Method for the selective enrichment of double-stranded DNA from nucleic acid mixtures

The present invention relates to a method for the selective enrichment of double-stranded DNA, in particular of supercoil plasmid DNA, from nucleic acid mixtures. The present invention is concerned with the stripping (removal) of single-stranded nucleic acids, such as for example ribonucleic acid (RNA), denatured genomic deoxyribonucleic acid (DNA) and/or partly denatured open circle plasmid DNA, from preparations containing double-stranded nucleic acids such as supercoil (sc) plasmid DNA.

In the prior art, there are numerous methods for isolating plasmid DNA for therapeutic applications, both on a kit and on a "high-throughput" (HT) scale, and on a production scale, which however in the majority of cases do not deal with a separation of genomic DNA (gDNA) (which under normal conditions is present in a double-stranded form) and/or open circle plasmid DNA (oc pDNA) from sc plasmid DNA (sc pDNA).

However, there are a series of publications, which deal specifically with this subject. In analytical applications, chromatographic methods are mainly to be found for the oc/sc separation of pDNA (e.g. TSKgel, DEAE-NPR and other products of the TSKgel series from Tosoh Biosciences), but capillary gel electrophoretic methods and a series of molecular biological techniques relating to sequence-specific hybridisations (e.g. those called *triple helix,* etc.) are also used.

Some chromatographic technologies (PlasmidSelect resin from GE Healthcare and other HIC resins), which however are characterised by complex handling, high capital costs and above all by low yield efficiencies, are described for preparative processing of pDNA on a pilot and a production scale.

Recently, there have also been publications relating to the use of two-phase separations for the preparative isolation of plasmid DNA. In the works mentioned hereinafter, however, the problem of separating single-stranded DNA from double-stranded DNA has not been described at all or not technically satisfactorily, cf. for example Kepka C, Rhodin J, Lemmens R, Tjerneld F, Gustavsson P-E., 2004, *Extraction of plasmid DNA from Escherichia coli cell lysate in a thermoseparating aqueous two-phase system*1*, Journal of Chromatography A 1024(1-2): 95-104.; Frerix, A., Müller, M., Kula. M.-R., and Hubbuch J. *Scalable recovery of*

plasmid DNA based on aqueous two-phase separation, Biotechnol. Appl. Biochem. (2005) 042, 57-66; Ribeiro S.C., Monteiro G.A., Cabral J.M.S., Prazeres D.M.F., 2002, *Isolation of plasmid DNA from cell lysates by aqueous two-phase systems*, Biotechnology and Bioengineering 78(4): 376-384.

For example, the described methods for oc/sc separation such as hydrophobic interaction chromatography (HIC) and thiophilic chromatography (Lemmens R., Olsson U., Nyhammar T., Stadler J., 2003, *Supercoiled plasmid DNA: selective purification by thiophilic/aromatic adsorption*, Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 784(2): 291-300) and counter-current chromatography (Kendall D., Booth A.J., Levy M.S., Lye G.J., 2001, *Separation of supercoiled and open-circular plasmid DNA by liquid-liquid counter-current chromatography*, Biotechnology Letters 23(8): 613-619) are very time-consuming and very expensive due to low capacities and/or lack of yield, which must be seen as a disadvantage of these methods.

The object of the present invention is therefore to specify a method in which it is made possible to separate selectively (partially) denatured gDNA and oc pDNA as well as other single-stranded nucleic acids from double-stranded nucleic acid(s) such as sc plasmid DNA, without having to accept the above-mentioned disadvantages of the known methods. Especially, the object is to be seen in enabling sc pDNA to be separated from other nucleic acids.

The invention achieves this object by means of the method specified in the independent Claim 1. Further advantageous embodiments of the method according to the invention ensue from the dependent claims, the description, the examples and the drawing.

