid DNA elution. In a 1-minute press Start. Eluted plasmid DNAs are ready to use.

Purified by this protocol plasmids had OD260/280 in a range 1.85-1.95, which indicates the plasmids are pure enough to be tested in the plasmid quality-sensitive downstream applications. Average yields of plasmids obtained were for pBR322 - 3±0.5 μg; pEGFP - 22±4 μg, pLacZ -30±3 μg. pLacZ plasmid yield from 2 ml overnight *E. coli* culture reach up to 30 μg, which will correspond to 3 mg of the plasmid being isolated from 200 ml bacterial culture by DM miniprep.

Devise-mediated minipreps are time efficient compare to standard column based plasmid minipreps

The steps of miniprep performed by newly developed device use the different setting of such parameters like time and speed of centrifugation, the intensity of vibration used for pellet resuspension and the oscillation amplitude and oscillation frequency of rotor motion for liquid mixing. To minimize the time of miniprep, the optimized miniprep procedure parameters settings were done and installed in the device. An optimization done ensures completeness of cell resuspension, bacterial lysis and solution mixing needed for miniprep performance. Fig. 1C shows the parameter settings for each step. After the step is complete, the device automatically goes to the next step highlighting it on the screen. To evaluate time efficiency, we compared the DM miniprep protocol versus SM one. The plasmid isolation using DM minipreps allows saving time up to 60% upon processing of 12 samples regarding the SM miniprep and requires 20 minutes to complete the procedure (Table 1). It should be noted that the recently published one-step miniprep method accomplishes one sample only for 26 minutes excluding the time for plasmid drying and dissolution (Lezin at al., 2011). Our DM miniprep excludes the manual handling of solution mixing and cell pellet re-suspension and decreases the total time of the minipreps procedure, making the plasmid isolation faster than ever.

The DM plasmid is pure for restriction digestion, and sequencing, is free of dccc DNA plasmid form and has lower amount of host genomic DNA

We compared the sensitivity of the plasmid DNA purified by either DM or SM minipreps in restriction enzyme digestion and sequencing reactions. Although, DM and SM minipreps produce almost similar amount of plasmid DNA, we see, among the three plasmids isolated by DM miniprep a sustainable tendency in increasing the yield and improving the quality of plasmid DNA in sense of plasmid topological structure and presence of chromosomal fraction: in pEGFP plasmid, often used in cell transfection experiments, we do not observe the dcccDNA plasmid form as well as we see some chromosomal fraction in SM pBR322 (Fig. 3A, B, C).

We also found that DM miniprep plasmid DNA is a robust template in sequencing reactions of *PPARg2* and *Oct4* promotor region fragments of 585 and 750 bp in size respectively, representing the bisulphite treated inserts used in DNA methylation analysis of the genes (Supplementary Fig. S1, S2). All plasmids used in the analysis of DNA methylation of distal and proximal Oct4 enhancers (Baryshev et al., 2018) and the proximal PPARg2 promoter (Baryshev et al., 2020) were obtained by the DM miniprep method. We have compared the presence of host genomic DNA in the plasmids obtained both by DM, SM minipreps and commercial non-miniprep kits recommended for purification of high quality plasmid DNA. From data presented in Fig. 4A, B, it can be seen that a small fraction of the host genomic DNA in the range of 100-150 pg per 50 ng of plasmid is present in all purified plasmids, and the DM preparation contains less chromosomal DNA than the SM. According to densitometry analysis, the amount of chromosomal fraction found in the plasmids is 60±5 pg

for QIAGEN Plasmid Midi Kit; 120 ±10 pg for ZymoPURE™ II Plasmid Midiprep Kit; 105± 10 for DM miniprep and 130 ±10 for SM miniprep (Fig. 4C, D). Thus, our results show that the hydrodynamic forces rising at a rotor oscillation frequency specified do not affect the chromosomal shearing in DM miniprep and the content of host genomic DNA in the final DM plasmid preparation is sustainable lower than in the SM isolated plasmid (Fig. 4A, B, C, D).

