nucleic acid purification, these methods may have one or more drawbacks including, for example, high cost, high complexity, slow speed, low yield, low purity, contamination, toxicity, and/or inefficiency. Magnetic particles have previously been used to bind and separate nucleic acids in various purification methods. These methods may have several advantages, such as eliminating centrifugation and/or vacuum processing steps, yielding a higher purity product, and or improving operator safety. However, methods employing magnetic particles can still suffer from various disadvantages, such as slow speed, high complexity, and/or poor overall yield.

[0005] Accordingly, it would be advantageous to provide magnetic particle-based methods and kits for nucleic acid purification that may be faster, less complex, less expensive, and/or improved in terms of product purity and/or yield. The resulting purified nucleic acids can be used in a wide variety of biochemical and diagnostic applications, such as polymerase chain reactions (PCR), cloning, transfection, restriction digest, and clinical diagnostics.

SUMMARY

[0006] The disclosure relates, in various embodiments, to methods for purifying nucleic acids, the methods comprising (a) combining a sample comprising at least one nucleic acid with a suspension buffer to form a suspension, (b) combining the suspension with a lysis buffer to form a lysate, (c) combining the lysate with a binding buffer to form a solution; (d) combining the solution with at least one magnetic particle to form a combined solution comprising at least one modified magnetic particle reversibly bound to the at least one nucleic acid, (e) separating the at least one modified magnetic particle from the combined solution, (f) washing the at least one modified magnetic particle with a first wash buffer and a second wash buffer, and (g) combining the modified magnetic particle with an elution buffer.

[0006a] In an aspect, there is provided a method for nucleic acid purification, comprising:

(a) combining a sample comprising at least one nucleic acid with a suspension buffer to form a suspension;

(b) combining the suspension with a lysis buffer to form a lysate;

(c) combining the lystate with a binding buffer to form a solution;

(d) combining the solution with at least one magnetic particle to form a combined solution comprising at least one modified magnetic particle, wherein the at least one modified magnetic particle is reversibly bound to the at least one nucleic acid;

(e) separating the at least one modified magnetic particle from the combined solution;

(f) washing the at least one modified magnetic particle with a first wash buffer and a second wash buffer; and

(g) combining the at least one modified magnetic particle with an elution buffer;

wherein:

the suspension buffer comprises at least one first ion chelating agent, present in a concentration ranging from about 1 mM to about 10 mM, and at least one first buffer compound, present in a concentration ranging from about 10 mM to about 100 mM;

the lysis buffer comprises at least one detergent chaotropic agent, present in a concentration ranging from about 1% to about 10% by weight, and at least one second buffer compound, present in a concentration greater than or equal to about 0.2 M;

the binding buffer comprises at least one first chaotropic agent, present in a concentration ranging from about 4 M to about 6 M, optionally at least one first salt, present in a concentration ranging from about 0.1 M to about 2 M, at least one first alcohol, present in a concentration ranging from about 1% to about 5% by volume, and at least one third buffer compound, present in a concentration ranging from about 0.25% to about 3% by weight;

the first wash buffer comprises at least one second chaotropic agent, present in a concentration ranging from about 4 M to about 6 M, at least one second ion chelating agent, present in an concentration ranging from about 1 mM to about 10

mM, at least one second alcohol, present in a concentration ranging from about 30% to about 50% by volume, and at least one fourth buffer compound, present in a concentration ranging from about 10 mM to about 100 mM; and

the second wash buffer comprises at least one third alcohol and optionally at least one second salt.

[0007] According to various embodiments, the suspension buffer can comprise at least one ion chelating agent, present in a concentration ranging from about 1 mM to about 10 mM, and at least one buffer compound, present in a concentration ranging from about 10 mM to about 100 mM. The lysis buffer may comprise at least one

detergent chaotropic agent, present in a concentration ranging from about 1% to about 10% by weight, and at least one buffer compound, present in a concentration greater than or equal to about 0.2 M. In non-limiting embodiments, the binding buffer can comprise at least one chaotropic agent, present in a concentration ranging from about 4 M to about 6 M, optionally at least one salt, present in a concentration ranging from about 0.1 M to about 2 M, at least one alcohol, present in a concentration ranging from about 1% to about 5% by volume, and at least one buffer compound, present in a concentration ranging from about 0.25% to about 3% by weight. According to additional embodiments, the first wash buffer can comprise at least one chaotropic agent, present in a concentration ranging from about 4 M to about 6 M, at least one ion chelating agent, present in a concentration ranging from about 1 mM to about 10 mM, at least one buffer compound present in a concentration ranging from about 10 mM to about 100 mM, and at least one alcohol, present in a concentration ranging from about 30% to about 50% by volume. In further embodiments, the second wash buffer may comprise at least one alcohol and optionally at least one salt. Magnetic particles can be chosen, for example, from carboxyl coated magnetic particles, silica-based magnetic particles, and combinations thereof. Also disclosed herein are nucleic acid purification kits comprising these buffers and magnetic particles.

