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Hofmeister series salts enhance purification of plasmid DNA by non-ionic detergents

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Abstract

Ion-exchange chromatography is the standard technique used for plasmid DNA purification, an essential molecular biology procedure. Non-ionic detergents (NIDs) have been used for plasmid DNA purification, but it is unclear whether Hofmeister series salts (HSS) change the solubility and phase separation properties of specific NIDs, enhancing plasmid DNA purification. After scaling-up NID-mediated plasmid DNA isolation, we established that NIDs in HSS solutions minimize plasmid DNA contamination with protein. In addition, large-scale NID/HSS solutions eliminated LPS contamination of plasmid DNA more effectively than Qiagen ion-exchange columns. Large-scale NID isolation/NID purification generated increased yields of high quality DNA compared to alkali isolation/column purification. This work characterizes how HSS enhance NID-mediated plasmid DNA purification, and demonstrates that NID phase transition is not necessary for LPS removal from plasmid DNA. Specific NIDs such as IGEPAL CA-520 can be utilized for rapid, inexpensive and efficient laboratory-based large-scale plasmid DNA purification, outperforming Qiagen-based column procedures.

Keywords

plasmid DNA purification; non-ionic detergents; Hofmeister salts

INTRODUCTION

Plasmid DNA purification is a critical and routine procedure in molecular biology laboratories. Here, we tested whether HSS can change the phase separation properties of specific NIDs, enhancing plasmid DNA purification. Plasmid DNA purification involves the removal of contaminating macromolecules from cellular extracts. These contaminants include chromosomal DNA, RNA, proteins, polysaccharides and lipopolysaccharides (LPS). LPS (endotoxins) are major components of the *E. coli* cell wall which often co-purify with DNA because they contain negatively charged phosphate groups (Magalhaes et al. 2007). Plasmid DNA contaminated with LPS may be toxic to cells in culture, and in animals activates inflammatory cascades that may lead to altered experimental responses. Currently, ion-exchange chromatography has replaced classic CsCl-ethidium bromide gradients for isolating nucleic acids of the highest purity (Yang et al. 2008).

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NIDs are soluble amphipathic molecules consisting of polar (hydrophilic) and nonpolar (hydrophobic) moieties (Table 1) (Garavito and Ferguson-Miller 2001). The hydrophile-lipophile balance (HLB) determines the water solubility of a detergent (Griffin 1954; Neugebauer 1990). A higher HLB value indicates that a detergent is more hydrophilic. At low concentrations, NIDs are dissolved in water as isolated molecules, but at higher concentrations they self-associate forming micelles. The hydrophobic tails of detergent molecules are gathered in the center of micelles forming a fat soluble core, while the hydrophilic heads face outward protecting the core from contact with water. Because of this peculiar structure, even relatively hydrophobic NIDs are soluble in water and may form transparent solutions at room temperature. At higher temperatures, micelles disorganize and NID solubility decreases. When the cloud point (CP) is reached, initially transparent solutions become turbid and emulsion-like because they split from a one-phase to a two-phase solution, in which the aqueous phase is depleted of NID (and any other substance included in micelles) and the organic phase is rich in NID. These NID characteristics suggest that removal of lipid-like molecules, such as LPS, from NID solutions can be achieved by separating the organic and aqueous phases (Bordier 1981; Cotten et al. 1994; Sanchez-Ferrer et al. 1994).

Dissolution of salts can dramatically change water properties, including surface tension, solubility, and stability of macromolecules. HSS is a ranking of salts based on their quantitative propensity to change water properties (Collins and Washabaugh 1985). Originally, this ranking referred to the minimum salt concentration required to precipitate (salting-out) egg protein from aqueous solution (Cacace et al. 1997). These properties are more pronounced for anionic salt components compared to cations, and ranking of the most common anions is as follows:

In this series, species following Cl^- are frequently referred as chaotropes (destabilizing structure, salting-in) (Hamaguchi and Geiduschek 1962), while those preceding Cl^- as kosmotropes (stabilizing structure, salting-out) (Collins and Washabaugh 1985). We reasoned that HSS may increase protein solubility not only in water solutions, but also in water/alcohol solutions, thereby facilitating precipitation of plasmid DNA free of protein.

Here, we have characterized how HSS change the solubility and phase separation properties of specific NIDs, and developed a novel and efficient plasmid DNA purification procedure.