The DM pEGFP plasmid has similar transfection efficiency compared to EndoZero and Midi kit purified plasmid

The transfection efficiency of pEGFP plasmid purified by DM approach was compared with that of the same plasmid purified with a commercial EndoZero and Quiagen Midi kits. The results presented in Fig. 5A show that plasmid purified by DM miniprep was able to transfect 35% of PC3 cells, whereas EndoZero and Quiagen Midi kits purified plasmids transfected 34% and 33 % of cells, consequently. Similar transfection efficiency observed among the three plasmids tested, proposed that DM miniprep plasmid DNA can successfully be used in the cell transfection experiments, allowing making these experiments more cost- and time-effective.

GFP-TAT binding

To test whether GFP-TAT fusion protein could bind to DNA in vitro, pcDNA 3.1/His/LacZ plasmid was mixed with various concentrations of GFP-TAT fusion. Formed complexes were analyzed by electrophoresis on an agarose gel and stained by ethidium bromide (Fig. 5B). As it seen in the figure, the migration of supercoiled DNA bands are shifted with increasing of amount of GFP-TAT fusion. These results suggested that positively charged TAT peptide might bind to plasmid DNA. This binding would be occurring between negative charges of plasmid DNA and positive charges of TAT protein transduction domain of fusion protein via electrostatic interactions.

Discussion

In spite, the plasmid DNA purification is well-established method in molecular biology, the technique is still developing for time-, cost saving and decreasing of laborious, especially upon processing of multiple samples. It was found that with a decrease in the time and speed of centrifugation of the bacterial culture, the time for resuspending the bacterial sediment using a vortex device is significantly reduced (Voo and Jacobsen, 1998). The plasmid DNA recovery in miniprep occurs by alkaline cells lysis followed by neutralization with an acidic high molar potassium acetate. After step of neutralization, the cell debris, high molecular weight chromosomal DNA and other impurities become trapped within the soft, buoyant and highly shear sensitive gel-matrix (Ciccolini at al. 1999). The separation of the plasmid-containing solution from the floating gel-matrix is carried out by high-speed centrifugation. The mixing conditions and the time incubation with alkaline are critical in plasmid minipreps. Many plasmid miniprep protocols indicate 5-minutes time incubation for the lysis of bacteria that were mixed with lysis buffer by vigorously inverting the tube. Thereafter, neutralization is carried out by adding the chilled neutralization buffer and inverting the tube, followed by incubation on ice for 5 minutes (Qiagen Plasmid Handbook. 2021). During the lysis step, intracellular constituents are released within a few seconds, and the chromosomal DNA is irreversibly denatured under extreme alkaline conditions, while plasmid DNA is capable of annealing after the

neutralization step. If alkaline environment, during the lysis exceeds the 0.15 ± 0.03 M (pH 12.9 ± 0.2), irreversible denaturation of the supercoiled plasmid DNA occurs (Meacle at al. 2004). Therefore, while the lysis buffer of 0.2 M NaOH range is mixed with the resuspended cells, some of the cells will experience of locally high pH leading to plasmid denaturation that is shown to increase with time of exposure, and as a result, denatured supercoiled plasmid DNA appears in the final eluate of SM Miniprep (Fig. 3A, B, C). Yu et al. (2008) showed that such alkaline denatured supercoiled DNA has a stable conformation with unregistered, topologically constrained double strands and intrastranded secondary structure. This form is not effective in gene transfer, and constitute important impurities due to their physical and chemical similarities with the supercoiled form (Prazeres at al. 1998). It has been shown that lysis of 4 ml *E. coli* suspension occurs within 30-40 seconds (Ciccolini at al. 1999) and that the pure supercoiled plasmid denatured rapidly in 0.2 M NaOH (Meacle at al. 2004).

