

Micronuclei DNA from peripheral red blood cells and uses thereof

TECHNICAL FIELD

[0001] The present disclosure relates to the fields of biology, medicine and bioinformatics. Particularly, the present disclosure relates to peripheral red blood cell micronuclei DNA and its application in cancer detection.

BACKGROUND

[0002] Cancer is one of the main diseases threatening human health and life. It is reported that, in 2018, there were 18.1 million new cancer cases and 9.6 million cancer deaths worldwide. Nearly half of new cancer cases and more than half of cancer deaths occurred in Asia (Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. Bray Freddie et al., CA: A Cancer Journal for Clinicians. 2018). Despite decades of continuous exploration, progress has been made in the diagnosis and treatment of cancer, but there is still a huge demand for cancer detection, especially the screening, diagnosis, classification and staging of cancer.

[0003] Blood continuously circulates in the body, and the total blood of normal adults accounts for about 8% of the body weight of men and about 7.5% of the body weight of women. Peripheral blood samples are easy to collect, store and transport and have high stability (Dagur, P.K. and J.J. McCoy, Collection, Storage, and Preparation of Human Blood Cells. Curr Protoc Cytom, 2015. 73: p. 5.1.1-16).

[0004] Micronuclei is usually considered as a small nuclear structure formed when chromosomes or chromosome fragments are not incorporated into one of the daughter nuclei during cell division, which is usually a sign of genotoxicity events and chromosome instability. Generally, it is a small nuclear structure formed outside the main nucleus which is independent of the main nucleus, due to the incorrect repair or unrepaired DNA breakage, or lagging asymmetric chromosome or chromatid fragment caused by chromosome non-separation (Liu, S., et al., Nuclear envelope assembly defects link mitotic errors to chromothripsis. Nature, 2018. 561(7724): p. 551-555).

[0005] Up to now, there is no report on the micronuclei DNA isolated or purified from peripheral red blood cells, and there is no report on the use of peripheral red blood cell micronuclei DNA for cancer detection.

SUMMARY

[0006] Generally, the present disclosure relates to micronuclei DNA isolated or purified from peripheral red blood cells, its extraction method, and its application in screening, diagnosis, typing and/or staging of diseases.

[0007] The first aspect of the present disclosure relates to micronuclei DNA isolated or purified from peripheral red blood cells.

[0008] In some embodiments, the micronuclei DNA isolated or purified from peripheral red blood cells does not contain or substantially does not contain nucleated cell genomic DNA.

[0009] In some embodiments, the peripheral blood is human peripheral blood. In a specific embodiment, the peripheral blood is fresh human peripheral blood.

[0010] In some embodiments, the micronuclei DNA is used for cancer detection, such as early screening, diagnosis, typing and/or staging of cancer. In some specific embodiments, the micronuclei DNA is used for diagnosis of pan-cancer patients, including but not limited to patients suffering from colorectal cancer (also referred to as "CRC" hereinafter), hepatocellular cancer (also referred to as "HCC" hereinafter) or lung cancer (also referred to as "LC" hereinafter).

[0011] In some embodiments, the micronuclei DNA is used for early screening, diagnosis, typing and/or staging of cervical cancer.

[0012] In some embodiments, the micronuclei DNA is used for early screening, diagnosis, typing and/or staging of cervical cancer, and the micronuclei DNA comprises a gene classifier shown in Table 2, 4 or 6.

[0013] In other embodiments, the micronuclei DNA is used for early screening, diagnosis, typing and/or staging of colorectal cancer.

[0014] In a further embodiment, the micronuclei DNA is used for early screening, diagnosis, typing and/or staging of colorectal cancer, and the micronuclei DNA comprises a gene classifier shown in Table 8 or 10.

[0015] In some further embodiments, the micronuclei DNA is used for early screening, diagnosis, typing and/or staging of hepatocellular cancer.

[0016] In some even further embodiments, the micronuclei DNA is used for early screening, diagnosis, typing and/or staging of lung cancer.

[0017] In some even further embodiments, the micronuclei DNA is used for discriminating power between each of the two cancer patient groups: CRC vs. HCC, LC vs. HCC, LC vs. CRC.

[0018] In some even further embodiments, the micronuclei DNA is used for the multiclass discrimination of different types of cancers. In a specific embodiment, the micronuclei DNA is used for the multiclass discrimination of HD ("health donors"), HCC, LC and CRC.

[0019] The second aspect of the present disclosure relates to a method for isolating or purifying micronuclei DNA from peripheral red blood cells, which comprises the following steps:

- a) providing peripheral blood samples;
- 5 b) isolating mononuclear cells and red blood cells from peripheral blood samples;
- c) collecting red blood cells;
- d) treating collected red blood cells with a red blood cell lysis buffer; and
- e) extracting micronuclei DNA from the lysed red blood cells.

10 In a specific embodiment, the collected red blood cells are subjected to two or more sequential filtrations, e.g., filtrations by cell strainers, e.g., filtrations by 10 μ m cell strainers.

[0020] In some embodiments, the red blood cell lysis buffer specifically lyses red blood cells by changing the osmotic pressure of cell suspension, but does not lyse nucleated cells.

[0021] In some embodiments, the red blood cell lysis buffer comprises NH₄Cl, NaHCO₃, EDTA or a combination thereof.

15 [0022] In some embodiments, micronuclei DNA is extracted from the lysed red blood cells by a DNA extraction reagent. In certain embodiments, the DNA extraction reagent comprises a protease, such as protease K. In certain specific embodiments, the DNA extraction reagent comprises protease K and EDTA.

20 [0023] In some embodiments, before step b), a step of diluting the peripheral blood sample is further included, for example, diluting with phosphate buffer solution in equal volume.

[0024] In some embodiments, in step b), the peripheral blood sample is subjected to density gradient centrifugation, such as Ficoll density gradient centrifugation, to obtain a mononuclear cell layer and a red blood cell layer.

25 [0025] A third aspect of the present disclosure relates to a method for constructing a gene classifier for cancer detection through peripheral red blood cell micronuclei DNA, which comprises:

- a) providing more than one class, wherein each class represents a group of subjects with common characteristics;
- 30 b) isolating or purifying peripheral red blood cell micronuclei DNA from peripheral red blood cells of each subject of each class;
- c) sequencing the whole genome of the peripheral red blood cell micronuclei DNA to obtain fragment sequence information of the micronuclei DNA;
- d) comparing the fragment sequence information of micronuclei DNA from
- 35 peripheral red blood cells in different classes of subjects;

e) training the characteristic DNA fragment set for specific cancers according to the differences in the distribution of fragment the sequence information of micronuclei DNA in peripheral red blood cells of different classes of subjects, thus obtaining a gene classifier for specific cancer detection.

5 [0026] In certain embodiments, the different classes are cancer subjects and non-cancer subjects for the same cancer.

[0027] In certain embodiments, the different classes are subjects with different types of the same cancer.

10 [0028] In certain embodiments, the different classes are subjects at different stages of the same cancer type.

[0029] The fourth aspect of the present disclosure relates to a gene classifier for cancer detection, which is constructed by peripheral red blood cell micronuclei DNA.

[0030] In certain embodiments, the gene classifier comprises the genes shown in Table 2, 4, 6, 8 or 10.

15 [0031] A fifth aspect of the present disclosure relates to a method of cancer detection for a test subject, comprising:

a) extracting micronuclei DNA in peripheral red blood cells of the test subject, wherein the extract does not contain or substantially does not contain nucleated cell genomic DNA;

20 b) sequencing the micronuclei DNA and sample-matched genomic DNA by whole genome sequencing to obtain signature of the micronuclei DNA from red blood cells in specific genomic elements or different bin size for the test subject;

25 c) comparing the sample-matched genomic DNA and micronuclei DNA in red blood cells or micronuclei DNA from different types of samples in step b) with the whole genome analysis, so as to classify the micronuclei DNA from genomic DNA and evaluate the difference of micronuclei DNA signature from types of samples;

30 d) comparing the signature information of the micronuclei DNA from different classes of cancer patients or healthy donors obtained in step b) with the gene classifier or other deep neural network classifier for cancer detection of the present disclosure, so as to classify the test subjects into one or more of the classes.

[0032] A sixth aspect of the present disclosure relates to a system for cancer detection of a test subject, which comprises a comparison means for comparing peripheral red blood cell micronuclei DNA from the test subject with the gene classifier of the present disclosure.

35 [0033] A seventh aspect of the present disclosure relates to the use of an agent for analyzing micronuclei DNA of peripheral red blood cells in the preparation of a detection device or a detection kit for cancer screening, diagnosing, typing and/or staging.

[0034] In some specific embodiments, the screening or diagnosis is early screening or diagnosis.

[0035] The eighth aspect of the present disclosure relates to peripheral red blood cell micronuclei DNA for use in cancer detection.

5 [0036] The ninth aspect of the present disclosure relates to a method for isolating peripheral red blood cells.

[0037] The tenth aspect of the present disclosure relates to the use of peripheral red blood cells in cancer detection.

[0038] The above content is summary in general, so it includes simplification, 10 generalization and omission of details when necessary. Therefore, those skilled in the art will recognize that this general summary is merely illustrative and is not intended to be limiting in any way. Other aspects, features and advantages of the methods, compositions and/or devices and/or other subjects described herein will become apparent under the teachings herein. A summary is provided to simplify the introduction of some selected concepts, which will be 15 further described in the following detailed description. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an auxiliary means to determine the scope of the claimed subject matter. In addition, the contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference in their entirety.

20 TECHNICAL EFFECTS OF THE INVENTION

[0039] The inventors extracted micronuclei DNA from peripheral red blood cells for the first time and performed high-throughput sequencing on the extracted micronuclei DNA. Through bioinformatics analysis, erythrocyte micronuclei DNA has been successfully used in cancer screening, diagnosis, risk ranking, typing and staging, which has important guiding 25 significance for cancer prevention, treatment and prognosis.

[0040] The invention has achieved superior technical effects in at least the following aspects.

[0041] Abundancy in sample sources

[0042] According to the invention, peripheral blood is used as a sample source, and the 30 source is abundant, stable, and easy to obtain, collect, store and transport.

[0043] Effectiveness in the isolation of micronuclei DNA from red blood cells

[0044] By the method disclosed in the present disclosure, micronuclei DNA in red blood cells can be effectively isolated from human peripheral blood. It has not been reported in the art that micronuclei DNA in red blood cells can be effectively isolated from human peripheral 35 blood.

[0045] Simple and fast operation

[0046] According to the present disclosure, only a small amount (for example, only 1ml) of peripheral blood needs to be collected from the subject, which may relieve the psychological pressure of the subject. Particularly, for the detection of cervical cancer, there is no need to collect cervical exfoliated cells of the subjects, which is easy to operate and can effectively reduce the psychological pressure of the subjects.

[0047] In addition, by high-throughput sequencing, micronuclei DNA can be quickly sequenced to obtain genetic information.

[0048] High sensitivity and specificity of cancer detection

[0049] Using micronuclei DNA obtained from peripheral red blood cells, cancer can be detected with extremely high sensitivity and specificity by the method of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] The present invention will be more apparent to those skilled in the art through the specific embodiments and examples described in the present disclosure, combined with the following drawings.

[0051] Figure 1 shows a schematic diagram of isolating peripheral blood cells by Ficoll density gradient centrifugation.

[0052] Figure 2 shows that mononuclear cells and red blood cells were collected after Ficoll density gradient centrifugation.

[0053] Figure 3 shows the flow chart of sample processing and high-throughput sequencing of peripheral blood mononuclear cell genomic DNA and erythrocyte micronuclei DNA.

[0054] Figure 4 shows the bioinformatics analysis algorithm logic.

[0055] Figure 5 shows hierarchical clustering of healthy individuals and cervical cancer patients.

[0056] Figure 6 shows hierarchical clustering of patients with different types of cervical cancer (squamous cell carcinoma and adenocarcinoma).

[0057] Figure 7 shows hierarchical clustering of cervical cancer patients at different stages.

[0058] Figure 8 shows the risk ranking of subjects and screening of cervical cancer patients by the gene classifier of the present disclosure.

[0059] Figure 9 shows the risk ranking of subjects by the gene classifier of the present disclosure, and patients with cervical squamous cell carcinoma from patients with cervical adenocarcinoma were distinguished.

[0060] Figure 10 shows hierarchical clustering of healthy individuals and patients with colorectal cancer.

[0061] Figure 11 shows hierarchical clustering of patients with different types of colorectal cancer (colon cancer and rectal cancer).

5 [0062] Figure 12 shows the risk ranking of subjects by the gene classifier of the present disclosure to screen colorectal cancer patients.

[0063] Figure 13 shows the risk ranking of subjects by the gene classifier of the present disclosure to differentiate colon cancer patients from rectal cancer patients.

[0064] Figure 14 shows the multiclass discrimination of HD, HCC, LC and CRC samples
10 in a training cohort (left), validation cohort (middle) and test cohort (right).

[0065] Figure 15 (Figure 15A-D) shows the characterization the profile of the red blood cell micronuclei DNA (i.e., a rbcDNA signature) in healthy donors and cancer patients.

DETAILED DESCRIPTION

[0066] While the present invention can be embodied in many different manners, specific
15 illustrative embodiments thereof which demonstrate the principles of the invention are disclosed herein. It should be emphasized that the present invention is not limited to the specific embodiments illustrated. In addition, any chapter titles used herein are for organizational purposes only and should not be interpreted as limiting the described subject matter.

20 [0067] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention will have the meanings commonly understood by those of ordinary skill in the art. In addition, unless the context requires otherwise, the singular term shall include the plural, and the plural term shall include the singular. More specifically, as used in this specification and the appended claims, unless the context clearly indicates otherwise, the
25 singular forms “a,” “an” and “the” include plural referents. Therefore, for example, reference to “a protein” may include a variety of proteins; and reference to “a cell” includes a mixture of cells, etc. In this application, unless otherwise stated, the use of the expression “or” refers to “and/or.” In addition, the use of the term “comprising” and other forms such as “comprise” and “comprises” is not limiting. Furthermore, the ranges provided in the description and the
30 appended claims include all values between endpoints and breakpoints.

[0068] Generally, the terms related to cell and tissue culture, molecular biology, immunology, microbiology, genetics, and protein as well as nucleic acid chemistry and hybridization described herein, and their techniques are well-known and commonly used in the art. Unless otherwise stated, the methods and techniques of the present invention are
35 generally carried out according to conventional methods known in the art, as described in various general and more specific references cited and discussed throughout this specification.

See, for example, Abbas et al., Cellular and Molecular Immunology, 6th ed., W.B. Saunders Company (2010); Sambrook J. & Russell D. Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Wiley, John & Sons, Inc. (2002); Harlow and Lane Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan et al., Short Protocols in Protein Science, Wiley, John & Sons, Inc. (2003). Terms related to analytical chemistry, synthetic organic chemistry and drugs and pharmaceutical chemistry described herein, as well as laboratory procedures and techniques, are well-known and commonly used terms in the field. In addition, any chapter titles used herein are for organizational purposes only and are not to be interpreted as limiting the described subject matter.