MATERIALS AND METHODS

NIDs, salts and endotoxin removal solution (ERS) were purchased from Sigma–Aldrich (St. Louis, MO) or Mallinckrodt Baker (Phillipsburg, NJ). Antibiotics were purchased from USB (Cleveland, Ohio), Sigma–Aldrich, and EMD/Calbiochem (Gibbstown, NJ). Bacterial media, including LB and Super Broth (SB) were obtained from Quality Biological (Gaithersburg, MD). DH5 α and XL1-Blue were supplied by Invitrogen (Carlsbad, CA) and Stratagene (La Jolla, CA), respectively. The plasmid pLTM 330 (6.5 Kb) was kindly provided by L. Tessarolo (NCI, Frederick, MD). pLTM 330 is a pBluescript-based plasmid and high-copy number. All enzymes were purchased from New England Biolabs (Ipswich, MA). Enzymatic reactions were performed at 37°C for 1 hour if not otherwise specified.

DNA quantification

DNA was quantified using a two wavelength spectrophotometric method on nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Protein quantification

The BCA Protein Assay Kit (Pierce, IL) was used for total protein quantification with the following modifications: the ratio of sample to working reagent was from 1:4 to 1:5. Samples were incubated at 75°C for 15 min. Readings were taken in the protein concentration range of 1–40 µg/ml. The following BSA standards were used in duplicate: 40 µg/ml, 20 µg/ml, 10 µg/ml, 5 µg/ml, 3 µg/ml, 2 µg/ml, 1 µg/ml, and 0 µg/ml. The average $\pm$ standard deviation for the linear correlation coefficient was 0.9 ± 0.003 .

LPS quantification

The ToxinSensor Assay Kit (Genscript, NJ) was used to determine LPS levels. The assay utilizes chromogenic substrate and LAL (*Limulus Amebocyte Lysate* from *Limulus polyphemus*) reagent clotting upon exposure to endotoxin. The absorbance of the released chromophore measures the endotoxin concentration. The test's results are expressed in endotoxin units (EU). According to the manufacturer's information, 10–15 EU corresponds to 1 ng of LPS.

Alkali DNA isolation and purification

For the alkali isolation/purification in Figure 1, the bacterial pellet was resuspended in 333 µl buffer P1 (Qiagen). Subsequently, 333 µl buffer P2 was added to the suspension and gently mixed. Finally, 333 µl buffer P3 was added and mixed. Cellular debris was removed by centrifugation and DNA was precipitated with 0.7 V of isopropanol. The DNA pellet was rinsed with 70% of ethanol.

The alkali isolation and purification for Figure 2 and Table 6 were performed using the QIAfilter Maxi kit (Qiagen, Germantown, MD) according to manufacturer's instructions. Alkali lysates were purified by Qiagen-tip 500 columns.

NID large-scale plasmid isolation procedure

Bacteria were harvested from LB/SB cultures by centrifugation. If the pellet was large, it was loosened by mixing with a small spatula and vortexing until a bacterial slurry was attained. The bacterial pellet was resuspended in the large-scale extraction buffer. Lysozyme (250 µg/ml), RNase A 25 µg/ml (optional), and 0.5% IGEPAL CA-630 (or Triton X-100) from 10% stock were added to the bacterial suspension. The suspension was mixed by gently inverting the tube to avoid unnecessary foaming. The suspension was incubated in a water bath at 65°C for 10 min for bacterial suspension volumes up to about 30 ml, or up to 30 min for larger volumes. The cellular debris was spun down at 30000 rpm for 30 min or until a compact pellet of bacterial debris was formed. 20–30 µg/ml RNase A was added to cleared extracts and incubated at 37°C for 15 min. The cleared extracts were precipitated with 0.6 volumes isopropanol at 3000–4000 rpm for 15 min at RT, and then the pellets were rinsed with 70% ethanol and dissolved in TE buffer. The composition of the large-scale extraction buffer was: 5 % sucrose, 50 mM EDTA, 50 mM Tris pH 8, 1 M KCl or 1.25 M NH₄Cl.

NID large-scale plasmid purification procedure

The bacterial strain XL1-Blue was used to identify the best purification conditions (1.5 ml for every 100 ml bacterial culture, using 0.2 M GuHCl, 100 mM Tris pH9 with either Triton X-114 or IGEPAL CA-520). Under these conditions, we confirmed similar results using DH5α. The procedure was performed according to the steps detailed in Table 2. Note that positive displacement devices are better for dispensing viscous NIDs.

RESULTS

Effective scaling-up of the NID isolation procedure

To assess how effectively the NID plasmid DNA isolation procedure could be scaled-up and how small a volume of lysis buffer can be used without loss of DNA quality and quantity, we analyzed the effects of increasing bacterial cell concentration on the amount and molecular forms of plasmid DNA extracted by alkali or NIDs. The pLTM 330 plasmid was used in these and all subsequent experiments. We used 333 μ l of each alkali method solution and 1 ml of the NID extraction buffer to achieve the same 1 ml final lysate volume because the NID method uses a single extraction buffer whereas the alkali method uses three solutions. For both methods, 1:10 lysis buffer to culture volume ratios produced the largest amount of DNA per culture volume (Figure 1, lanes 1,2), whereas increasing bacterial culture volumes from 10 ml to 40 ml decreased the yield of DNA, including fast migrating and slow migrating DNA forms, per culture volume unit (Figure 1, lanes 3–8). However, the yield of fast migrating forms was far higher with the NID method at any culture volume. There was almost no fast migrating DNA when a 40 ml culture volume was used in the alkali method (Figure 1, lane 7), but a 1:20 ratio still produced sufficient amount of DNA in the NID method (Figure 1, lane 4). These results show that compared to alkali, the NID method is more effective in large-scale plasmid isolation procedures over a wide range of bacterial cell concentrations.