Taking into account these data and our observation that the cell suspension obtained under the conditions of steps1 and 2 (Fig. 1C) of the DM miniprep method is lysed immediately (becomes clear) after the addition of the lysis solution, we exclude the incubation time in alkali and the mixing as well, and add a neutralizing solution at once. This will minimize the expose of the plasmid to alkaline environment, its denaturation, and, we assume, will fall off the effect of a shear in genomic DNA. Due to the change in the rheological properties of the lysate during the lysis steps and especially the neutralization step, it is difficult to achieve gentle and efficient mixing even in miniprep format, with regard to shearing genomic DNA, to avoid the presence of this contaminant in the low copy number plasmid obtained by the standard miniprep method (Fig. 3A). The addition of a denser (d = 1.16 g / cm^3) neutralizing solution settle it to the bottom of the tube, and to ensure faster renaturation of denatured plasmids, rapid intensive mixing could be a correct strategy. Therefore, we have introduced the rotor oscillation-driving mixing in miniprep procedure. With these modifications, we were able to demonstrate that the mixing efficiency of solution affects the plasmid quality and yield (Fig. 2). We determined that the combination of oscillation amplitude 45 degrees, oscillation frequency 400 rpm ensures homogenous admixing of solutions during minipreps in a 5 second, and, yielding the amount of plasmid DNA exceeding that of the SM isolated plasmid (Fig. 2A, C, D). We further revealed that the use of DM mixing options has a great advantage for low copy number plasmid pBR322 production compared to SM method. Any of DM mixing parameters studied are able to produce predominantly monomeric CCC form of the plasmid, avoiding the presence of visible chromosomal fraction on the gel, in contrast to SM method (Fig. 3A, B). Meanwhile, we did not observed the same effect for high copy number plasmids.

Taking into consideration the hydrodynamic aspects of fluid mixing at a relatively high rotor osculation frequency, which can lead to a force generated by the liquid due to turbulence and might have an effect on chromosomal shearing, we have assessed the presence of the host genomic DNA in plasmid preparations (Fig. 4). Unexpectedly, our results show that the action of fluid hydrodynamic stresses caused by the movement of the rotor with the indicated oscillation frequency does not affect the chromosomal shearing in the DM miniprep, and the content of the host genomic DNA in the final plasmid preparation is consistently lower than in the isolated SM. plasmid (Fig. 4A, B, C ,D). This is consistent with the finding that efficient mixing during neutralization achieved with a Rushton turbine and agitation speed in the range of 190-1200 rpm does not lead to contamination of the plasmid DNA with the chromosomal fraction (Chamsart at al. 2001). However, isolated plasmid DNAs studied are mainly found in the form of OC, even with gentle mixing.

It seems that prolonged incubation in alkali and a 5-minute neutralization step promote the turning of CCC to the OC isomer of the plasmid. In addition, we provide evidence that the presence of host genomic DNA is observed even upon using the plasmid isolation kit other than the miniprep format. From the data presented in Fig. 4C, D, one can conclude that a small fraction of host genomic DNA in the range of 60-150 pg per 50 ng of plasmid, representing an impurity of plasmid preparations and detected in plasmid samples obtained by both DM, SM minipreps and by commercial non-miniprep kits, using alkaline lysis of bacterial cells, arise due to the shearing of the host chromosome during alkaline lysis of cells. An improving the selective cccDNA plasmid/chromosomal DNA binding/elution conditions to silica membrane, can help to avoid the plasmid preparation to be contained the host genomic DNA fraction.