DEFINITION

[0069] In order to better understand the present invention, definitions and interpretations of related terms are provided as follows.

[0070] In the context of the present disclosure, the term “DNA” refers to deoxyribonucleic acid.

[0071] In the context of the present disclosure, the term “micronuclei” is intended to refer to a small nuclear structure containing DNA in a specific cell other than the nucleus. There is no nucleus in peripheral red blood cells, so there is only micronuclei structure.

[0072] In the context of the present disclosure, the term “cervical cells” include cells located at any part of the cervix and cells detached from any part of the cervix that can be diseased. In one embodiment, cervical cells are cells isolated from tissues exfoliated from that inner wall of the cervix in a natural or artificial way, also called “cervical exfoliated cells.”

[0073] In the context of the present disclosure, a “subject” refers to a subject to be tested. In certain embodiments, the “subject” is a human subject.

[0074] In the context of the present disclosure, a “patient” refers to a subject suffering from a certain disease, such as cervical cancer.

[0075] In the context of the present disclosure, “cancer” is a general term for malignant tumors. “Tumor” refers to the abnormal proliferation of cells in local tissues under the influence of various tumorigenic factors.

[0076] In the context of the present disclosure, a “cancer subject” or a “cancer patient” are used interchangeably, referring to a subject suffering from a certain cancer, such as cervical cancer or colorectal cancer.

[0077] In the context of the present disclosure, a “non-cancer subject” refers to a subject who does not suffer a certain cancer. For example, “non-cervical cancer subject” refers to a subject without cervical cancer. In the specific embodiments and examples of the present disclosure, a “non-cancer subject” is also referred to as a “healthy individual,” and likewise,
5 it refers to that the individual or subject does not have such cancer.

[0078] In the context of the present disclosure, the term “cancer detection” refers to detecting the condition of a subject suffering from cancer. “Detecting” includes but is not limited to screening, diagnosis, typing and staging. “Screening” refers to preliminarily detecting whether there is cancer or the risk of cancer. “Diagnosis” or “medical diagnosis”
10 refers to assessing the patient’s condition from a medical point of view. “Typing” refers to further dividing the same kind of cancer into specific subtypes. For example, cervical cancer can be classified into cervical squamous cell carcinoma and cervical adenocarcinoma. “Staging” refers to predicting, assessing or dividing the stage of a cancer. For example, cervical cancer (squamous cell carcinoma) can be divided into three stages: low
15 differentiation, low-medium differentiation, medium differentiation and high differentiation.

[0079] In the context of the present disclosure, the term “nucleated cell” refers to a cell in which a nucleus exists. For peripheral blood, the term “nucleated cell” is the general term for a granulocyte, a monocyte and a lymphocyte.

[0080] In the context of the present disclosure, the term “genome” refers to the sum of all genetic information in a cell, especially a complete set of haploid genetic material in a cell.
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[0081] In the context of the present disclosure, the term “nucleated cell genomic DNA,” “nucleated cell nucleus genome,” or “nucleated cell nucleus genomic DNA” are used interchangeably, meaning all genetic information contained in nuclear chromosomes.

[0082] In the context of the present disclosure, the term “gene classifier” or “classifier”
25 can be used interchangeably, referring to a group of DNA fragments or a group of genes in genomic DNA or micronuclei DNA that are specific for a specific disease.

[0083] In the context of the present disclosure, the term “DNA fragment library” or “DNA library” can be used interchangeably, which refers to double-stranded DNA obtained by completing the ends of a sample DNA fragment, adding a phosphate group at the 5' end,
30 adding an adenine nucleotide (A) at the 3' end, and connecting an adapter and a sample barcode at both ends.

[0084] In the context of the present disclosure, the term “micronuclei DNA from red blood cells” and “erythrocyte micronuclei DNA” are used interchangeably, and is intends to refer micronuclei DNA isolated from red blood cells. In a specific embodiment, the red blood
35 cells are peripheral red blood cells. Accordingly, in the context of the present disclosure, “peripheral red blood cells micronuclei DNA,” “peripheral erythrocyte micronuclei DNA”, and “micronuclei DNA from peripheral red blood cells” are used interchangeably. In a specific embodiment, micronuclei DNA is isolated or purified from peripheral red blood cells.

[0085] In the context of the present disclosure, the term “high-throughput sequencing” (also known as Next-Generation Sequencing (NGS)) refers to DNA sequencing technology that simultaneously sequences thousands (even millions) of DNA templates in a single chemical reaction.

- 5 [0086] In the context of the present disclosure, the term “reads” refers to the sequence of a sample DNA fragment in a DNA fragment library measured by high-throughput sequencing, with the sequence linked in the library preparation stage removed.

[0087] In the context of the present disclosure, the term “coverage depth” refers to an effective nucleic acid sequencing fragment for base recognition in a specific region, also
10 known as the number of reads.

[0088] In the context of the present disclosure, the term “sequence alignment” refers to the alignment of reads to a reference genome (e.g., a human reference genome) by the principle of sequence identity.

[0089] In the context of the present disclosure, the term “reference genome” is the whole
15 genome sequence of an organism of the same species as the sample DNA, which can be obtained from a public database. In one embodiment, the reference genome is a human reference genome. The public database is not particularly limited. In some embodiments, the public database is GenBank database of NCBI.

[0090] In the context of the present disclosure, the term “sensitivity” refers to the
20 percentage of samples with positive tests in the total number of patients. In medical diagnosis, sensitivity can be expressed by the following formula, reflecting the ratio of correctly diagnosing patients:

[0091] $\text{Sensitivity} = \text{true positive number} / (\text{true positive number} + \text{false negative number}) \times 100\%$.

25 [0092] In short, if “true positive,” “false positive,” “true negative” and “false negative” are represented by “a”, “b”, “c” and “d”, respectively, the relationship among sensitivity, specificity, missed diagnosis rate, misdiagnosis rate and accuracy can be shown as follows.

Screening method	Pathological diagnosis (gold standard)		Sum
	Diseased	Non-diseased	
Positive (+)	True positive (a)	False positive (b)	a+b
Negative (-)	False negative (c)	True negative (d)	c+d
Sum	a+c	b+d	a+b+c+d

[0093] Among the cases with positive screening results by this method, “true positive (a)” refers to the number of cases diagnosed as diseased by pathology, and the result of a
30 method is also positive; “false positive (b)” refers to the number of cases diagnosed as non-

diseased by pathology, and the result of a method is positive; “false negative (c)” refers to the number of cases diagnosed as diseased by pathology and the result of a method is negative; and “true negative (d)” refers to the number of cases diagnosed as non-diseased by pathology and the result of a method is negative.

5 [0094] Sensitivity (sen) = $a/(a+c)$;

[0095] Specificity (sep) = $d/(b+d)$;

[0096] Missed diagnosis rate = $c/(a+c)$;

[0097] Misdiagnosis rate = $b/(b+d)$;

[0098] Accuracy = $(a+d)/(a+b+c+d)$

10 [0099] As known by those skilled in the art, the higher the value of sensitivity and specificity, the better; and the lower the missed diagnosis rate and misdiagnosis rate, the better.

[00100] In the context of the present disclosure, the term “specificity” refers to the percentage of samples with negative tests in healthy people in the total number of healthy people. In medical diagnosis, “specificity” can be expressed by the following formula, which reflects the ratio of correct diagnosis of non-patients:

[00101] Specificity = true negative number / (true negative number + false positive number) $\times 100\%$.

[00102] In the context of the present disclosure, the term “missed diagnosis rate,” also known as false negative rate, refers to the percentage of patients who are actually diseased when screening or diagnosing a disease in a population, but are determined as non-patients according to the diagnostic criteria. In medical diagnosis, the missed diagnosis rate can be expressed by the following formula:

20 [00103] Missed diagnosis rate = false negative number / (true positive number + false negative number) $\times 100\%$.

[00104] In the context of the present disclosure, the term “misdiagnosis rate,” also known as false positive rate, refers to the percentage of subjects who do not actually suffer from a disease when screening or diagnosing a disease in a population, but are determined as patients with such a disease according to the diagnostic criteria. In medical diagnosis, the misdiagnosis rate can be expressed by the following formula:

30 [00105] Misdiagnosis rate = false positive number / (true negative number + false positive number) $\times 100\%$.

[00106] In the context of the present disclosure, the expression “about” refers to that the deviation does not exceed plus or minus 10% of a specified value or range.

[00107] **Peripheral blood**

[00108] In the present disclosure, “peripheral blood” refers to blood released into the circulatory system by hematopoietic organs and participating in the circulation. “Peripheral blood” is different from immature blood cells in hematopoietic organs such as bone marrow.

5 In the present disclosure, peripheral blood can be collected by reference to known methods in the art such as venous blood collection, fingertip blood collection or earlobe blood collection.

[00109] Generally, peripheral blood consists of plasma and blood cells, wherein the blood cells further include white blood cells (also called “leukocytes”), red blood cells and platelets. By volume, red blood cells account for about 45%, plasma accounts for about 54.3%, and
10 white blood cells account for about 0.7% of the total peripheral blood. Leukocytes are nucleated cells, which are the general term of granulocytes, monocytes and lymphocytes. Normal red blood cells have no nucleus, no genomic DNA, and are nuclear-free cells.

[00110] In the context of the present disclosure, a “peripheral blood mononuclear cell” (PBMC) refers to a cell with a single nucleus in peripheral blood, including monocytes and
15 lymphocytes.

[00111] **Separation of peripheral blood cells**

[00112] The separation methods of peripheral blood cells include natural sedimentation, differential sedimentation, sodium chloride separation, density gradient centrifugation and so on.

20 [00113] Different components of peripheral blood can be separated by using the density difference between different components of peripheral blood. For example, different components of peripheral blood can be separated by Ficoll density gradient centrifugation or Percoll method.

[00114] In a specific embodiment of the present disclosure, peripheral blood is separated
25 by Ficoll density gradient centrifugation. Specifically, it is carried out in the following ways:

[00115] 1. Peripheral blood collection and sample preparation

[00116] Peripheral blood is obtained from a subject and diluted appropriately. For example, it can be diluted by adding phosphate buffer solution (PBS). In certain embodiments, about 1-5ml of fresh peripheral blood is obtained from a subject and diluted by adding an equal
30 volume of PBS to obtain a diluted blood sample. In a specific embodiment, 1ml of fresh peripheral blood is obtained from a subject, and 1×PBS is added for equal volume dilution to obtain diluted peripheral blood samples.

[00117] 2. Density gradient centrifugation of peripheral blood samples

[00118] Initially, an appropriate amount of Ficoll density gradient centrifuge is added into
35 the density gradient centrifuge tube, and then the diluted peripheral blood sample as described above is added thereto. In certain embodiments, an appropriate amount of Ficoll

density gradient centrifuge is added to the density gradient centrifuge tube in a ratio of the volume of peripheral blood collected from the subject to the volume of Ficoll density gradient centrifuge of about 1:3 to 1:10. For example, in a specific embodiment, 1ml of fresh peripheral blood is obtained from a subject, and 5ml of Ficoll density gradient centrifuge (Stemcell, Lymphoprep™ 07801) is added to the density gradient centrifuge tube.

[00119] Then, the diluted peripheral blood sample was slowly added onto the Ficoll density gradient centrifuge in the Ficoll density gradient centrifuge tube for density gradient centrifugation. Density gradient centrifugation can be carried out for about 10-15 minutes at about 15-25°C and at about 1000-1500g g. In a specific embodiment, the density gradient centrifugation is performed by 1200g centrifugation at 18°C for 15 minutes.

[00120] After density gradient centrifugation, it is divided into three layers: the upper layer is plasma, the middle layer is PBMC layer, and the bottom layer is RBC layer.

[00121] Collection of PBMC and RBC respectively. For example, the middle and upper layer liquid in the density gradient centrifuge tube is sucked by a suction means (such as a straw), and PBMC is separated and collected. An extraction means (such as a needle tube) is used to extract bottom red blood cells from the bottom of the density gradient centrifuge tube, and RBCs are separated and collected. In a specific embodiment, the bottom red blood cells are extracted from the bottom of the density gradient centrifuge tube by using a needle tube to a 1.5ml centrifuge tube, with 1×PBS added up to a volume of 1 ml. Centrifugation is conducted at room temperature for 10min at 300g, and red blood cells at the bottom of the tube are collected. The collected RBCs were then subject to two sequential filtrations by 10µm cell strainers to remove potential contamination of nucleated cells.

[00122] **Isolation of micronuclei DNA from peripheral red blood cells**

[00123] According to the inventor's knowledge, there is no report on isolating micronuclei DNA from human peripheral red blood cells in the prior art. Unexpectedly, the inventors found that the micronuclei DNA of peripheral red blood cells can be separated simply and efficiently by the method of the present disclosure. In certain embodiments, the collected red blood cells are first lysed and then centrifuged. Thereafter, micronuclei DNA was extracted from the supernatant after centrifugation. In certain embodiments of the present disclosure, "peripheral red blood cell micronuclei DNA" includes all DNA present in peripheral red blood cells. In a specific embodiment of the present disclosure, the isolated "peripheral red blood cell Micronuclei DNA" does not contain nucleated cell genomic DNA. In another specific embodiment of the present disclosure, isolated "peripheral red blood cell micronuclei DNA" substantially does not contain nucleated cell genomic DNA.

[00124] The inventors also unexpectedly found that micronuclei DNA isolated from peripheral red blood cells can be used to detect various cancers.

[00125] Lysis of red blood cells

[00126] In some embodiments, the collected red blood cells are lysed by adding a red blood cell lysis buffer. Erythrocyte lysis buffer can lyse erythrocytes while hardly damaging nucleated cells (such as PBMC). It can lyse erythrocytes effectively by slightly changing the osmotic pressure of cell suspension without affecting all nucleated cells. The red blood cell lysis buffer commonly used in the art contains NH_4Cl , NaHCO_3 , EDTA or other combinations, for example, NH_4Cl , NaHCO_3 and EDTA. For example, every 1000ml of red blood cell lysis buffer contains 8.3g NH_4Cl , 1.0g NaHCO_3 , 1.8 ml of 5% EDTA and ultra-pure water.

10 [00127] The red blood cell lysis buffer can be, for example, a red blood cell lysis buffer (Biosharp, Cat No./ID: BL503B), a red blood cell lysis buffer (Solarbio, Cat No./ID: R1010) or a BD FACS Lysing Solution red blood cell lysis buffer (BD, Cat No./ID: 349202). In a specific embodiment, 10 ml of red blood cell lysis buffer (Biosharp, Cat No./ID: BL503B) is added to the collected red blood cells, and the collected red blood cells are lysed for 20
15 minutes at room temperature in the dark.

