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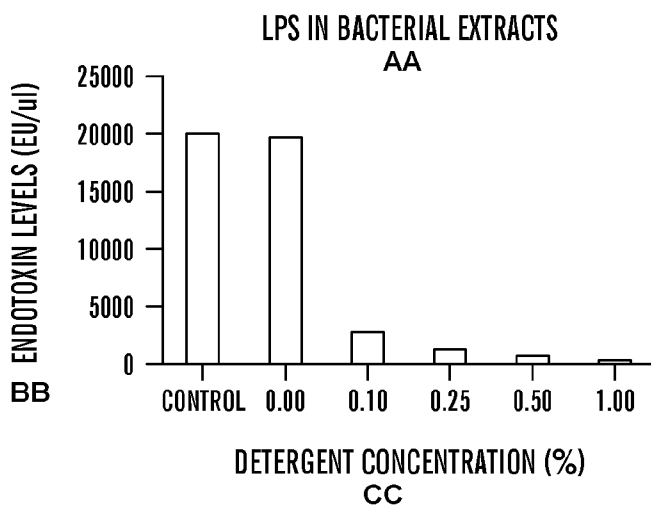
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(54) **Title:** HIGH-THROUGHPUT PLASMID DNA PREPARATION



**FIG. 1**

(57) **Abstract:** The present invention provides for compositions and methods of high-throughput plasmid DNA purification from *E. coli* bacteria. More specifically, an acidic, zwitterionic detergent solution provides for superior removal of contaminating endotoxins and improves transfectability of plasmid DNA.



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## HIGH-THROUGHPUT PLASMID DNA PREPARATION

### CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit under 35 U.S.C. § 119(e) of the U.S. Provisional Application No. 61/046,186 filed April 18, 2008, the contents of which are incorporated herein by reference in its entirety.

### FIELD OF THE INVENTION

[0002] The present invention relates to high throughput screening and molecular genetics. The present invention relates to compositions and methods for rapid, high-quality purification of plasmid DNA from e.g., *E. coli*.

### BACKGROUND OF THE INVENTION

[0003] Over the past few years, plasmid DNA has been recognized as a powerful tool in several biotechnological methods. Based on its molecular properties (e.g., closed circular molecule, easily cleaved by restriction enzymes, harboring an ideal marker gene for selection and rapidly amplifiable in a host system independently), plasmid DNA has been at the center of the most recent and advanced technologies in many disciplines including medicine, agriculture, molecular biology, industry, and biocontrol. In research, plasmid DNA is used as a vector allowing the study and generation of Genetically Modified Organisms (GMO), i.e. the transfer and the subcloning of transgenes across boundaries of species, and functional characterizations of several genes (genomics) within a species.

[0004] The use of high quality plasmid DNA often determines the success in various manipulations of genetic material during routine applications such as polymerase chain reaction (PCR) amplification, DNA sequencing, and subcloning of transgenes. Therefore, protocols for extraction of plasmid DNA with high yield and quality have earned serious attention. Several methods for plasmid DNA preparation, including those referred to as plasmid mini-prep, or plasmid DNA miniprep, and commercial kits have been made available. Some of these methods give relatively low yield, however, and are time consuming especially when carrying out molecular analysis of a large number of samples.

[0005] In practice, problems are often linked to the challenges of isolating pure (high quality) plasmid DNA. These problems often arise due to contamination by phenolic

compounds and polysaccharides, particularly bacterial lipopolysaccharides (endotoxin). For example, acidic polysaccharides are potential inhibitors of Hind III restriction enzyme and they also inhibit classical primer PCR by inhibiting Taq DNA polymerase activity. These contaminants may distort the results in many analytical applications, and may lead to incorrect interpretations.

**[0006]** In typical plasmid DNA purification systems, high concentrations of endotoxins are released into the bacterial lysate. Like DNA, endotoxins are negatively charged and co-purify with plasmid DNA in both silica-based and anion-exchange-based purification steps. Endotoxins can also form large aggregates resulting in co-purification in size exclusion columns and cesium chloride density gradients. Therefore, current plasmid purification systems can result in high levels of endotoxin contaminating the purified plasmid DNA.

### SUMMARY OF THE INVENTION

**[0007]** Laboratories often engage in a large number of plasmid preparations for purposes such as transgene subcloning, PCR amplification, transformation or transfection, gene cloning and the screening of positive clones, require rapid and efficient protocols for plasmid preparation.

**[0008]** The present invention provides for a rapid and efficient protocol for plasmid preparation, enabling the processing of many samples in one experiment, and increasing the number of replicates per day. The protocol described herein is relatively simple and rapid. It provides high yield, high quality plasmid DNA. The DNA obtained using the compositions and methods described herein is consistently superior for transfection, restriction, amplification by PCR, and is suitable for cloning and for sequencing. Additionally, the yield of the plasmid DNA using this approach is higher than that obtained with commercial kits.

**[0009]** An embodiment of the invention provides methods for rapid plasmid purification from *E. coli* including selective removal of capsular polysaccharide and lipopolysaccharide by extraction using an acidic detergent solution. This provides a way to reduce endotoxin (lipopolysaccharide), a compound that reduces the efficiency of transfection when present as a contaminant in DNA preparations. The methods and compositions described herein also provide a way of reducing the biomass of lysates during plasmid DNA preparation, thus preventing clogging of filters used to clarify lysates. The method is particularly useful in the preparation of high yield, high quality plasmid DNAs for transfection.

**[0010]** The method can also be used in column chromatography of plasmid DNA to remove polysaccharide contaminants.

[0011] The method can also be used in the purification of endotoxin-free proteins from *E. coli*.

[0012] One aspect of the methods described herein relates to a method for the purification of plasmid DNA from *E. coli* bacteria comprising the steps of: (a) contacting *E. coli* bacteria comprising plasmid DNA with an acidic, zwitterionic detergent solution comprising 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7; (b) incubating the bacteria in the acidic, zwitterionic detergent solution for at least 1 minute; and (c) purifying the plasmid DNA.

[0013] In one embodiment of this aspect and all other aspects described herein, the zwitterionic detergent is added to culture medium of the *E. coli* bacteria.

[0014] In another embodiment of this aspect and all other aspects described herein, incubating is performed for 1 minute to 60 minutes.

[0015] In another embodiment of this aspect and all other aspects described herein, incubating is performed at a temperature of 0°C to 45°C.

[0016] In another embodiment of this aspect and all other aspects described herein, the plasmid DNA is purified using a method comprising alkaline lysis of the *E. coli* bacteria.

[0017] In another embodiment of this aspect and all other aspects described herein, the acidic, zwitterionic detergent consists essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6;

[0018] In another aspect the methods described herein relate to a method for the purification of plasmid DNA from *E. coli* bacteria, the method comprising the steps of: (a) preparing a lysate of the bacteria; (b) adding an acidic, zwitterionic detergent solution to the lysate to a final concentration of 0.1% to 5% zwitterionic detergent; and (c) purifying plasmid DNA from the lysate.

[0019] In one embodiment of this aspect and all other aspects described herein, purifying comprises binding plasmid DNA to a solid support.

[0020] In another embodiment of this aspect and all other aspects described herein, purifying comprises binding the plasmid DNA to a silica matrix or to an anion exchange material.

[0021] In another embodiment of this aspect and all other aspects described herein, the method further comprises the step of subjecting the lysate to a vacuum of -100 to -1050 mBar prior to centrifugal clarification of the lysate.

[0022] Another aspect described herein relates to a method for the purification of plasmid DNA from *E. coli* bacteria comprising the steps of: (a) culturing the *E. coli* bacteria in liquid

culture media; (b) adding to the culture media a diatomaceous filter aid; (c) removing the media from the bacteria; (d) incubating the bacteria in an acidic, zwitterionic detergent solution; (e) removing the acidic, zwitterionic detergent solution; (f) incubating the bacteria with a lysis solution; (g) filtering the bacterial lysis solution and collecting the filtered lysate; (h) adding a guanidine, detergent solution to the lysate; (i) loading the lysate into a matrix to which the plasmid DNA binds; and eluting the plasmid DNA from the matrix.

**[0023]** In one embodiment of this aspect and all other aspects described herein, the acidic, zwitterionic detergent solution consists essentially of 100 mM sodium citrate, 5 mM EDTA, and 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH 4.5.

**[0024]** In another embodiment of this aspect and all other aspects described herein, the incubation in acidic, zwitterionic detergent solution is conducted at 30°C to 37°C for about 30 minutes with shaking.

**[0025]** In another embodiment of this aspect and all other aspects described herein, the method further comprises the step of incubating the filtered lysates with a zwitterionic detergent solution prior to the loading step.

**[0026]** In another embodiment of this aspect and all other aspects described herein, the method further comprises the step of washing the loaded matrix with a zwitterionic detergent solution prior to the eluting step.

**[0027]** Another aspect described herein is a method for the reducing the amount of endotoxin from *E. coli* bacterial cells comprising the steps of: (a) contacting the *E. coli* bacterial cells with acidic, zwitterionic detergent solution consisting essentially of 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7; (b) incubating the bacterial cells in the acidic, zwitterionic detergent solution at temperatures from 0°C to 45°C for a duration of 1 minute to 60 minutes; and (c) collecting the bacterial cells from the solution pursuant to standard techniques.