Surprisingly, all plasmid purified by DM method and stored at + 4 degrees for one years showed differences in stability of CCC form plasmid DNA (Fig. 3B). If plasmids obtained by the SM and DM (oscillation amplitude 45 degrees, oscillation frequency 400 rpm) methods retained the CCC form as predominant, then supercoiling of high copy number plasmids isolated by DM at 270 degrees and low copy number plasmids isolated at 180 and 90 degrees of mixing options, was almost completely lost, turning into an OC isoform (Fig. 3B). In *E. coli*, topological state of plasmid DNA is under control of four DNA topoisomerases that able alter negative supercoils from CCC form of DNA (Higgins and Vologodskii, 2015). It seems that the observation of changes in the topological behavior of plasmids that we observe after long-term storage is mediated by the co-purification of Topo I / Topo III of type I enzymes, which break one strand at a time, converting the supercoiled form to the OC isoform. Thus, the DM method can differentiate plasmid isolation relative to possible co-purification of the CCC plasmid-Topo I / Topo III complexes and may be of interest for supercoiling studies.

Here we have introduced the new DM miniprep method. We assume that an increase in the yield of plasmid DNA and their higher quality achieved with DM miniprep is associated with a short exposure time to an alkaline environment, faster step of neutralization of minipreps, and more efficient mixing of solutions using rotor oscillations. The plasmid DNAs purified using the DM method and the GeneJET miniprep kit are free of the dcccDNA plasmid topological invariant and contain less of genomic DNA. We expanded the field of application of the DM miniprep plasmids and showed that the quality of the plasmids is high enough to be used in studies of protein / peptide-DNA binding, and that plasmids purified by DM method can be widely used in DNA methylation analysis. Our DM method can differentiate plasmid isolation relative to possible co-purification of the CCC plasmid-Topo I / Topo III complexes and may be of interest for supercoiling studies. With the DM method, miniprep is less labor procedure for plasmid miniprep with minimal manipulation, and can be considered as “semi-automatic” method, allowing up to 60% timesaving when processing 12 samples compared to SM. The above approach can be especially useful for researchers, who have limited access to laboratory supplies and equipment and require a rapid method and apparatus for isolating plasmid DNA.

Declarations

Data availability

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This work was supported by an internal grant from Rīga Stradiņš University No. 132311.

Author information

Affiliations

Institute of Microbiology and Virology, Riga Stradins University, Riga, Latvia

Mikhail Baryshev

ELMI Ltd, Riga, Latvia

Dmitrijs Merkulov & Ivan Mironov

Contributions

DM and IM designed and constructed the device for miniprep. MB conducted experiments and analyzed the data. MB wrote the manuscript. All authors read and approved the manuscript.

Corresponding author

Correspondence to Mikhail Baryshev.

Ethics declarations

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest

The authors declare that they have no known competing interests.

References

1. Altschuler M, Heddens DK, Diveley R.R.Jr, Kresheck GC (1994) Plasmid DNA isolation utilizing a novel nonionic detergent. Biotechniques 17:434–436
2. Birnboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513–1523
3. Baryshev M, Inashkina I, Salmina K, Jackson TR, Erenpreisa J (2018) DNA methylation of the Oct4A enhancers in embryonal carcinoma cells after etoposide treatment is associated with alternative splicing and altered pluripotency in reversibly senescent cells. Cell Cycle, 2018, 17(3), pp. 362–366
4. Baryshev M, Petrov N, Ryabov V, Popov B (2020) Transient expression of inactive RB in mesenchymal stem cells impairs their adipogenic potential and is associated with hypermethylation of the PPARγ2 promoter. Genes Dis in press