Hofmeister series salts decrease plasmid DNA protein contamination

We tested which HSS most effectively purifies plasmid DNA from protein contamination in large-scale preps. We determined protein concentration in the DNA sample after treatment with RNase and the first isopropanol DNA precipitation (IDP) (Figure 2, row 1). Next, we compared the ability of some chaotropic (Figure 2, rows 2–3), neutral (Figure 2, rows 4–7), or kosmotropic salts (Figure 2, row 8) to decrease protein co-precipitation from plasmid DNA/salt/isopropanol solutions. For all salts, protein contamination decreased after the third IDP (Figure 2, rows 2–8). Neutral salts (LiCl) removed protein more effectively (Figure 2, rows 4–7). In particular, 4.5 M LiCl at pH 9 eliminated protein most efficiently (Figure 2, row 5), while chaotropic and kosmotropic salts removed protein less efficiently (Figure 2, rows 2, 3 and 8). We also tested the combination of a relatively low salt concentration second IDP (0.5 M NaCl) with a high salt concentration third IDP (4.5 M LiCl), and found that this approach is effective in removing protein (Figure 2, row 5 vs. 9). These results suggest that several HSS, but especially some neutral ones such as LiCl, effectively purify plasmid DNA from protein contamination.

Hofmeister series salts regulate the phase separation properties and CP of NIDs

To test how HSS regulate NID-dependent phase separation, we measured CP and used a fat-soluble tracking dye (Sudan IV) and the re-clouding test. We concluded that phase separation is incomplete when either Sudan IV is visible in the aqueous phase or the solution becomes cloudy upon re-heating. Addition of neutral or chaotropic HSS to high HLB NIDs, such as Tween 20, produced either unstable phase separation or no phase separation (results not shown), which precluded using these combinations. Addition of kosmotropic HSS (NaAc and NH₄Ac) to Tween 20 decreased CP and produced a stable but incomplete phase separation, as shown by the tracking dye and the re-clouding tests (NaAc, Table 3A, row 3), or the re-clouding test (NH₄Ac, Table 3A, row 5). Triton X-114, a lower HLB NID, had a lower CP and stable and complete phase separation in kosmotropic (results not shown), neutral (LiCl) or chaotropic (GuaHCl) HSS (Table 3A, rows 9, 11). Another lower HLB NID, IGEPAL CA-520, has limited solubility in water, but strong chaotropes such as GuaSCN made it soluble in this solution with a CP of 34°C (Table 3A, row 14). At higher GuaSCN concentrations, CP increased from 34°C to >100°C, virtually abolishing phase

separation of IGEPAL CA-520 (Table 3A, row 15). Neutral or chaotropic HSS increased the volume of the organic phase, but trace chloroform improved phase separation and increased the volume of the aqueous phase (Table 3B). These results show that HSS modulate the solubility and phase separation of NIDs.

Lower HLB NIDs in neutral/chaotropic HSS solutions minimize LPS contamination

As expected from the previous sets of experiments, LPS contamination was quite high with high HLB NIDs (Tween 20) and kosmotropic HSS (Table 4A). Moreover, kosmotropic HSS, such as NH_4Ac or NaAc , increased LPS contamination with any NID, although to a lower degree with low HLB NIDs (Table 4B, C). Lower HLB NIDs, such as Triton X-100, Triton X-114 and IGEPAL CA-520, minimized LPS contamination when mixed with either neutral (LiCl) or chaotropic (GuaHCl) HSS (Table 4B, C). DNA contamination with LPS did not increase when trace chloroform was used to recover the aqueous phase after purification by lower HLB NIDs in neutral or chaotropic HSS solutions (Table 4C). These results show that low HLB NIDs and neutral/chaotropic HSS effectively purify plasmid DNA from LPS.