**[0028]** In one embodiment of this aspect and all other aspects described herein, the acidic, zwitterionic detergent consists essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6;

**[0029]** In another embodiment of this aspect and all other aspects described herein, the zwitterionic detergent is selected from the group consisting of n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, CHAPS, 3-(decyldimethylammonio)-propane-sulfonate inner salt, 3-(N,N-dimethyloctadecylammonio) propanesulfonate, 3-(N,N-dimethyloctylammonio) propanesulfonate inner salt, 3-(N,N-dimethylpalmitylammonio) propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, BigCHAP, CHAPSO, DDMAU.

LADAO, Zwittergent® 3-08, Zwittergent® 3-10, Zwittergent® 3-14, Zwittergent® 3-16, and N-Dodecyl-N,N-dimethylglycine.

**[0030]** Another aspect described herein is a solution for the removal of endotoxin from *E. coli* bacteria comprising an acidic, zwitterionic detergent solution consisting essentially of 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7.

**[0031]** In another aspect, described herein is a solution for the removal of endotoxin from *E. coli* bacteria comprising an acidic, zwitterionic detergent solution consisting essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6.

**[0032]** Another aspect described herein relates to a composition comprising a cultured *E. coli* bacterium comprising plasmid DNA or an *E. coli* bacterial lysate comprising plasmid DNA, and an acidic, zwitterionic detergent, wherein the zwitterionic detergent present at a concentration of 0.1% to 5% (w/v).

**[0033]** In one embodiment of this aspect and all other aspects described herein, the lysate is a clarified bacterial lysate.

**[0034]** In another embodiment of this aspect and all other aspects described herein, the lysate was prepared by alkaline lysis.

**[0035]** In another embodiment of this aspect and all other aspects described herein, the acidic, zwitterionic detergent solution consists essentially of 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH4.5.

**[0036]** In another embodiment of this aspect and all other aspects described herein, the solid support is selected from silica and a solid support comprising an anion exchange material.

**[0037]** In another embodiment of this aspect and all other aspects described herein, the composition further comprises a lysate of *E. coli* bacteria comprising the plasmid DNA.

**[0038]** In another embodiment of this aspect and all other aspects described herein, the zwitterionic detergent is present at a concentration of 0.01% to 5.0%.

**[0039]** In another embodiment of this aspect and all other aspects described herein, the zwitterionic detergent is present at a concentration of 0.1% to 1.0%

**[0040]** In another embodiment of this aspect and all other aspects described herein, the pH of the acidic, zwitterionic detergent solution is between 3.0 and 6.99, inclusive.

**[0041]** In another embodiment of this aspect and all other aspects described herein, the pH of the acidic, zwitterionic detergent solution is between 4.0 and 6.0, inclusive.

**[0042]** In another aspect the present invention relates to a composition comprising a solid support comprising plasmid DNA and an acidic, zwitterionic detergent solution.

[0043] In one embodiment of this aspect and all other aspects described herein, the acidic, zwitterionic detergent solution consists essentially of 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH 4.5.

[0044] In another embodiment of this aspect and all other aspects described herein, the solid support is selected from silica and a solid support comprising an anion exchange material.

[0045] In another embodiment of this aspect and all other aspects described herein, the composition further comprises a lysate of *E. coli* bacteria comprising the plasmid DNA.

[0046] In another embodiment of this aspect and all other aspects described herein, the zwitterionic detergent is present at a concentration of 0.01% to 5.0%. In another embodiment of this aspect and all other aspects described herein, the zwitterionic detergent is present at a concentration of 0.1% to 1.0%.

[0047] In another embodiment of this aspect and all other aspects described herein, the pH of the acidic, zwitterionic detergent solution is between 3.0 and 6.99, inclusive.

[0048] In another embodiment of this aspect and all other aspects described herein, the pH of the acidic, zwitterionic detergent solution is between 4.0 and 6.0, inclusive.

[0049] In another embodiment of this aspect and all other aspects described herein, the zwitterionic detergent is selected from the group consisting of n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, CHAPS, 3-(decyldimethylammonio)-propane-sulfonate inner salt, 3-(N,N-dimethyloctadecylammonio) propanesulfonate, 3-(N,N-dimethyloctylammonio) propanesulfonate inner salt, 3-(N,N-dimethylpalmitylammonio) propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, BigCHAP, CHAPSO, DDMAU, LADAO, Zwittergent® 3-08, Zwittergent® 3-10, Zwittergent® 3-14, Zwittergent® 3-16, and N-Dodecyl-N,N-dimethylglycine.

[0050] Also described herein is a high-throughput method for purifying plasmid DNA from *E. coli* bacteria comprising the steps of: (a) contacting a plurality of *E. coli* bacteria samples comprising plasmid DNA with an acidic, zwitterionic detergent solution comprising 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7; (b) incubating the plurality of samples in the acidic, zwitterionic detergent solution for at least 1 minute; and (c) purifying the plasmid DNA from the plurality of samples in parallel.

[0051] In another embodiment of this aspect and all other aspects described herein, the high-throughput method is automated.

[0052] In another embodiment of this aspect and all other aspects described herein, the method comprises a robotic liquid handling system.

**[0053]** As used herein, the term “solid support comprising plasmid DNA” comprises any solid support to which plasmid DNA is physically, but non-covalently bound. Plasmid DNA bound on such a solid support can be eluted from the support under conditions that vary depending upon the support or matrix. As a common, but non-limiting example, DNA can be bound by ionic interactions. Generally, the term “solid support comprising plasmid DNA” refers to a solid support to which plasmid DNA is bound for the purpose of or in the process of purifying or isolating the plasmid DNA. Non-limiting examples of solid supports include silica or ground glass, and solid supports, e.g., polymeric supports, comprising anion exchange materials, including, but not limited to DEAE cellulose, DEAE dextran or DEAE Sepharose<sup>TM</sup>, among others. A “solid support” can be in the form of a column matrix or resin, but need not be packed in a column – that is, batch processing by contacting a lysate with a suspension of column matrix or resin is encompassed by the term.

**[0054]** As used herein, the term “clarified lysate” of bacteria refers to a bacterial lysate from which bulk protein and genomic DNA has been removed, e.g., by filtration or by precipitation, with or without filtration. A clarified lysate generally comprises less than 20% of the amount of bacterial protein and genomic DNA than a lysate that has not been clarified.

**[0055]** As used herein, the term “alkaline lysis” refers to bacterial lysis under alkaline conditions as described, for example, by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, or bacterial lysis under conditions substantially similar thereto.

**[0056]** As used herein, the term “purifying plasmid DNA” refers to the isolation of plasmid DNA from a bacterial lysate at a purity suitable for efficient *in vitro* restriction endonuclease digestion (e.g., at least 75% of molecules with a restriction site are cleaved, preferably at least 85%, at least 90%, at least 95% or more). Any method known in the art can be applied to purify plasmid DNA after *E. coli* cells, a lysate thereof, or solid support-bound plasmid DNA therefrom has been treated with an acidic, zwitterionic detergent solution as described herein. It is preferred that purified plasmid DNA is sufficiently pure for transfection of eukaryotic cells, and more preferred that the DNA comprises substantially less bacterial endotoxin than plasmid DNA purified from bacteria, lysates or surface-bound plasmid DNA not treated with an acidic, zwitterionic detergent solution. By “substantially less” in this context is meant at least 50% less, preferably at least 75% less, more preferably at least 85%, 90%, 95%, 99% or even 100% (i.e., absent) compared to the amount present in a corresponding plasmid DNA preparation prepared without use of an acidic zwitterionic detergent solution. It is also preferred that the purified plasmid DNA is more efficiently

transfected into eukaryotic, e.g., mammalian cells than plasmid DNA prepared without the use of an acidic zwitterionic detergent solution. By "more efficiently" in this context is meant at least 5% greater transfection efficiency, relative to the efficiency for a preparation of the same plasmid prepared without the use of an acidic zwitterionic detergent solution, and preferably at least 7%, 10%, 15%, 18%, 20% or greater more efficient.

**[0057]** As used herein, the term "high throughput" refers to concurrent, or "parallel" plasmid DNA purification from a plurality (i.e., at least two) of different *E. coli* bacterial cultures; preferably 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, 100, or 1000 or more plasmid preparations can be purified simultaneously. The plasmid preparations can be of a single plasmid or multiple, different plasmids, and can be purified from one or more bacterial strains. The term "high-throughput" encompasses automation of plasmid purification using e.g., robotic pipettors, robotic samplers, robotic shakers, data processing and control software, liquid handling devices, incubators, and/or detectors etc.. For the purposes of automation, it is preferred that the number of *E. coli* bacterial cultures for plasmid purification corresponds to the number of wells in a standard multi-well plate (e.g. 6-well plate, 12-well plate, 96-well plate, 384-well plate, etc.).

**[0058]** As used herein, the term "reducing the amount of endotoxin" means that there is at least 50% less endotoxin present in the cells, lysate, or plasmid DNA following treatment with a zwitterionic detergent than in cells, lysate, or plasmid DNA prepared without contacting the cells, lysate or DNA with an acidic zwitterionic detergent; preferably endotoxin is at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, or even 100% (i.e., no detectable endotoxin) lower in plasmid DNA prepared in the presence of a zwitterionic detergent than that of plasmid DNA prepared in the absence of the zwitterionic detergent. Endotoxin can be measured by any means known in the art. For the avoidance of doubt, however, the endotoxin assay described herein can be used to determine the extent of endotoxin removal as that term is used herein.

**[0059]** Preparations are also referred to herein as "preps."

**[0060]** As used herein, the term "cultured *E. coli* bacteria" refers to a substantially homogeneous (e.g., clonal) population of an *E. coli* bacterium, grown under laboratory conditions.

**[0061]** As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the invention, yet open to the inclusion of unspecified elements, whether essential or not.

