agarose electrophoresis. FIG. 1 shows the electrophoresis results. Referring to FIG. 1, the left lane (M) is the electrophoresis results of a 1 kbp Ladder DNA marker, and the right lane (S) is the electrophoresis results of the eluate. As shown in FIG. 1, the eluate contained merely DNA of a long chain length, and particularly a band smaller than a 1 kbp marker was not observed at all. From these results, it was demonstrated that long-chain nucleic acids are selectively adsorbed and short-chain nucleic acids are not adsorbed in the case where nucleic acids are adsorbed by a nucleic acid adsorbent from the mixture of the first buffer and the whole blood sample used in the nucleic acid recovery method according to the present embodiment. That is, it was found that there is no risk of unintentional recovery of short-chain nucleic acids including circular DNA in the step (a) of the nucleic acid recovery method according to the present embodiment.

[0070]

[Reference Example 4]

The effect of alcohol concentration on the strength of DNA of 200 bp, 500 bp or 1000 bp to adsorb onto a nucleic acid adsorbent was examined. dsDNA fragment standard samples of 200 bp, 500 bp and 1000 bp used in Reference Example 1 were employed as the dsDNA. In addition, alcohol-free buffer 4-1 (3 mol/L of guanidine hydrochloride, and 10 volume% of Triton X), 5 volume% alcohol-containing buffer 4-2 (3 mol/L of guanidine hydrochloride, 10 volume% of Triton X, and 5 volume% of isopropanol), and 10 volume% alcohol-containing buffer 4-3 (3 mol/L of guanidine hydrochloride, 10 volume% of Triton X, and 10 volume% of isopropanol) were used as a buffer for dissolving DNA.

[0071]

Specifically, a DNA solution where 1000 ng of DNA had been dissolved in buffer 4-1, 4-2 or 4-3 was passed through a silica membrane filter to adsorb the DNA

CLAIMS

1. (Currently amended) A method of recovering short-chain nucleic acids having a length of 1000 bases or less from a whole blood sample, comprising:

a step (a) of bringing a mixture having an alcohol concentration of 0 to 5 volume%, the mixture being prepared by mixing a whole blood sample with a first buffer containing one or more members selected from the group consisting of a chaotropic agent and a surfactant, into contact with a first nucleic acid adsorbent to adsorb nucleic acids derived from the whole blood sample onto the first nucleic acid adsorbent, and then separating and removing the first nucleic acid adsorbent to recover liquid components;

a step (b) of bringing a mixture having an alcohol concentration of 10 volume% or higher, the mixture being prepared by mixing the liquid components recovered in the step (a) with a second buffer containing an alcohol and at least one of a chaotropic agent and a surfactant, into contact with a second nucleic acid adsorbent to adsorb short-chain nucleic acids having a length of 1000 bases or less derived from the whole blood sample onto the second nucleic acid adsorbent, and then separating and recovering the second nucleic acid adsorbent from the recovered liquid components; and

a step (c) of eluting and recovering nucleic acids from the second nucleic acid adsorbent recovered in the step (b), wherein

when the first buffer contains the alcohol, the alcohol contained in the first buffer is at least one of branched or linear alkanols having 1 to 5 carbon atoms,

the alcohol contained in the second buffer is at least one of branched or linear alkanols having 1 to 5 carbon atoms,

the chaotropic agent contained in the first buffer is one or more compounds selected from a guanidine salt, sodium iodide, and sodium perchlorate, and

the chaotropic agent contained in the second buffer is one or more compounds selected from a guanidine salt, sodium iodide, and sodium perchlorate.

2. (Original) The method of recovering short-chain nucleic acids according to Claim 1, wherein a nucleic acid having a length of 5000 bases or more derived from the whole blood sample is separated and removed by adsorbing the nucleic acid having a length of 5000 bases or more onto a nucleic acid adsorbent in the step (a).

