applications such as nucleic acid amplifications methods such as RT-PCR, qPCR, sequencing methods such as Next Generation Sequencing, cloning methods, or the like. Especially, there is a need for methods that allow rapid nucleic acid isolation from samples without the need to apply a time-consuming enzymatic digestion step.

[006] The present invention addresses these needs by providing compositions, uses and methods that are suitable among other things for a very fast and reliable isolation of nucleic acids with sufficient purity and integrity from different cell samples.

[007] Within the scope of the inventor's research activities, various novel lysis methods for the isolation of nucleic acids were developed. Surprisingly, an enzyme-free (purely chemical) method based on reducing agents such as for example the chemical TCEP (or similar phosphine compounds) and anionic detergents such as SDS at elevated temperatures was identified, which has both a low toxicity of the chemicals involved, is extremely efficient, fast and easy to perform and results in nucleic acids with sufficient purity and integrity. This new method reduces the time for nucleic acid isolation to less than 15 minutes, which results in massive advantages for the end user compared to the state of the art.

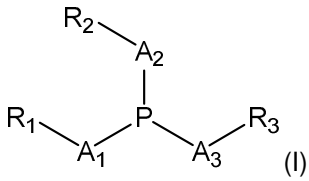
[008] The solution of the present invention is described in the following, exemplified in the examples, illustrated in the Figures and reflected in the claims.

SUMMARY

[009] The present ~~invention~~disclosure relates to a use of a "solution" (in the following also denoted as "solution of the ~~invention~~disclosure"), which comprises

(a) a buffering substance, preferably for buffering the solution at a pH of about 1 to 13, more preferably at a pH of about 4-11 or 4-10, and even more preferably at a pH of about 6-9; and

(b) a reducing agent according to formula (I)



(I)

wherein

R_1, R_2 and R_3 are independently selected from the group consisting of

-H, $-OR_4$, $-COOR_5$, $-P(O)(OR_6)OR_7$, $-N(R_8)R_9$, $-S(O)_{0-2}R_{10}$, and $-SO_3H$;

R_4 to R_{10} are independently selected from the group consisting of

-H, and $-(C_1-C_{15})$alkyl;

A_1, A_2 and A_3, are independently selected from the group consisting of

$-(C_1-C_{15})$alkylene-, $-(C_3-C_{10})$cycloalkylene-, $-(C_2-C_{15})$alkenylene-;

A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected

from -OR_4, -$COOR_5$, and -(C_1-C_{15})alkyl; and a salt thereof; and

(c) preferably an anionic detergent,

for isolating nucleic acids from a sample at a temperature of at least about 60°C, preferably for at least 10 seconds.

[0010] The present ~~invention~~disclosure also relates to an *in vitro* method for isolating nucleic acids from a sample, wherein said method makes use of the solution of the ~~invention~~disclosure.

[0011] The present ~~invention~~disclosure also relates to a use of the solution of the ~~invention~~disclosure, which optionally further comprises (d) less than 1 M of a chaotropic salt (CAO) and/or urea and/or thiourea; for isolating nucleic acids from a sample. Said solution of the ~~invention~~disclosure which optionally further comprises (d) less than 1 M of a chaotropic salt (CAO) and/or urea and/or thiourea may also be employed in an *in vitro* method of the ~~invention~~disclosure which is for isolating nucleic acids from a sample.

[0012] The present ~~invention~~disclosure also relates to the solution of the ~~invention~~disclosure as defined herein and further to the solution of the ~~invention~~disclosure as defined herein for use in a method or use as disclosed herein.

[0013] Further, the present ~~invention~~disclosure relates to the solution of the ~~invention~~disclosure as defined herein for use in a method or use as disclosed herein, wherein said solution of the ~~invention~~disclosure may optionally further comprise less than 1 M of a chaotropic salt (CAO) and/or urea and/or thiourea and wherein the solution has a temperature of at least about 60 °C.

[0014] The present ~~invention~~disclosure also relates to a kit comprising a solution as described herein, particularly the solution of the ~~invention~~disclosure.

[0014a] In one aspect, the present disclosure relates to a use of a solution, which comprises (a) a buffering substance; (b) a reducing agent according to formula (I) wherein R_1, R_2 and R_3 are independently selected from the group consisting of -H, -OR_4, -$COOR_5$, -$P(O)(OR_6)OR_7$, -$N(R_8)R_9$, -$S(O)_{0-2}R_{10}$, and -SO_3H; R_4 to R_{10} are independently selected from the group consisting of -H, and -(C_1-C_{15})alkyl; A_1, A_2 and A_3, are independently selected from the group consisting of -(C_1-C_{15})alkylene-, -(C_3-C_{10})cycloalkylene-, -(C_2-C_{15})alkenylene-; A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from -OR_4, -$COOR_5$, and -(C_1-C_{15})alkyl; and a salt thereof; and (c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS); for isolating nucleic acids from a sample at a temperature of at least about 60 °C.