[0062] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[0063] The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

### BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1.** Increasing concentrations of Zwittergent® 3-14 were used to extract *E. coli* bacteria strain DH5  $\alpha$ , and the LPS content of the bacteria after extraction was measured. Control: unextracted bacteria; 0.00% detergent: bacteria extracted with acidic solution lacking zwitterionic detergent.

**Figure 2.** Yield of plasmid DNA from samples treated as in Figure 1.

**Figure 3.** Comparison of the transfection ability of *Gaussia* luciferase reporter plasmid DNA obtained using commercial purification kits without (Regular) or with (Extracted) extraction with the acidic, zwitterionic detergent solution of an embodiment of the present invention.

**Figure 4.** Carbohydrate analysis of identical bacterial samples extracted with acidic, zwitterionic detergent solution (0.1% detergent); with acidic, zwitterionic detergent solution lacking detergent (Citrate Buffer); or with 0.1M NaCl (Saline). The extracted bacteria were pelleted by centrifugation and the resulting pelleted bacteria (Bacteria) and extracted supernatants (Extract) were assayed for total carbohydrates.

**Figure 5.** Electron micrograph of *E. coli* bacteria extracted with the acidic, zwitterionic detergent solution. Inset shows intact "triple layer" bacterial wall.

**Figure 6.** Transfection efficiency of column-purified plasmid DNA. Equivalent amounts of plasmid DNAs were transfected into Huh7 cells and luciferase expression assayed by standard procedures. Results are normalized relative to the luciferase expression from columns lacking the wash procedure. Wash Conditions: A, no wash; B, wash buffer 40%

isopropanol, 4M guanidine; C, wash buffer 40% isopropanol, 4M guanidine, 0.1% Zwittergent® 3-14; D, wash buffer 60% isopropanol, 10mM Tris-Cl pH8.0; E, wash buffer 60% isopropanol, 10mM Tris-Cl pH8.0, 0.1% Zwittergent® 3-14; F, wash buffer 2M guanidine; G, wash buffer 2M guanidine, 0.1% Zwittergent® 3-14; H, wash buffer 2M guanidine, 1% Zwittergent® 3-14.

**Figure 7.** Following alkaline lysis of *E. coli* carrying plasmids having a luciferase reporter gene, lysates containing 2M guanidine were left untreated (10 ul No Deterg), or amended with Zwittergent® 3-14 to 0.1% (10ul + Deterg). The lysates were then placed on a silica column and washed twice with 80% ethanol and the DNAs eluted in TE. Equivalent amounts of plasmid DNA were transfected into Huh7 cells and the resulting luciferase expression assayed.

**Figure 8.** High-throughput comparison of commercial plasmid preparation kits with (Modified) or without (Plain) the use of acidic, zwitterionic detergent solution.

**Figure 9.** A lysate prepared using a conventional method is shown at left, while a lysate prepared using a zwitterionic detergent solution as described herein combined with vacuum treatment of lysate prior to centrifugation is shown at right. It can be readily observed that the flask on the right does not contain cellular debris floating at the top of the tube while the flask on the left contains a large amount of cellular debris on the top.

## DETAILED DESCRIPTION OF THE INVENTION

**[0064]** It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

**[0065]** As used herein and in the claims, the singular forms include the plural reference and vice versa unless the context clearly indicates otherwise. Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.” The term “about” when used in connection with percentages may mean  $\pm 1\%$ . Further, where ranges are recited, e.g., ranges of pH, temperature, purity, etc., it should be understood that the range includes any integer value within the range as if it were explicitly

recited, and that sub-ranges within the range are likewise included as if they were specifically recited.

**[0066]** All patents and other publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

**[0067]** Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present application are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.*, Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, (2d ed., Cold Spring Harbor Lab. Press, Cold Spring Harbor, N.Y., 1989) and Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIO. (Greene Pub. Assoc., 1992). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. Standard techniques can be used for chemical syntheses, chemical analyses, formulations, and preparations.

**[0068]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as those commonly understood to one of ordinary skill in the art to which this invention pertains. Although any known methods, devices, and materials may be used in the practice or testing of the invention, the methods, devices, and materials in this regard are described herein. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

**[0069]** The present invention provides for composition and methods for high throughput plasmid preparation from *E. coli*. A particular embodiment provides for a method of removal

of lipopolysaccharide (endotoxin) from cultures of *E. coli* by treatment with an acidic solution containing a zwitterionic detergent.

**[0070]** Currently, there are a number of methods to reduce endotoxin in DNA preparations. These include the use of column chromatography using anion exchange, hydrophobic interaction, and other resins (J. Gene Med., 2004 Feb;6 Suppl 1:S54-66); Triton®X-114 two-phase extraction; and treatment with various endotoxin removal resins (e.g., polymyxin B resin). The major disadvantage of these methods is that they are laborious. Also, because they are used in downstream processes, these existing methods do not result in reduction of biomass of lysates and thus do not address the problem of clogged filters during lysate clarification. An advantage provided by compositions and methods of the present invention is the reduction in biomass, which thus allows greater yields of DNA to be obtained. The approach provided herein is also easier and less expensive than other current approaches.

**[0071]** Purified (endotoxin-free) plasmid DNA is used in a variety of processes. In research, plasmid DNA preparations are conventionally done as either large scale (“maxi”), medium scale (“midi”), or small scale (“mini”) preparations. Each of these methods of preparation are commonly done using DNA purification kits, and the approach provided herein is useful in each method. Moreover, plasmid DNA minipreps are increasingly commonly performed in high-throughput microplate format for e.g., transfection, a technique that requires consistent high yields of highly purified DNA. The present approach is particularly useful in this high-throughput format. Additionally, endotoxin-free DNA is also used clinically for gene therapy trials, such as DNA vaccines, and the present invention is useful in this context as well.

**[0072]** Zwitterionic detergents contain both a positive and negative charge in their hydrophilic headgroup. These compounds are therefore electrically neutral, like the nonionic detergents, but can efficiently disrupt protein-protein interactions like the ionic detergents; therefore they tend to be intermediate in their mildness. Current plasmid purification approaches do not use acidic, zwitterionic detergent solutions for the removal of endotoxin contaminants as provided by embodiments of the present invention.

**[0073]** The use of acidic, zwitterionic solution as described herein may be used in conjunction with conventional means or standard techniques discussed above. For example, standard techniques include approaches that take advantage of filtering techniques, such as silica matrix columns. Lakshmi et al., 272 Anal. Biochem. 109-12 (1999); Tianen et al., 1149(2) J. Chromatography, 158-68 (2007). Such standard techniques also include various

high-throughput approaches, including robotic approaches. *See, e.g.,* Itoh et al., 9(5) Genome Res. 463-70 (1999); Kachel et al., 6(9) BMC Biotechnol. (2006).

**[0074]** Briefly, for example, according to an embodiment of the invention, bacteria are pelleted by traditional methods (e.g., 4000g x 10 min) in a centrifuge. The bacterial pellet is then resuspended in a volume equal to half of the original culture volume in a acidic, zwitterionic detergent solution prewarmed to 37°C. The solution contains 0.1% n-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (also known as 3-(N,N-Dimethyltetradecylammonio) propanesulfonate; 3-(Myristyldimethylammonio) propanesulfonate; N-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate; Myristyl sulfobetaine; SB3-14; or Zwittergent® 3-14 (Calbiochem-Novabiochem Corp., San Diego, CA), 100 mM sodium citrate, 5 mM EDTA, pH 4.5 in water. The resuspended bacteria are then mixed at 350 rpm in an orbital shaker at 37°C for 30 minutes to extract the polysaccharides. The extracted bacteria are then pelleted in a centrifuge by traditional methods (e.g., 4000g x 10 min). The supernatant containing the extracted endotoxin is discarded and the pelleted bacteria are then processed to obtain plasmid DNA by conventional means.

**[0075]** The acidic, zwitterionic detergent solution may contain the detergent in concentrations ranging from 0.01% to 5% (for example, from 0.1% to 1%). The pH of the solution may range from pH 3 to pH 7 (for example, from pH 4 to pH 6). The extraction may occur at temperatures from 0°C to 45°C (for example, from 20°C to 37°C) and the duration of extraction may be from 1 minute to 60 minutes (for example, from 5 minutes-30 minutes). The acidic, zwitterionic detergent may also consist of one or more of other zwitterionic detergents such as CHAPS, 3-(Decyldimethylammonio)-propane-sulfonate inner salt, 3-(N,N-Dimethyloctadecylammonio) propanesulfonate, 3-(N,N-Dimethyloctylammonio) propanesulfonate inner salt, 3-(N,N-Dimethylpalmitylammonio) propanesulfonate, N-Dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, BigCHAP, CHAPSO, DDMAU, LADAO, Zwittergent® 3-08 (Calbiochem-Novabiochem Corp., San Diego, CA), Zwittergent® 3-10, Zwittergent® 3-14, Zwittergent® 3-16, or EMPIGEN® BB (N-Dodecyl-N,N-dimethylglycine, Allbright & Willson, Warley, West Midlands, UK).

**[0076]** In an alternative embodiment, the bacteria are concentrated upon a permeable membrane (e.g., by vacuum) and exposed to the acidic, zwitterionic detergent solution by flow across the membrane. The bacteria may also be exposed to the acidic, zwitterionic detergent solution by direct addition of the acidic detergent to the culture medium without prior pelleting of the bacteria.