NID-purified large-scale plasmid DNA contains low levels of protein and LPS

Next, we tested different combinations of plasmid DNA isolation/purification procedures in a larger scale format (100 ml culture). LPS levels prior to the purification procedure were similar in the alkali and NID procedure: $698,600 \pm 13,400$ EU/ml and $732,100 \pm 22,900$ EU/ml, respectively. However, protein concentration prior to the purification procedure was higher in the NID compared to the alkali procedure (1045 ± 45 $\mu\text{g/ml}$ vs. 518 ± 60 $\mu\text{g/ml}$). Samples isolated and purified by NIDs had a low protein level, but samples isolated by alkali and purified by either IGEPAL CA-520 or column contained the lowest protein contamination (Table 5, rows 2, 3 vs. 4, 5, 8). Column purification was ineffective in removing protein from NID-extracted samples (Table 5, rows 6, 8). Adjustment of the NID cleared lysates to pH 7 (pH for the column washing buffer) did not reduce DNA contamination by either protein or LPS (Table 5, row 7). To facilitate comparison of the data, we converted the LPS units (EU/ml DNA solution) into the units used by other investigators (EU/ $6\mu\text{g}$ DNA) (Cotten et al. 1994), or by Qiagen (EU/ $1\mu\text{g}$ DNA) (EndoFree Plasmid Purification Handbook, Qiagen). Importantly, the LAL activity of our assay kit was 10–15 EU/1 ng LPS, the kit used by Cotton et al. had similar LAL activity (8–10 EU/1 ng LPS), while the LAL activity of the Qiagen kit was about 6.9 times lower (1.8 EU/1 ng LPS). To convert our data to the units reported by Cotton et al., the Avg LPS (EU/ml) was divided by the DNA concentration ($\mu\text{g/ml}$) and multiplied by 6. To convert our data to the units used in the Qiagen kit, the Avg LPS (EU/ $6\mu\text{g}$ DNA) was divided initially by 6.9 and then by 6. The endotoxin removal solution (ERS) was not efficient in LPS removal from NID DNA (Table 5, row 1). The lowest amount of LPS contamination was detected in samples purified by NIDs after either alkali or NID isolation (Table 5, rows 2–5). Taken together, these data show that large-scale NID isolation and purification are highly efficient for protein and LPS removal, and demonstrate that specific NIDs such as IGEPAL CA-520 are more effective than columns for the purification of plasmid DNA from LPS.

NID isolation and purification generates higher yields of high quality DNA compared to alkali isolation and column purification

To evaluate the effects of various plasmid DNA isolation/purification combinations on the degree of bacterial chromosomal contamination and plasmid DNA molecular structure, we studied the DNA reported in Table 6. As some NIDs absorb at 280nm (Grant and Hjerten 1977), we included 260/280 ratios to ensure that no significant NID contamination occurred and that DNA spectrophotometric quality was similar using different methods. Isolation by the alkali method and purification by column produced various plasmid DNA molecular

forms (Table 5, Row 8 and Figure 3A, lanes 1, 2). We used various endonucleases to identify these forms. DNA digestion by exonuclease λ showed that the upper band represents bacterial genomic DNA (Figure 3A, lane 3). The lower “irreversibly denatured” band represented single stranded DNA (Birnboim and Doly 1979), as identified by elimination of this band after treatment with Mung bean nuclease (Figure 3A, lane 4) (Kedzierski and Laskowski 1973). The most intense band represented covalently closed circular (CCC) DNA because it disappeared after treatment with nicking endonuclease (Figure 3A, lane 5). This treatment also showed that the upper portion of the middle band in lane 4 represents plasmid DNA in a relaxed form. Finally, treatment with restriction endonuclease showed that the lower portion of the middle band in lane 4 most likely represents linearized plasmid DNA (Figure 3A, lane 6). Similar DNA forms were present when DNA was isolated by alkali and purified by NIDs, specifically IGEPAL CA-520 (Table 5, Row 5 and Figure 3B, lanes 1–4). DNA isolated by NIDs (IGEPAL CA-630) and purified by NIDs (IGEPAL CA-520) had less genomic DNA and did not contain “irreversibly denatured” DNA (Table 5, Row 3 and Figure 3C, lanes 1–4). In addition, it was enriched in total DNA, including CCC DNA and DNA in the relaxed form (Figure 3C, lanes 1–4). Enrichment with total DNA was also evident in the NID isolated DNA compared to alkali DNA when Triton X-114 was used for purification (Table 5, Row 2 and Figure 3D, lanes 1, 2 vs. lanes 3, 4). High salt concentrations used in NID isolation/purification procedures did not inhibit linearization of plasmid DNA by the salt-sensitive endonuclease Age I (Figure 3A, B, C, lanes 4). Overall, these experiments show that plasmid DNA isolation and purification using specific NIDs generate higher yields of high quality DNA compared to alkali isolation and Qiagen column purification.