[0014b] In another aspect, the present disclosure refers to an *in vitro* method for isolating nucleic acids from a sample, the method comprising (i) contacting the sample with a solution, which comprises (a) a buffering substance; (b) a reducing agent according to formula (I) wherein R_1, R_2 and R_3 are independently selected from the group consisting of

-H, -OR_4, -$COOR_5$, -$P(O)(OR_6)OR_7$, -$N(R_8)R_9$, -$S(O)_{0-2}R_{10}$, and -SO_3H; R_4 to R_{10} are independently selected from the group consisting of -H, and -(C_1-C_{15})alkyl; A_1, A_2 and A_3, are independently selected from the group consisting of -(C_1-C_{15})alkylene-, -(C_3-C_{10})cycloalkylene-, -(C_2-C_{15})alkenylene-; A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from -OR_4, -$COOR_5$, and -(C_1-C_{15})alkyl; and a salt thereof; and (c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS); (ii) incubating the solution including the sample at a temperature of at least about 60°C.

[0014c] In another aspect, the present disclosure refers to a solution for use in a method or use according to the disclosure herein, the solution comprising (a) a buffering substance (BU); (b) a reducing agent (RA) according to formula (I): wherein R1, R2 and R3 are independently selected from the group consisting of
-H, -OR4, -COOR5, -P(O)(OR6)OR7, -N(R8)R9, -S(O)0-2R10, and -SO3H; R4 to R10 are independently selected from the group consisting of -H, and -(C1-C15)alkyl; A1, A2 and A3, are independently selected from the group consisting of -(C1-C15)alkylene-, -(C3-C10)cycloalkylene-, -(C2-C15)alkenylene)-; A1, A2 and A3 optionally are further substituted with one or more substituents selected from -OR4, -COOR5, and -(C1-C15)alkyl; and a salt thereof; and (c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS); wherein the solution has a temperature of at least about 60 °C.

[0014d] In another aspect, the present disclosure refers to a solution comprising a sample for use in a method or use according to the disclosure herein, wherein the solution comprises (a) a buffering substance (BU); (b) a reducing agent (RA) according to formula (I): wherein R_1, R_2 and R_3 are independently selected from the group consisting of -H, -OR_4, -$COOR_5$, -$P(O)(OR_6)OR_7$, -$N(R_8)R_9$, -$S(O)_{0-2}R_{10}$, and -SO_3H; R_4 to R_{10} are independently selected from the group consisting of -H, and –(C_1-C_{15})alkyl; A_1, A_2 and A_3, are independently selected from the group consisting of –(C_1-C_{15})alkylene-, -(C_3-C_{10})cycloalkylene-, -(C_2-C_{15})alkenylene-; A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from -OR_4, -$COOR_5$, and –(C_1-C_{15})alkyl; and a salt thereof; and (c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS); wherein the solution has a temperature of at least about 60 °C.

[0014e] In yet another aspect, the present disclosure refers to an In vitro method for isolating nucleic acids from a sample, the method comprising (i) contacting the sample with a solution, which comprises (a) a buffering substance; (b) a reducing agent according to formula (I) wherein R1, R2 and R3 are independently selected from the group consisting of

-H, -OR4, -COOR5, -P(O)(OR6)OR7, -N(R8)R9, -S(O)0-2R10, and -SO3H; R4 to R10 are independently selected from the group consisting of -H, and -(C1-C15)alkyl; A1, A2 and A3, are independently selected from the group consisting of -(C1-C15)alkylene-, -(C3-C10)cycloalkylene-, -(C2-C15)alkenylene-; A1, A2 and A3 optionally are further substituted with one or more substituents selected from -OR4, -COOR5, and -(C1-C15)alkyl; and a salt thereof; and (c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS); wherein the solution including the sample has a temperature of at least about 60°C.

[0014f] In yet another aspect, the present disclosure refers to a kit comprising (a) a solution according to the disclosure herein, (b) optionally means for enzymatic digestion; preferably an enzyme and/or (c) optionally means for separating of non-nucleic acid components from nucleic acids, preferably a matrix for separating of non-nucleic acid components from nucleic acids.