[00128] Centrifugation

[00129] Thereafter, supernatant and precipitate (cell debris) are separated by centrifugation. In a specific embodiment, centrifugation is performed at 3000g at room temperature for 10 minutes, and then the supernatant is taken.

20 [00130] Isolation of micronuclei DNA

[00131] Then, micronuclei DNA is extracted from the supernatant. In certain embodiments, the DNA contained in the supernatant is pretreated by adding EDTA and protease K. EDTA is added in the digestion process with protease K to inhibit the influence of Mg^{2+} -dependent nuclease. In a specific embodiment, the supernatant is incubated with 10 mm EDTA
25 (Solarbio Cat No./ID: E1170), 200 ug/ul protease K (ProteinaseK, Ambion, Cat No./ID: AM2548) at 56 °C for 8 hours.

[00132] After incubation, commercial kits or reagents are used to extract micronuclei DNA. Examples of commercial kits include but are not limited to QIAamp DNA Blood Mini Kit, DNAzol reagent, PureLink™ Pro 96 Genomic DNA Purification Kit (Thermo, Cat
30 No./ID: K182104A), blood genomic DNA extraction system (0.1-20 ml) (TIANGEND, Cat No./ID: P349), HiPure Blood DNA Midi Kit III(Magen, Cat No./ID: D3114). In a specific embodiment, erythrocyte micronuclei DNA is extracted using QIAamp DNA Blood Mini Kit (Qiagen, Cat No./ID: 51106).

[00133] **Extraction of genomic DNA from peripheral blood mononuclear cells**

35 [00134] The genomic DNA of peripheral blood mononuclear cells can be extracted by commercial kits. In a specific embodiment, for peripheral blood mononuclear cell samples

obtained after density gradient centrifugation, genomic DNA is extracted using QIAamp DNA Blood Mini Kit (Qiagen, Cat No./ID: 51106).

[00135] **Whole genome amplification**

[00136] Whole-genome amplification (WGA) is non-selective amplification of the whole genome sequence. Its main purpose is to maximize the amount of DNA on the basis of faithfully reflecting the whole genome, and to amplify the whole genome DNA of micro tissues and single cells without sequence bias.

[00137] Whole-genome amplification methods are mainly divided into the following types: first, amplification technology based on thermal cycles and PCR; second, amplification technology based on isothermal reaction and not based on PCR; and the third is MALBAC (Multiple Annealing and Looping-based Amplification Cycles). The WGA technology based on PCR includes degenerate oligonucleotide primer PCR (DOP-PCR), linker-adaptor PCR (LA-PCR), interspersed repeat sequence PCR (IRS-PCR), tagged random primer PCR (T-PCR), primer extension preamplification PCR (PEP-PCR), among others. WGA based on isothermal reaction includes multiple displacement amplification (MDA), primase-based whole genome amplification (pWGA) and so on.

[00138] The methods of amplifying the whole genome DNA of a single cell mainly include MDA, MALBAC and DOP-PCR. These amplification methods can amplify pg-level or fg-level DNA in cells to µg-level which can satisfy sequencing.

20 [00139] Multiple displacement amplification (MDA)

[00140] Multiple displacement amplification (MDA) was first proposed by Dr. Lizardi of Yale University in 1998. This method is a constant temperature amplification method based on the principle of strand displacement amplification. Phage Φ29 DNA polymerase was used in multiple displacement amplification. PhageΦ29 DNA polymerase has a strong binding ability to DNA template, which can continuously amplify 100 Kb DNA template without dissociation from the template. At the same time, the enzyme has 3'-5' exonuclease activity and low amplification error rate.

[00141] Multiple displacement amplification has the following advantages:

- samples need not be purified;
- 30 - stable yield;
- uniform amplification of the genome;
- amplification with high fidelity;
- simple operation, independent of PCR reaction.

[00142] Commercial kits for MDA include REPLI-g series kits (Qiagen Inc), GenomiPhi series kits (GE Healthcare Inc), among others.

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[00143] MALBAC (Multiple Annealing and Looping-based Amplification Cycles)

[00144] MALBAC is different from non-linear or exponential amplification, but uses special primers to make the ends of amplicon complementary to each other. In this technique, the unique DNA polymerase with strand displacement activity is used for quasi-linear whole
5 genome pre-amplification, and then exponential amplification is performed by PCR technology, which provides sufficient experimental materials for downstream analysis. In 2012, Science magazine published two articles related to this technology (C. Zong et al., Science 2012: 1622-1626; S. Lu et al., Science: 1627-1630).

[00145] MALBAC has the following advantages:

- 10 - high amplification success rate;
 - good uniformity;
 - high coverage.

[00146] Commercial kits for MALBAC include MALBAC®single cell amplification kit from YiKon.

15 [00147] Degenerate oligonucleotide primer PCR(DOP-PCR)

[00148] The difference between DOP-PCR and conventional PCR is that it uses a single semi-degenerate primer and low renaturation temperature, has no species specificity, has no relation with the complexity of DNA, and can uniformly amplify the whole genome.

[00149] Commercial kits for DOP-PCR include PicoPlex series kits (Rubicon Genomics
20 Inc), GenomePlex series kits (Sigma Aldrich Inc), SurePlex series kits (BlueGnome, which has been acquired by Illumina) and so on.

[00150] In the present disclosure, PBMC genomic DNA and RBC micronuclei DNA can be amplified by the whole genome amplification methods known in the art. In a specific embodiment, PBMC genomic DNA and RBC micronuclei DNA are amplified by MDA.
25 Specifically, for PBMC genomic DNA and RBC micronuclei DNA extracted by QIAamp DNA Blood Mini Kit (Qiagen, Cat No./ID: 51106), MDA was performed by using REPLI-g Single Cell Kit (Qiagen, Cat No./ID: 150345), respectively. And the amplified DNA sample is obtained.

[00151] The REPLI-g Single Cell Kit adopts multiple displacement amplification (MDA)
30 technology, which can uniformly amplify single cell or purified genomic DNA, and can cover all loci of genome. All buffers and reagents are produced through a strictly controlled process to avoid DNA contamination and ensure reliable results for each experiment.

[00152] **Library construction**

[00153] A library is constructed by fragmenting the genomic DNA into short DNA
35 molecules, then connecting the fragmented genomic DNA to universal adaptors, and then generating millions or even more single-molecule multi-copy PCR clone arrays.

[00154] In the present disclosure, any conventional method in the field can be used to fragment the amplified DNA and construct a DNA fragment library. For example, a commercially available kit can be used to fragment genomic DNA and construct a library of DNA fragments.

5 [00155] In certain embodiments, the process of fragmenting genomic DNA and constructing a DNA fragment library by using a kit may include:

(i) performing fragmentation on genomic DNA;

(ii) carrying out terminal modification on the obtained DNA fragments:

- End Repair,
- 10 • adding a phosphate group to the 5' end of the DNA fragment repaired as above, and
- adding an adenine nucleotide (A) to the 3' end of the DNA fragment repaired as above (A-tailing);

(iii) ligating an adaptor and a sample barcode at the ends of the DNA fragment modified as above;

(iv) Fragment Selection: agarose gel electrophoresis is performed on the ligation products as above, and the DNA fragments (i.e., DNA fragment library) correctly ligated with the adaptor and the sample barcode were recovered by using any commercially available kit.

(v) Library Amplification: the DNA fragments with the adaptor and the sample barcode correctly ligated as above is amplified by polymerase chain reaction (PCR).

[00156] In a specific embodiment of the present disclosure, after MDA, the amplified DNA samples are subjected to secondary sequencing library construction using TruePrep DNA Library Prep Kit V2 for Illumina (Vazyme, TD503).

[00157] **High throughput sequencing**

25 [00158] In the present disclosure, as long as the high-throughput sequencing of the DNA fragment library can be realized, there is no special restriction on the sequencing method and apparatus adopted. In certain embodiments, the library of DNA fragments is high-throughput sequenced using a commercially available sequencer. For example, the high-throughput sequencing of DNA fragment library can be performed by using a sequencer from Illumina, a sequencer from Apply Biosystems (ABI), a sequencer from Roche, a sequencer from Helicos, or a sequencer from Complete Genomics.

30 [00159] In a specific embodiment, the genomic DNA of peripheral blood mononuclear cells and erythrocyte micronuclei DNA are sequenced by Novo-seq platform (NovaSeq 6000, from Novogene, Beijing), with 10× sequencing depth and 30G data volume.

[00160] In the specific embodiment of the present disclosure, the original sequencing files for sequencing the erythrocyte micronuclei DNA and the genomic DNA of peripheral blood mononuclear cells are stored in FASTQ files. FASTQ is a standard text-based format to save biological sequences (usually nucleic acid sequences) and their sequencing quality information.

[00161] **Bioinformatics analysis**

[00162] After high-throughput sequencing, bioinformatics analysis of the obtained sequencing results generally includes quality control, data comparison, post-alignment processing, among others.

10 [00163] In certain embodiments of the present disclosure, quality control is performed on the original sequencing files of erythrocyte micronuclei DNA, and the sequencing data passing the quality control is compared with the reference genome, and then post-processing is performed.

15 [00164] In a further embodiment of the present disclosure, quality control is performed on genomic DNA of peripheral blood mononuclear cells, and sequencing data passing the quality control is compared with a reference genome.

[00165] Quality control

20 [00166] The sequencing data is quality controlled by data quality control software. The process of quality control includes adapter removal, filtering of low-quality reads, removal of low-quality 3' and 5' ends, removal of reads with more N, inspection of data quality, etc. Commonly used data software includes FastQC, Fastx_toolkit, Trimmomatic and so on.

[00167] As the most classic quality control software, FastQC can make quick statistics on gene information of high-throughput sequencing data and give corresponding chart reports. The software can be obtained at the following website:
25 <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.

[00168] In addition, Fastx_toolkit software can be obtained at the following website: http://hannonlab.cshl.edu/fastx_toolkit/; and Trimmomatic software can be obtained through the following website: <http://www.usadellab.org/cms/?page=trimmomatic>.

30 [00169] In a specific embodiment of the present disclosure, the original sequencing files of erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells are subjected to adaptor removal by cutadapter software (Kong, Y., Btrim: a fast, lightweight adaptor and quality trimming program for next-generation sequencing technologies. Genomics, 2011. 98(2): p. 152-3), and the quality control is carried out by FastQC software.

[00170] Data comparison

35 [00171] After quality control, the data passed the quality control will be compared to the reference genome by a software. Sequencing data comparison software commonly used in

this field includes BWA, Bowtie, Maq, Novoalign, etc., which can be obtained from the following website:

BMA: <http://bio-bwa.sourceforge.net>

Bowtie: <http://bowtie-bio.sourceforge.net>

5 Maq: <http://maq.sourceforge.net>

Novoalign: <http://www.novocraft.com/products/novoalign/>

[00172] In certain embodiments of the present disclosure, the sequencing data of erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells can be compared to reference genomes, such as human genomes, respectively, through data comparison software in the field. In a specific embodiment of the present disclosure, the sequencing data of erythrocyte micronuclei DNA and peripheral blood mononuclear cell genome DNA were compared to human genome (GenBank) by BWA software.

[00173] Post-alignment processing of data

[00174] Post-alignment processing may include the following situations, such as removing duplicate reads, Indel local re-alignment, re-proofreading of base mass values, and so on. Whether or not to carry out post-alignment processing is determined according to actual needs. The commonly used post-alignment processing includes removing duplicate reads. Different reads aligned onto the same position of the reference genome may be considered as duplication due to quality problems, sequencing errors, alignment errors, alleles, among others.

[00175] In some embodiments of the present disclosure, post-alignment processing is performed by removing duplicate reads. In a specific embodiment of the present disclosure, improper alignment and repeated reads are removed by Picard software (Weisenfeld, N.I., et al., Direct determination of diploid genome sequences. *Genome Res*, 2017. 27(5): p. 757-767). Picard software can be obtained from the following website: <http://broadinstitute.github.io/picard/>

[00176] Data analysis

[00177] After data processing, the sequencing data obtained are analyzed.

[00178] *Comparison and counting of reads*

[00179] In certain embodiments of the present disclosure, whether there are significant differences in the fragmentation degree of DNA fragments in red blood cells of different types of subjects is compared. For example, the reads of sequencing fragments existing in micronuclei DNA of samples can be counted by software for reads counting (such as HTseq-count、featureCounts、BEDTools、Qualimap、Rsubread、GenomicRanges, etc.). Variance analysis (such as ANOVA test) is applied to judge whether there is a significant difference therebetween.

[00180] In certain specific embodiments of the present disclosure, the reads of small sequencing fragments existing in erythrocytes micronuclei DNA are counted corresponding to the gene regions of human genome by HTseq-count software (Anders, S., P.T. Pyl and W. Huber, HTSeq--a Python framework to work with high-throughput sequencing data. 5 Bioinformatics, 2015. 31(2): p. 166-9).

[00181] In a specific embodiment of the present disclosure, one class is peripheral red blood cell micronuclei DNA from cervical cancer patients and the other class is peripheral red blood cell micronuclei DNA from healthy individuals.

[00182] In another specific embodiment of the present disclosure, one class is peripheral 10 red blood cell micronuclei DNA from patients with cervical adenocarcinoma and the other class is peripheral red blood cell micronuclei DNA from cervical squamous cell carcinoma.

[00183] In another specific embodiment of the present disclosure, one class is peripheral red blood cell micronuclei DNA from medium-differentiated patients in cervical squamous cell carcinoma, and the other class is peripheral red blood cell micronuclei DNA from low- 15 medium differentiated or low differentiated patients in cervical squamous cell carcinoma.

[00184] In a further embodiment of the present disclosure, one class is peripheral red blood cell micronuclei DNA from colorectal cancer patients and the other class is peripheral red blood cell micronuclei DNA from healthy individuals.

[00185] In a further embodiment of the present disclosure, one class is peripheral red 20 blood cell micronuclei DNA from colon cancer patients and the other class is peripheral red blood cell micronuclei DNA from rectal cancer.

[00186] Data classification and classifier construction

[00187] Classification is an important method of data mining. Based on the existing data, a classification function is learned or a classification model is constructed, which also called a 25 classifier. Classifiers can map data records in the database to a given class, which can be applied to data prediction. Classification methods include decision tree, selection tree, logistic regression, Naive Bayes and deep neural network.

[00188] In certain embodiments of the present disclosure, genes with significant differences are selected as features, and a classifier is constructed for known classified 30 samples based on support vector machine (SVM) to predict the specific disease classification of unknown samples (Huang, M.W., et al., SVM and SVM Ensembles in Breast Cancer Prediction. PLoS One, 2017. 12(1): p. e0161501). In some specific embodiments of the present disclosure, through the hierarchical clustering-based support vector machine algorithm, a classifier composed of a group of genes corresponding to DNA fragments is 35 constructed. In a specific embodiment of the present disclosure, two types of samples are randomly clustered according to Pearson correlation to construct a classifier composed of a group of genes.