[0077] *E. coli* bacteria which have been treated with the acidic detergent solution as described herein contain reduced amounts of contaminating lipopolysaccharide (LPS, endotoxin) as shown in Figure 1. Increasing concentrations of Zwittergent® 3-14 were used to extract *E. coli* bacteria strain DH5  $\alpha$ , and the LPS content of the bacteria after extraction was measured using a commercial kit (Cambrex Corp., East Rutherford, NJ). Control bacteria denotes bacteria which were unextracted, and 0.00% detergent denotes bacteria extracted with acidic solution lacking the zwitterionic detergent. These data demonstrate that extraction of bacteria with acidic zwitterionic detergent may result in removal of over 99% of bacterial LPS from the bacterial sample.

[0078] Measurement of plasmid DNA purified from samples treated identically in parallel demonstrated that the acidic, zwitterionic detergent solution treatment resulted in only minor decreases in plasmid DNA yields despite the more dramatic reductions of LPS (Figure 2). Thus, this approach allows for purification of DNA samples containing vastly reduced amounts of LPS.

[0079] When the approach of the present invention is applied to bacteria prior to using a conventional DNA plasmid preparation, the reduced endotoxin allows isolation of DNA which has increased transfection ability (Figure 3). For example, the present approach was applied to one-half of an *E. coli* culture containing a *Gaussia* luciferase reporter plasmid, while the other half of the culture was untreated as a control. The reporter gene plasmid DNA was then isolated from each culture using a commercial plasmid DNA isolation kit (HiSpeed Kit, Qiagen, Valencia, CA) and equivalent amounts of each DNA sample were used to transfect Huh-7 cells (human hepatoma cell line, Nakabayashi et al., 42 Cancer Res. 3858-63 (1982)). The *Gaussia* luciferase activity was then measured from each culture after transfection with the control DNA luciferase activity normalized to 100%. The results, depicted in Figure 3, show that the acidic, zwitterionic detergent solution extraction produced DNA that transfected approximately 18% better than the control DNA under these conditions.

[0080] In addition to the ability to prepare DNA with reduced endotoxin and increased transfectability, the approach of the present invention allows removal of polysaccharides from bacterial samples prior to downstream processing. Polysaccharide removal from the bacterial samples is advantageous when polysaccharides may hinder downstream isolation of plasmid DNA or proteins from the samples. Figure 4 demonstrates the removal of carbohydrates from bacterial samples using an approach of the present invention. In this

example, identical bacterial samples were extracted with either acidic, zwitterionic detergent solution (0.1% detergent), acidic solution lacking detergent (Citrate Buffer) or with 0.1M NaCl (Saline). The extracted bacteria were pelleted by centrifugation (4000g, 10min) and the resulting pelleted bacteria and extracted supernatants were assayed for total carbohydrates using an acid phenol assay (Dubois et al., 28 Anal. Chem. 350-56 (1956)). Polysaccharides in the extracted supernatants were concentrated by precipitation with ethanol prior to assay. The results demonstrate that extraction with acidic, zwitterionic detergent solution containing 0.1% detergent resulted in extraction of nearly 50% of total bacterial carbohydrates. Much of the extracted carbohydrate was recovered in the extracted fraction after ethanol precipitation. Thus, this embodiment of the present invention provides for an approach to isolate carbohydrates from bacterial cultures (e.g., for vaccine production).

**[0081]** Treatment of *E. coli* bacteria with the acidic, zwitterionic detergent solution does not significantly alter the size or shape of the bacteria as revealed by electron microscopy, Figure 5. Moreover, the “triple layer” bacterial wall remains intact (Figure 5, inset).

**[0082]** Treatment of bacteria with the acidic, zwitterionic detergent solution according to the present invention not only results in removal of carbohydrate, but also allows more efficient downstream processing of the bacteria. For this purpose, a zwitterionic detergent solution as described herein can be added directly to the culture (e.g., culture medium) of the *E. coli* bacteria. In conventional plasmid DNA purification procedures bacteria are subjected to alkaline lysis followed by neutralization to effect precipitation of bacterial protein and genomic DNA. The resulting precipitate is then separated from the desired plasmid DNA by filtration or centrifugation. Bacterial samples subjected to extraction with the acidic, zwitterionic detergent solution followed by conventional alkaline lysis produce a finer precipitate that is more easily filtered. More specifically, in this experiment, bacteria were subjected to the acidic, zwitterionic detergent solution as described and then pelleted by centrifugation. The data showed that the extracted bacteria are significantly lighter in color than untreated control bacteria. After alkaline lysis, the resulting precipitate from the extracted bacteria exhibits a much finer and slightly smaller precipitate that is more easily filtered than the untreated sample. The better filtration properties of bacteria treated with the acidic, zwitterionic detergent solution of the present invention facilitates the use of larger quantities of bacteria in filtration methods of DNA purification as well as providing more consistent DNA yields in microplate-based DNA minipreps. Hence, this approach is especially useful in the context of high-throughput sample preparation.

**[0083]** The acidic, zwitterionic detergent solution of the present invention is also useful in column washes during plasmid DNA purification. Thus, in addition to removing endotoxin from bacteria, the acidic, zwitterionic detergent solution may also be used to solubilize endotoxin and other contaminants during plasmid DNA preparation by inclusion in buffers used to wash columns containing bound plasmid DNAs. The washed DNA can then be eluted from the columns by standard procedures. For example, plasmid DNA encoding a luciferase reporter gene was prepared from bacterial cultures by standard alkaline lysis procedures. The plasmid DNA was then bound to a silica matrix by addition of 8M Guanidine to a final concentration of 2M guanidine. The DNA/guanidine solution was added to the silica column and centrifuged (2000g, for 5 minutes) to bind the DNA to the column. The DNA bound to the column was then washed with a variety of different buffers either lacking or containing zwitterionic detergent. The columns were then washed twice with 80% ethanol and the DNAs eluted with TE (10mM Tris pH8, 1mM EDTA). Equivalent amounts of plasmid DNAs were then used to transfect Huh7 cells and the amount of *Gaussia* luciferase produced was assayed by standard procedures. The results are shown in Figure 6 and are normalized relative to the luciferase expression from columns lacking the wash procedure. The wash conditions shown are: A, no wash; B, wash buffer 40% isopropanol, 4 M guanidine; C, as B but with 0.1% Zwittergent® 3-14; D, 60% isopropanol, 10 mM Tris-Cl pH 8.0; E, same as D but with 0.1% Zwittergent® 3-14; F, 2M guanidine; G, as F but with 0.1% Zwittergent® 3-14; H, same as F but with 1% zwittergent 3-14. These results indicate inclusion of zwitterionic detergents at 0.1% concentration allows isolation of plasmid DNA with improved transfection ability.

**[0084]** Additionally, the acidic, zwitterionic detergents described herein may be used in lysates during plasmid DNA purification. More specifically, for example, the addition of zwitterionic detergent to lysates made during alkaline lysis of bacteria yields plasmid DNA with increased transfection ability. Bacteria containing a luciferase reporter plasmid were grown by standard procedures and subjected to alkaline lysis. The resulting lysates containing 2 M guanidine were then either left untreated or amended with Zwittergent® 3-14 to a final concentration of 0.1%. The lysates were then placed on a silica column and washed twice with 80% ethanol and the DNAs eluted in TE. Equivalent amounts of the plasmid DNA containing a luciferase reporter gene were transfected onto Huh7 cells and the resulting luciferase was assayed by conventional methods. The results, shown in Figure 7, indicate that lysates containing 0.1% zwitterionic detergent yield over five-fold higher amounts of transfection under these conditions. These data demonstrate that addition of zwitterionic

detergents to bacterial lysates prior to column chromatography can give DNAs with improved ability for transfection.

**[0085]** It is also contemplated herein that the use of a zwitterionic detergent solution for the purification of a plasmid can be combined with the application of a vacuum to a lysate prior to centrifugation to prepare a clarified lysate. In one embodiment, a lysate is subjected to a vacuum in the range of -100 to -1050 mbars prior to lysate clarification. In specific embodiments, a lysate is subjected to a vacuum for a time period in the range of 0.5 minutes to 60 minutes, inclusive.

The present invention may be as defined in any one of the following numbered paragraphs.

1. A method for the purification of plasmid DNA from *E. coli* bacteria comprising the steps of:
  - (a) contacting *E. coli* bacteria comprising plasmid DNA with an acidic, zwitterionic detergent solution comprising 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7;
  - (b) incubating the bacteria in the acidic, zwitterionic detergent solution for at least 1 minute; and
  - (c) purifying the plasmid DNA.
2. The method of paragraph 1, wherein the zwitterionic detergent is added to culture medium of the *E. coli* bacteria.
3. The method of paragraph 1 or 2, wherein the incubating is performed for 1 minute to 60 minutes.
4. The method of paragraph 1, 2, or 3, wherein the incubating is performed at a temperature of 0°C to 45°C.
5. The method of paragraph 1, 2, 3 or 4, wherein the plasmid DNA is purified using a method comprising alkaline lysis of the *E. coli* bacteria.
6. The method of any one of paragraphs 1-5, wherein the acidic, zwitterionic detergent consists essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6;