[0015] The Figures show:

[0016] Fig. 1 shows the gel electrophoresis performed with the different samples used in Example 1, which are depicted in **Fig. 1**. The gel in **Fig. 1A** reflects nucleic acids obtained by sample 1 and 2. The gel depicted in **Fig. 1B** shows nucleic acids obtained by sample 3, 4, 5, 6, and 7. As evident from **Fig. 1**, all samples provide for a high-molecular-weight band with no low-molecular-weight smears indicating that only low or no amounts of degraded nucleic acids are present in the tested samples.

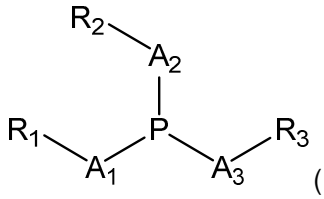
[0017] Fig. 2 shows the gel electrophoresis on the nucleic acids obtained in Example 2. The gel obtained by gel electrophoresis performed with the different samples used in this experiment is depicted in **Fig. 2** (nucleic acids obtained by sample 1, 2, 3, 4, 5, and 6 in the respective lanes). As evident from **Fig. 2** all samples provide for a high-molecular-weight band indicating that intact (non-degraded) nucleic acids are present in the tested samples. The low-molecular-weight smears seem to be higher in the acidic conditions than in the

Claims:

1. Use of a solution, which comprises

(a) a buffering substance;

(b) a reducing agent according to formula (I)



(I)

wherein

R_1, R_2 and R_3 are independently selected from the group consisting of

-H, $-OR_4$, $-COOR_5$, $-P(O)(OR_6)OR_7$, $-N(R_8)R_9$, $-S(O)_{0-2}R_{10}$, and $-SO_3H$;

R_4 to R_{10} are independently selected from the group consisting of

-H, and $-(C_1-C_{15})$alkyl;

A_1, A_2 and A_3, are independently selected from the group consisting of

$-(C_1-C_{15})$alkylene-, $-(C_3-C_{10})$cycloalkylene-, $-(C_2-C_{15})$alkenylene-;

A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from $-OR_4$, $-COOR_5$, and $-(C_1-C_{15})$alkyl;

and a salt thereof; and

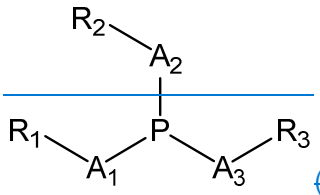
(c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS);

for isolating nucleic acids from a sample at a temperature of at least about 60 °C.

~~2. Use of a solution, which comprises~~

~~(a) a buffering substance;~~

~~(b) a reducing agent according to formula (I)~~



~~(I)~~

~~wherein~~

~~R_1, R_2 and R_3 are independently selected from the group consisting of~~

~~-H, $-OR_4$, $-COOR_5$, $-P(O)(OR_6)OR_7$, $-N(R_8)R_9$, $-S(O)_{0-2}R_{10}$, and $-SO_3H$;~~

~~R_4 to R_{10} are independently selected from the group consisting of~~

~~-H, and $-(C_1-C_{15})$alkyl;~~

~~A_1, A_2 and A_3, are independently selected from the group consisting of~~

~~-(C_1-C_{15})alkylene-, -(C_3-C_{10})cycloalkylene-, -(C_2-C_{15})alkenylene-;~~

~~A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from -OR_4, -$COOR_5$, and -(C_1-C_{15})alkyl;~~

~~and a salt thereof; and~~

~~(c) an anionic detergent;~~

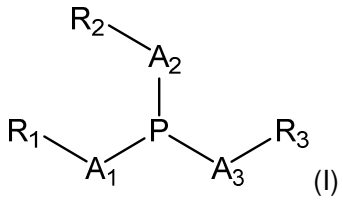
~~for isolating nucleic acids from a sample.~~

23. *In vitro* method for isolating nucleic acids from a sample, the method comprising

(i) contacting the sample with a solution, which comprises

(a) a buffering substance;

(b) a reducing agent according to formula (I)



(I)

wherein

R_1, R_2 and R_3 are independently selected from the group consisting of

-H, -OR_4, -$COOR_5$, -P(O)(OR_6)OR_7, -N(R_8)R_9, -S(O)$_{0-2}R_{10}$, and -SO_3H;

R_4 to R_{10} are independently selected from the group consisting of

-H, and -(C_1-C_{15})alkyl;

A_1, A_2 and A_3, are independently selected from the group consisting of

-(C_1-C_{15})alkylene-, -(C_3-C_{10})cycloalkylene-, -(C_2-C_{15})alkenylene-;

A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from -OR_4, -$COOR_5$, and -(C_1-C_{15})alkyl;

and a salt thereof; and

(c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS);

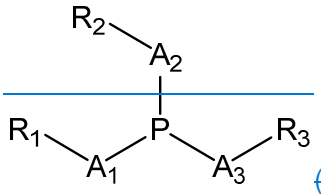
(ii) incubating the solution including the sample at a temperature of at least about 60°C.