The invention relates to a method for the specific stripping of single-stranded nucleic acids, such as for example RNA, denatured genomic DNA and partly denatured open circle plasmid DNA, from preparations which likewise contain double-stranded nucleic acids such as supercoil plasmid DNA. In a special case, for example, the present invention enables selective denaturing of gDNA and oc pDNA as well as their subsequent separation by extraction in a two-phase system. This therefore involves polishing right down to double-stranded nucleic acids, such as sc pDNA for example. The method is distinguished by the fact that portions of double-stranded nucleic acids, such as genomic DNA, loop-forming RNA and native double-stranded oc-pDNA, can be completely or partially selectively converted into individual

strands by denaturing, and subsequently selectively separated from double-stranded nucleic acid, such as sc Plasmid-DNA, with high efficiency and capacity in an aqueous two-phase system. The denaturing step can preferably be induced by strongly alkaline conditions (e.g. addition of NaOH, KOH etc.) or heat incubation (e.g. heating to ≥70 °C, in particular ≥80 °C, depending on the GC content of the nucleic acid). In comparison with existing conventional (e.g. chromatographic) methods, the advantages of the invention presented lie particularly in the considerably lower costs, the significantly faster speed of execution and the ability to more easily automate the methodology.

The present invention relates to a method for the selective stripping of partially and completely denatured nucleic acids from double-stranded nucleic acids, in particular sc pDNA. The method is particularly suitable for the manufacture of sc pDNA preparations on a pilot and production scale, e.g. for the manufacture of sc plasmid DNA for human genetic vaccination or for gene therapeutic applications, but because of its simplicity is also suitable for use in manual kit and automated high-throughput (HT) applications, e.g. in diagnostics. The method according to the invention is particularly well suited for the selective stripping of single-stranded nucleic acids and open circle (oc) plasmid DNA from preparations containing supercoil plasmid DNA.

When purifying plasmid molecules for clinical or diagnostic use, the process development is focused on the product quality to be achieved on the one hand and on the resulting preparation costs (*cost-of-goods*, COGs) in relation to this on the other. In this connection, the objective with regard to the required purity of the target molecule (e.g. pDNA) can differ considerably. The desired and necessary degrees of purity in clinical applications are considerably higher by comparison than those required in most diagnostic applications, for example. However, in both fields, the objective of process optimisation is to reduce the costs of cleaning to a minimum in order to make commercial applications possible. This objective can only be achieved by the development of high-resolution purification methods, as these at the same time enable the number of necessary purification steps to be kept as low as possible.

This objective (i.e. The most efficient purification of plasmid DNA possible) becomes all the more difficult the more physico-chemically similar the components to be stripped out are to the target molecule. What is termed open circle (oc) plasmid DNA differs essentially from supercoil (sc) plasmid DNA only by a strand break or a plurality of strand breaks in one

strand or both strands of the double helical structure of the plasmid molecule, which consequently also leads to steric differences between the two topological forms. oc pDNA is produced from sc pDNA predominantly by enzymatic or mechanical nicking of the sc pDNA which is mainly present in vivo. In doing so, an sc pDNA is produced if, before the closing of two individual strands to form one double strand, one of the two strands or both strands are twisted so that, after closing to form the double strand, loops ("supercoils") are formed due to the resulting stresses.

The present invention for the first time enables the desired separation of nucleic acids to be achieved not only highly selectively but also extremely easily, quickly and in particular cost effectively. In doing so, an almost quantitative separation of (partially) single-stranded DNA (e.g. denatured oc pDNA) from double-stranded DNA (e.g. sc pDNA) is achieved after gentle treatment to obtain single and double-stranded nucleic acids in a subsequent two-phase separation, such as is described in WO 2004/106516 A1. This is achieved by means of inexpensive additives, which can be disposed of without any problems, with at the same time high specific capacity of the described invention.

The present invention therefore describes the specific complete or partial denaturing of double-stranded nucleic acids, for instance by the effect of alkaline pHs of 11 or higher, or by means of heat. Such denaturing has the consequence that single-stranded nucleic acids, such as DNA or RNA, follow different distribution coefficients in phase systems compared with double-stranded nucleic acids, such as DNA, due to modified dissolution characteristics.