DISCUSSION

Here, we have described an inexpensive, rapid and efficient approach to purify plasmid DNA using NIDs and HSS in large-scale procedures. Several pieces of data support these conclusions. First, NID isolation could be effectively scaled-up. Second, HSS regulated the phase separation properties of NIDs. Third, NIDs in HSS solutions minimized plasmid DNA protein contamination. Fourth, large-scale NID purification was more effective than columns for the purification of plasmid DNA from LPS. Lastly, large-scale NID isolation/NID purification generated increased yields of high quality DNA compared to alkali isolation/column purification. This work establishes a new approach for laboratory-based large-scale plasmid DNA purification which outperforms current Qiagen-based column procedures. This investigation does not address industrial level of purification scaling-up, which clearly will represent an important and interesting focus for future studies.

Protein

The most important factors improving protein purification were increased ionic strength of the salt in the DNA solution, number of IDPs and basic pH (Figure 2). Importantly, 98% protein purification was achieved with three IDPs, and no further decrease of protein contamination was obtained by performing additional IDPs (G.L. personal observation). The degree of purification reported here for the NID procedure is similar to that achieved with the alkali procedure using 2.5 M ammonium sulfate (Freitas et al. 2006) or 1.4 M CaCl_2 (Eon-Duval et al. 2003).

Figure 2 represents results obtained when crude NID extracts were precipitated with isopropanol, while Table 4 was obtained after crude NID extracts were purified by NIDs before isopropanol precipitation. Nonetheless, protein contamination after purification with 0.75 M LiCl and Triton X-100 in table 4 are similar to row 5 in figure 2 (using the same solution for the third IDP). This suggests that the protein in the DNA/protein pellet generated by IGEPAL CA-630 or Triton X-100 plasmid extraction cannot be resolubilized

during purification with Triton X-100. On the other hand, using a low HLB NID during the purification step probably resolubilizes some proteins from the DNA-protein pellet which leads to increased protein contamination. Along these lines, it would seem better to use low HLB NIDs for extraction, and high HLB NIDs for purification. However, the combination of Triton X-114 for extraction and Triton X-100 for purification is not practical because of the low CP of Triton X-114 in the extraction buffer and incomplete phase separation of Triton X-100 during purification. Therefore, we suggest using Triton X-114 or IGEPAL CA-520 for LPS purification with subsequent saturation of plasmid DNA solution with chloroform to remove trace protein. In fact, we have shown that DNA purification with IGEPAL CA-520 and subsequent isopropanol/LiCl precipitation results in a protein contamination that is approximately 20 times lower (for DNA isolated by NIDs) or >300 lower (for DNA isolated by alkali) compared to the DNA purified by ERS (Table 5).

Protein contamination of plasmid DNA isolated by NIDs is slightly higher compared to alkali isolation (Table 5). This can be understood in the context of the Melander-Horvath theory, which suggests that protein solubility is influenced not only by hydrophobic, but also electrostatic forces (Melander and Horvath 1977). At higher salt concentrations, protein surface charges are screened out making protein molecules virtually neutral dipoles, and protein solubility is directly proportional to the product $D \times \mu \times m$, where D is the relative dielectric constant of the solvent; μ is the dipole moment of a protein molecule; and m is the molarity of the salt. SDS is believed to elongate protein molecules (Reynolds and Tanford 1970; Shirahama et al. 1974), whereas NIDs preserve the compact globular form of proteins. Most SDS is probably removed in the alkali procedure because it forms low-soluble salts with potassium acetate. However, some traces of SDS are likely to remain in DNA solutions even after IDPs. In addition, proteins are only marginally stable at room temperature because the free energy of protein conformational transition from the native to the denatured state ($\Delta G_{\text{unfold}} = G_{\text{denatured}} - G_{\text{native}}$) is typically low, about 5–20 kcal/mol protein (Privalov and Tsalkova 1979). Therefore, most globular denatured proteins are either irreversibly denatured or refold slowly, suggesting that in the alkali procedure most proteins retain an elongated shape and higher dipole moment compared to the NID-treated proteins.

LPS

The original description of LPS removal from aqueous solutions using Triton X-114 used two phase transitions: from two-phase solution to one-phase solution during incubation on ice, and vice-versa during incubation at 37°C (Aida and Pabst 1990). However, our NID purification procedure works in the two-phase solution temperature range (Table 3A, rows 8, 9, 12, 13). Only Triton X-114 mixed in 0.2 M GuaHCl may undergo a short-term one-phase transition (Table 3A, row 10). Thus, our data demonstrate that phase transition is not necessary for LPS removal. Importantly, LPS content in the NID-purified plasmid DNA is decreased compared to previously reported column purification data, and provides the same low level of LPS reported by combining two purification methods such as CsCl centrifugation (or Nucleobond ion-exchange columns) and polymyxin B resin (Table 5, rows 3–5) (Cotten et al. 1994).