[00189] In certain embodiments of the present disclosure, specific regions of erythrocyte micronuclei DNA are further selected before constructing the classifier.

[00190] In certain embodiments of the present disclosure, macs2 software is used to search for the fragments of erythrocyte micronuclei DNA which are mainly enriched in a specific region relative to the genome DNA sequencing reads of peripheral blood mononuclear cells, and to remove the peak areas which are more enriched by peripheral blood mononuclear cells relative to peripheral blood mononuclear cells *per se* as a whole. Compared with peripheral blood mononuclear cells, genome information annotation and pathway enrichment (KEGG, gene ontology) were performed on red blood cell-specific fragments (Chen, L., et al., Gene Ontology and KEGG Pathway Enrichment Analysis of a Drug Target-Based Classification System. PLoS One, 2015. 10(5): p. e0126492.).

[00191] *Application of Classifier*

[00192] On the basis of the classifier constructed in the present disclosure, the present invention can be widely used in biological research, medical research, clinical diagnosis and other fields by isolating peripheral blood micronuclei DNA from subjects in the manner described in the present disclosure and performing biological analysis. The invention has important value in scientific research and medical fields.

APPLICATION OF THE INVENTION

[00193] The inventors have successfully isolated erythrocyte micronuclei DNA from peripheral blood and applied it to cancer detection for the first time, including screening, diagnosis, typing and staging of cancer.

[00194] Among cancers, cervical cancer and colorectal cancer account for a large proportion of new cases and fatal cases.

[00195] **Cervical cancer**

[00196] Cervical cancer is one of the most common gynecological tumors, and its incidence is increasing year by year. According to the statistics of the World Health Organization (WHO), there are an average of 530,000 new cases of cervical cancer every year, and about 250,000 women die from cervical cancer, among which developing countries account for 80% of the global total cases (Schiffman, M., et al., Carcinogenic human papillomavirus infection. Nat Rev Dis Primers, 2016. 2: p. 16086). In China, there are about 140,000 new cases of cervical cancer and about 37,000 deaths every year. Therefore, early screening and clinical staging of cervical cancer patients are of great significance to the treatment of cervical cancer.

[00197] Pathogenic factors of cervical cancer

[00198] Pathogenic factors of cervical cancer include but are not limited to the following aspects:

[00199] Virus infection

[00200] HPV infection is the main pathogenic factor of cervical cancer. There are many subtypes of HPV, about 40 of which are related to reproductive tract infection. Continuous infection by high-risk HPV subtypes (subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 69), especially HPV subtypes 16 and 18 can cause cervical cancer.

[00201] Sexual behavior and number of deliveries

[00202] Other biological factors

[00203] Chlamydia trachomatis, herpes simplex virus type II, trichomoniasis and other pathogens have synergistic effects in the pathogenesis of cervical cancer caused by high-risk HPV infection.

[00204] Other behavioral factors

[00205] Smoking as a synergistic factor of HPV infection can increase the risk of cervical cancer. In addition, malnutrition and poor sanitation can also affect the occurrence of diseases.

[00206] Early screening method for cervical cancer in the prior art

[00207] At present, the early screening of cervical cancer is mainly carried out by virus detection and cytological detection. Among them, virus detection is mainly human papillomavirus (HPV) detection, while cytological detection mainly includes Pap smear and TCT detection.

[00208] (1) HPV detection

[00209] HPV can cause squamous epithelial proliferation of human skin mucosa. According to its pathogenicity, it can be divided into low-risk type and high-risk type. Low risk infection can cause common warts, genital warts (condyloma acuminatum) and other symptoms. Persistent high-risk human papillomavirus (HPV) infection is the main cause of cervical cancer. Molecular epidemiological analysis shows that some types of human papillomavirus (HPV) are the main causes of invasive cervical cancer and cervical intraepithelial neoplasia. At present, more than 80 types of HPV have been found, and about 40 of them can infect reproductive tract [Schiffman, M., et al., Carcinogenic human papillomavirus infection. Nat Rev Dis Primers, 2016. 2: p. 16086; Munoz, N., et al., Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med, 2003. 348(6): p. 518-27.]. Among them, high-risk HPV (such as HPV 16 and HPV 18) is often associated with invasive cervical cancer. The detection methods of high-risk HPV mainly include morphological observation, immunohistochemistry, dot-blot hybridization, in situ blotting hybridization, PCR/RFLP, PCR/Southern and so on.

[00210] Screening cervical cancer by HPV virus detection can identify more than 95% of precancerous cervical lesions, but it is mainly aimed at patients with cervical intraepithelial neoplasia (CIN) grade 2 or more, while the specificity for CIN2 negative patients is relatively

low, because most women have spontaneous clearance after transient HPV infection, and hardly progress to CIN3 and cancer (Cook, D.A., et al., Evaluation of a validated methylation triage signature for human papillomavirus positive women in the HPV FOCAL cervical cancer screening trial. *Int J Cancer*, 2018). HPV detection can only determine whether women are infected with carcinogenic HPV, but cannot determine the risk of individual cancer, and there are still few HPV negative cervical cancer patients. Therefore, there may be false positives in HPV testing. On the basis of HPV detection, it is usually necessary to combine other clinical detection indications for subsequent diagnosis.

[00211] (2) Pap smear

[00212] Pap Smear, also known as cervical smear or Pap test, is a traditional and most commonly used screening method for cervical cancer. In this method, the cervical exfoliated cells are collected, stained and microscopically observed to test whether there are precancerous cells or cancer cells on the cervix, which has always been regarded as the “gold standard” for cervical cancer detection (Rodríguez, A.C. and J. Salmeron, Cervical cancer prevention in upper middle-income countries. *Prev Med*, 2017. 98: p. 36-38).

[00213] Combined with pathological observation, Pap smear can clearly identify the development of cervical cancer, but this method can only detect about 50% of cervical precancerous lesions. The difference in sample collection quality, insufficient cell collection, fewer abnormal cells, and the shielding of abnormal cells by blood or inflammatory cells will affect smear observation, resulting in poor detection sensitivity (Cook, D.A., et al., Evaluation of a validated methylation triage signature for human papillomavirus positive women in the HPV FOCAL cervical cancer screening trial. *Int J Cancer*, 2018). At the same time, due to the limitation of sampling, it is difficult to have regular detection and trace cases.

[00214] (3) TCT detection

[00215] TCT test, also named as liquid-based thin-layer cytology test, collects cervical cell samples through a special sampler, but does not directly carry out smear observation, and instead puts the collector into a culture bottle filled with cell preservation solution for rinsing to obtain enough cell samples (Massad, L.S., et al., 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. *Obstet Gynecol*, 2013. 121(4): p. 829-46). After that, the cell sample bottles were sent for laboratory inspection, and the cell samples were dispersed and filtered by automatic cell detector, so as to reduce the interference of blood, mucus and inflammatory tissues and obtain a thin cervical cell layer for further microscopic detection and diagnosis.

[00216] TCT detection is an optimized detection scheme for pap smear of cervical cancer developed in recent decades. Compared with the traditional Pap smear of cervical cancer, TCT detection significantly improved the satisfaction of specimens and the detection rate of abnormal cells of cervical cancer. The detection rate of cervical cancer cells by TCT was 100%, and some precancerous lesions could also be found (Andy, C., L.F. Turner and J.O.

Neher, Clinical inquiries. Is the ThinPrep better than conventional Pap smear at detecting cervical cancer? J Fam Pract, 2004. 53(4): p. 313-5). However, the detection rate of TCT for cervical precancerous lesions is still low, and the sensitivity for early screening and detection of cervical cancer is low, and there are still many atypical squamous cells (ASC-US) and
5 atypical glandular cells (AGC) with unknown meanings.

[00217] The above methods still have some limitations. First of all, for the above-mentioned methods, it is often necessary to use combined screening methods in clinical use (Zigras, T., et al., Early Cervical Cancer: Current Dilemmas of Staging and Surgery. Curr Oncol Rep, 2017. 19(8): p. 51). Secondly, at present, the samples used for cervical cancer
10 screening by the above method are cervical exfoliated cells, and the sampling method will inevitably cause damage and psychological burden to patients, and at the same time, there are certain restrictions on sampling requirements, and the quality of samples is difficult to control. In addition, screening for cervical cancer often requires regular detection. According to FDA standards, for women over 21 years old, regular detection should be conducted every 3 years
15 to assess the risks. The large fluctuation of sampling quality stability may lead to the loss of long-term regression testing. Therefore, a more reliable and stable sample source is needed to provide a more dynamic, accurate and instructive monitoring method and system for cervical cancer screening.

[00218] In the context of the present disclosure, "cervical cancer" includes any type of
20 cervical cancer.

[00219] Classification and staging of cervical cancer

[00220] The occurrence and development of cervical cancer has a gradual evolution process, which can last from several years to several decades. It is generally considered that the evolution can be divided into several stages: mild intraepithelial neoplasia (CINI),
25 moderate intraepithelial neoplasia (CINII), severe intraepithelial neoplasia (CINIII) and invasive cancer.

[00221] Cervical cancer can be classified into different types according to different standards.

[00222] According to whether cancer has metastasized or not, cervical cancer can be
30 divided into cancer in situ and invasive cancer. Cancer in situ is more common in women aged 30-35, while invasive cancer is more common in women aged 45-55. Lymphatic metastasis may occur in patients with severe cervical cancer. After local infiltration, the cancer invaded lymphatic vessels to form tumor plugs, which were drained into local lymph nodes with lymph fluid and spread in lymphatic vessels.

35 [00223] According to pathological types, cervical cancer can be divided into three types: squamous cell carcinoma, adenocarcinoma and adeno-squamous carcinoma.

[00224] Cervical squamous cell carcinoma is the main type of cervical cancer. According to histological differentiation, it can be divided into three grades: Grade I is highly differentiated squamous cell carcinoma, Grade II is medium differentiated squamous cell carcinoma (non-keratinized large cell type), and Grade III is low-medium differentiated and low differentiated squamous cell carcinoma (small cell type).

[00225] Cervical adenocarcinoma includes mucinous adenocarcinoma type and malignant adenoma type. Mucinous adenocarcinoma originates from columnar mucous cells of cervical canal, and the glandular structure can be seen under microscope. The hyperplasia of glandular epithelial cells is multilayer, the dysplasia is obvious, and mitosis is seen. The cancer cells protrude into the glandular cavity in mastoid shape. Malignant adenoma is a highly differentiated adenocarcinoma of cervical canal mucosa. There are many cancerous glands with different sizes and varied shapes, which extend into the deep cervical stroma in a punctate way. The glandular epithelial cells are atypical and often have lymph node metastasis.

[00226] Unexpectedly, the inventors found that peripheral red blood cell micronuclei DNA can be used for screening and diagnosing cervical cancer. The inventors further unexpectedly found that the micronuclei DNA of peripheral red blood cells can be used to distinguish the types of cervical cancer, which can be divided into squamous cell carcinoma and adenocarcinoma. The inventor further unexpectedly found that, peripheral red blood cell micronuclei DNA can stage cervical cancer, for example, cervical squamous cell carcinoma can be divided into high-differentiated type, medium-differentiated type, and low-medium-differentiated and low-differentiated type. It is of great significance for the early diagnosis, screening, classification and staging of cervical cancer.

[00227] Colorectal cancer

[00228] Colorectal cancer (CRC) is a cancer that arises from the colon or rectum. It is one of the most common malignant tumors in the gastrointestinal tract. The early symptoms are not obvious. The symptoms and signs shown with the increase of the cancer can include blood in stools, weight loss, and constant fatigue (General Information About Colon Cancer. NCI. May 12, 2014. Archived from the original on July 4, 2014. Retrieved June 29, 2014).

[00229] There are approximately 1.4 million new cases of colorectal cancer each year. Colorectal cancer ranks third among newly diagnosed cancers, and it is also the fourth cause of death from cancer. Studies have shown that by 2030, the number of global colorectal cancer cases is expected to increase by 60%, with more than 2.2 million new cases per year and approximately 1.1 million deaths per year (Global patterns and trends in colorectal cancer incidence and mortality. M, et al. Gut. 2017; 66:683-91).

[00230] Globally, colorectal cancer is the third most common cancer, accounting for about 10% of all cancer cases. It is especially common in developed countries, where more than 65% of cases are found to be CRC, and it is usually less common in women than in men

(Forman D, Ferlay J (2014). "Chapter 1.1: The global and regional burden of cancer". In Stewart BW, Wild CP (eds.). World Cancer Report. the International Agency for Research on Cancer, World Health Organization. pp. 16–53. ISBN 978-92-832-0443-5).

[00231] With the improvement of people's living standards in China, the incidence of colorectal cancer is on the rise. The latest statistics show that the incidence and mortality of colorectal cancer (CRC) in China have maintained an upward trend. Cancer statistics in China in 2015 show that the incidence and mortality of colorectal cancer in my country rank fifth among all malignant tumors, with 376,000 new cases and 191,000 deaths. Among them, the amount in urban areas is much higher than that in rural areas, and the incidence of colon cancer has increased significantly. Most patients are already in the middle and late stages when found. Early diagnosis of colorectal cancer is extremely important, and early diagnosis can significantly increase the possibility of successful treatment (5. Standards for Diagnosis and Treatment of Colorectal Cancer in China (2017 Edition) [J]. Chinese Journal of Medical Frontiers (Electronic Edition), 2018, 10(3): 1-21).

[00232] Causes of disease

[00233] Most colorectal cancers are caused by factors like aging and lifestyle, and only a few cases are caused by potential hereditary diseases. Risk factors include diet, obesity, smoking and lack of physical activity. Another risk factor is inflammatory bowel disease, including Crohn's disease and ulcerative colitis. Some hereditary diseases lead to colorectal cancer, including familial adenomatous polyposis and hereditary nonpolyposis colon cancer. CRC usually begins with benign tumor and appears as polyp, which may become cancerous with time.

[00234] Classification

[00235] *Classification according to causes*

[00236] According to the causes, colorectal cancer can be divided into three classes, two of which have genetic factors:

- sporadic colorectal cancer (50% to 60%);
- familial colorectal cancer (30% to 40%);
- hereditary colorectal cancer (4% to 6%).

[00237] Sporadic colorectal cancer: Sporadic colorectal cancer is the most common type, with 90% of patients diagnosed at the age of 50 and above. It is not directly related to genetics or family history. About one in every 20 Americans has this type of CRC.

[00238] Familial colorectal cancer: Some families are prone to CRC. If more than one person in the family suffers from CRC, especially before the age of 50, attention must be paid to it. If immediate family members (parents, siblings or children) have colorectal cancer, the risk of such family members will double.