7. A method for the purification of plasmid DNA from *E. coli* bacteria, the method comprising the steps of:
  - (a) preparing a lysate of the bacteria;
  - (b) adding an acidic, zwitterionic detergent solution to the lysate to a final concentration of 0.1% to 5% zwitterionic detergent; and
  - (c) purifying plasmid DNA from the lysate.
8. The method of paragraph 7 wherein the purifying comprises binding plasmid DNA to a solid support.
9. The method of paragraph 7 or 8, wherein the purifying comprises binding the plasmid DNA to a silica matrix or to an anion exchange material.
10. The method of paragraph 7, 8, or 9, further comprising the step of subjecting the lysate to a vacuum of -100 to -1050 mBar prior to centrifugal clarification of the lysate.
11. A method for the purification of plasmid DNA from *E. coli* bacteria comprising the steps of:
  - (a) culturing the *E. coli* bacteria in liquid culture media;
  - (b) adding to the culture media a diatomaceous filter aid;
  - (c) removing the media from the bacteria;
  - (d) incubating the bacteria in an acidic, zwitterionic detergent solution;
  - (e) removing the acidic, zwitterionic detergent solution;
  - (f) incubating the bacteria with a lysis solution;
  - (g) filtering the bacterial lysis solution and collecting the filtered lysate;
  - (h) adding a guanidine, detergent solution to the lysate;
  - (i) loading the lysate into a matrix to which the plasmid DNA binds; and

- eluting the plasmid DNA from the matrix.
12. The method of paragraph 11, wherein the acidic, zwitterionic detergent solution consists essentially of 100 mM sodium citrate, 5 mM EDTA, and 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH 4.5.
  13. The method of paragraph 11 or 12, in which the incubating in acidic, zwitterionic detergent solution is conducted at 30°C to 37°C for about 30 minutes with shaking.
  14. The method of paragraph 11, 12, or 13, further comprising the step of incubating the filtered lysates with a zwitterionic detergent solution prior to the loading step.
  15. The method of any one of paragraph 11-14, further comprising the step of washing the loaded matrix with a zwitterionic detergent solution prior to the eluting step.
  16. A method for the reducing the amount of endotoxin from *E. coli* bacterial cells comprising the steps of:
    - (a) contacting the *E. coli* bacterial cells with acidic, zwitterionic detergent solution consisting essentially of 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7;
    - (b) incubating the bacterial cells in the acidic, zwitterionic detergent solution at temperatures from 0°C to 45°C for a duration of 1 minute to 60 minutes; and
    - (c) collecting the bacterial cells from the solution pursuant to standard techniques.
  17. The method of paragraph 16, wherein the acidic, zwitterionic detergent consists essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6;
  18. The method of any one of paragraphs 1-17, wherein the zwitterionic detergent is selected from the group consisting of n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, CHAPS, 3-(decyldimethylammonio)-propane-sulfonate inner salt, 3-(N,N-dimethyloctadecylammonio) propanesulfonate, 3-(N,N-dimethyloctylammonio) propanesulfonate inner salt, 3-(N,N-dimethylpalmitylammonio) propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, BigCHAP, CHAPSO, DDMAU, LADAO, Zwittergent® 3-08,

Zwittergent® 3-10, Zwittergent® 3-14, Zwittergent® 3-16, and N-Dodecyl-N,N-dimethylglycine.

18. A solution for the removal of endotoxin from *E. coli* bacteria comprising an acidic, zwitterionic detergent solution consisting essentially of 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7.
19. A solution for the removal of endotoxin from *E. coli* bacteria comprising an acidic, zwitterionic detergent solution consisting essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6.
20. A composition comprising a cultured *E. coli* bacterium comprising plasmid DNA or an *E. coli* bacterial lysate comprising plasmid DNA, and an acidic, zwitterionic detergent, wherein the zwitterionic detergent present at a concentration of 0.1% to 5% (w/v).
21. The composition of paragraph 20 wherein the lysate is a clarified bacterial lysate.
22. The composition of paragraph 20 or 21, wherein the lysate was prepared by alkaline lysis.
23. The composition of paragraph 20, 21, or 22 wherein the acidic, zwitterionic detergent solution consists essentially of 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH4.5.
24. The composition of any one of paragraphs 20-23 wherein the solid support is selected from silica and a solid support comprising an anion exchange material.
25. The composition of any one of paragraphs 20-24 further comprising a lysate of *E. coli* bacteria comprising the plasmid DNA.
26. The composition of any one of paragraphs 20-25 wherein the zwitterionic detergent is present at a concentration of 0.01% to 5.0%.
27. The composition of any one of paragraphs 20-26, wherein the zwitterionic detergent is present at a concentration of 0.1% to 1.0%

28. The composition of any one of paragraphs 20-27 wherein the pH of the acidic, zwitterionic detergent solution is between 3.0 and 6.99, inclusive.
29. The composition of any one of paragraphs 20-28 wherein the pH of the acidic, zwitterionic detergent solution is between 4.0 and 6.0, inclusive.
30. A composition comprising a solid support comprising plasmid DNA and an acidic, zwitterionic detergent solution.
31. The composition of paragraph 30 wherein the acidic, zwitterionic detergent solution consists essentially of 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH4.5.
32. The composition of paragraph 30 or 31, wherein the solid support is selected from silica and a solid support comprising an anion exchange material.
33. The composition of paragraph 30, 31, or 32, further comprising a lysate of *E. coli* bacteria comprising the plasmid DNA.
34. The composition of any one of paragraphs 30-33, wherein the zwitterionic detergent is present at a concentration of 0.01% to 5.0%.
35. The composition of any one of paragraphs 30-34, wherein the zwitterionic detergent is present at a concentration of 0.1% to 1.0%
36. The composition of any one of paragraphs 30-35 wherein the pH of the acidic, zwitterionic detergent solution is between 3.0 and 6.99, inclusive.
37. The composition of any one of paragraphs 30-36 wherein the pH of the acidic, zwitterionic detergent solution is between 4.0 and 6.0, inclusive.
38. The solution of any one of the preceding paragraphs, wherein the zwitterionic detergent is selected from the group consisting of n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, CHAPS, 3-(decyldimethylammonio)-propane-sulfonate inner salt, 3-(N,N-dimethyloctadecylammonio) propanesulfonate, 3-(N,N-dimethyloctylammonio) propanesulfonate inner salt, 3-(N,N-dimethylpalmitylammonio) propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, BigCHAP, CHAPSO, DDMAU, LADAO, Zwittergent® 3-08,

Zwittergent® 3-10, Zwittergent® 3-14, Zwittergent® 3-16, and N-Dodecyl-N,N-dimethylglycine.

39. A high-throughput method for purifying plasmid DNA from *E. coli* bacteria comprising the steps of:
- (a) contacting a plurality of *E. coli* bacteria samples comprising plasmid DNA with an acidic, zwitterionic detergent solution comprising 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7;
  - (b) incubating the plurality of samples in the acidic, zwitterionic detergent solution for at least 1 minute; and
  - (c) purifying the plasmid DNA from the plurality of samples in parallel.
40. The method of paragraph 39, wherein the high-throughput method is automated.
41. The method of paragraph 39, where the method comprises a robotic pipettor.

## EXAMPLES

### **Example 1. High-throughput plasmid preparation from *E. coli***

**[0086]** Bacterial glycerol stock (5µl) was spotted onto an OMNI PLATE® (Nalgene, Rochester, NY) containing the appropriate antibiotics. A Biomek® FX robot (Beckman Coulter, Inc., Fullerton CA) may be used for the spotting step. The bacterial culture was grown overnight at 37°C. Alternatively, the glycerol stocks can be inoculated directly into TB medium.

**[0087]** TB medium was prepared by dissolving 12 g Bacto-Tryptone (BD Diagnostic Systems, Sparks, MD), 24 g Yeast Extract (Sigma-Aldrich, St. Louis, MO), and 8 ml 50% glycerol (Sigma-Aldrich) in a total volume of 900 ml with double-distilled water (ddH<sub>2</sub>O), and then autoclaved. After this mix has cooled, 100 ml KPI salts (0.17M KH<sub>2</sub>PO<sub>4</sub> and 0.72 M K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O) were added, bringing the total volume to 1 L. An antibiotic can also be added to the medium for selection of bacteria carrying a particular plasmid. With regard to the antibiotics, carbenicillin has produced a higher yield than ampicillin.

**[0088]** The overnight culture was then inoculated into two deep well blocks each containing 1.5 ml/well TB with appropriate antibiotics using a 96-pin tool. This was grown 24 hrs, at 37°C, shaking 800 rpm in a Multitron (Rose Scientific, Edmonton, Alberta, Canada) or similar shaker. Alternatively, four blocks of 0.75 ml/well TB can be grown in a 300 rpm shaker.

**[0089]** 100 µl/well of 1% Celpure® 300 diatomite filter aid (Advanced Minerals, Santa Barbara, CA; Sigma, St. Louis, MO) was added to each block. Plates were spun at 5000 g for 10 minutes in swinging bucket centrifuge (for example, a Sorvall RC12BP at 4000 rpm). Plates were decanted by inverting into a large plastic beaker. Plates were placed, still inverted, on paper towels to remove residual media. The Celpure® 300 filter aid solution was made by suspending 5 g powder in 500 ml ultrafiltered distilled H<sub>2</sub>O and 1 ml 0.5M EDTA, and was stored at 4 °C. The Celpure® filter aid was mixed thoroughly to resuspend it before use. Pipeting Celpure® filter aid solutions may be eased by cutting off the ends of the pipet tips with a sharp scissors to enlarge the tip opening.

**[0090]** Bacterial pellets were resuspended using 500 µl/well acidic, zwitterionic detergent solution, prewarmed to 37 °C. The plates were then sealed tightly with foil, with care as the detergent may cause leakage if the wells are not sealed well, or if the plates are vortexed too violently. The pellets were resuspended by vortexing briefly, then the plates were shaken at 32°C or 37°C (i.e., in a warm room) at 300 rpm-350 rpm for 30 minutes. For this experiment, the acidic, zwitterionic detergent solution consisted of 100 mM sodium citrate, 5 mM EDTA, and 0.1% n-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, in ultrafiltered distilled water (dH<sub>2</sub>O), pH 4.5. Lysis buffer is stored at 4°C, but pre-warmed to 37°C before use.