In contrast to oc pDNA, for example, sc pDNA also denatures during the denaturing phase, but re-natures completely back to the supercoil double helix structure owing to the three-dimensional topology and the resulting steric stabilisation of what is called the supercoil, e.g. after neutralising or cooling. Compared with double-stranded DNA, single-stranded DNA and RNA have a more hydrophobic surface, which is due to the presence of free bases. Owing to the different re-naturing and denaturing characteristics and the resulting structural characteristics, hydrophobicity and charge densities of oc and sc pDNA for example, a highly selective separation of the two plasmid topoisomers can be achieved by extraction in aqueous two-phase systems.

In the present invention, this mechanism is intentionally used to considerably amplify the usually extremely small differences between the surface characteristics of sc pDNA and oc pDNA as well as gDNA by deliberate selective denaturing, as a result of which a later separation can be carried out highly efficiently.

For the present invention, a buffer is added in step (d). A potassium phosphate buffer is preferably used here. In this case, the buffer particularly preferably contains a mixture of K_2HPO_4 and KH_2PO_4. The buffers according to the invention are preferably used with a pH in the range from pH 5.8 to pH 8.5, and particularly preferably with a pH in the range from pH 6.5 to pH 8. For example, a mixture of the stock solution of 3.83 M K_2HPO_4 and 2.45 M KH_2PO_4 and a PEG 800 concentration of 75% w/w (resulting in a pH of ca. 7) can be particularly preferably used in the method according to the invention. In this case, K_2HPO_4 and KH_2PO_4 are used, for example in a total concentration of 5 – 30% (w/w) based on the two-phase system, preferably in a total concentration of 10 - 25% (w/w), and particularly preferably in a total concentration of 20% (w/w). The potassium phosphate is usually added in a temperature range between ice-cold and room temperature. Room temperature as defined by the present invention designates a temperature range of 18 to 25°C. Preferably, an ice-cooled phosphate buffer is used in the method according to the invention. Advantageously, incubation is not necessary after adding the potassium phosphate; mixing which is as complete and uniform as possible of the solution after adding the buffer is the decisive factor. If incubation should be carried out, however, the incubation period is usually about 1 to 15 minutes. Preferably, as mentioned above, the preparation is agitated, for example vigorously shaken, stirred or similar, during and/or after adding the salt components.

The polymer component which is used according to the invention is preferably polyethylene glycol (PEG). The polyethylene glycol is preferably used with a molecular weight having an arithmetic mean of 600 to 1000 g/mol, more preferably having an arithmetic mean of 700 - 900 g/mol and particularly preferably having an arithmetic mean of 750 - 880 g/mol, as one of the two components of the two-phase system. The PEG used in the present invention preferably consists of a mixture of polyethylene glycol with an average molecular weight of 600 g/mol (PEG 600) and polyethylene glycol with an average molecular weight of 1000 g/mol (PEG 1000). Both polyethylene glycols are commercially available (e.g. Fluka, Buchs, Switzerland). In this case, the ready-to-use PEG mixture consists, for example, of 30 – 50% (w/w) PEG 600 and 50 – 70% (w/w) PEG 1000, preferably 33 – 45% (w/w) PEG 600 and 55

– 67% (w/w) PEG 1000, particularly preferably of 36 – 40% (w/w) PEG 600 and 60 – 64% (w/w) PEG 1000 and very particularly preferably of 38% (w/w) PEG 600 and 62% (w/w) PEG 1000.