[00239] Hereditary colorectal cancer: At present, many hereditary diseases have been found to be related to CRC, including hereditary nonpolyposis colon cancer (HNPCC), also known as Lynch syndrome; Familial adenomatous polyposis (FAP); Attenuated familial adenomatous polyposis (AFAP); APCI 1307K; Potts-Jaggers syndrome; MYH-associated polyposis (MAP); Juvenile polyposis; Hereditary polyposis.

[00240] *Classification according to focus of cancer*

[00241] According to the focus of cancer, colorectal cancer can be divided into colon cancer and rectal cancer.

[00242] Importance of early screening

10 [00243] Lifestyles such as high-fat diet, smoking and alcoholism may increase the risk of colorectal cancer. More than 90% of colorectal cancer patients are over 50 years old. Usually, the best treatment period is missed because of neglecting the early symptoms of the disease, including bloody feces or changes in defecation habits. Early diagnosis can significantly increase the possibility of successful treatment.

15 [00244] In recent years, in the United States, the incidence rate and mortality rate of CRC are gradually decreasing. The microscopic simulation model MISCAN-Colon suggests that the observed mortality rate of CRC is decreasing, and about 53% of it may benefit from CRC screening. In 2012, 65.1% of adults at the age of 50-75 in the United States had been screened for CRC, and 27.7% had never been screened. Colonoscopy is the most frequently
20 used screening examination (nearly 62%). From 2002 to 2010, the screening rate increased from 52.3% to 65.4%. With the improvement of screening rate, early treatment and intervention for risk individuals significantly reduced the incidence rate and mortality rate of CRC (Cronin KA, Lake AJ, Scott S, et al. Annual Report to the Nation on the Status of Cancer, part I: National cancer statistics. Cancer 2018; 124:2785).

25 [00245] Early screening and diagnosis methods of colorectal cancer in the prior art

[00246] Early screening and diagnosis of colorectal cancer mainly include the following ways:

[00247] (1) Colonoscopy

[00248] Colonoscopy is the most accurate and universal diagnostic examination of CRC,
30 which can locate lesions in the whole large intestine and perform biopsy to find simultaneous tumors and remove polyps. Observed under endoscope, most colon cancer and rectal cancer are intraluminal masses which originate from mucosa and protrude into the lumen. Tumors can be exophytic or polypoid. Bleeding (blood oozing or obvious bleeding) may be observed in fragile, necrotic or ulcerated lesions. A few gastrointestinal tumor lesions (both
35 asymptomatic and symptomatic individuals) are non-polypoid. A study found that, non-polypoid colorectal tumors are more prone to carcinogenesis than polypoid tumors. Compared with polypoid lesions, cancer caused by non-polypoid adenoma may be more

difficult to be detected under colonoscopy, but colonoscopy is more sensitive to this situation than barium enema or CT colonography. When experienced endoscopy operators use colonoscopy to examine asymptomatic patients, the missed diagnosis rate of CRC is 2%-6%.

[00249] (2) Flexible sigmoidoscopy

5 [00250] It has been observed that in the past 50 years, the proportion of right colon cancer or proximal colon cancer in the United States and around the world is gradually increasing, and the incidence of tumors originating from cecum is increasing with the fastest speed. In view of this, and considering the high incidence of simultaneous CRC, for patients with suspected CRC, flexible sigmoidoscopy is generally considered not to be an appropriate
10 diagnostic examination, unless the tumor is palpable in rectum. In this case, total colonoscopy is still needed to assess whether the rest of the colon has simultaneous polyps and cancer. However, flexible sigmoidoscopy is used to screen CRC. It is one of the few methods that have been proved by randomized controlled trials to reduce the incidence and morbidity of CRC.

15 [00251] (3) CT colonography

[00252] CT colonography, also known as virtual colonoscopy or CT colonography, can provide a computer-simulated intraluminal perspective for the inflated colon. This technology uses traditional spiral CT scanning or MRI to obtain a large number of continuous data, and uses complex post-processing software to generate images, which can enable operators to
20 walk and pass in any selected direction in the clean colon cavity. CT colonography needs mechanical bowel preparation similar to barium enema, because feces can be similar to polyps in image, causing interference. CT colonography can also detect extracolonic lesions, which can provide information on the causes of symptoms and tumor staging, but it may also lead to anxiety and increase costs due to unnecessary examinations. And its detection rate for
25 clinically important lesions may also be low.

[00253] Compared with colonoscopy, CT colonography is an alternative with similar sensitivity and less trauma for patients with CRC. However, considering that colonoscopy can remove/biopsy the lesions and simultaneous cancers or polyps seen during the operation, colonoscopy is still considered as the gold standard for CRC symptoms. When the use of
30 colonoscopy is limited, CT colonography is preferred over barium enema (Mulder SA, Kranse R, Damhuis RA, et al. Prevalence and prognosis of synchronous colorectal cancer: a Dutch population-based study. Cancer Epidemiol 2011; 35:442).

[00254] However, due to the particularity of sampling and testing methods, the above screening methods will inevitably lead to psychological burden and local injury of some
35 screeners, which is also an influencing factor that limits the long-term and large-scale screening, and it is necessary to consider the adaptability of patients' age and screening methods.

[00255] (4) Guaiac-based Faecal Occult Blood Test (gFOBT)

[00256] This test detects whether there is blood in a patient's stool sample. But blood stool test is not 100% accurate, because not all cancers cause bleeding, or they may not bleed all the time. Therefore, this test can give false negative results. Blood may also be present due to other diseases or conditions, such as hemorrhoids. The method of detecting fecal hemoglobin by guaiac is an indirect method for detecting peroxidase activity. There are non-hemoglobin peroxidase catalytic components in various foods, which may cause false positive, thus limiting the application of this method. Its advantage lies in the convenience and rapidity of initial detection and screening, which has certain guiding significance for further detection and diagnosis, but its accuracy is relatively low.

[00257] (5) Immunochemical test (Faecal Immunochemical Test, FIT)

[00258] This test uses antibodies to detect fecal occult blood. FIT uses monoclonal or polyclonal antibodies to directly detect hemoglobin in human feces, which is not affected by dietary. In qualitative FIT, color change is visible after the hemoglobin content in feces exceeds a certain threshold. While quantitative FIT can measure the value: when it exceeds a certain normal range, it is defined as positive. Compared with gFOBT, immunochemical test requires less stool samples, and there is no dietary restriction before collecting stool samples, but only one or two stool samples are collected each time (Mettle Kalager, et al. Overdiagnosis in Colorectal Cancer Screening: Time to Acknowledge a Blind Spot[J]. Gastroenterology, 2018 August 01). Even if there is merely occult blood in a sample, occult blood can also be detected. Occult blood in a sample indicates intestinal bleeding. This method has relatively high specificity, but poor sensitivity, and there may also be false positive or negative results due to interference from other diseases, which makes it impossible to make a definite diagnosis.

[00259] (6) Fecal DNA test

[00260] Colorectal cancer generally occurs in colorectal epithelial tissue, and first grows into intestinal cavity. During its growth, tumor cells are constantly shed into intestinal cavity and discharged with feces. The shed tumor cells in feces contain special components (such as mutated and methylated human genes), which can be used as tumor markers. Fecal DNA test analyzed several DNA markers of colon cancer or precancerous polyp cells flowing into feces. Patients can be provided with a kit containing instructions on how to collect stool samples at home, and then send it to the laboratory for detection and analysis. This test is more accurate for detecting colon cancer than polyp, but it cannot detect all DNA mutations that indicate the existence of tumor. The value of fecal gene detection lies in early diagnosis, which can prompt the occurrence of colorectal cancer, find precancerous adenoma and help patients to find colorectal cancer at an earlier stage (Imperiale, T.F., et al., Multitarget Stool DNA Testing for Colorectal-Cancer Screening. New England Journal of Medicine, 2014. 370(14): p. 1287-1297). However, fecal genetic testing can only be used as an auxiliary

diagnostic method. If there is a positive result, it must be confirmed and intervened by colonoscopy. However, due to the complexity of fecal DNA, its low specificity and low success rate of fecal DNA preparation will lead to insufficient cost-effectiveness, which greatly hinders its practical application.

5 [00261] The above methods are relatively convenient for sampling, and non-invasive. Non-invasive detection is more acceptable to patients, which may be used as an indicator of CRC screening. However, due to the specificity and sensitivity of the methods, most of them can only be used as an auxiliary means of diagnosis, and other means such as colonoscopy are still needed for diagnosis and intervention. Meanwhile, for stool sampling and treatment,
10 the psychological burden to a certain extent, as well as the complexity and pollution of stool samples, also cause problems in the stability and repeatability of sample detection (Brenner, H., et al., Prevention, Early Detection, and Overdiagnosis of Colorectal Cancer Within 10 Years of Screening Colonoscopy in Germany. Clinical Gastroenterology and Hepatology, 2015. 13(4): p. 717-723). Therefore, a more reliable and stable sample source is needed to
15 provide a more dynamic, accurate and instructive monitoring system for CRC screening.

[00262] Surprisingly, the inventors found that peripheral red blood cell micronuclei DNA can be used to screen and diagnose colorectal cancer. The inventors further surprisingly found that micronuclei DNA of peripheral red blood cells can be used to distinguish the types of colorectal cancer, which can be divided into colon cancer and rectal cancer. It is of great
20 significance for early diagnosis, screening and risk ranking of colorectal cancer.

[00263] Lung cancer

[00264] Lung cancer is the most common cancer type worldwide in terms of both incidence and mortality. The key cause of lung cancer is tobacco smoking, which is responsible for 63% of overall global deaths from lung cancer and for more than 90% of lung
25 cancer deaths in countries where smoking is prevalent in both sexes. Causes of lung cancer also including: secondhand smoke, family history of lung cancer, exposed to asbestos, arsenic, chromium, beryllium, nickel, soot, or tar in the workplace, air pollution, etc.

[00265] Classification according to causes:

[00266] According to the causes, lung cancer can be divided into two main classes are
30 small-cell lung carcinoma (SCLC) and non-small-cell lung carcinoma (NSCLC).

[00267] **SCLC** (10%-15%): This type of lung cancer is the most aggressive and rapidly growing of all the types. SCLC is strongly related to cigarette smoking. SCLCs metastasize rapidly to many sites within the body and are most often discovered after they have spread extensively.

35 [00268] **NSCLC** (85%): NSCLC has three main types designated by the type of cells found in the tumor. They are:

- Adenocarcinomas (40%), while adenocarcinomas are associated with smoking like

other lung cancers, this type is also seen in non-smokers -- especially women -- who develop lung cancer. Adenocarcinoma in situ (previously called bronchioloalveolar carcinoma) is a subtype of adenocarcinoma that frequently develops at multiple sites in the lungs and spreads along the preexisting alveolar walls. It may also look like pneumonia on a chest X-ray. It is increasing in frequency and is more common in women. People with this type of lung cancer tend to have a better prognosis than those with other types of lung cancer;

- Squamous cell carcinomas (25%-30%), squamous cell cancers arise most frequently in the central chest area in the bronchi. This type of lung cancer most often stays within the lung, spreads to lymph nodes, and grows quite large, forming a cavity;
- Large cell carcinomas (10%-15%), this type of cancer has a high tendency to spread to the lymph nodes and distant sites.

Other types of cancers can arise in the lung; these types are much less common than NSCLC and SCLC and together comprise only 5%-10% of lung cancers.

[00269] *The diagnosis of lung cancer is mainly focused on Imaging examination:*

[00270] (1) X-ray inspection: X-ray examination can understand the location and size of lung cancer, and may see local emphysema, atelectasis, or infiltrating lesions or pulmonary inflammation near the lesion due to bronchial obstruction. (2) Bronchoscopy: the bronchoscope can directly observe the pathological conditions of the bronchial lining and lumen. Tumor tissue can be taken for pathological examination, or bronchial secretions can be drawn for cytological examination to confirm the diagnosis and determine the histological type. (3) Cytological examination: sputum cytology is a simple and effective method for general screening and diagnosis of lung cancer. Most patients with primary lung cancer can find shed cancer cells in the sputum. The positive rate of sputum cytology for central lung cancer can reach 70% to 90%, while that for peripheral lung cancer is only about 50%. (4) ECT inspection: ECT bone imaging can detect bone metastases earlier. Both X-ray film and bone imaging have positive findings. If the osteogenesis reaction of the lesion is static and the metabolism is not active, the bone imaging is negative and the X-ray film is positive. The two complement each other and can improve the diagnosis rate. (6) Mediastinoscopy: mediastinoscopy is mainly used for patients with mediastinal lymph node metastasis, which is not suitable for surgical treatment, and other methods cannot obtain pathological diagnosis.

[00271] Surprisingly, the inventors found that peripheral red blood cell micronuclei DNA (rbcDNA) can be used to screen and diagnose lung cancer. The inventors further surprisingly found that rbcDNA signature is of great significance for early diagnosis, screening and risk ranking of lung cancer.

[00272] **Hepatocellular cancer**

[00273] Hepatocellular cancer (HCC) is the fifth most common cause of cancer, and the incidence is increasing globally due to the spread of hepatitis B and C virus infections, causes

also including: cirrhosis, heavy drinking, obesity and diabetes, abusive anabolic steroids, iron storage disease and aflatoxin. If caught early, it can sometimes be cured by surgery or transplantation. In more severe cases, it cannot be cured.

[00274] Detection of serum bio-markers for hepatocellular cancer

- 5 [00275] (1) The determination of serum alpha-fetoprotein (AFP) is relatively specific for the diagnosis of this disease. Immunoassay measures continuous serum $\text{AFP} \geq 400 \mu\text{g/L}$, and can rule out pregnancy, active liver disease, etc., then the diagnosis of liver cancer can be considered. However, approximately 30% of patients with liver cancer are clinically negative for AFP. (2) Blood enzymology and other tumor marker examinations. The levels of γ -glutamyl transpeptidase and its isoenzymes, abnormal prothrombin, alkaline phosphatase, and
10 lactate dehydrogenase isoenzymes in the serum of patients with liver cancer may be higher than normal, but it lacks specificity.

[00276] Imaging examination

- [00277] (1) Ultrasound examination can show the size, shape, location of the tumor and
15 whether there are tumor thrombi in the hepatic vein or portal vein, and the diagnostic coincidence rate can reach 90%. (2) CT examination has a high resolution, and the diagnostic coincidence rate for liver cancer can reach more than 90%, and it can detect small cancer foci with a diameter of about 1.0 cm. (3) The diagnostic value of MRI is similar to that of CT. It is better than CT in distinguishing benign and malignant intrahepatic lesions, especially from
20 hemangioma. (4) Selective angiography of celiac artery or hepatic arteriography. For cancers with abundant blood vessels, but the low-resolution limit for small liver cancers tumor volume less than 2.0cm, the positive rate can reach 90%. (5) Needle aspiration cytology for liver puncture, needle aspiration under the guidance of B-mode ultrasound can help increase the positive rate in cancer diagnosis, but with invasive tissue damage.