**[0091]** Duplicate plates were pooled into a single plate and covered with foil. Plates were spun at 5000g at 4°C for 10 minutes in a swinging bucket centrifuge (e.g., Sorvall 4000 rpm). The plates were then decanted, and inverted on paper towels to remove residual supernatant.

**[0092]** A solution (250µl) containing 50mM Tris-Cl, pH8.0 and 10mM EDTA, was added, the plates were covered with foil to seal tightly, and pellets were resuspended by vortexing carefully ~1minute to get complete resuspension. Thorough resuspension is important. A microplate mixing device such as a Mixmate™ (Eppendorf AG, Westbury, NY) may be used. Centrifuging for 5sec-10sec in a table-top centrifuge (quickspin) briefly before removing foil removes residual liquid from the foil.

**[0093]** Next, a solution (250  $\mu$ l) containing 2% SDS and 0.2 N NaOH, was added and the plates were covered with foil seal and mixed by inverting five times. The plates were incubated at room temperature for 5-10 minutes and quickspun briefly before removing foil.

**[0094]** Then, 250  $\mu$ l of a solution containing glacial acetic acid, potassium acetate, and 2% Celpure® 300 filter aid, was added quickly and vigorously. The plates were covered with foil seal and mixed by inverting five times. This solution is made by bringing 72 ml of 17.4 M glacial acetic acid, 122.5 g potassium acetate, and 10 g Celpure® filter aid to 500 ml with dH<sub>2</sub>O. The final pH should be ~pH4.8. This was quickspun briefly, then the foil removed and replaced with gas permeable seal. The plates were then placed in a vacuum chamber and degassed for 5 minutes.

**[0095]** Plates were then spun at 5000g at 4°C for 5 minutes in a swinging bucket centrifuge (e.g., a Sorvall at 4000 rpm).

**[0096]** A filter plate (Seahorse Bioscience Inc., North Billerica, MA) was prepared on a fresh deep well block (Riplate, RK Manufacturing, Inc., Jackson, MS). A FX robot with filter tips was employed to transfer the lysate to the filter plate/deep well block stack. The plates were spun at 750 g (Sorvall 1500 rpm), at 4°C, for 5 minutes in swinging bucket centrifuge. The filter plate was then discarded. A 250 $\mu$ l aliquot of a solution of 8 M guanidine and 0.4% detergent was added to the cleared lysates in the deep well block.

**[0097]** A silica filter plate was prepared by adding 400 $\mu$ l of 0% Celpure® 300 filter aid to a deep well plate, for example a Seahorse GF 1.2  $\mu$ m plate. This was placed briefly on vacuum to remove liquid. Lysates were transferred to the silica plate, which was then spun at 750 g for 5 minutes to load lysates. Alternatively, the lysates may be loaded onto the silica plate and vacuum adjusted so that it takes 1-2 minutes for the liquid to pass through. The silica plate was then washed with 1ml of a solution of 4 M guanidine, 40% isopropanol, and 0.1% detergent. The plate was then spun at 750 g for 5 minutes, and the wash repeated. Alternatively, the washes may be done with vacuum.

**[0098]** Next, the silica plate was washed with 1 ml 80% ethanol, spun at 750 g for 5 minutes, and this wash repeated. The plate was then spun at 2000 g (Sorvall 2500 rpm) for 5 minutes. The first wash can be done with vacuum but it is advisable to spin after the second wash to remove all traces of liquid from the plate. The plates were then dried by placement in a vacuum chamber until the silica turned white, about 5-10minutes.

**[0099]** The samples were then eluted into fresh 300 $\mu$ l conical polypropylene plates (Greiner Bio-One No. Am., Monroe, NC) with 150 $\mu$ L of TE (10 mM Tris, pH 8.0 and 1 mM

EDTA), and spun at 750g for 5 minutes. A second elution was performed by addition of another 150  $\mu$ L of TE, followed by centrifugation at 2000g for 5 minutes. The eluate was collected in the same collection plate.

**Example 2. Comparison of commercial kits and acidic, zwitterionic detergent buffer**

**[00100]** High-throughput comparison of commercial plasmid preparation kits with Qiagen QIAprep 96 Turbo and QIAwell 96 Ultra plasmid preparation kits were used in the protocol from Example 1, with or without the use of acidic, zwitterionic detergent solution (100 mM sodium citrate, 5 mM EDTA, and 0.1% n-Tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, in ultrafiltered distilled water (dH<sub>2</sub>O), pH 4.5). As shown in Figure 8, plasmid preparations with the acidic, zwitterionic detergent solution (Modified) had significantly higher plasmid yields in the high-throughput environment compared to those without (Plain) the buffer. These data are also reflected in Table 1:

Table 1. Histogram of Yields

ng DNA	Turbo Plain	Turbo Modified	Ultra Plain	Ultra Modified
2500	53	0	74	0
5000	42	0	21	1
7500	0	0	0	26
10000	0	3	0	53
12500	0	31	0	9
15000	0	53	0	6
17500	0	8	0	0
20000	0	0	0	0

**Example 3: Use of Zwitterionic detergent with vacuum to obtain clear lysates**

**Method:**

**[00101]** Bacteria Preparation: A starter culture of *E. coli* containing plasmid pSK2.0 was used to inoculate three identical 2-liter Erlenmeyer flasks; each flask contained 250 ml TB medium. The cultures were grown for 27 hours and then pooled together into a single flask so that a uniform culture of bacteria was used as a starting material. The culture was aliquoted into two identical centrifuge bottles each containing 175 mL of culture. The cultures were spun at 6000 rpm for 10 minutes to pellet the bacteria and the supernatant decanted. One bottle containing untreated bacteria (“culture A”) was stored at -60°C until further use. The bacteria in the other bottle (“culture B”) were resuspended in 50 ml of zwitterionic solution containing 0.1% zwittergent (without CellPure<sup>®</sup>) which was prewarmed

to 37°C. The bacteria were then incubated with shaking at 37°C for 30 min. The bacteria were then pelleted at 8000 rpm for 10 min, the supernatant decanted, and the bacteria frozen until later use.

**[00102]** DNA preparation: The two cultures described above were then thawed and subjected to alkaline lysis. The cultures were each resuspended in 10 ml of Qiagen Maxi prep<sup>®</sup> solution 1 with vigorous pipetting and vortexing. 10 ml of Qiagen Maxi prep<sup>®</sup> Solution 2 was then added and the bottles mixed gently by inversion and incubated at room temperature for 5 minutes. 10 ml of Qiagen Maxi prep<sup>®</sup> Solution 3 was added to culture A and mixed gently per standard methods. 10 ml of Solution 3 containing 2% (w/v) Celpure<sup>®</sup> 300 was added to culture B and mixed gently per standard methods. The lysates from each culture were then transferred to clear polycarbonate centrifuge tubes for better visualization. Culture B lysate was then subjected to vacuum of approximately -800 mbar (range, -100 to -1050 mbars) for 10 minutes (range, 0.5 minute to 60 minutes). The tube can be covered with a gas-permeable sealing film (e.g., Axygen BF-400) during the vacuum treatment to prevent spillover. The cultures were then centrifuged at 8000 rpm for 10 minutes to pellet the bacterial debris.

**[00103]** This method allows generation of *E. coli* lysates that are substantially free from floating material after centrifugation, as shown herein in Figure 9. Since the floating material contains bacterial debris, the lysates obtained by this method are more pure than those obtained via conventional methods. The lysates obtained by this method are more easily decanted than those obtained via conventional methods. The lysates obtained by this method can be subjected to aspiration by liquid handling robots from above without aspiration of the bacterial debris. Thus, this combination of zwitterionic solution with vacuum treatment of lysates is ideally suited for robotic preparation of plasmid DNAs in high throughput microplate format.

#### **Example 4: Adding zwitterionic detergent solution directly to culture media**

**[00104]** In order to reduce the number of centrifugation steps necessary during plasmid purification, the zwitterionic detergent solution can be added directly to the culture medium, prior to the first centrifugation step to pellet the bacterial culture. Since it is necessary for the methods and compositions described herein to produce a change in pH,

addition of the zwitterionic detergent directly to the culture media is contemplated only for media that are not highly pH buffered. Some exemplary non-buffered media include LB, 2X YT, M9, or SDCAS, among others (O'Kennedy RD 2000 J Biotech 76: 175-183). The TB media used herein in the Examples buffer is a pH buffered medium and is therefore not contemplated for use with this method.

**[00105]** Specifically, the method is to add 0.2 volumes of a solution of 1% Zwittergent in 100 mM citric acid pH 2.0 to 1 volume of culture containing E coli bacteria. The resulting pH of the culture should be between 3.5 and 5.5. The cultures are then treated as described herein for plasmid purification (e.g., incubated at 37 degrees for 30 min, pelleted, and then subjected to alkaline lysis, etc.).

