Fundamentals and applications." Journal of Chromatography A., Vol.1184 (2008)p.504-541

Summary of Invention

Problem to be solved by the Invention

[0007] The method of the past using phenol/chloroform/ethanol had a necessity of using harmful organic solvents and it took trouble for centrifugation and the like. The method using a column, filter, or the like, for adsorbing nucleic acids had a problem that the column, filter, or the like is prone to clogging, and it also had a problem in terms of ease of operation.

[0008] Accordingly, a main object of the present disclosure is to provide a method of electrophoresing nucleic acids, which is capable of highly efficiently concentrating and purifying nucleic acids in a short time by simple operation.

Means for solving the Problem

[0009] In order to solve the problems described above, the present disclosure provides a method of electrophoresing nucleic acids, which method includes electrophoresing a nucleic acid in which an intercalator having an anionic functional group has been inserted.

[0009a] In one aspect, there is provided a method of electrophoresing nucleic acids, the method comprising: electrophoresing a nucleic acid in a polymer gel comprising an anionic functional group having a pKa of from 1 to 5, in which an intercalator having an anionic functional group has been inserted in said nucleic acid.

In this step of the method of electrophoresing nucleic acids, it is desirable that an acid

group has been inserted.

[0009b] In another aspect, there is provided a method of concentrating and purifying nucleic acids, the method comprising: electrophoresing a nucleic acid in a polymer gel comprising an anionic functional group having a pKa of from 1 to 5, and wherein an intercalator having an anionic functional group has been inserted in said nucleic acid.

[0010] ~~Still~~In a further aspect, the present disclosure provides a cartridge for nucleic acid electrophoresis, including a channel formed to be capable of introducing a liquid; a damming portion, which dams substances, disposed at a predetermined position of the channel; electrodes disposed at both ends of the channel; an intercalator that can be inserted to a nucleic acid, having an anionic functional group; and a buffer; which intercalator and the buffer are housed between the damming portion and a negative-electrode end.

[0011] In yet another aspect, there is provided a method of producing a cartridge for nucleic acid electrophoresis, the method comprising: allowing a monomer solution, introduced in a channel formed on a substrate, to gel into a predetermined shape, by photopolymerization, to form a polymer gel, said polymer gel comprising an anionic functional group having a pKa of from 1 to 5 ; and housing an intercalator that can be inserted to a nucleic acid, having an anionic functional group, into the channel. Furthermore, the present disclosure provides a method of producing a cartridge, which method includes allowing a monomer solution, introduced in a channel formed on a substrate, to gel into a predetermined shape, by

photopolymerization, to form the polymer gel formation or disposing a dialysis membrane; and housing an intercalator that can be inserted to a nucleic acid, having an anionic functional group, into the channel.

[0012] In order to solve the problems described above, the present disclosure provides a method of electrophoresing nucleic acids, which method includes mixing a sample containing a nucleic acid, a compound having a functional group which undergoes dehydration condensation reaction with a carboxyl group included in

a substance contained in the sample and a condensing agent of the dehydration condensation reaction; and electrophoresing the nucleic acid.

[0012a] In another aspect, there is provided a method of electrophoresing nucleic acids, the method comprising: mixing a sample containing a nucleic acid, a compound having a functional group which undergoes dehydration condensation reaction with a carboxyl group included in a substance contained in the sample, and a condensing agent of the dehydration condensation reaction; and electrophoresing the nucleic acid in a polymer gel comprising an anionic functional group having a pKa of from 1 to 5, in which an intercalator having an anionic functional group has been inserted in said nucleic acid.

In these steps of the method of electrophoresing nucleic acids, it is desirable that the functional group be an amino group.

Further, in this method of electrophoresing nucleic acids, it is desirable that the compound be ethanolamine or ethylenediamine.

Still further, in this method of electrophoresing nucleic acids, it is desirable that the condensing agent include at least one selected from 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride salt, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride, dicyclohexylcarbodiimide and diisopylcarbodiimide.