- 25 [00278] Surprisingly, the inventors found that peripheral red blood cell micronuclei DNA (rbcDNA) can be used to screen and diagnose hepatocellular cancer. The inventors further surprisingly found that rbcDNA signature is of great significance for early diagnosis, screening and risk ranking of hepatocellular cancer.

[00279] **Combined application of the invention and other methods**

- 30 [00280] In certain embodiments, the methods of the present disclosure can also be combined with other methods for screening, diagnosing or risk ranking of cancer. Those skilled in the art can select suitable other methods in the prior art as required.

- [00281] In certain embodiments, methods related to cervical cancer that can be combined with the methods of the present disclosure include, for example, detection of high-risk HPV and cytological
35 examination of cervical exfoliated cells. In an embodiment, the detection methods for high-risk HPV include morphological observation method, immunohistochemistry method, dot-blot hybridization method, blotting hybridization in situ, PCR/RFLP method, PCR/Southern method and the like. In one embodiment, the cytological examination of cervical exfoliated cells includes TCT, Pap smear, etc.

[00282] In certain embodiments, methods related to colorectal cancer that can be combined with the methods of the present disclosure include, for example, colonoscopy, flexible sigmoidoscopy, CT colonography, fecal occult blood test, immunochemical test, fecal DNA test, and the like.

5

EXAMPLES

[00283] In the following section, the present invention is further illustrated by examples. Examples are provided by way of illustration, but the present invention is not limited to the following examples. In the following examples, the subjects are all human subjects.

[00284] Example 1: Density gradient centrifugation of peripheral blood

10 [00285] Through the following steps, the peripheral blood samples of each subject were subjected to density gradient centrifugation.

[00286] Step 1. 1 ml fresh peripheral blood was obtained from a subject, and 1×PBS was added in equal volume to prepare a diluted blood sample.

15 [00287] Step 2. 5ml Ficoll density gradient centrifuge (Stemcell, Lymphoprep™ 07801) was added into the density gradient centrifuge tube.

[00288] Step 3. The diluted blood sample prepared in step 1 was slowly added to the density gradient centrifuge tube in Step 2. Density gradient centrifugation was performed at 1200g at 18°C for 15 minutes.

20 [00289] After density gradient centrifugation, the sample was divided into three layers: the upper layer was plasma, the middle layer was peripheral blood mononuclear cells (PBMC), and the bottom layer was red blood cells (as shown in Figure 1).

[00290] Example 2: Separation of blood cells

[00291] After density gradient centrifugation in Example 1, peripheral blood mononuclear cells and red blood cells were separated.

25 [00292] Specifically, as shown in Figure 2, the middle-upper layer liquid in the density gradient centrifuge tube was sucked through a straw, and the peripheral blood mononuclear cell sample was separated and collected. Red blood cells were extracted from the bottom of density gradient centrifuge tube via a needle tube, added into a 1.5ml centrifuge tube. 1×PBS was added into the centrifuge tube to a volume of 1ml liquid. Centrifugation was proceeded
30 at room temperature for 10min at 300g, and red blood cells at the bottom of the tube were collected. The collected RBCs were then subject to two sequential filtrations by 10μm cell strainers to remove potential contamination of nucleated cells.

[00293] Example 3: DNA extraction

35 [00294] In this example, the genome of peripheral blood mononuclear cells and erythrocyte micronuclei DNA were extracted, respectively.

[00295] 3.1 Extraction of genomic DNA from peripheral blood mononuclear cells

[00296] Genomic DNA was extracted from the peripheral blood mononuclear cell sample obtained in Example 2 using QIAamp DNA Blood Mini Kit (Qiagen, Cat No./ID: 51106), as shown in Figure 3.

5 [00297] 3.2 Extraction of erythrocyte micronuclei DNA

[00298] Red blood cells obtained in Example 2 were lysed by a red blood cell lysis buffer. Specifically, 10ml of red blood cell lysis buffer (Biosharp, Cat No./ID: BL503B) was added to the red blood cells collected in Example 2, lysed for 20 minutes at room temperature in the dark, and centrifuged at 3000g at room temperature for 10 minutes. Supernatant was taken
10 and incubated with 10mm EDTA (Solarbio Cat No./ID: E1170) and 200ug/ul protease K (Ambion, Cat No./ID: AM2548) at 56°C for 8 hours. Erythrocyte micronuclei DNA was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Cat No./ID: 51106).

[00299] **Example 4: DNA amplification, library construction and sequencing**

[00300] Genomic DNA of peripheral blood mononuclear cells and erythrocyte micronuclei
15 DNA extracted in Example 3 were amplified, library constructed and sequenced, respectively.

[00301] 4.1 DNA amplification

[00302] Genomic DNA of peripheral blood mononuclear cells and erythrocyte Micronuclei DNA prepared in Example 3 were subjected to multiple displacement amplification (MDA) using REPLI-g Single Cell Kit(Qiagen, Cat No./ID: 150345), to obtain
20 amplified DNA samples.

[00303] 4.2 Library construction

[00304] After MDA, the amplified DNA samples were subjected to second-generation sequencing library construction using TruePrep DNA Library Prep Kit V2 for Illumina (Vazyme, TD503).

25 [00305] 4.3 High-throughput sequencing

[00306] Genomic DNA of peripheral blood mononuclear cells and erythrocyte micronuclei DNA were sequenced by Novo-seq platform, with 10× sequencing depth and 30G data.

[00307] **Example 5: Bioinformatics analysis of erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells**

30 [00308] Bioinformatics analysis was made on micronuclei DNA information in red blood cells by the following steps (see Figure 4 for the logic of bioinformatics analysis algorithm):

[00309] 1. Quality control. Quality control on the original sequencing files of double-ended sequencing of erythrocyte micronuclei DNA and peripheral blood mononuclear cell genome DNA respectively through FastQC software.

- [00310] 2. Adaptor removal. Adaptor removal in the original sequencing file through cutadapter software (Kong, Y., Btrim: a fast, lightweight adapter and quality trimming program for next-generation sequencing technologies. Genomics, 2011. 98(2): p. 152-3). According to sequencing quality, the reads of small fragments with appropriate length and accurate pairing were reserved.
- [00311] 3. Data alignment. Sequenced fragments of red blood cell micronuclei DNA and peripheral blood mononuclear cell genome DNA were analyzed by bwa software (<http://bio-bwa.sourceforge.net>) was aligned to the human genome, and inappropriate and repeated reads were removed by Picard (Weisenfeld, N.I., et al., Direct determination of diploid genome sequences. Genome Res, 2017. 27(5): p. 757-767).
- [00312] 4. Comparison and counting of reads. The reads of sequenced small fragments in erythrocyte micronuclei DNA were counted corresponding to the gene regions of human genome using htseq-count software (Anders, S., P.T. Pyl and W. Huber, HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics, 2015. 31(2): p. 166-9), to compare whether there were significant differences in the degree of DNA fragmentation in red blood cells of healthy individuals and cancer patients.
- [00313] 5. Peak Calling. Searching for the fragments of red blood cell micronuclei DNA which were mainly enriched in a specific genetic region relative to the genome DNA sequencing reads of peripheral blood mononuclear cells through macs2 software, and removing the peak areas which were more enriched by peripheral blood mononuclear cells relative to PBMC *per se* as a whole.
- [00314] 6. Genome information annotation and pathway enrichment of specific broken fragments in erythrocyte micronuclei DNA. Compared with peripheral blood mononuclear cells, genome information annotation and pathway enrichment (KEGG, Gene Ontology) were performed on specific broken fragments of erythrocytes (Chen, L., et al., Gene Ontology and KEGG Pathway Enrichment Analysis of a Drug Target-Based Classification System. PLoS One, 2015. 10(5): p. e0126492), the specific broken genes in erythrocyte micronuclei DNA were obtained.
- [00315] 7. Data classification and classifier construction. Differentiated genes were select as features to construct classifiers for known classified samples based on support vector machine (SVM), and to predict unknown samples (Huang, M.W., et al., SVM and SVM Ensembles in Breast Cancer Prediction. PLoS One, 2017. 12(1): p. e0161501).
- [00316] 7.1 Data classification
- [00317] Specifically, the reads count in gene regions of “n” experimental samples and “m” control samples were selected every time, wherein “n” and “m” refers to the number of samples). The differentiated genes (also called “characteristic genes”) were screened out by ANOVA test to distinguish the two types of samples.

[00318] 7.2 Classifier construction

[00319] Classifier parameter adjustment. Based on the characteristic genes screened in step 7.1, the training group ($n=100$) was determined by using the algorithm SVM/LOOCV (leave-one-out cross-validation support vector machine). First, the true labels of all samples were set (e.g., the sample in the experimental group was recorded as 1, and the sample in the control group was recorded as 0). One sample was picked out at a time as a test set, and all other samples ($n-1$) were used to build a model and test the "test set". The test set traversed all samples to complete n rounds of cross-validation, and the test results for each sample were obtained. Based on the whole test results and the real label of each sample, the accuracy, sensitivity and specificity were calculated, so as to adjust the best parameters of the model and construct the training model. In this study, the parameters of SVM were set as $C=100$ and $\gamma=10^{-4}$, wherein C is the penalty coefficient, that is, the tolerance of errors; γ is a default parameter when RBF function is selected as kernel.

[00320] 7.3 Prediction on unknown samples

[00321] Based on the training model obtained in the previous step, unknown samples (i.e., test sets) that did not participate in the training were used to predict the test set samples through the classifier constructed in the previous step, to obtain the prediction results of the test set and the real labels of the samples, and to present the proportion of each prediction result in the two classes (i.e., risk assessment index). Unknown samples were predicted and to show the results of binary classification.

[00322] **Example 6: Construction of a classifier for clustering healthy individuals and cervical cancer patients using erythrocyte micronuclei DNA**

[00323] In this example, there were 15 subjects, including:

[00324] Experimental group: 9 patients diagnosed with cervical cancer by other methods

[00325] Control group: 6 healthy individuals (non-cervical disease individuals).

[00326] The peripheral blood samples from patients with cervical cancer were expressed in the form of "P" plus patient number. For example, "P1" represented a peripheral blood sample from the first cervical cancer patient ("Patient 1"), "P2" represented a peripheral blood sample from the second cervical cancer patient ("Patient 2"), and so on. In addition, peripheral blood samples from healthy individuals were expressed in the form of "H" plus individual number. For example, "H1" represents the peripheral blood sample from the first healthy individual, "H2" represents the peripheral blood sample from the second healthy individual, and so on.

[00327] The basic information of 9 patients with cervical cancer is shown in Table 1. "cervical cancer type" refers to the type of cervical cancer diagnosed by other methods.

Table 1

Patient number	Age	HPV Detected value	Maximum diameter of tumor (cm)	Type of cervical cancer	Existence of lymph node metastasis or not
1	65	25.55	3.3	Medium differentiated squamous cell carcinoma	No
2	36	50.13	4.2	Low-medium differentiated adenocarcinoma	No
3	36	916.-3	4.0	CINIII budding growth (early invasion of cancer)	Not dissected
4	43	240.09	4.1	Low differentiated squamous cell carcinoma	Yes (without surgery)
5	50	3.67	3.7	Non-invasive carcinoma	Not dissected
6	56	503.61	3.2	Low-medium differentiated squamous cell carcinoma	No
7	71	1212.8	2.6	Medium differentiated squamous cell carcinoma	Yes (without surgery)
8*	37	No	5.4	Low-medium differentiated squamous cell carcinoma	Yes
9	44	68.36	2.1	Medium-high differentiated adenocarcinoma	No

*: Patient 8 is HPV negative.

[00328] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and
5 bioinformatics analysis was carried out as described in Example 5.

[00329] Specifically, 9 samples of primary cervical cancer and 6 samples of healthy women were selected for the reads counting, and 2306 differential genes were screened out

by ANOVA test to distinguish the two classes of samples. Then, according to Pearson correlation, the two classes of samples were clustered in unsupervised hierarchy, showing that there were significant differences between the two classes of samples.

[00330] As shown in Figure 5, the erythrocyte micronuclei DNA from the peripheral blood samples of cervical cancer patients and the erythrocyte micronuclei DNA from the peripheral blood samples of healthy individuals were clustered to obtain 2,306 differential genes (forming a classifier for distinguishing healthy individuals from cervical cancer patients). In Figure 5, each row represents a differential gene, and each column represents a patient.

[00331] The list of 2,306 differential genes is shown in Table 2. Each gene corresponds to each row from top to bottom in Figure 5.

Table 2

1	A2MP1	577	HIST1H4F	1153	NANOS1	1729	TAS2R3
2	ABCA11P	578	HLA-DOA	1154	NANP	1730	TAS2R30
3	ABCA9-AS1	579	HLA-DOB	1155	NAT1	1731	TAS2R39
4	ABCB7	580	HLA-DPB1	1156	NAT16	1732	TAS2R42
5	ABCG4	581	HLA-DQB2	1157	NAV2-AS4	1733	TATDN2
6	ABCG5	582	HLA-F	1158	NBPF13P	1734	TBPL1
7	ABHD3	583	HLTF	1159	NCALD	1735	TBR1
8	ABRAXAS1	584	HMGAI1P7	1160	NCAPD3	1736	TCAMIP
9	ACADM	585	HMGN5	1161	NCAPG	1737	TCEAL3
10	ACAN	586	HMGXB3	1162	NCEP2L	1738	TCEAL4
11	ACAP2	587	HNF1A	1163	NCK1-AS1	1739	TCEANC
12	ACAT2	588	HNRNPA1P10	1164	NCKIPSD	1740	TCF24
13	ACKR3	589	HNRNPA3	1165	NCL	1741	TCHH
14	ACKR4	590	HNRNPD	1166	NDST3	1742	TCHHL1
15	ACOD1	591	HNRNPDL	1167	NDUFA4	1743	TCOF1
16	ACP7	592	HNRNPL	1168	NDUFB8	1744	TCP11L1
17	ACSM5	593	HNRNPU	1169	NDUFV2-AS1	1745	TCP11L2
18	ACTR1B	594	HOOK3	1170	NECTIN3-AS1	1746	TDRD6
19	ACTR3C	595	HOTAIRM1	1171	NEDD9	1747	TDRKH-AS1
20	ACTR6	596	HOXC8	1172	NEFM	1748	TEAD3
21	ADAM21	597	HPCAL4	1173	NEGR1-IT1	1749	TEAD4
22	ADAM30	598	HPS6	1174	NEK8	1750	TEFM
23	ADAT1	599	HRASLS	1175	NELFCD	1751	TET2
24	ADCY2	600	HRASLS2	1176	NETO2	1752	TEX101
25	ADCY6	601	HRC	1177	NEUROD6	1753	TFB2M
26	ADCY9	602	HS3ST3B1	1178	NFAT5	1754	TFE3
27	ADD3-AS1	603	HSD3BP4	1179	NFE2L3	1755	TFF2
28	ADPGK	604	HSD52	1180	NFIA-AS2	1756	TGFA
29	ADRB1	605	HSDL1	1181	NFIB	1757	TGFB3
30	AFDN-DT	606	HSDL2	1182	NFIC	1758	THAP2
31	AFG1L	607	HSF2	1183	NHLH1	1759	THEM4
32	AFTPH	608	HSPA14	1184	NHLRC3	1760	TIGD4
33	AGAP4	609	HSPA9	1185	NIFK	1761	TMM21