NIDs with HLBs >12.5 (Tween 20) demonstrate an incomplete phase separation and higher LPS contamination compared to low HLB NIDs, which provide complete phase separation (Tables 3A and 4). These data demonstrate that the degree of NID phase separation is important for LPS purification. However, LPS purification also depends on how HSS change micelle characteristics, as suggested by the higher LPS contamination when low HLB NIDs are mixed with kosmotropic compared to neutral/chaotropic HSS (Table 4). Kosmotropic solutions increase hydrophobic effects (i.e. self-isolation of non polar solute molecules and polar water molecules), which leads simultaneously to decreased solubility of NID nonpolar moieties, decreased CP, increased micelle compactness, and decreased

volume of the organic phase, while the opposite occurs with chaotropes (Deguchi and Meguro 1975; Meyer et al. 2006). As examples, isopropanol and ethanol are not soluble in concentrated NaCl, NaCitr or Na₂SO₄ solutions due to the increasing hydrophobic effect of kosmotropes, but butanol and amylc alcohols are soluble in GuaSCN solutions because of the decreased hydrophobicity of chaotropes. Therefore, we propose that kosmotropes lead to increased LPS contamination by impeding inclusion of LPS into micelles.

Our data also suggest that NID micelle-independent mechanisms are critical to decrease LPS contamination. In fact, IGEPAL CA-520 in LiCl solutions provided the highest degree of LPS purification despite its limited solubility in water (Tables 3A, 4 and 5). Under these conditions, LPS may localize along the NID/water interface with its lipophilic components submerged in the NID organic phase, and its hydrophilic components sticking out into the water phase. Also in this instance, specific HSS modulate how well IGEPAL CA-520 purifies plasmid DNA (Table 4).

DNA

In the large-scale plasmid isolation procedure, increasing cell crowding is less deleterious for the NID method compared to the alkali procedure (Figure 1). In fact, large scale NID DNA preps contained less genomic DNA and more total DNA, including CCC and relaxed form DNA (Figure 3 and Table 5). In Figure 1, NID DNA has higher quality compared to alkali DNA probably because the NID method uses only one solution, with no need to mix three solutions, including denaturation and neutralization buffers. Cell concentrations higher than recommended probably lead to deterioration of both denaturation and neutralization, which are necessary for plasmid recovery. This is consistent with our observation that “irreversibly denatured” DNA, the fastest migrating form DNA commonly seen after the alkali procedure (Birnboim and Doly 1979), is present in lane 1 but not in lane 3, 5 and 7 (Figure 1). Since the original observation by Birnboim and Doly in 1979, this DNA is considered evidence of overexposure of DNA to alkali, suggesting that the interaction between alkali and DNA was significantly decreased at higher cell concentrations (although still enough to remove most chromosomal DNA).

About 35% of NID extracted DNA is lost during the NID purification procedure (Table 5, compare DNA concentration in samples extracted and purified by NID with samples extracted by NID but purified by ERS), but the remaining DNA has comparable quality to the alkali isolated and column purified DNA.

Conclusions

In this report, we have characterized how HSS change NID properties, making specific NIDs suitable for laboratory-based large-scale plasmid DNA purification procedures. This NID approach outperforms current Qiagen-based column procedures not only in LPS purification but also in DNA quality. To minimize LPS and protein contamination, we advice implementing the NID purification procedure in 1.5 ml for every 100 ml bacterial culture using 0.2 M GuaHCl, 100 mM Tris pH9, and either Triton X-114 or IGEPAL CA-520. Because of its low cost, simplicity and efficiency, this NID/HSS purification procedure represents a new standard in laboratory-based large-scale plasmid DNA purification techniques.

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Figure 1.

The NID method increases the yield of plasmid DNA over a wider range of bacterial cell concentrations compared to the alkali method. Cells were harvested from 10 ml (lanes 1,2), 20 ml (lanes 3,4), 30 ml, (lanes 5,6) and 40 ml (lanes 7,8) bacterial cultures. Alkali prep DNA=lanes 1,3,5,7. NID prep DNA=lanes 2,4,6,8. A 5 μ l aliquot of either undiluted (10 ml culture), 2-fold diluted (20 ml culture), 3-fold diluted (30 ml culture), or 4-fold diluted (40 ml culture) plasmid DNA was loaded.

Figure 2.

Hofmeister series salts purify plasmid DNA from protein contamination. Cleared lysates from 25 ml LB cultures were generated according to the NID large-scale procedure. The lysate was treated with RNase A 20 µg/ml for 15 min at 37°C, followed by incubation for 15 min at 55°C. Lysates were precipitated with isopropanol (1st precipitation). Pellets were reconstituted in 0.5 ml of the different salt solutions and precipitated further (2nd and 3rd precipitation). Pellets were rinsed with 70% ethanol before dissolving them in 100 µl TE. All insoluble residues were removed before measuring protein concentration.

Figure 3.