~~4. *In vitro* method for isolating nucleic acids from a sample, the method comprising~~

~~(i) contacting the sample with a solution, which comprises~~

~~(a) a buffering substance;~~

~~(b) a reducing agent according to formula (I)~~



~~(I)~~

~~wherein~~

~~R_1, R_2 and R_3 are independently selected from the group consisting of~~

~~-H, $-OR_4$, $-COOR_5$, $-P(O)(OR_6)OR_7$, $-N(R_8)R_9$, $-S(O)_{0-2}R_{10}$, and $-SO_3H$;~~

~~R_4 to R_{10} are independently selected from the group consisting of~~

~~-H, and -$(C_1$-$C_{15})$alkyl;~~

~~A_1, A_2 and A_3, are independently selected from the group consisting of~~

~~-$(C_1$-$C_{15})$alkylene-, -$(C_3$-$C_{10})$cycloalkylene-, -$(C_2$-$C_{15})$alkenylene-;~~

~~A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from $-OR_4$, $-COOR_5$, and -$(C_1$-$C_{15})$alkyl;~~

~~and a salt thereof; and~~

~~(c) an anionic detergent.~~

35~~.~~ The method or use of any one of the preceding claims, wherein the method or use does not include a step of contacting the sample with an enzyme.

46~~.~~ The method or use of any one of the preceding claims, wherein the nucleic acids remain in solution during all method steps.

57~~.~~ The method or use of any one of the preceding claims, wherein the isolated nucleic acids are analyzed by PCR, next generation sequencing, SNP genotyping or RT-PCR.

68~~.~~ The method or use of any one of the proceedings claims, wherein

i) R_1, R_2 and R_3 are independently selected from the group consisting of -H, $-OR_4$, $-COOR_5$, $-P(O)(OR_6)OR_7$, $-S(O)_{0-2}R_{10}$, and $-SO_3H$,
preferably from H, -OH, and -COOH and/or

ii) R_4 to R_{10} are -H, and/or

iii) A_1, A_2 and A_3, are independently selected from the group consisting of
-$(C_1$-$C_9)$alkylene-, -$(C_3$-$C_9)$cycloalkylene-, -$(C_2$-$C_9)$alkenylene-;
preferably -$(C_1$-$C_5)$alkylene-, -$(C_3$-$C_6)$cycloalkylene-, -$(C_2$-$C_5)$alkenylene- and/or

iv) A_1, A_2 and A_3, are independently selected from the group consisting of
-$(C_1$-$C_9)$alkylene- and -$(C_2$-$C_9)$alkenylene-,
preferably -$(C_1$-$C_5)$alkylene-, and -$(C_2$-$C_5)$alkenylene~~)~~-,
more preferably -$(C_1$-$C_3)$alkylene-, and -$(C_2$-$C_3)$alkenylene- and/or

v) A_1, A_2 and A_3 optionally are further substituted with one or more substituents

selected from -OR_4 or (C_1-C_{15})alkyl, preferably (C_1-C_{15})alkyl.

79. The method or use of any one of the preceding claims, wherein the solution further comprises a complexing agent.

~~10. The method or use of of any one of the preceding claims, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS), preferably sodium dodecyl sulfate (SDS).~~

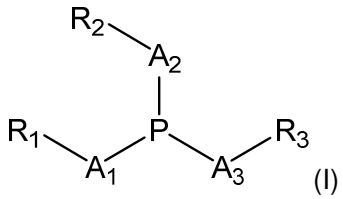
811. The method or use of any one of the preceding claims, wherein the method or use further comprises a step of contacting the sample with a clearing solution, wherein the clearing solution comprises a cationic ion, preferably the cationic ion is K^+, Rb^+, Cs^+, Mg^{++}, Ca^{++}, Sr^{++} or Ba^{++}.

912. The method or use of any one of the preceding claims, wherein the isolating of nucleic acids from a sample is at a temperature of at least about 80 °C for at least 10 seconds or wherein the incubating is at a temperature of at least about 80 °C for at least 10 seconds.