34	AGFG1	610	HTATIP2	1186	NIPBL-AS1	1762	TMM8A
35	AGO2	611	HTR1A	1187	NIPSNAP3A	1763	TMM9
36	AGPAT1	612	HTR1D	1188	NIPSNAP3B	1764	TKTL2
37	AGPAT3	613	HTR3D	1189	NKAIN3	1765	TLCD2
38	AIMP1	614	HYALP1	1190	NKAPL	1766	TLE3
39	AIPL1	615	HYKK	1191	NKRF	1767	TLR1
40	AK4	616	IAPP	1192	NKX1-2	1768	TLR4
41	AKAIN1	617	ICE1	1193	NLGN2	1769	TLX1NB
42	AKAP11	618	IDS	1194	NLRP2B	1770	TM6SF1
43	AKAP5	619	IFT20	1195	NLRP7	1771	TMC01
44	AKIRIN1	620	IFT88	1196	NNT-AS1	1772	TMEM128
45	AKR1C4	621	IGFBP1	1197	NOL3	1773	TMEM132E
46	AKTIP	622	IGFL3	1198	NOS2	1774	TMEM14EP
47	ALDH3A2	623	IGLL5	1199	NOV	1775	TMEM155
48	ALKBH8	624	IGSF9B	1200	NPTN	1776	TMEM169
49	AMMECR1	625	IL10RB-AS1	1201	NPVF	1777	TMEM170A
50	AMPH	626	IL13	1202	NR2F1-AS1	1778	TMEM177
51	ANGPT2	627	IL17RC	1203	NRG3-AS1	1779	TMEM200C
52	ANKRD13C	628	IL17RE	1204	NRIP2	1780	TMEM209
53	ANKRD18DP	629	IL20RB	1205	NRN1	1781	TMEM220
54	ANKRD20A19P	630	IL21	1206	NSMCE3	1782	TMEM225
55	ANKRD36BP1	631	IL33	1207	NT5E	1783	TMEM229B
56	ANKRD62P1-PARP4P3	632	IL4R	1208	NTM-AS1	1784	TMEM235
57	ANKS4B	633	IL6	1209	NTS	1785	TMEM254
58	ANXA2P1	634	IL6R	1210	NUBP1	1786	TMEM255B
59	AP3D1	635	INE2	1211	NUDT5	1787	TMEM53
60	AP3M2	636	ING4	1212	NUFIP1	1788	TMEM62
61	AP4S1	637	INHBA	1213	NUGGC	1789	TMEM65
62	APOBEC1	638	IPO8	1214	NUF188	1790	TMEM81
63	APOBEC4	639	IQCA1	1215	NXF4	1791	TMEM86A
64	APOL4	640	IQCF2	1216	NXFE3	1792	TMEM9
65	APOL6	641	IQGAP2	1217	OCLM	1793	TMEM99
66	APOLD1	642	IRAK1BP1	1218	ODAM	1794	TMIGD3
67	ARAP1	643	IRS2	1219	ODF4	1795	TMOD4
68	ARF4-AS1	644	ISLR2	1220	OGT	1796	TMPPE
69	ARFGAP2	645	ISM1	1221	OLFM5P	1797	TMPRSS4
70	ARFGEF1	646	ISOC1	1222	OLMALINC	1798	TMSB15A
71	ARHGAP22-IT1	647	ITFG1-AS1	1223	OMG	1799	TNFSF13B
72	ARHGAP26-AS1	648	ITFG2	1224	OR10A7	1800	TNK2-AS1
73	ARHGAP36	649	ITGAL	1225	OR10AD1	1801	TNPO1
74	ARHGEF11	650	ITGAV	1226	OR10AG1	1802	TNRC17
75	ARHGEF7	651	ITGB3BP	1227	OR10H2	1803	TNRC6C
76	ARID1B	652	IWS1	1228	OR10Q1	1804	TOMM34
77	ARL11	653	JADE3	1229	OR10R2	1805	TOPIP2
78	ARL2BP	654	JAM3	1230	OR10S1	1806	TOPORS
79	ARL6IP1	655	JMJD1C-AS1	1231	OR10T2	1807	TP53
80	ARMC6	656	JRK	1232	OR11H6	1808	TPCN2
81	ARMCX5	657	KANSL3	1233	OR11H7	1809	TPD52L3

82	ARPC1B	658	KATNBL1P6	1234	OR12D2	1810	TP11P2
83	ARPP19	659	KBTBD13	1235	OR14J1	1811	TPRG1-AS1
84	ARSJ	660	KC6	1236	OR1A2	1812	TRAF6
85	ASB10	661	KCNA3	1237	OR1E3	1813	TRAMI
86	ASB3	662	KCNH1-IT1	1238	OR1F2P	1814	TRAMIL1
87	ASIC4	663	KCNJ1	1239	OR1J1	1815	TRDN-AS1
88	ATP13A3	664	KCNJ13	1240	OR1K1	1816	TRIB1
89	ATP2B1-AS1	665	KCNK3	1241	OR1S1	1817	TRIB2
90	ATP5F1C	666	KCNK5	1242	OR2A25	1818	TRIM23
91	ATP5PO	667	KCNMB3	1243	OR2AG1	1819	TRIM3
92	ATP6V0A4	668	KCNS1	1244	OR2AG2	1820	TRIM32
93	ATP7A	669	KCTD21	1245	OR2AJ1	1821	TRIM51
94	ATXN7L3B	670	KCTD9	1246	OR2C3	1822	TRIM58
95	AUNIP	671	KDELC1	1247	OR2D2	1823	TRIM69
96	AWAT1	672	KDM4A-AS1	1248	OR2F1	1824	TRIQK
97	AXIN2	673	KIAA0754	1249	OR2M1P	1825	TRMT5
98	B3GALNT1	674	KIAA0895L	1250	OR2T27	1826	TRNT1
99	B3GNT5	675	KIAA2026	1251	OR2T7	1827	TRPC7-AS2
100	BAG2	676	KIF20A	1252	OR2V1	1828	TSC22D1
101	BBS1	677	KIF4B	1253	OR2V2	1829	TSGA13
102	BBS2	678	KIF6	1254	OR2W5	1830	TSHR
103	BBS9	679	KIRREL3-AS3	1255	OR4C12	1831	TSPAN2
104	BEX1	680	KLF11	1256	OR4C13	1832	TSPYL4
105	BEX5	681	KLF12	1257	OR4C3	1833	TSR1
106	BFSP1	682	KLF13	1258	OR4E2	1834	TSSK1B
107	BHLHE40-AS1	683	KLF3	1259	OR4F6	1835	TSSK4
108	BICRAL	684	KLHDC1	1260	OR4K13	1836	TTBK1
109	BIRC6-AS2	685	KLHL34	1261	OR4Q2	1837	TTC8
110	BLOC1S2	686	KLK11	1262	OR4S1	1838	TTF1
111	BLVRB	687	KLK2	1263	OR51E1	1839	TTL
112	BMP15	688	KLLN	1264	OR51I2	1840	TPA
113	BMS1	689	KLRB1	1265	OR51T1	1841	TUBB4A
114	BORA	690	KLRC1	1266	OR52B6	1842	TUBG1
115	BRMS1L	691	KLRF2	1267	OR52E2	1843	TWF1
116	BRWD1-AS1	692	KLRG1	1268	OR52E4	1844	TXNDC2
117	BSND	693	KMT2E-AS1	1269	OR52N2	1845	TYMS
118	BTBD3	694	KPNA6	1270	OR52N5	1846	TYMSOS
119	BTBD9-AS1	695	KPRP	1271	OR52W1	1847	UBA5
120	BTG1	696	KRBA2	1272	OR52Z1	1848	UBA6
121	BTG2	697	KRCC1	1273	OR5AC2	1849	UBA7
122	BTG4	698	KRIT1	1274	OR5B3	1850	UBE2D1
123	BTN2A2	699	KRT10	1275	OR5K3	1851	UBE2G1
124	BTN3A2	700	KRT13	1276	OR5M8	1852	UBE2L6
125	BTNL2	701	KRT25	1277	OR6B1	1853	UBE2MP1
126	BUD31	702	KRT4	1278	OR6C2	1854	UBE2N
127	C11orf143	703	KRT6B	1279	OR6C68	1855	UBE2Q2
128	C11orf44	704	KRT83	1280	OR6C74	1856	UBR7
129	C11orf53	705	KRTAP12-2	1281	OR6K3	1857	UCA1

130	C11orf54	706	KRTAP17-1	1282	OR6S1	1858	UGDH
131	C12orf10	707	KRTAP19-2	1283	OR7A5	1859	UGT1A1
132	C12orf45	708	KRTAP20-1	1284	OR7C1	1860	UGT1A10
133	C12orf71	709	KRTAP4-5	1285	OR7C2	1861	UNC45B
134	C12orf75	710	KRTAP5-2	1286	OR7D2	1862	UOX
135	C14orf39	711	KRTAP5-AS1	1287	OR7E24	1863	UPF1
136	C16orf89	712	KRTAP9-4	1288	OR7G1	1864	UPF3B
137	C17orf75	713	KRTDAP	1289	OR8A1	1865	USP15
138	C18orf54	714	LAMP5	1290	OR8B4	1866	USP20
139	C19orf66	715	LAMP5-AS1	1291	OR8I3	1867	USP30
140	C1GALT1	716	LAMTOR5-AS1	1292	OR9A4	1868	USP40
141	C1QTNF6	717	LAPTM4B	1293	OSTF1	1869	USP51
142	C1S	718	LATS1	1294	OSTM1	1870	UTP14C
143	C1orf68	719	LCORL	1295	OTOR	1871	UTP3
144	C21orf59	720	LCP2	1296	OTUB2	1872	VAR3
145	C2orf48	721	LDLRAD4-AS1	1297	OTUD1	1873	VCAM1
146	C2orf49	722	LEF1-AS1	1298	OTUD6B-AS1	1874	VGLL1
147	C2orf74	723	LGALS13	1299	OVAAL	1875	VMA21
148	C2orf78	724	LGALS17A	1300	PABPC5-AS1	1876	VPS37C
149	C3orf18	725	LGALS3	1301	PACRG-AS1	1877	VPS51
150	C3orf38	726	LGALS8-AS1	1302	PACS1	1878	VPS53
151	C4orf45	727	LIMD1-AS1	1303	PACSIN2	1879	VSIG1
152	C4orf47	728	LIMK2	1304	PADI2	1880	VSIG10L
153	C5orf24	729	LINC00161	1305	PAFAH2	1881	VSTM2L
154	C5orf64	730	LINC00216	1306	PAGE2	1882	VSTM5
155	C5orf66	731	LINC00240	1307	PAK1IP1	1883	VSX1
156	C6orf99	732	LINC00308	1308	PAPD7	1884	VWA5B2
157	C7orf13	733	LINC00309	1309	PAPSS2	1885	VWC2
158	C7orf26	734	LINC00317	1310	PARP3	1886	WASF3
159	C8orf31	735	LINC00331	1311	PARP8	1887	WBP11
160	C8orf48	736	LINC00339	1312	PATE3	1888	WDFY4
161	C9orf170	737	LINC00363	1313	PAWR	1889	WDR45
162	C9orf40	738	LINC00392	1314	PAX2	1890	WDR55
163	C9orf43	739	LINC00440	1315	PAX6	1891	WDR92
164	C9orf66	740	LINC00467	1316	PAXBPI-AS1	1892	WFDC2
165	C9orf92	741	LINC00491	1317	PBDC1	1893	WFDC5
166	CA2	742	LINC00520	1318	PBX1	1894	WFIKK2
167	CA8	743	LINC00539	1319	PCDH9-AS3	1895	WISP3
168	CACNA1I	744	LINC00548	1320	PCDHA11	1896	WNT10B
169	CACNB3	745	LINC00615	1321	PCDHA13	1897	WNT9B
170	CADM4	746	LINC00620	1322	PCDHA2	1898	WSB2
171	CALCRL	747	LINC00626	1323	PCDHA5	1899	WTAP
172	CAMK1G	748	LINC00628	1324	PCDHAC1	1900	XCR1
173	CASC1	749	LINC00641	1325	PCDHAC2	1901	XK
174	CASC3	750	LINC00668	1326	PCDHB1	1902	XKR4
175	CASC9	751	LINC00674	1327	PCDHB12	1903	XKR6
176	CAVIN4	752	LINC00837	1328	PCDHB15	1904	XKR9
177	CBWD5	753	LINC00844	1329	PCDHB16	1905	XXYLT1-AS2