The concentration of the PEG in the aqueous two-phase system according to the invention is chosen so that two phases form together with the salt components, wherein however the PEG concentration at which the double-stranded DNA, e.g. plasmid DNA, changes from the lower phase, in which it can be found at lower concentrations, to the upper phase is not exceeded. Preferably, the PEG content in the overall mixture is at least 10% (w/w) and is limited in an upwards direction by the concentration of PEG at which the double-stranded DNA (e.g. plasmid DNA) changes from the lower phase, in which it can be found at lower concentrations, to the upper phase. After adding PEG, the solution should preferably have a temperature of about 10 to 50°C, particularly preferably a temperature of about 15 to 40°C. After the formation of the phases, which can take from several minutes to hours depending on the volume of the batch, the double-stranded DNA (e.g. plasmid DNA) will be found in the saline lower phase. Formation of the phases can optionally be accelerated by centrifuging the batch, as a result of which, advantageously, the time required for the method according to the invention is further reduced. The conditions under which such a centrifugation step is carried out are familiar to those skilled in the art.

Compared with solid adsorptive phases, aqueous two-phase systems have the advantage that they have a considerably higher capacity for the double-stranded DNA (such as plasmid DNA) to be purified, which in practice is only limited by the solubility in the phases. Furthermore, the method can be scaled almost at will due to the very simple equipment necessary. But not only automation, but also independently thereof a production on an industrial scale, for example for the production of >> 2 g highly purified cleaned plasmid DNA per batch, can only be achieved easily with the simplifications described here. Likewise, with the present invention, the double-stranded DNA (such as plasmid DNA) can advantageously be freed to a very large extent from RNA and denatured gDNA, which in many applications, particularly in clinical applications, is a to some extent regulatory requirement. Plasmid DNA, which is polished using the method according to the invention, particularly after a primary purification step (for example by means of QIAGEN resin, QIAGEN, Hilden, Germany), is within the approval specifications currently accepted in gene therapy or genetic vaccination. In this way, large quantities of highly pure plasmid DNA can

advantageously be produced with very little outlay on equipment while using non-toxic substances and with comparatively low costs. In this regard, it should be mentioned that, in comparison with other isolation methods, such as for example CsCl density gradient centrifugation or phenol extraction, the substances used in the two-phase system according to the invention are ecologically harmless and can be completely and easily removed from the purified plasmid DNA.

In the method according to the invention, the lower phase, which is produced in step (e) and which contains the double-stranded DNA, is separated from the upper phase, which contains the undesired nucleic acids, whereupon the desired double-stranded DNA can be obtained in enriched form from the lower phase (step (f)). Although a high degree of stripping can be achieved with just a single phase separation, the efficiency of the method according to the invention can be further increased by repeating steps (d) to (f) once to several times or by carrying out the extractions using the counter-current principle. It is expedient, for example, to repeat method steps (d), (e) and (f) one to three times. For extremely highly enriched double-stranded nucleic acid(s), such as sc plasmid DNA, steps (d) to (f) can also be repeated more than three times until the required purity is achieved. The double-stranded DNA (e.g. plasmid DNA) is to be found in the lower phase in each case. Carrying out this optional step leads to a repeated purification of the double-stranded DNA (e.g. plasmid DNA) and therefore to a further stripping of contaminants, such as RNA for example, from the double-stranded DNA (e.g. plasmid DNA).

Subsequently to the method according to the invention, it is expedient to isolate the double-stranded DNA (such as sc plasmid DNA), which is to be found in the lower phase. The isolation and desalination of the (plasmid) DNA from the lower phase produced in step (e) can be carried out by ultrafiltration, diafiltration or gel filtration for example. However, for the purpose of the present invention, any other method known to those skilled in the art can be used for isolation and/or desalination of the (plasmid) DNA from the lower phase.

The aqueous low-molarity buffer used in step (b) is preferably a weak buffer which has only a low ionic strength. The molarity of the buffer used is preferably not more than 100 mM, more preferably not more than 50 mM, and in particular not more than 10 mM.

Examples of suitable aqueous low-molarity buffer systems are a Tris buffer, a Tris/EDTA buffer, phosphate-buffered saline solution (PBS) or a citrate buffer, as well as other buffer systems, which appear to be suitable to those skilled in the art.