[0007a] In another aspect, there is provided a kit for nucleic acid purification, comprising:

(a) a suspension buffer comprising at least one first ion chelating agent, present in a concentration ranging from about 1 mM to about 10 mM, and at least one first buffer compound, present in a concentration ranging from about 10 mM to about 100 mM;

(b) a lysis buffer comprising at least one detergent chaotropic agent, present in a concentration ranging from about 1% to about 10% by weight, and at least one second buffer compound, present in a concentration greater than or equal to about 0.2 M;

(c) a binding buffer comprising at least one first chaotropic agent, present in a concentration ranging from about 4 M to about 6 M, optionally at least one first salt, present in a concentration ranging from about 0.1 M to about 2 M, at least one first alcohol, present in a concentration ranging from about 1% to about 5% by volume, and at least one third buffer compound, present in a concentration ranging from about 0.25% to about 3% by weight;

(d) a first wash buffer comprising at least one second chaotropic agent, present in a concentration ranging from about 4.5 M to about 6 M, at least one second ion chelating agent, present in an concentration ranging from about 1 mM to about 10 mM, at least one second alcohol, present in a concentration ranging from about 30% to about 50% by volume, and at least one fourth buffer compound, present in a concentration ranging from about 10 mM to about 100 mM;

(e) a second wash buffer comprising at least one third alcohol and optionally at least one second salt; and

(f) at least one magnetic particle.

[0007b] In another aspect, there is provided a kit for nucleic acid purification, comprising:

(a) a suspension buffer comprising at least one first ion chelating agent chosen from EDTA, isomers thereof, and combinations thereof, present in a concentration ranging from about 8 mM to about 10 mM, and at least one first buffer compound chosen from Tris, Tris-HCl and combinations thereof, present in a concentration ranging from about 10 mM to about 40 mM;

(b) a lysis buffer comprising at least one detergent chaotropic agent chosen from SDS, SDBS, and combinations thereof, present in a concentration ranging from about 1% to about 3% by weight, and at least one second buffer compound chosen from akali hydroxides and combinations thereof, present in a concentration greater than or equal to about 0.2 M;

(c) a binding buffer comprising at least one first chaotropic agent chosen from GuHCl, GuSCN, and combinations thereof, present in a concentration ranging from about 4 M to about 4.5 M, at least one first salt chosen from ammonium sulfate,

ammonium acetate, sodium acetate, and combinations thereof, present in a concentration ranging from about 0.3 M to about 1.5 M, from about 1% to about 2% by volume of isopropanol, and at least one third buffer compound chosen from glacial acetic acid, hydrochloric acid, and combinations thereof, present in a concentration ranging from about 1% to about 3% by weight;

(d) a first wash buffer comprising at least one second chaotropic agent chosen from GuHCl, GuSCN, and combinations thereof, present in a concentration ranging from about 4.5 M to about 5 M, at least one second ion chelating agent chosen from EDTA, isomers thereof, and combinations thereof, present in an concentration ranging from about 1 mM to about 3 mM, from about 30% to about 40% by volume of isopropanol, and at least one fourth buffer compound chosen from Tris, Tris-HCl, and combinations thereof, present in a concentration ranging from about 20 mM to about 50 mM;

(e) a second wash buffer comprising at least about 70% by volume of ethanol and at least one second salt chosen from ammonium sulfate, ammonium acetate, sodium acetate, and combinations thereof, present in a concentration ranging from about 80 mM to about 100 mM; and

(f) at least one silica-based magnetic particle.

[0008] Additional features and advantages of the disclosure will be set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the embodiments of the disclosure as described herein, including the detailed description which follows, the claims, and the appended drawings.

[0009] It is to be understood that both the foregoing general description and the following detailed description present various embodiments of the disclosure, and are intended to provide an overview or framework for understanding the nature and character of the claims. The accompanying drawings are included to provide a further understanding of the disclosure, and are incorporated into and constitute a part of this specification. The drawings illustrate various embodiments of the

disclosure and together with the description serve to explain the principles and operations of the embodiments disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The following detailed description can be best understood when read in conjunction with the following drawings, where like structures are indicated with like reference numerals and in which:

[0011] **FIG. 1** is a flow diagram illustrating a nucleic acid purification method according to one embodiment of the disclosure;

[0012] **FIG. 2** is a graph illustrating pDNA yield for methods according to various embodiments of the disclosure as compared to a prior art method;

[0013] **FIG. 3** is a graph illustrating transfection efficiency for methods according to various embodiments of the disclosure as compared to a prior art method;

[0014] **FIG. 4** illustrates an agarose gel electrophoresis analysis of pDNA (cut or uncut with *EcoRI*) purified using methods according to various embodiments of the disclosure and using a prior art method;

[0015] **FIG. 5** is a graph illustrating pDNA yield for methods according to various embodiments of the disclosure as compared to a prior art method; and

[0016] **FIG. 6** is a graph illustrating transfection efficiency for methods according to various embodiments of the disclosure as compared to a prior art method.

DETAILED DESCRIPTION

Methods

[0017] Disclosed herein are methods for nucleic acid purification, the methods comprising (a) combining a sample comprising at least one nucleic acid with a suspension buffer to form a suspension, (b) combining the suspension with a lysis buffer to form a lysate, (c) combining the lysate with a binding buffer to form a solution; (d) combining the solution with at least one magnetic particle to form a combined solution comprising at least one modified magnetic particle reversibly bound to the at least one nucleic acid, (e) separating the at least one modified