5. Chamsart S, Patel H, Hanak JA, Hitchcock AG, Nienow AW (2001) The impact of fluid-dynamic-generated stresses on chDNA and pDNA stability during alkaline cell lysis for gene therapy products Biotechnol Bioeng. Nov 20; 75(4)
6. Cheng L, Li TY, Zhang Y (2004) Rapid preparation of total nucleic acids from E. coli for multi-purpose applications. J Biochem Mol Biol 37:351–355
7. Chowdhury EH, Akaike T (2005) Rapid isolation of high quality, multimeric plasmid DNA using zwitterionic detergent. J Biotechnol. 119:343–347
8. Ciccolini LAS, Ayazi Shamlou P, Titchener-Hooker NJ, Ward JM & Dunnill P (1999) Rheological properties of chromosomal and plasmid DNA during alkaline lysis reaction. Bioprocess Eng 21:231–237
9. Diogo MM, Queiroz JA, Monteiro GA, and Prazeres DMF (1999) Separation and Analysis of Plasmid Denatured Forms Using Hydrophobic Interaction Chromatography. Anal. Biochem 275, 122–124
10. Engebrecht Jo, Brent R, and Kaderbhai MA (1991) Curr Protoc Mol Biol 1.6.1-1.6.10
11. Frerix A, Geilenkirchen P, Muller M, Kula MR, Hubbuch J (2007) Separation of Genomic DNA, RNA, and Open Circular Plasmid DNA From Supercoiled Plasmid DNA by Combining Denaturation, Selective Renaturation and Aqueous Two-Phase Extraction. Biotechnol. Bioeng. 2007;96: 57–66
12. He M, Wilde A, Kaderbhai MA (1990) A simple single-step procedure for small scale preparation of Escherichia coli plasmids. Nucleic Acids Res 18:1660
13. Higgins NP, and Vologodskii AV (2015) Topological Behavior of Plasmid DNA. Microbiol Spectr. 2015 April; 3(2)
14. Kovalenko SA, Tanaka M, Ozawa T (1994) Simple methods for preparation of plasmid DNA yielding long and accurate sequence data. Nucleic Acids Res 22:5771–5772
15. Kuhnert P, Nicolet J, & Frey J (1995) Rapid and accurate identification of Escherichia coli K-12 strains. Appl Environ Microbiol 61:4135–4139
16. Lezin G, Kosaka Y, Yost HJ, Kuehn MR, Brunelli L (2011) A One-Step Miniprep for the Isolation of Plasmid DNA and Lambda Phage Particles. PLoS ONE 6(8):e23457. doi:10.1371/journal.pone.0023457
17. Meacle FJ, Lander R, Ayazi Shamlou P, Titchener-Hooker NJ (2004) Impact of Engineering Flow Conditions on Plasmid DNA Yield and Purity in Chemical Cell Lysis Operations.
18. Biotechnol Bioeng. 2004 Aug 5; 87(3)
19. Prazeres DMF, Schluep T, Cooney C (1998) Preparative purification of supercoiled plasmid DNA using anion-exchange. J Chromatogr A. May 8;806(1):31–45
20. Qiagen Plasmid Handbook. 2021
21. Sato M, Akasaka E, Saitoh I, Ohtsuka M, Nakamura S, Sakurai T, and Watanabe S (2012) A simplified protocol for the semi-large scale recovery of plasmids from *Escherichia coli* grown on agar plates. J Biomed Sci Eng **5**, 406–408
22. Sato M, Inada E, Saitoh I, Matsumoto Y (2014) Microbial and enzyme technology: An efficient and convenient method for MiniPrep analysis of recombinant plasmids.<bvertical-align:super;> </bvertical-align:super;>J Biomed Sci Eng **7**, 105–107

23. Sousa F, Duarte MF, Prazeres Jo˜ao Queiroz1 A (2009) Improvement of transfection efficiency by using supercoiled plasmid DNA purified with arginine affinity chromatography J Gene Med 11:79–88
24. Vinograd J, Lebowitz J (1966) Physical and topological properties of circular DNA. J Gen Physiol 49:103–125
25. Voo KS, Jacobsen BM (1998) Rapid resuspension of pelleted bacterial cells for miniprep plasmid DNA isolation. Biotechniques 24:240–3
26. Yu J, Zhang Z, Cao K, Huang X (2008) Visualization of alkali-denatured supercoiled plasmid DNA by atomic force microscopy. Biochem Biophys Res Commun. 2008 Sep 26;374(3):415–8
27. https://tools.thermofisher.com/content/sfs/manuals/MAN0012655_GeneJET_Plasmid_Miniprep_UG.pdf

Table

Table 1

Time efficiency of devise-mediated vs standard minipreps.