For creating denaturing conditions in step (c), the pH of the solution can be increased to 11 or above, or the temperature can be increased to 70°C or above. The pH can be increased in a known manner by adding a strong base such as NaOH or KOH. When increasing the temperature, it is of advantage if the chosen temperature lies between about 70 and 95°C, in particular about 80 and 95°C. The optimum temperature here depends on the GC content of the nucleic acids present.

It has been shown to be particularly advantageous when the method according to the invention is carried out subsequently to a preliminary purification or preliminary separation, wherein any known purification method can be used for the preliminary purification/preliminary separation. A particularly suitable preliminary purification/preliminary separation is an aqueous two-phase separation, for example, such as is known from WO 2004/106516 A1. With the appropriate procedure, a very high degree of purification can be achieved. For instance, in this way it is possible to increase the content of sc pDNA in a sample, based on the existing total quantity of nucleic acid, to 90% and more overall, in particular to 95% and more and even to 99% and more. Another especially suitable method for the preliminary purification/preliminary separation is anion exchange chromatography in which comparable percentage sc pDNA contents can be achieved in a sample.

The present invention is explained in more detail below with reference to examples.

Example 1

This example concerns the denaturing of oc pDNA under alkaline conditions and stripping in the two-phase system.

A pre-purified and concentrated plasmid DNA preparation containing oc pDNA and sc pDNA as well as the further nucleic acids RNA and partially double-stranded gDNA is incubated at a strongly alkaline pH (> 11). Under these conditions, a denaturing (strand separation) of the

DNA double helix and the double-strand RNA (e.g. tRNAs) is achieved, which can only be reversed in a limiting region with intact supercoil DNA. After transfer of the denaturing batch to a buffered phase system which is optimised for the separation of oc/sc pDNA topoisomers, an efficient and high resolution separation of the oc pDNA, gDNA and the partially double-stranded RNA from the sc pDNA target molecule takes place.

This was carried out as follows. 5 g NaOH (0.4 M NaOH) were added to 15 g of a plasmid-containing starting solution (36 µg/ml, determined by means of HPLC). The reaction batch was mixed and incubated at room temperature for 5 min. 20 g of potassium phosphate buffer (50% w/w, pH 7.4) and 10 g PEG 800 (75% w/w) were then added. This composition was in turn mixed well. After mixing, a typical clouding of the batch occurred. The settling of the upper and lower phase can be accelerated by centrifugation (e.g. 5 min at 2000g). The separated lower phase contains the purified sc pDNA while denatured DNA (oc pDNA and gDNA) can be seen as a white "smear" in the phase boundary (interphase, between lower and upper phase).

The results can be seen in the 0.8% agarose gel depicted in Fig. 1. The following can be seen in the figure:

Lanes	Sample
1+2	Starting solution containing plasmid
3+4	Plasmid in desalinated lower phase following alkaline denaturing and two-phase extraction
5	Interphase from the aqueous two-phase separation, resuspended in TE (10mM Tris/Cl, 1mM EDTA, pH 8.0)
6	gDNA, pDNA standard

Samples 1 and 2, and 3 and 4 were applied in duplicate before, and after purification, respectively in identical volumetric ratios. It can be clearly seen from the agarose gel shown in Fig. 1 that the proportion of oc pDNA (third band from the bottom) in the nucleic acids in lanes 3 and 4, which have been subjected to a method according to the invention and which have been removed from the lower phase after aqueous two-phase separation, is greatly reduced compared with lanes 1 and 2, which represent the starting solution. The sc pDNA target molecule (second band from the bottom) is present in a highly purified form. It can also be seen from lanes 3 and 4 of Fig. 1 that the low molecular weight RNA residues (bottom band) of the sample have been practically quantitatively removed as a result of the treatment according to the invention of the nucleic acid sample. Finally, in lane 5 (interphase), only the

low molecular weight RNA residues (bottom band) and denatured DNA can be seen in the form of pocket contamination.

