

CLAIMS

【Claim 1】

A composition for determining whether a nucleic acid to be determined is methylated, comprising a primer set for amplifying a target site of the nucleic acid to be determined,

wherein the primer set comprises

(1) a methylation forward primer, an unmethylation forward primer, and a counter direction primer; or

(2) a methylation reverse primer, an unmethylation reverse primer, and a counter direction primer,

wherein the methylation forward primer and the methylation reverse primer comprise a CpG recognition site which recognizes a CpG sequence of the nucleic acid to be determined to be determined,

wherein the unmethylation forward primer and the unmethylation reverse primer comprise a TpG recognition site which recognizes a TpG sequence of the nucleic acid to be determined, and

wherein the TpG sequence is converted from the CpG sequence of the nucleic acid to be determined.

【Claim 2】

The composition according to claim 1, wherein the TpG sequence is converted by an agent which modifies a methylated nucleic acid to be determined and an unmethylated nucleic acid to be determined differently each other.

【Claim 3】

The composition according to claim 2, wherein the agent converts an unmethylated cytosine residue into thymine.

【Claim 4】

The composition according to claim 2, wherein the agent is at least one selected from the group consisting of sulfurous acid, bisulfite, hydrogen sulfite and disulfite.

【Claim 5】

The composition according to claim 1, wherein the CpG recognition site or the TpG recognition site is located within 40 bases from the 3' end of the methylation forward primer, the methylation reverse primer, the unmethylation forward primer, or the unmethylation reverse primer.

【Claim 6】

The composition according to claim 1, wherein the target site comprises a CpG sequence.

【Claim 7】

The composition according to claim 1, wherein the target site is 50 to 150 bp in size.

【Claim 8】

The composition according to claim 1, wherein the primer has a size of comprising 15 to 40 bases.

【Claim 9】

The composition according to claim 1, wherein the counter direction primer

comprises 5 or less of CpG or TpG recognition sites of the nucleic acid to be determined.

【Claim 10】

The composition according to claim 1, wherein the T_m difference of the methylation forward primer and unmethylation forward primer of (1); or the T_m difference of the methylation reverse primer and unmethylation reverse primer of (2) is 15°C or less.

【Claim 11】

The composition according to claim 1, wherein
the methylation forward primer and unmethylation forward primer of (1); or
the methylation reverse primer and unmethylation reverse primer of (2)
are comprised at a concentration ratio of 100:1 to 1:100.

【Claim 12】

The composition according to claim 1, wherein the nucleic acid to be determined is comprised in a biological sample.

【Claim 13】

The composition according to claim 12, wherein the biological sample comprises at least one selected from the group consisting of blood, serum, tissue, cells, feces and urine.

【Claim 14】

The composition according to claim 12, wherein the biological sample comprises a methylated nucleic acid to be determined and an unmethylated nucleic acid to be determined.

【Claim 15】

The composition according to claim 14, wherein the concentration of the methylated nucleic acid to be determined is 75% or less of the concentration of the unmethylated nucleic

acid to be determined.

【Claim 16】

The composition according to claim 1, wherein the nucleic acid to be determined comprises a biomarker for diagnosing cancer.

【Claim 17】

The composition according to claim 16, wherein the cancer comprises at least one selected from the group consisting of liver cancer, large intestine cancer, esophageal cancer, stomach cancer, rectal cancer, colorectal cancer, oral cancer, pharyngeal cancer, laryngeal cancer, lung cancer, non-small cell lung cancer, colon cancer, breast cancer, cervical cancer, endometrial cancer, ovarian cancer, prostate cancer, testis cancer, bladder cancer, kidney cancer, liver cancer, pancreatic cancer, biliary tract cancer, bone cancer, connective tissue cancer, skin cancer, melanoma, brain cancer, head and neck cancer, thyroid cancer, leukemia, Hodgkin disease, lymphoma, urinary tract cancer, and multiple myeloma blood cancer.

【Claim 18】

The composition according to claim 1, wherein the target site is a biomarker located in a CpG island.

【Claim 19】

A method for determining whether a nucleic acid to be determined is methylated, comprising

a step of modifying a methylated nucleic acid to be determined and an unmethylated nucleic acid to be determined differently from each other, wherein the nucleic acid to be determined is comprised in a biological sample; and

a step of amplifying a target site by treating the biological sample with the composition according to any one of claims 1 to 18.

【Claim 20】

The method according to claim 19, wherein the modifying is performed by treating the biological sample with an agent which modifies a methylated nucleic acid to be determined and an unmethylated nucleic acid to be determined differently from each other.

【Claim 21】

The method according to claim 19, wherein the biological sample is at least one selected from the group consisting of blood, serum, tissue, cells, feces and urine.

【Claim 22】

The method according to claim 19, further comprising a step of quantifying methylation level of the nucleic acid to be determined.

【Claim 23】

The method according to claim 22, wherein the step of quantifying is quantifying the methylation level of the biological sample, by comparing AUC (Area under the curve) of a normalized melt curve of the biological sample, a sample in which the nucleic acid to be determined is 100% methylated, and a sample in which the nucleic acid to be determined is 100% unmethylated.

【Claim 24】

The method according to claim 19, further comprising
a step of obtaining a melt curve of the biological sample;
a step of obtaining a normalized melt curve of a biological sample in which the

methylation ratio of the nucleic acid to be determined is identified; and

a step of quantifying methylation level of the biological sample, by comparing the melt curve of the biological sample and the normalized melt curve.

【Claim 25】

The method according to claim 19, wherein the target site comprises a biomarker located in a CpG island.

【Claim 26】

A method for providing information for diagnosing cancer, comprising
a step of modifying a methylated nucleic acid to be determined and an unmethylated nucleic acid to be determined differently from each other, in a nucleic acid to be determined comprised in a biological sample;

a step of amplifying by treating the biological sample with the composition according to any one of claims 1 to 18; and

a step of determining whether a nucleic acid to be determined is methylated,
wherein the nucleic acid to be determined comprises a biomarker for diagnosing cancer.

【Claim 27】

The method according to claim 26, wherein the biomarker nucleic acid for diagnosing cancer is methylated or unmethylated in a cancer patient.

【Claim 28】

The method according to claim 26, further comprising a step of comparing the methylation level of the nucleic acid to be determined with the methylation level of a control

group.

【Claim 29】

The method according to claim 26, wherein the cancer comprises one or more selected from the group consisting of

liver cancer, large intestine cancer, esophageal cancer, stomach cancer, rectal cancer, oral cancer, pharyngeal cancer, laryngeal cancer, lung cancer, colon cancer, breast cancer, cervical cancer, endometrial cancer, ovarian cancer, prostate cancer, testis cancer, bladder cancer, kidney cancer, liver cancer, pancreatic cancer, bone cancer, connective tissue cancer, skin cancer, brain cancer, thyroid cancer, leukemia, Hodgkin disease, lymphoma, and multiple myeloma blood cancer.

【Claim 30】

The method according to claim 26, wherein the biomarker for diagnosing cancer is located in a CpG island.

【Claim 31】

A kit for determining whether a nucleic acid to be determined is methylated, comprising the composition according to any one of claims 1 to 18.

【Claim 32】

A composition for diagnosing cancer, comprising the composition according to any one of claims 1 to 18, wherein the nucleic acid to be determined comprises a cancer biomarker.

【Claim 33】

A kit for diagnosing cancer, comprising the composition according to claim 32.