## CLAIMS

1. A method for the purification of plasmid DNA from *E. coli* bacteria comprising the steps of:
  - (a) contacting *E. coli* bacteria comprising plasmid DNA with an acidic, zwitterionic detergent solution comprising 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7;
  - (b) incubating said bacteria in said acidic, zwitterionic detergent solution for at least 1 minute; and
  - (c) purifying said plasmid DNA.
2. The method of claim 1, wherein said zwitterionic detergent is added to culture medium of said *E. coli* bacteria.
3. The method of claim 1 or 2, wherein said incubating is performed for 1 minute to 60 minutes.
4. The method of claim 1, 2, or 3, wherein said incubating is performed at a temperature of 0°C to 45°C.
5. The method of claim 1, 2, 3 or 4, wherein said plasmid DNA is purified using a method comprising alkaline lysis of said *E. coli* bacteria.
6. The method of any one of claims 1-5, wherein said acidic, zwitterionic detergent consists essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6;
7. A method for the purification of plasmid DNA from *E. coli* bacteria, the method comprising the steps of:
  - (a) preparing a lysate of said bacteria;
  - (b) adding an acidic, zwitterionic detergent solution to said lysate to a final concentration of 0.1% to 5% zwitterionic detergent; and

- (c) purifying plasmid DNA from said lysate.
8. The method of claim 7 wherein said purifying comprises binding plasmid DNA to a solid support.
9. The method of claim 7 or 8, wherein said purifying comprises binding said plasmid DNA to a silica matrix or to an anion exchange material.
10. The method of claim 7, 8, or 9, further comprising the step of subjecting said lysate to a vacuum of -100 to -1050 mBar prior to centrifugal clarification of said lysate.
11. A method for the purification of plasmid DNA from *E. coli* bacteria comprising the steps of:
- (a) culturing said *E. coli* bacteria in liquid culture media;
  - (b) adding to said culture media a diatomaceous filter aid;
  - (c) removing said media from said bacteria;
  - (d) incubating said bacteria in an acidic, zwitterionic detergent solution;
  - (e) removing said acidic, zwitterionic detergent solution;
  - (f) incubating said bacteria with a lysis solution;
  - (g) filtering said bacterial lysis solution and collecting the filtered lysate;
  - (h) adding a guanidine, detergent solution to said lysate;
  - (i) loading said lysate into a matrix to which said plasmid DNA binds; and  
eluting said plasmid DNA from said matrix.
12. The method of claim 11, wherein said acidic, zwitterionic detergent solution consists essentially of 100 mM sodium citrate, 5 mM EDTA, and 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH 4.5.
13. The method of claim 11 or 12, in which said incubating in acidic, zwitterionic detergent solution is conducted at 30°C to 37°C for about 30 minutes with shaking.

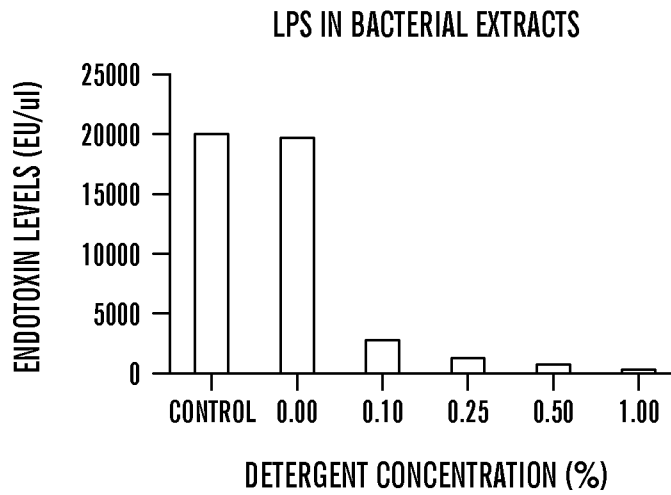
14. The method of claim 11, 12, or 13, further comprising the step of incubating said filtered lysates with a zwitterionic detergent solution prior to said loading step.
15. The method of any one of claim 11-14, further comprising the step of washing said loaded matrix with a zwitterionic detergent solution prior to said eluting step.
16. A method for the reducing the amount of endotoxin from *E. coli* bacterial cells comprising the steps of:
  - (a) contacting said *E. coli* bacterial cells with acidic, zwitterionic detergent solution consisting essentially of 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7;
  - (b) incubating said bacterial cells in said acidic, zwitterionic detergent solution at temperatures from 0°C to 45°C for a duration of 1 minute to 60 minutes; and
  - (c) collecting said bacterial cells from said solution pursuant to standard techniques.
17. The method of claim 16, wherein said acidic, zwitterionic detergent consists essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6;
18. The method of any one of claims 1-17, wherein said zwitterionic detergent is selected from the group consisting of n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, CHAPS, 3-(decyldimethylammonio)-propane-sulfonate inner salt, 3-(N,N-dimethyloctadecylammonio) propanesulfonate, 3-(N,N-dimethyloctylammonio) propanesulfonate inner salt, 3-(N,N-dimethylpalmitylammonio) propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, BigCHAP, CHAPSO, DDMAU, LADAO, Zwittergent® 3-08, Zwittergent® 3-10, Zwittergent® 3-14, Zwittergent® 3-16, and N-Dodecyl-N,N-dimethylglycine.
18. A solution for the removal of endotoxin from *E. coli* bacteria comprising an acidic, zwitterionic detergent solution consisting essentially of 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7.

19. A solution for the removal of endotoxin from *E. coli* bacteria comprising an acidic, zwitterionic detergent solution consisting essentially of 0.1% to 1.0% zwitterionic detergent at a pH range from pH 4 to pH 6.
20. A composition comprising a cultured *E. coli* bacterium comprising plasmid DNA or an *E. coli* bacterial lysate comprising plasmid DNA, and an acidic, zwitterionic detergent, wherein said zwitterionic detergent present at a concentration of 0.1% to 5% (w/v).
21. The composition of claim 20 wherein said lysate is a clarified bacterial lysate.
22. The composition of claim 20 or 21, wherein said lysate was prepared by alkaline lysis.
23. The composition of claim 20, 21, or 22 wherein said acidic, zwitterionic detergent solution consists essentially of 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH4.5.
24. The composition of any one of claims 20-23 wherein said solid support is selected from silica and a solid support comprising an anion exchange material.
25. The composition of any one of claims 20-24 further comprising a lysate of *E. coli* bacteria comprising said plasmid DNA.
26. The composition of any one of claims 20-25 wherein said zwitterionic detergent is present at a concentration of 0.01% to 5.0%.
27. The composition of any one of claims 20-26, wherein said zwitterionic detergent is present at a concentration of 0.1% to 1.0%
28. The composition of any one of claims 20-27 wherein the pH of said acidic, zwitterionic detergent solution is between 3.0 and 6.99, inclusive.
29. The composition of any one of claims 20-28 wherein the pH of said acidic, zwitterionic detergent solution is between 4.0 and 6.0, inclusive.
30. A composition comprising a solid support comprising plasmid DNA and an acidic, zwitterionic detergent solution.

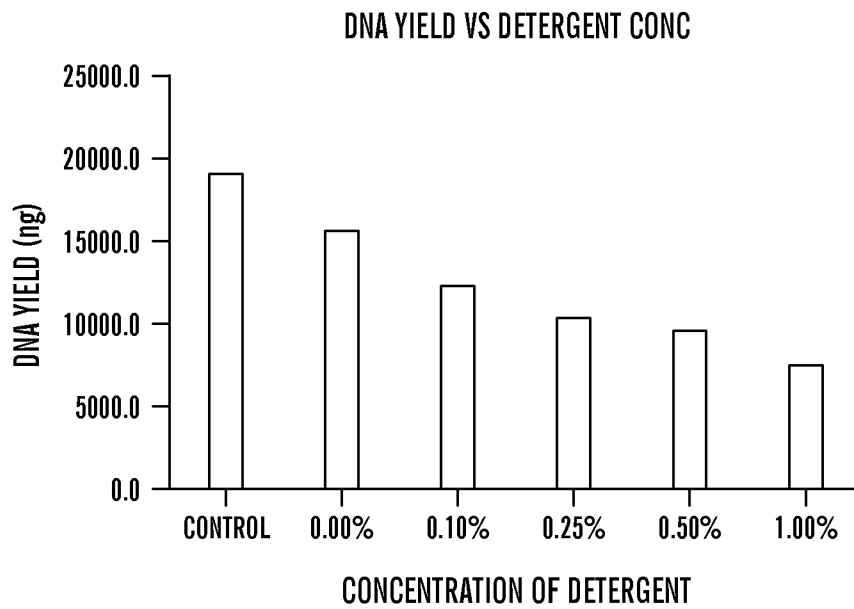
31. The composition of claim 30 wherein said acidic, zwitterionic detergent solution consists essentially of 0.1% n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, pH4.5.
32. The composition of claim 30 or 31, wherein said solid support is selected from silica and a solid support comprising an anion exchange material.
33. The composition of claim 30, 31, or 32, further comprising a lysate of *E. coli* bacteria comprising said plasmid DNA.
34. The composition of any one of claims 30-33, wherein said zwitterionic detergent is present at a concentration of 0.01% to 5.0%.
35. The composition of any one of claims 30-34, wherein said zwitterionic detergent is present at a concentration of 0.1% to 1.0%
36. The composition of any one of claims 30-35 wherein the pH of said acidic, zwitterionic detergent solution is between 3.0 and 6.99, inclusive.
37. The composition of any one of claims 30-36 wherein the pH of said acidic, zwitterionic detergent solution is between 4.0 and 6.0, inclusive.
38. The solution of any one of the preceding claims, wherein said zwitterionic detergent is selected from the group consisting of n-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, CHAPS, 3-(decyldimethylammonio)-propane-sulfonate inner salt, 3-(N,N-dimethyloctadecylammonio) propanesulfonate, 3-(N,N-dimethyloctylammonio) propanesulfonate inner salt, 3-(N,N-dimethylpalmitylammonio) propanesulfonate, N-dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, BigCHAP, CHAPSO, DDMAU, LADAO, Zwittergent® 3-08, Zwittergent® 3-10, Zwittergent® 3-14, Zwittergent® 3-16, and N-Dodecyl-N,N-dimethylglycine.
39. A high-throughput method for purifying plasmid DNA from *E. coli* bacteria comprising the steps of:

- (a) contacting a plurality of *E. coli* bacteria samples comprising plasmid DNA with an acidic, zwitterionic detergent solution comprising 0.01% to 5.0% zwitterionic detergent at a pH range from pH 3 to pH 7;
  - (b) incubating said plurality of samples in said acidic, zwitterionic detergent solution for at least 1 minute; and
  - (c) purifying said plasmid DNA from said plurality of samples in parallel.
40. The method of claim 39, wherein said high-throughput method is automated.
41. The method of claim 39, where said method comprises a robotic pipettor.