Plasmid DNA isolated and purified by NIDs is enriched in total DNA, including CCC DNA. Plasmid DNA was obtained by 5 different methods, with 2 replicas shown for each method. (A) Lanes 1–6: isolation by alkali and purification by column. (B) Lanes 1–4: isolation by alkali and purification by NID (IGEPAL CA-520). (C) Lanes 1–4: isolation by NID (IGEPAL CA-630) and purification by NID (IGEPAL CA-520). (D) Lanes 1–2: isolation by NID (IGEPAL CA-630) and purification by NID (Triton X-114). Lanes 3–4: isolation by alkali and purification by NID (Triton X-114). Bacterial genomic DNA was identified as the slow migrating band lost following digestion with Exonuclease λ (A, lane 3). Denatured single stranded DNA was identified as the fast migrating form lost upon treatment with Mung bean nuclease (A, lane 4; B, lane 3). CCC plasmid DNA was identified as the intense form lost by treatment with nicking endonuclease Nt.BbvCI (A, lane 5). Linear DNA (A, lane 4; B, lane 3; C, lane 3) was identified as the form migrating similar to DNA linearized using restriction endonuclease Age I (A, lane 6; B, lane 4; C, lane 4).

Table 1

Chemical structure and basic physicochemical properties of NIDs.

Name	Chemical structure	HLB	CP (°C)	specific density
AcPOESEs	Tween 20	CO ₂ R=laurate	16.7	76
	Tween 80	CO ₂ R=oleate	15	65
rOPPOEEt	IGEPAL CA-720	n=12.5	14	
	Triton X-100	n= 9.6	13.5	65
	IGEPAL CA-630	n=9	13	63–67
	Triton X-114	n=7–8	12.4	22
	IGEPAL CA-520	n=5	10	N/A

Table 2

NID large-scale plasmid purification procedure. The parts of the procedure in dashed boxes are optional.

Hofmeister series salts regulate the phase separation properties of NIDs. (A) 0.5 ml of different NIDs stained with Sudan IV were tested with or without co-solutes. Cellular extracts were prepared from 40 ml LB cultures according to the NID large-scale isolation procedure. The Tween 20 solutions had pH 7 and low HLB NID solutions had pH 9 (adjusted with 100 mM Tris). CP was determined visually by monitoring the transition temperature between one and two-phase state of the solution. Both the dye and the re-clouding tests were performed by heating NID solutions 15°C above CP followed by centrifugation. a= phase separation is not stable upon cooling; b= no one-phase state observed between 4–100°C; c= no two-phase state observed between 4–100°C. NS= non-soluble particles do not allow test. (B) Chloroform increases the volume of the aqueous phase when low HLB NIDs are mixed with neutral or chaotropic HSS.

Table 3

Table 3A						
Solutes	Row	Co-solutes		CP (°C)	Tests of phase separation	
		Cell Extra ct	HSS		Tracking dye	Re-clouding
Tween 20	1	–	None	88–90	a	a
	2	–	1.5 M NaAc	61–63	+	++
	3	+	1.5 M NaAc	NS	+++	+++
	4	–	5 M NH ₄ Ac	36–38	–	+
	5	+	5 M NH ₄ Ac	NS	–	+
Triton X-114	6	–	None	23–24		
	7	+	None	NS		
	8	–	0.75 M LiCl	15–16		
	9	+	0.75 M LiCl	NS	–	–
	10	–	0.2 M GuaHCl	26–27		
IGEPAL CA-520	11	+	0.2 M GuaHCl	NS	–	–
	12	+	0.75 M LiCl	b	–	–
	13	+	0.2 M GuaHCl	b	–	–
	14	–	2.5 M GuaSCN	34–36		
	15	–	3.5 M GuaSCN	c		

Table 3B

Solute	Co-solutes		Weight of collected aqueous phases, mg	
	Cell extract	HSS	Without chloroform	With chloroform
Triton X-114	–	None	370	410
	+	0.75 M LiCl	420	450
	+	0.2 M GuaHCl	380	440
IGEPAL CA-520	+	0.75 M LiCl	410	430
	+	0.2 M GuaHCl	370	430

Table 4

The addition of neutral or chaotropic HSS to low HLB NIDs minimizes LPS contamination in plasmid DNA. LPS (EU/ml, numerator) and protein concentration ($\mu\text{g/ml}$, denominator) were determined in DNA generated by NID isolation and purified by NIDs and HSS. Five replicas of each sample were prepared from 30 ml LB cultures according to the NID large-scale isolation procedure followed by NID purification. Prior to the second IDP, salt concentrations in DNA solutions were adjusted as follows:

NH_4Ac solutions to 2 M, NaAc solutions to 0.75 M, and 0.2 M GuaHCl solutions to 0.5 M NaCl (0.75 M LiCl solutions remained unchanged). The third IDP was performed in 4.5 M LiCl, 100 mM Tris pH 9 solutions for all samples. The sign + or – in the first column indicates whether the sample was treated with chloroform.