Still further, in this method of electrophoresing nucleic acids, it is desirable that the compound have at least a first cationic functional group which undergoes dehydration

condensation reaction with a carboxyl group and a second cationic functional group which exists in an ionic state during the electrophoresis.

Still further, in this method of electrophoresing nucleic acids, it is desirable to include the steps of applying a voltage across a channel filled with a

buffer so that the nucleic acid introduced at a predetermined position in the channel is electrophoresed; and damming the nucleic acid moving to a positive-electrode end. It is desirable that a pH of the buffer be 8 or less.

Still further, in this method of electrophoresing nucleic acids, it is desirable that the sample include at least one of a living body-originated sample containing at least one of throat swab, nasal swab, vaginal swab, blood, plasma, serum, tissue, tissue fragment, sputum, spinal fluid, urine, sweat, and feces; or a sample containing at least one of cell culture liquid, bacterial broth, liquid in food, and soil.

Still further, the present disclosure provides a method of concentrating and purifying nucleic acids, which method includes mixing a sample containing a nucleic acid, a compound having a functional group which undergoes dehydration condensation reaction with a carboxyl group included in a substance contained in the sample and a condensing agent of the dehydration condensation reaction; and electrophoresing the nucleic acid.

[0012b] In a further aspect, there is provided a method of concentrating and purifying nucleic acids, the method comprising: mixing a sample containing a nucleic acid, a compound having a functional group which undergoes dehydration condensation reaction with a carboxyl group included in a substance contained in the sample and a condensing agent of the dehydration condensation reaction; and electrophoresing the nucleic acid in a polymer gel comprising an anionic functional group having a pKa of from 1 to 5, in which an intercalator having an anionic functional group has been inserted in said nucleic acid.

[0013] Still further, the present disclosure provides a cartridge for nucleic acid electrophoresis, including a channel formed to be capable of introducing

a liquid; a polymer gel disposed at a predetermined position of the channel; electrodes disposed at both ends of the channel; a compound having a functional group which undergoes dehydration condensation reaction with a carboxyl group; a condensing agent of the dehydration condensation reaction; and a buffer; which compound, the condensing agent and the buffer are housed in the channel.

[0013a] In yet another aspect, there is provided a cartridge for nucleic acid electrophoresis, comprising: a channel formed to be capable of introducing a liquid; a damming portion, which dams substances, disposed at a predetermined position of the channel; electrodes disposed at both ends of the channel; an intercalator that can be inserted to a nucleic acid, the intercalator having an anionic functional group; a compound having a functional group which undergoes dehydration condensation reaction with a carboxyl group; a condensing agent of the dehydration condensation reaction; and a buffer; the compound, the condensing agent, the intercalator and the buffer being housed between the damming portion and a negative-electrode end.

[0014] In another aspect, there is provided a method of producing a cartridge for nucleic acid electrophoresis, the method comprising: allowing a monomer solution, introduced in a channel formed on a substrate, to gel into a predetermined shape, by photopolymerization, to form a polymer gel comprising an anionic functional group having a pKa of from 1 to 5; and housing a compound having a functional group which undergoes dehydration condensation reaction with a carboxyl group, a

condensing agent of the dehydration condensation reaction, an intercalator that can be inserted to a nucleic acid, the intercalator having an anionic functional group, and a buffer, into the channel. Furthermore, the present disclosure provides a method of producing a chip for concentrating and purifying nucleic acids, which method includes allowing a monomer solution, introduced in a channel formed on a substrate, to gel into a predetermined shape, by photopolymerization, to form the polymer gel or dispose a dialysis membrane; and housing a compound having a functional group which undergoes dehydration condensation reaction with a carboxyl group, a condensing agent of the dehydration condensation reaction, and a buffer, into the channel.

Effect of the Invention

[0015] According to the present disclosure, there is provided a method of electrophoresing nucleic acids, which is capable of concentrating and recovering nucleic acids with high efficiency in a short time by simple operation.

Brief Description of Drawings