Example 2

This example concerns the denaturing of oc pDNA by heat incubation and stripping in the two-phase system. A total of 350 µl containing pDNA (100 µg/ml) and gDNA (49 µg/ml) were prepared in TE. The batch was then heated to temperatures of 70 to 95°C (in 5°C steps), incubated for 5 min in each case, and subsequently cooled on ice for 5 min in each case. 300 mg of the samples were then mixed with 300 mg TE, and 600 mg phosphate buffer (50%, w/w) and 300 mg PEG (75%, w/w) were added and mixed. Following this, the preparation was centrifuged. The total volume was 1.2 ml, of which 650 µl were accounted for by the lower phase, which contained the purified sc DNA.

The results can be seen in the 0.8% agarose gel depicted in Fig. 2. The following can be seen in the figure:

Lanes	Sample
1	pDNA and gDNA at RT (standard (Std), corresponding to 100% yield)
2	pDNA and gDNA at RT, subsequently aqueous two-phase system
3	5 min at 70°C; subsequently on ice and aqueous two-phase system
4	5 min at 75°C; subsequently on ice and aqueous two-phase system
5	5 min at 80°C; subsequently on ice and aqueous two-phase system
6	5 min at 85°C; subsequently on ice and aqueous two-phase system
7	5 min at 90°C; subsequently on ice and aqueous two-phase system
8	5 min at 95°C; subsequently on ice and aqueous two-phase system

The bottom band of the agarose gel shown in Fig. 2 shows sc pDNA. It can also be seen from the gel that, under the given conditions and for the plasmid pCMVβ used, a denaturing temperature of 80°C with 5 min incubation is sufficient to achieve a practically quantitative separation of gDNA and oc pDNA from sc pDNA.

Example 3

This example relates to the oc pDNA stripping ("polishing") of a vaccination plasmid by means of alkaline denaturing and an aqueous two-phase system following primary purification by means of anion exchange chromatography.

The manufacture of plasmid DNA for gene therapeutic and genetic vaccinations is subject to stringent regulations and exacting specifications. Certain plasmid sequences tend to be "nicked" in preparation, i.e. to acquire single-strand breaks, and therefore to form high proportions of oc pDNA. The resulting proportions of oc pDNA must be stripped after initial purification. In the present case, this stripping was carried out by means of anion exchange chromatography.

The test was carried out as follows. 59.24 g of a plasmid solution (4 mg/ml) were added to 540.8 g of a Tris/EDTA buffer (pH 8). 200 g NaOH (0.4 M) were then added and mixed. The mixture was then incubated for 5 minutes at room temperature. 700 g potassium phosphate buffer (50% w/w, pH 7.4) and 400 g PEG 800 (75% w/w, 60°C) were subsequently added to the mixture. This was again mixed and centrifuged at 3000 x g for 10 min. The lower phase (ca. 900 ml) contains purified sc pDNA, which is converted to suitable formulation solutions by means of ultrafiltration or gel filtration for example.

The results can be seen in the 0.8% agarose gel depicted in Fig. 3. The following can be seen in the figure:

Lane 1 (before "polishing"):	Total pDNA: 236.6 mg;		
	Pocket:	4.1%	mainly gDNA;
	oc:	28.4%	(absolute: 67.2 mg oc pDNA);
	sc:	67.5%	(absolute: 159.7 mg sc pDNA);
Lane 2 (after "polishing")	Total pDNA: 168 mg		
	Pocket:	No detectable signals;	
	oc:	14.9%	(absolute: 25 mg oc pDNA)
	sc:	85.1%	(absolute: 142 mg sc pDNA)

As is shown by a comparison of the two lanes 1 and 2 of the gel shown in Fig. 3, the oc pDNA band after the purification step (after "polishing", see lane 2) is considerably weaker than before the purification step (before "polishing", see lane 2). The quantitative results relating to this can be seen in the above table.