Devise-mediated MiniPrep Standard MiniPrep Time

Number of preps	Total time, min	Operator'time min	Total time, min	Operator'time min	efficiency, %
2	12	4	17	5	42
4	14	7	21	9	50
8	17	10	26	14	53
12	20	13	32	20	60

Figures

Figure 1

Schematic view of a miniprep assisting instrument assembly. **A** Centrifuge/mixer- based apparatus. B Two-part rotor. C Pre-set parameters for each step of device-mediated miniprep showing the different setting of such parameters as time and speed of centrifugation, intensity of vibration for pellet resuspension and oscillation frequency of rotor motion at amplitude 45 degree for liquid mixing.

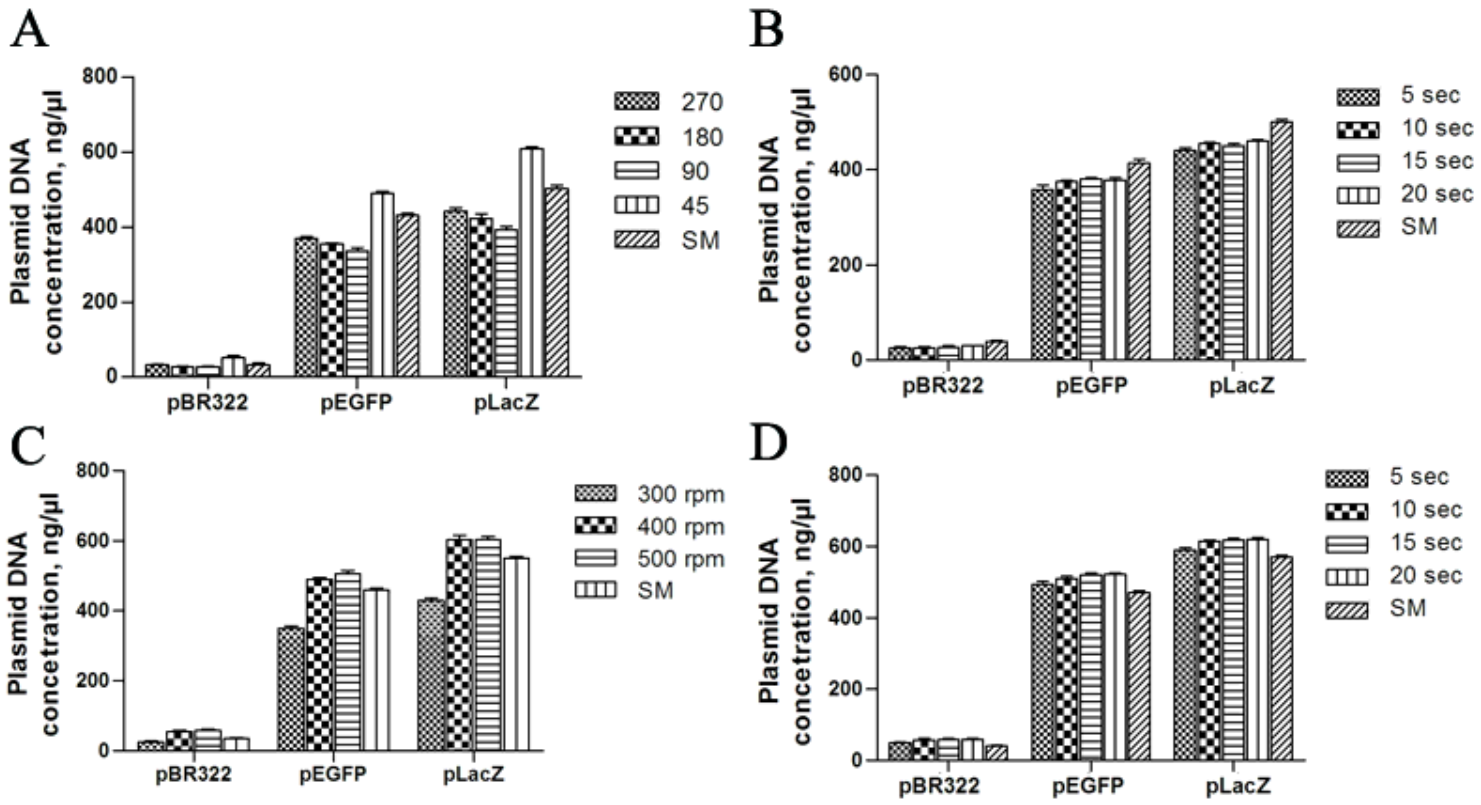

Figure 2

Admixing efficiency of solutions performed by rotor oscillation affects the plasmid DNA yields. **A** Effect of oscillation amplitude of 5 second oscillation-driving mixing in DM minipreps at oscillation frequency 400 rpm on plasmid yields. **B** Effect of time oscillation driving mixing in DM minipreps at oscillation amplitude 270 degrees and oscillation frequency 400 rpm on plasmid yields. **C** Effect of oscillation frequency of 5 second oscillation driving mixing in DM minipreps at oscillation amplitude 45 degrees on plasmid yields. **D** Effect of time oscillation driving mixing at oscillation amplitude 45 degrees and oscillation frequency 400 rpm in DM minipreps on plasmid yields. Assays were done in triplicate, and error bars represent standard deviation.

Figure 3