3. (Currently amended) The method of recovering short-chain nucleic acids according to Claim 1 or 2, wherein the first buffer contains guanidine hydrochloride as the chaotropic agent and a surfactant, a concentration of guanidine hydrochloride is higher than or equal to 1 mol/L and lower than or equal to a maximum solubility, and a concentration of the surfactant is 1 to 10 volume%.

4. (Currently amended) The method of recovering short-chain nucleic acids according to any one of Claims 1 to 3, wherein the alcohol contained in the first buffer and the alcohol contained in the second buffer are isopropanol or ethanol, and

an alcohol concentration in the mixture of the liquid components recovered in the step (a) and the second buffer in the step (b) is 10 to 60 volume%.

5. (Currently amended) The method of recovering short-chain nucleic acids according to any one of Claims 1 to 3, wherein the alcohol contained in the first buffer and the alcohol contained in the second buffer are isopropanol, and an isopropanol concentration in the mixture of the liquid components recovered in the step (a) and the second buffer in the step (b) is 10 to 40 volume%.

6. (Currently amended) The method of recovering short-chain nucleic acids according to any one of Claims 1 to 5, wherein the second buffer contains one or more chaotropic agents selected from the group consisting of guanidine thiocyanate and guanidine isothiocyanate as the chaotropic agent and a surfactant, a concentration of the chaotropic agent is higher than or equal to 0.5 mol/L and lower than or equal to a maximum solubility, and a concentration of the surfactant is 3 to 15 volume%.

7. (Original) The method of recovering short-chain nucleic acids according to any one of Claims 1 to 6, wherein the second buffer contains 10 to 200 mmol/L of a cation salt.

8. (Original) The method of recovering short-chain nucleic acids according to any one of Claims 1 to 7, wherein the step (b) is

a step (b') of adding a proteolytic enzyme to the liquid components recovered in the step (a) to carry out an enzymatic reaction, and

a step (b") of bringing the mixture of the liquid components and the second buffer into contact with the second nucleic acid adsorbent to adsorb the short-chain nucleic acids having a length of 1000 bases or less derived from the whole blood sample onto the second nucleic acid adsorbent, and then separating and recovering the second nucleic acid adsorbent, after the step (b').

9. (Original) The method of recovering short-chain nucleic acids according to any one of Claims 1 to 8, wherein the first nucleic acid adsorbent or the second nucleic acid adsorbent is one or more members selected from the group consisting of a silicic acid-containing material, zeolite, aluminum oxide, titanium dioxide, zirconium dioxide, kaolin, magnetic particles, ceramics, and polystyrene.

10. (Original) The method of recovering short-chain nucleic acids according to Claim 9, wherein the silicic acid-containing material is silica, magnetic silica, glass, or silicon dioxide.

11. (Original) The method of recovering short-chain nucleic acids according to any one of Claims 1 to 10, wherein the short-chain nucleic acid is circulating DNA.

12. (Original) A method of purifying short-chain nucleic acids from a whole blood sample, using the method of recovering short-chain nucleic acids according to any one of Claims 1 to 11.

13. (Currently amended) A kit of recovering short-chain nucleic acids used in the method for recovering short-chain nucleic acids according to any one of Claims 1 to 11, comprising:

a first buffer which at least one of a chaotropic agent and a surfactant;

a second buffer which contains an alcohol and at least one of a chaotropic agent and a surfactant; and

nucleic acid adsorbents, wherein

the alcohol contained in the second buffer is at least one of branched or linear alkanols having 1 to 5 carbon atoms,

the chaotropic agent contained in the first buffer is one or more compounds selected from a guanidine salt, sodium iodide, and sodium perchlorate, and

the chaotropic agent contained in the second buffer is one or more compounds selected from a guanidine salt, sodium iodide, and sodium perchlorate.

14. (Original) The kit of recovering short-chain nucleic acids according to Claim 13, which is configured to recover circulating DNA from a whole blood sample.