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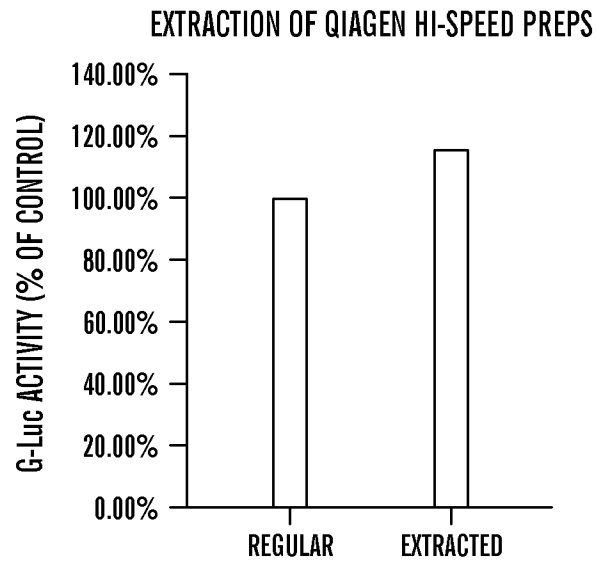


**FIG. 1**

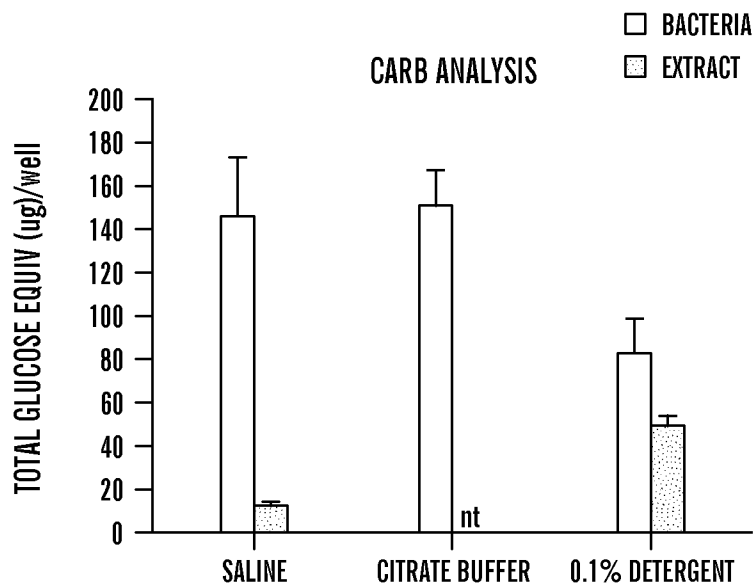


**FIG. 2**

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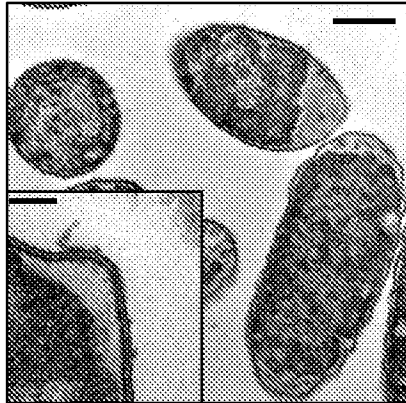


**FIG. 3**

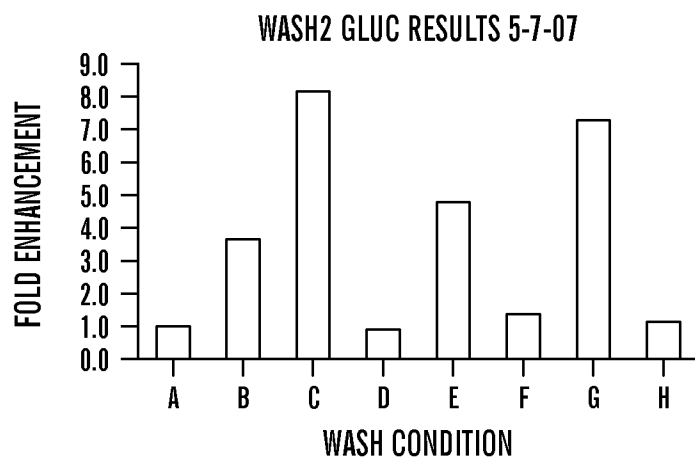


**FIG. 4**

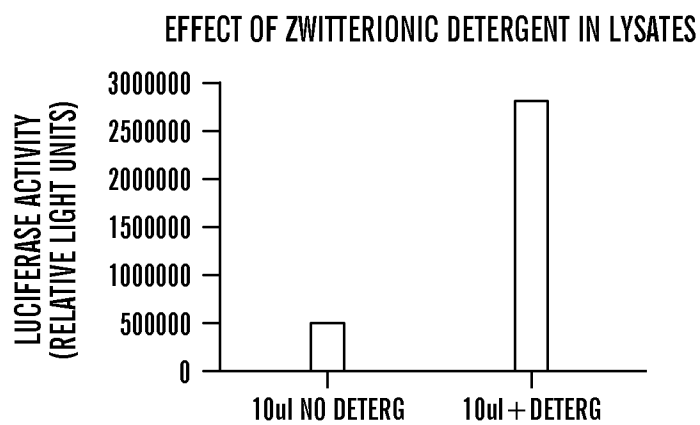
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**FIG. 5**

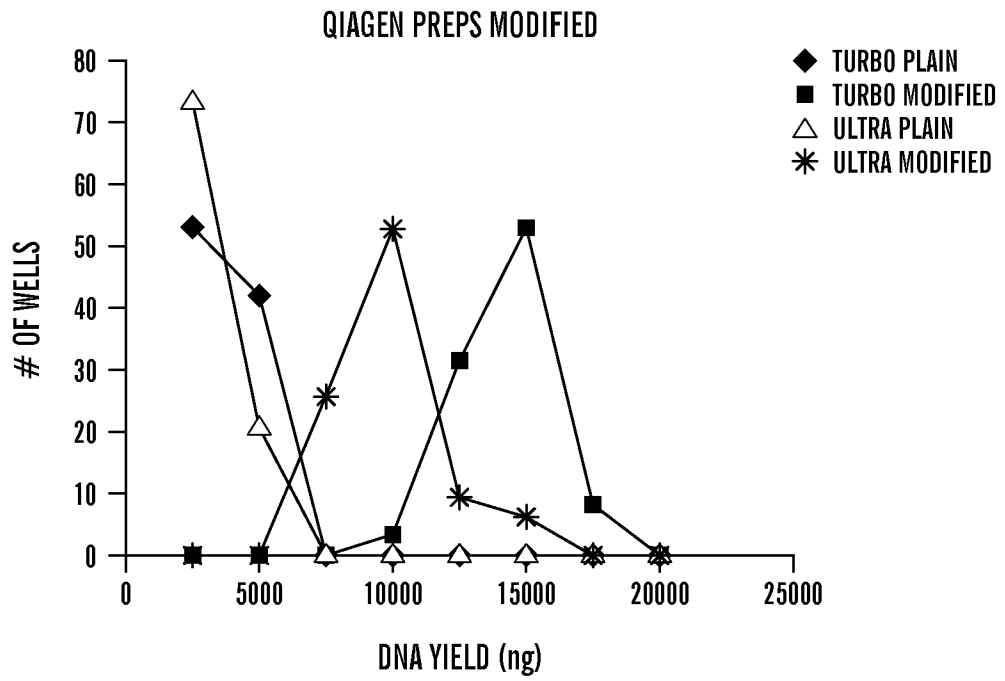


**FIG. 6**

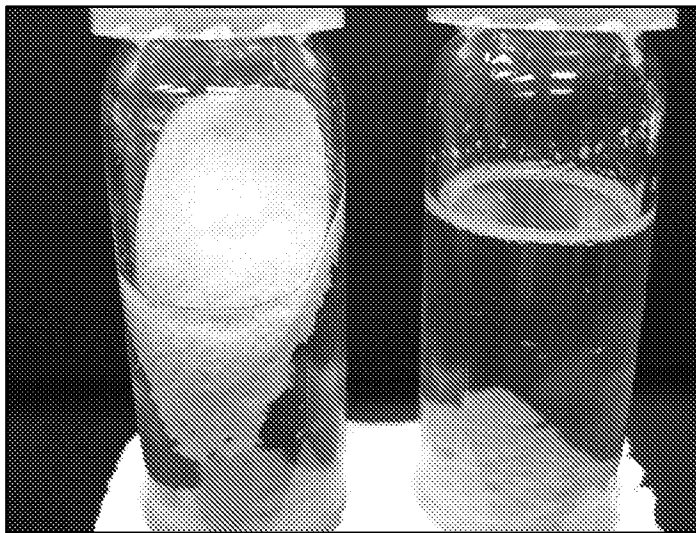


**FIG. 7**

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**FIG. 8**



**FIG. 9**