1013. Solution for use in a method or use of any one of the preceding claims, the solution comprising

(a) a buffering substance (BU);

(b) a reducing agent (RA) according to formula (I):



(I)

wherein

R_1, R_2 and R_3 are independently selected from the group consisting of

-H, -OR_4, -$COOR_5$, -$P(O)(OR_6)OR_7$, -$N(R_8)R_9$, -$S(O)_{0\text{-}2}R_{10}$, and -SO_3H;

R_4 to R_{10} are independently selected from the group consisting of

-H, and -(C_1-C_{15})alkyl;

A_1, A_2 and A_3, are independently selected from the group consisting of

-(C_1-C_{15})alkylene-, -(C_3-C_{10})cycloalkylene-, -(C_2-C_{15})alkenylene~~)~~-;

A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from -OR_4, -$COOR_5$, and -(C_1-C_{15})alkyl; and a salt thereof; and

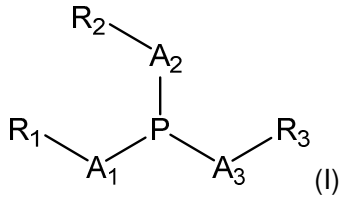
(c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS);

wherein the solution has a temperature of at least about 60 °C.

~~14~~11. Solution comprising a sample for use in a method or use of any one of the preceding claims, wherein the solution comprises

(a) a buffering substance (BU);

(b) a reducing agent (RA) according to formula (I):



(I)

wherein

R_1, R_2 and R_3 are independently selected from the group consisting of

-H, -OR_4, -$COOR_5$, -$P(O)(OR_6)OR_7$, -$N(R_8)R_9$, -$S(O)_{0-2}R_{10}$, and -SO_3H;

R_4 to R_{10} are independently selected from the group consisting of

-H, and -(C_1-C_{15})alkyl;

A_1, A_2 and A_3, are independently selected from the group consisting of

-(C_1-C_{15})alkylene-, -(C_3-C_{10})cycloalkylene-, -(C_2-C_{15})alkenylene~~)~~-;

A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from -OR_4, -$COOR_5$, and -(C_1-C_{15})alkyl; and a salt thereof; and

(c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS);

wherein the solution has a temperature of at least about 60 °C.

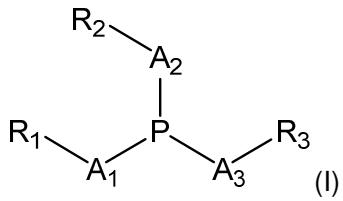
~~15. The solution for use in a method or use of claim 13 or the solution comprising a sample for use in a method or use of claim 14, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS), preferably sodium dodecyl sulfate (SDS).~~

~~16~~12. *In vitro* method for isolating nucleic acids from a sample, the method comprising

(i) contacting the sample with a solution, which comprises

(a) a buffering substance;

(b) a reducing agent according to formula (I)



(I)

wherein

R_1, R_2 and R_3 are independently selected from the group consisting of
-H, $-OR_4$, $-COOR_5$, $-P(O)(OR_6)OR_7$, $-N(R_8)R_9$, $-S(O)_{0-2}R_{10}$, and $-SO_3H$;
R_4 to R_{10} are independently selected from the group consisting of
-H, and $-(C_1-C_{15})$alkyl;
A_1, A_2 and A_3, are independently selected from the group consisting of
$-(C_1-C_{15})$alkylene-, $-(C_3-C_{10})$cycloalkylene-, $-(C_2-C_{15})$alkenylene-;
A_1, A_2 and A_3 optionally are further substituted with one or more substituents selected from $-OR_4$, $-COOR_5$, and $-(C_1-C_{15})$alkyl;
and a salt thereof; and

(c) an anionic detergent, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS);

wherein the solution including the sample has a temperature of at least about 60°C.

~~17. The method of claim 16, wherein the anionic detergent is sodium dodecyl sulfate (SDS) or lithium dodecyl sulfate (LiDS), preferably sodium dodecyl sulfate (SDS).~~

~~18~~13. The method of claim 12 ~~16 or claim 17~~, wherein the method further comprises a step of contacting the sample with a clearing solution, wherein the clearing solution comprises a cationic ion, preferably the cationic ion is K^+, Rb^+, Cs^+, Mg^{++}, Ca^{++}, Sr^{++} or Ba^{++}.

~~19~~14. A kit comprising

(a) a solution of any one of the preceding claims,
(b) optionally means for enzymatic digestion; preferably an enzyme and/or
(c) optionally means for separating of non-nucleic acid components from nucleic acids, preferably a matrix for separating of non-nucleic acid components from nucleic acids.