As can be seen from what is presented above, the quantity of oc plasmid pDNA in a biological sample can be reduced from more than 67 mg to 25 mg by means of the method according to the invention, i.e. a reduction of about 62% can be achieved. On the other hand, 142 mg of the 159.7 mg sc pDNA in the initial sample were recovered after carrying out the method according to the invention, which corresponds to a yield of about 89%.

Claims

1. Method for removing single-stranded nucleic acids from double-stranded nucleic acids, characterised by the following steps:

   (a) providing a mixture containing completely and/or partly double-stranded nucleic acids and optionally single-stranded nucleic acids;

   (b) resuspending the mixture from step (a) in an aqueous, low-molarity buffer system with low ionic strength and weak buffer effect;

   (c) adjusting conditions in the mixture from step (b), which lead to a reversible denaturing of a specified double-stranded nucleic acid or a plurality of specified double-stranded nucleic acids, wherein another nucleic acid or a plurality of other nucleic acids are irreversibly denatured;

   (d) further addition of buffer and a polymer component to the mixture from step (c);

   (e) incubation of the mixture from step (d) for a time which is sufficient for the formation of an aqueous two-phase system having an upper and a lower phase; and

   (f) removing the interphase and the upper phase containing the single-strand nucleic acid, and collection of the double-strand nucleic acid from the lower phase.

2. Method according to Claim 1, characterised in that the mixture from step (a) contains supercoil (sc) plasmid DNA.

3. Method according to Claim 1 or 2, characterised in that the specified double-stranded nucleic acid from step (c), which can be reversibly denatured, is supercoil (sc) plasmid DNA.

4. Method according to one of Claims 1 to 3, characterised in that the mixture from step (a) contains open circle (oc) plasmid DNA.

5. Method according to one of Claims 1 to 4, characterised in that, in step (f), single-stranded oc plasmid DNA in the upper phase is separated from double-stranded sc plasmid DNA in the lower phase.

6. Method according to one of the preceding claims, characterised in that the aqueous low-molarity buffer system according to step (b) with a low ionic strength and a weak buffer effect has a molarity of up to 100 mM.

7. Method according to one of the preceding claims, characterised in that the aqueous low-molarity buffer system according to step (b) is a Tris buffer, a Tris/EDTA buffer, phosphate-buffered saline solution (PBS), or a citrate buffer.

8. Method according to one of the preceding claims, characterised in that the denaturing conditions according to step (c) are produced by increasing the pH to 11 or higher with subsequent sufficient incubation.

9. Method according to one of the preceding claims, characterised in that the denaturing conditions according to step (c) are produced by increasing the temperature to 70°C or above and that immediate cooling takes place on completion of incubation.

10. Method according to one of the preceding claims, characterised in that the additional buffer added according to step (d) is a potassium phosphate buffer.

11. Method according to one of the preceding claims, characterised in that the polymer added according to step (d) is a polyethylene glycol (PEG), preferably a PEG with a molecular weight of 600 to 1000 g/mol, more preferably of 700 to 900 g/mol, and in particular of 750 to 880 g/mol, in each case based on the arithmetic mean.

12. Method according to one of the preceding claims, characterised in that the enriched double-stranded nucleic acid from the lower phase is further concentrated by ultrafiltration or gel filtration.

13. Method according to Claim 12, characterised in that the enriched double-stranded nucleic acid in the lower phase is sc plasmid DNA.

14. Method according to one of the preceding claims, characterised in that steps (d), (e) and (f) are repeated at least once.

15. Method according to Claim 14, characterised in that steps (d), (e) and (f) are repeated three times.

16. Method according to one of the preceding claims, characterised in that only the lower phase in step (e) contains sc plasmid DNA.

17. Method according to one of the preceding claims, characterised in that the method is carried out subsequently to a preliminary separation/preliminary purification.

18. Method according to Claim 17, characterised in that the preliminary separation/preliminary purification is an aqueous nucleic acid two-phase separation or an anion exchange chromatography.