DM minipreps plasmids are purer compared to SM one. **A** Comparing of DM and SM minipreps plasmids conformational state. **B** Comparing of DNA stability of DM and SM minipreps plasmids after one year's storage at + 4 degrees. **C** Comparing of pEGFP of DM (D) and SM (S) minipreps plasmids quality and capability of cleavage by restriction endonuclease in comparison to ZymoPURE-EndoZero Midiprep (Endo) and QIAGEN Midi Kits (Midi) isolated plasmid. 1 kb plus DNA Ladder was electrophoresed as DNA size marker. Presence of the host genomic DNA in SM isolated pBR322 is depicted by asterisk. dcccDNA,is indicated by arrowhead and is resistant to restriction enzyme digestion.

Figure 4

DM minipreps plasmids have lower amount of host genomic DNA. **A** The amount of host genomic DNA in DM and SM plasmids; lane 1-4 - reference interval of quantities' DNA for semi-quantitative analysis; P, E, L – pBR322, pEGFP, LacZ plasmids purified by DM at oscillation amplitude 45 degrees and 3, 4, 5 - 300, 400, 500 rpm oscillation frequency; SM^P, SM^E, SM^L - pBR322, pEGFP, LacZ plasmids purified by SM method. **B** Densitometry analysis of amplified products is presented. **C** The amount of host genomic DNA in pEGFP plasmid DNA purified either by SM (S) or DM (D) minipreps and ZymoPURE-EndoZero (Endo) Midiprep or QIAGEN Midi kit (Midi). **D** Densitometry analysis of amplified products is presented. Assays were done in triplicate, and error bars represent standard deviation.

