

length of 5000 bases or more onto a nucleic acid adsorbent in the step (a).

[3] The first buffer may contain guanidine hydrochloride and a surfactant, a concentration of guanidine hydrochloride may be higher than or equal to 1 mol/L and lower than or equal to a maximum solubility, and a concentration of the surfactant may be 1 to 10 volume%.

[4] The alcohol may be 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) may be 10 to 60 volume%.

[5] The alcohol may be 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) may be 10 to 40 volume%.

[6] The second buffer may contain one or more chaotropic agents selected from the group consisting of guanidine thiocyanate and guanidine thiocyanate and a surfactant, a concentration of the chaotropic agent may be higher than or equal to 0.5 mol/L and lower than or equal to a maximum solubility, and a concentration of the surfactant may be 3 to 15 volume%.

[7] The second buffer may contain 10 to 200 mmol/L of a cation salt.

[8] The step (b) may be 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 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] The first nucleic acid adsorbent or the second nucleic acid adsorbent may be

incorporated into such a blood-collected organism, or an artificial or synthetic nucleic acid. Examples of the artificial or synthetic nucleic acid include cosmids, vectors, cDNAs, and fragments thereof.

[0051]

5 The nucleic acid recovery method according to the present embodiment can efficiently recover short-chain nucleic acids having a length of 1000 bases or less.

 With respect to the nucleic acid recovery method according to the present embodiment, a desired nucleic acid to be recovered is preferably a short-chain nucleic acid having a length of 800 bases or less, more preferably a short-chain nucleic acid
10 having a length of 500 bases or less, or still more preferably a short-chain nucleic acid having a length of 200 bases. The nucleic acid recovery method according to the present embodiment is suitable for concentrating and purifying circulating DNAs in whole blood, non-coding RNAs, miRNAs, short-chain synthetic nucleic acids, and the like.

15 [0052]

 The nucleic acid recovery method according to the present embodiment is particularly suitable for purifying circulating DNAs in whole blood. In a conventional method, tumor-derived nucleic acids freely circulating in peripheral blood have been purified and recovered from the serum or plasma, whereas in the nucleic acid recovery
20 method according to the present embodiment, freely circulating tumor-derived nucleic acids can be purified directly from a whole blood sample, taking advantage of the selectivity of a nucleic acid to be adsorbed onto a nucleic acid adsorbent. In addition, the nucleic acid recovery method according to the present embodiment can also be automated, since the operation of individual steps is relatively simple. Further,
25 circulating DNAs recovered by the nucleic acid recovery method according to the present

3. The method of recovering short-chain nucleic acids according to Claim 1 or 2, wherein the first buffer contains guanidine hydrochloride 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. The method of recovering short-chain nucleic acids according to any one of Claims 1 to 3, wherein the alcohol is 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. The method of recovering short-chain nucleic acids according to any one of Claims 1 to 3, wherein the alcohol is 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. 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 thiocyanate 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. The method of recovering short-chain nucleic acids according to any one of

length of 5000 bases or more onto a nucleic acid adsorbent in the step (a).

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10 mixture of the liquid components recovered in the step (a) and the second buffer in the step (b) may be 10 to 40 volume%.

[6] The second buffer may contain one or more chaotropic agents selected from the group consisting of guanidine thiocyanate and guanidine isothiocyanate and a surfactant, a concentration of the chaotropic agent may be higher than or equal to 0.5
15 mol/L and lower than or equal to a maximum solubility, and a concentration of the surfactant may be 3 to 15 volume%.

[7] The second buffer may contain 10 to 200 mmol/L of a cation salt.

[8] The step (b) may be 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'')
20 of bringing the mixture of the liquid components and the second buffer into contact with the 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, after the step (b').

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4. The method of recovering short-chain nucleic acids according to any one of Claims 1 to 3, wherein the alcohol is 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. The method of recovering short-chain nucleic acids according to any one of Claims 1 to 3, wherein the alcohol is 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. 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 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%.
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