A		
Salt	4 M NH_4Ac	2 M NaAc
NID		
<i>Tween 20/+</i>	$\frac{39,370 \pm 11,310}{3.6 \pm 0.6}$	$\frac{24,380 \pm 7920}{3.7 \pm 1.1}$

B			
Salt	NaAc		
NID			
	0.25 M	0.75 M	1.5 M
<i>Triton X-100/+</i>	$\frac{2.6 \pm 0.6}{4.0 \pm 1.1}$	$\frac{2.9 \pm 1.3}{2.7 \pm 0.9}$	$\frac{447 \pm 125}{5.7 \pm 1.0}$
	LiCl		
	0.25 M	0.75 M	1.5 M
<i>Triton X-100/+</i>	$\frac{2.1 \pm 0.8}{7.4 \pm 3.2}$	$\frac{3.1 \pm 2.2}{3.8 \pm 1.5}$	$\frac{3.4 \pm 1.4}{5.7 \pm 1.3}$

C				
Salt	3 M NH_4Ac	1 M NaAc	0.75 M LiCl	0.2 M GuaHCl
NID				
<i>Triton X-114/+</i>	$\frac{251.8 \pm 69.8}{13.9 \pm 4.9}$	$\frac{63.7 \pm 15.6}{14.4 \pm 3.9}$	$\frac{2.3 \pm 0.33}{19.6 \pm 5.4}$	$\frac{4.1 \pm 2.6}{13.2 \pm 4.0}$
<i>Triton X-114/-</i>	$\frac{140.26 \pm 32.7}{12.3 \pm 2.0}$	$\frac{69.5 \pm 13}{14.8 \pm 3.3}$	$\frac{2.1 \pm 0.21}{14.5 \pm 3.8}$	$\frac{3.8 \pm 1.5}{15.3 \pm 4.2}$
<i>IGEPAL CA-520/+</i>	$\frac{20.2 \pm 5.8}{16.3 \pm 9.9}$	N/A	$\frac{1.94 \pm 0.63}{23.0 \pm 6.7}$	$\frac{1.8 \pm 0.9}{16.1 \pm 7.4}$
<i>IGEPAL CA-520/-</i>	N/A	N/A	$\frac{2.78 \pm 0.5}{18.1 \pm 6.7}$	$\frac{1.9 \pm 0.4}{16.7 \pm 4.8}$

NID-purified large scale plasmid DNA contains low levels of protein and LPS. 100 ml LB culture bacteria were lysed either by alkali (according to the QIAfilter maxi kit) or the NID large-scale isolation procedure. Alkali lysates were purified by Qiagen-tip 500 columns or NID purification. NID lysates were purified by ERS, NID purification procedure, or Qiagen-tip 500 columns. The NID lysates were adjusted to pH 7 before column loading. NID purification was implemented in a 1.5 ml final volume (0.2 M GuaHCl, 100 mM Tris pH 9 with either Triton X-114 or IGEPAL CA-520). Before the second IDP, 0.5 M NaCl was added to NID purified samples. The third IDP was performed in 4.5 M LiCl, 100 mM Tris pH 9 solutions. All DNA was dissolved in 250 µl TE and aliquots used to measure LPS levels, total protein and DNA concentrations.

Table 5

Row	Isolation	Purification	Avg DNA ±SD µg/ml	Avg OD ₂₆₀ ±SD	Avg LPS±SD (EU/ml)	Avg LPS ^a (EU/6 µg DNA)	Avg LPS ^b (EU/1 µg DNA)	Protein (µg/ml)
1	NID	ERS	2174±237	1.86±0.004	6647±1174	18.3	0.44	403±60
2	NID	Triton X-114 *	1384±70	1.89±0.01	15.6±4.5	0.7	0.002	38.4±5.4
3	NID	IGEPAL CA-520 *	1370±25	1.88±0.01	7.1±3.2	0.03	0.0007	21.5±2.8
4	Alkali	Triton X-114 *	967±121	1.89±0.02	5.1±3.1	0.03	0.0008	2.6±0.5
5	Alkali	IGEPAL CA-520 *	782±97	1.87±0.01	5.6±1.2	0.04	0.001	1.2±0.8
6	NID	Columns	901±264	1.86±0.02	2584±2493	17.2	0.41	356±114
7	NID, pH 7	Columns	1301±516	1.87±0.0	6196±808	28.6	0.69	489±120
8	Alkali	Columns	727±29	1.88±0.01	1099±895	9.1	0.21	0.7±0.2

Legend:

* Either XL1-Blue or DH5α were used for these experiments and gave similar results.

Avg LPS_a = LPS units according to Cotton M. *et al.* (Cotten et al. 1994), LAL 10 EU/1 ng LPS;

Avg LPS_b = LPS units according to the Qiagen's handbook, LAL 1.8 EU/1 ng LPS.