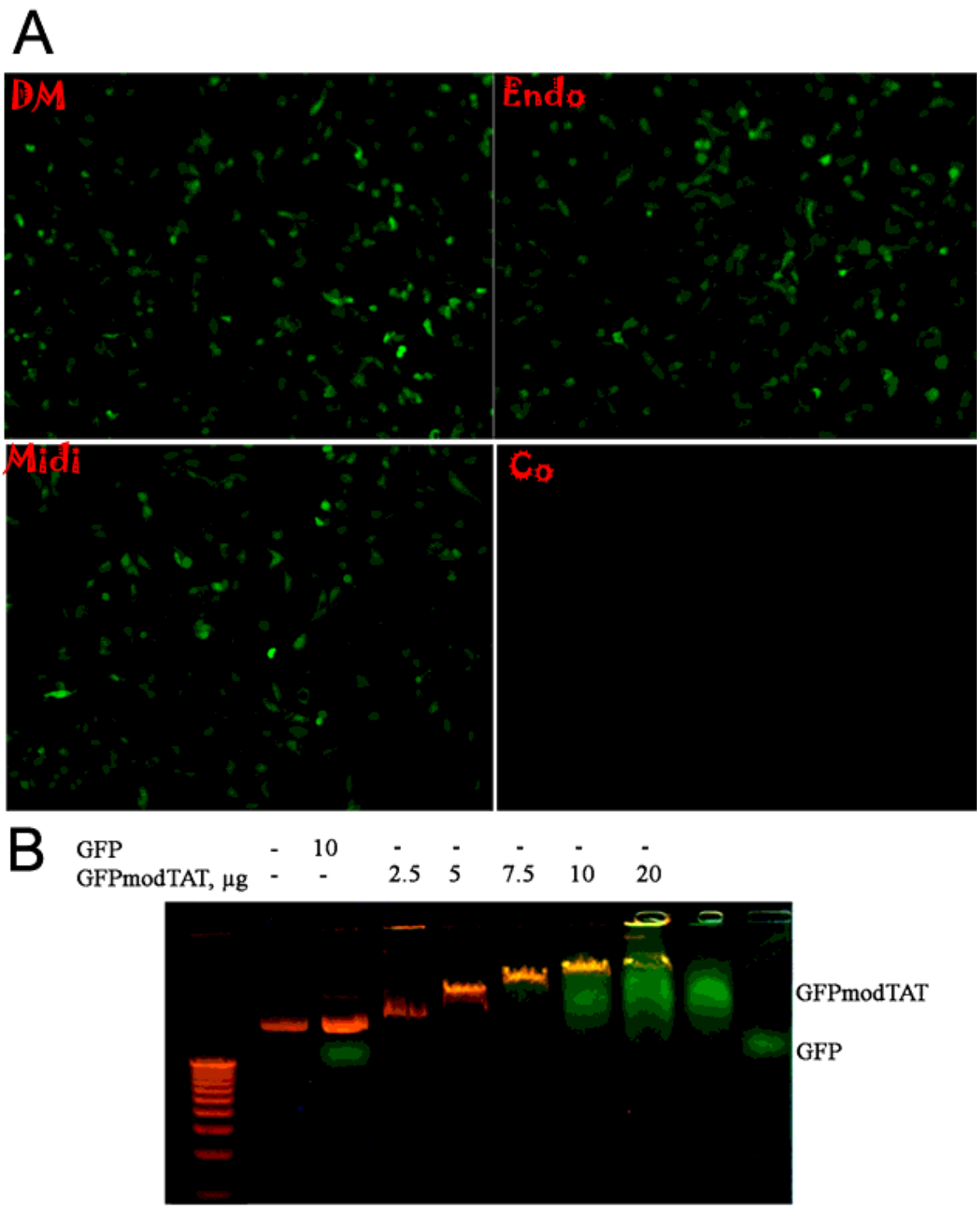

Figure 5

DM miniprep plasmids can efficiently be used in DNA quality-sensitive applications.

A Transfection efficiency of PC3 prostate cancer cells with DM minipreps plasmid is similar to ZymoPURE-EndoZero Midiprep and QIAGEN Midi Kits derived results. One of three independent experiments is reported. **B** pcDNA 3.1/His/LacZ plasmid purified with DM minipreps method forms GFP-TAT-fusion protein complexes. Samples were resolved by agarose gel electrophoresis.