178	CBX2	754	LINC00862	1330	PCDHB4	1906	XYLT2
179	CBX3	755	LINC00889	1331	PCDHB8	1907	YBEY
180	CBX5	756	LINC00901	1332	PCDHGA12	1908	YBX1
181	CC2D1B	757	LINC00924	1333	PCDHGA2	1909	YBX3P1
182	CCDC120	758	LINC00926	1334	PCDHGA5	1910	YES1
183	CCDC121	759	LINC00970	1335	PCDHGA8	1911	YIPF4
184	CCDC152	760	LINC00987	1336	PCDHGA9	1912	YIPF7
185	CCDC17	761	LINC00999	1337	PCDHGB4	1913	YTHDF1
186	CCDC18-AS1	762	LINC01012	1338	PCDHGB5	1914	ZBED2
187	CCDC186	763	LINC01016	1339	PCDHGB6	1915	ZBED3
188	CCDC89	764	LINC01036	1340	PCDHGB8P	1916	ZBED6CL
189	CCEPR	765	LINC01037	1341	PCDHGC4	1917	ZBTB24
190	CCIN	766	LINC01039	1342	PCDHGC5	1918	ZBTB41
191	CCL11	767	LINC01049	1343	PCGF3	1919	ZBTB8B
192	CCL22	768	LINC01099	1344	PCK2	1920	ZC2HC1A
193	CCL5	769	LINC01121	1345	PCMTD1	1921	ZC3H15
194	CCNG1	770	LINC01135	1346	PCOTH	1922	ZC3HAV1L
195	CCNI2	771	LINC01140	1347	PCTP	1923	ZCCHC10
196	CCNT2	772	LINC01191	1348	PDAP1	1924	ZCCHC12
197	CCNT2-AS1	773	LINC01202	1349	PDC	1925	ZDHH2
198	CCR5	774	LINC01209	1350	PDE5D	1926	ZDHH2
199	CCT2	775	LINC01224	1351	PDK4	1927	ZFAND2B
200	CCT6A	776	LINC01243	1352	PDLIM1	1928	ZFP14
201	CCT8	777	LINC01305	1353	PDPK1	1929	ZFP91
202	CD160	778	LINC01340	1354	PDZD8	1930	ZNF155
203	CD28	779	LINC01370	1355	PECR	1931	ZNF19
204	CD300LB	780	LINC01416	1356	PELO	1932	ZNF195
205	CD33	781	LINC01432	1357	PEX2	1933	ZNF207
206	CD37	782	LINC01461	1358	PEX5	1934	ZNF211
207	CD3E	783	LINC01474	1359	PFDN4	1935	ZNF234
208	CD40	784	LINC01476	1360	PFKFB1	1936	ZNF235
209	CD40LG	785	LINC01478	1361	PFN4	1937	ZNF254
210	CD58	786	LINC01486	1362	PGBD2	1938	ZNF260
211	CD72	787	LINC01499	1363	PGK1	1939	ZNF275
212	CDC40	788	LINC01564	1364	PGLYRP1	1940	ZNF280A
213	CDCA3	789	LINC01565	1365	PGM2L1	1941	ZNF280C
214	CDCP1	790	LINC01580	1366	PGM5P2	1942	ZNF284
215	CDH5	791	LINC01586	1367	PGR-AS1	1943	ZNF30-AS1
216	CDK13	792	LINC01594	1368	PGRMC2	1944	ZNF33BP1
217	CDKN2B	793	LINC01600	1369	PHC1	1945	ZNF34
218	CD8N	794	LINC01623	1370	PHF20L1	1946	ZNF362
219	CDV3	795	LINC01628	1371	PHF24	1947	ZNF384
220	CENPL	796	LINC01657	1372	PHLDA3	1948	ZNF398
221	CEP78	797	LINC01682	1373	PHYHD1	1949	ZNF432
222	CEP83-AS1	798	LINC01683	1374	PI4K2B	1950	ZNF44
223	CETN1	799	LINC01695	1375	PIGBOS1	1951	ZNF460-AS1
224	CFAP126	800	LINC01698	1376	PIGZ	1952	ZNF503
225	CFAP77	801	LINC01721	1377	PIN1P1	1953	ZNF529-AS1

226	CFAP97	802	LINC01723	1378	PIN4P1	1954	ZNF559
227	CFDP1	803	LINC01725	1379	PISRT1	1955	ZNF57
228	CFHR4	804	LINC01737	1380	PITRM1	1956	ZNF571
229	CHAMP1	805	LINC01846	1381	PITRM1-AS1	1957	ZNF577
230	CHIC1	806	LINC01851	1382	PITX2	1958	ZNF595
231	CHMP4B	807	LINC01877	1383	PKD2L1	1959	ZNF611
232	CHRNA10	808	LINC01878	1384	PLA2G4F	1960	ZNF619
233	CKAP5	809	LINC01907	1385	PLAGL1	1961	ZNF665
234	CLDN19	810	LINC01915	1386	PLCL2	1962	ZNF691
235	CLDN22	811	LINC01933	1387	PLEKHA1	1963	ZNF692
236	CLDN7	812	LINC01934	1388	PLEKHA4	1964	ZNF70
237	CLDND1	813	LINC01939	1389	PLEKHB2	1965	ZNF710
238	CLEC1B	814	LINC01959	1390	PLIN1	1966	ZNF770
239	CLEC2B	815	LINC01975	1391	PLPP1	1967	ZNF804A
240	CLEC5A	816	LINC01985	1392	PLPP3	1968	ZNF804B
241	CLK2P1	817	LINC01986	1393	PLPPR1	1969	ZNF805
242	CLRN1	818	LINC01990	1394	PLPPR2	1970	ZNF823
243	CLRN3	819	LINC01991	1395	PLRG1	1971	ZNF843
244	CLSTN2-AS1	820	LINC02009	1396	PLSCR2	1972	ZNF847P
245	CLTA	821	LINC02010	1397	PLUT	1973	ZNF850
246	CLTB	822	LINC02020	1398	PMCH	1974	ZRANB1
247	CLUL1	823	LINC02041	1399	PMS2P1	1975	ZSCAN2
248	CMC1	824	LINC02049	1400	PNLIPRP3	1976	ZSCAN20
249	CMC2	825	LINC02071	1401	PNMA8B	1977	ZSWIM9
250	CNBP	826	LINC02106	1402	PNRC2	1978	ZXDA
251	CNDP2	827	LINC02120	1403	POLG2	1979	ZYG11A
252	CNGA1	828	LINC02135	1404	POLN	1980	AAMP
253	CNGA4	829	LINC02149	1405	POM121L12	1981	ABCC5-AS1
254	CNIH4	830	LINC02158	1406	POM121L2	1982	ADGRE5
255	CNKSRL3	831	LINC02167	1407	POMP	1983	ADH1A
256	CNOT6L	832	LINC02185	1408	POPDC3	1984	AJAP1
257	CNP	833	LINC02210	1409	POU2F3	1985	ALAD
258	COG4	834	LINC02217	1410	PPARG	1986	ALG1
259	COG8	835	LINC02226	1411	PPBPP2	1987	ALG14
260	COL6A4P1	836	LINC02233	1412	PPIE	1988	ANKRD39
261	COMMD6	837	LINC02278	1413	PPMIE	1989	APOA5
262	COPB2	838	LINC02287	1414	PPMIF	1990	AQP3
263	COX7A2L	839	LINC02292	1415	PPMIN	1991	ARHGEF25
264	CPB1	840	LINC02303	1416	PPP1R10	1992	ARID5B
265	CPEB1	841	LINC02314	1417	PPP1R14D	1993	ARL4D
266	CPHXL	842	LINC02324	1418	PPP1R15A	1994	ATE1-AS1
267	CPLX2	843	LINC02339	1419	PPP1R7	1995	ATP10B
268	CPNE6	844	LINC02363	1420	PPP1R9B	1996	ATXN7L1
269	CREB1	845	LINC02365	1421	PPP4R3C	1997	B3GNT2
270	CRH	846	LINC02376	1422	PPWD1	1998	B9D2
271	CRIP1	847	LINC02380	1423	PRDM13	1999	BLK
272	CRISPLD1	848	LINC02382	1424	PRKAB1	2000	BRD9
273	CRNN	849	LINC02387	1425	PRKAR2A-AS1	2001	BRINP2

274	CRYBG2	850	LINC02414	1426	PRKX-AS1	2002	BYSL
275	CRYM	851	LINC02431	1427	PRLHR	2003	C12orf43
276	CSFIR	852	LINC02454	1428	PRMT6	2004	C12orf50
277	CST1	853	LINC02485	1429	PRMT9	2005	C12orf77
278	CST3	854	LINC02488	1430	PROK2	2006	C14orf144
279	CST9	855	LINC02523	1431	PROKR2	2007	C16orf90
280	CT62	856	LINC02542	1432	PROSER1	2008	C22orf24
281	CTHRC1	857	LINC02544	1433	PRPF38A	2009	C4orf3
282	CTIF	858	LINC02565	1434	PRPS1	2010	C8orf74
283	CTSO	859	LIPJ	1435	PRR15L	2011	CADM2-AS2
284	CTSV	860	LIVAR	1436	PRR23B	2012	CCAR1
285	CTXND1	861	LMCD1-AS1	1437	PRR23C	2013	CCDC9
286	CTXND2	862	LMNTD1	1438	PRR26	2014	CD2
287	CUL7	863	LMO4	1439	PRRG4	2015	CD68
288	CXCL3	864	LNK1-AS1	1440	PRRT4	2016	CD82
289	CXCL5	865	LOC100128594	1441	PRSS22	2017	CD8A
290	CXCL6	866	LOC100129138	1442	PRSS30P	2018	CEACAM19
291	CXCL9	867	LOC100129148	1443	PRSS46P	2019	CFB
292	CXCR4	868	LOC100129297	1444	PSD2-AS1	2020	CHCHD5
293	CYB5D2	869	LOC100129636	1445	PSG3	2021	CHRNA2
294	CYP11B1	870	LOC100129935	1446	PSG8	2022	CIDECP
295	CYP27C1	871	LOC100130075	1447	PSMA3	2023	CIT
296	CYP4V2	872	LOC100130298	1448	PSMC2	2024	CLDN11
297	CYP4Z1	873	LOC100132831	1449	PSMD10	2025	CMTM7
298	CYP4Z2P	874	LOC100192426	1450	PSMD12	2026	CNGB1
299	CYP8B1	875	LOC100240728	1451	PSMD9	2027	CNPPD1
300	CYTH3	876	LOC100270746	1452	PSTK	2028	COL9A3
301	CYTH4	877	LOC100270804	1453	PTAFR	2029	CPA2
302	CYYR1	878	LOC100272217	1454	PTBP3	2030	CSTA
303	DACT1	879	LOC100287467	1455	PTGDR	2031	CWC25
304	DACT3-AS1	880	LOC100287497	1456	PTPN2	2032	DDX47
305	DAGLB	881	LOC100288637	1457	PTPRA	2033	DEFB115
306	DARS-AS1	882	LOC100288842	1458	PTPRG-AS1	2034	DGKK
307	DAZL	883	LOC100289230	1459	PTTG1	2035	DIAPH2-AS1
308	DBF4	884	LOC100289495	1460	PURG	2036	DISC1-IT1
309	DBT	885	LOC100335030	1461	PUS3	2037	DNAH17-AS1
310	DBX2	886	LOC100500773	1462	PXN	2038	DNAJC12
311	DCAF12L1	887	LOC100505978	1463	PYCARD-AS1	2039	DPH3P1
312	DCAF12L2	888	LOC100506098	1464	QKI	2040	DPRX
313	DCK	889	LOC100506302	1465	RAE23	2041	DPYD-AS1
314	DCLRE1A	890	LOC100506358	1466	RABIF	2042	DYNC1I2
315	DCPS	891	LOC100506497	1467	RAD51	2043	EEF1AKMT2
316	DCTN1-AS1	892	LOC100506548	1468	RAD51AP1	2044	EIF2B2
317	DDB2	893	LOC100506682	1469	RAET1G	2045	EIF4E2
318	DDI1	894	LOC100507002	1470	RAG1	2046	F3
319	DDX42	895	LOC100507388	1471	RAP1B	2047	FADS2
320	DEFA5	896	LOC100508631	1472	RAPGEF3	2048	FAM214B
321	DEFA9P	897	LOC100996635	1473	RASL11B	2049	FBXO9

322	DENND1B	898	LOC100996664	1474	RASSF10	2050	FBXW4
323	DENND1C	899	LOC101805491	1475	RASSF4	2051	FCFIP2
324	DENND2A	900	LOC101926943	1476	RASSF6	2052	FRG1DP
325	DEPDC1-AS1	901	LOC101927045	1477	RBM11	2053	FRY-AS1
326	DEPDC1B	902	LOC101927142	1478	RBM15B	2054	FXYD5
327	DGCR5	903	LOC101927196	1479	RBM26-AS1	2055	GABARAPL1
328	DGUOK	904	LOC101927314	1480	RBM3	2056	GDF11
329	DHRS4-AS1	905	LOC101927391	1481	RBM44	2057	GGA3
330	DIRC3	906	LOC101927437	1482	RBM46	2058	GID4
331	DIS3	907	LOC101927516	1483	RBM48	2059	GNG7
332	DKFZP586I1420	908	LOC101927550	1484	RBMS3-AS3	2060	GPATCH1
333	DKK2	909	LOC101927560	1485	RBMX	2061	GPATCH4
334	DLG3	910	LOC101927661	1486	RBMXL2	2062	GPC6
335	DMWD	911	LOC101927708	1487	RBPJL	2063	GPR153
336	DNAAF3	912	LOC101927919	1488	RC3H2	2064	GPX3
337	DNAI1	913	LOC101927972	1489	RCAN1	2065	GRAMD2A
338	DNAJA3	914	LOC101928131	1490	REG1CP	2066	GRB10
339	DNAJC27	915	LOC101928134	1491	RERG	2067	GRM7
340	DNAJC5G	916	LOC101928140	1492	RFC3	2068	HAX1
341	DNAJC6	917	LOC101928143	1493	RFESD	2069	HMGB1
342	DNM1P46	918	LOC101928253	1494	RFPL1	2070	HNRNPH1
343	DNM3-IT1	919	LOC101928307	1495	RFPL18	2071	HSD17B2
344	DNMT1	920	LOC101928336	1496	RGR	2072	IDH3B
345	DNPH1	921	LOC101928370	1497	RGS12	2073	INO80D
346	DPM1	922	LOC101928429	1498	RGS13	2074	INTS14
347	DPP10-AS1	923	LOC101928443	1499	RGS18	2075	IVD
348	DPPA2P3	924	LOC101928495	1500	RHOBTB1	2076	JAKMIP1
349	DPRXP4	925	LOC101928517	1501	RHOJ	2077	KANSL1-AS1
350	DPY19L2P1	926	LOC101928530	1502	RHOJ	2078	KATNAL1
351	DPYD-AS2	927	LOC101928535	1503	RIF1	2079	KCNA4
352	DPYS	928	LOC101928622	1504	RIOK2	2080	KCNK10
353	DR1	929	LOC101928797	1505	RLIM	2081	KCTD2
354	DRAIC	930	LOC101928847	1506	RLN2	2082	KDM4D
355	DSTYK	931	LOC101928858	1507	RNASE11	2083	KLHL29
356	DTX3L	932	LOC101928936	1508	RNASE2	2084	KRBOX4
357	DUOXA1	933	LOC101928940	1509	RNASE3	2085	KRT18
358	DUSP18	934	LOC101929353	1510	RNASE4	2086	LAIR1
359	DUSP21	935	LOC101929372	1511	RNASE7	2087	LHFPL3-AS1
360	DUSP5	936	LOC101929517	1512	RNF103	2088	LIN9
361	DYNCILI2	937	LOC101929529	1513	RNF135	2089	LINC00114
362	DYNLRB2	938	LOC101929541	1514	RNF152	2090	LINC00342
363	DYRK2	939	LOC101929577	1515	RNF157-AS1	2091	LINC00347
364	EAF2	940	LOC101929586	1516	RNF167	2092	LINC00433
365	ECHDC2	941	LOC101929633	1517	RNF168	2093	LINC00443
366	EDRF1	942	LOC101929662	1518	RNF24	2094	LINC00536
367	EFNA2	943	LOC101929709	1519	RNF31	2095	LINC00599
368	EIF2D	944	LOC101929753	1520	RNF34	2096	LINC00694
369	EIF2S2	945	LOC101930085	1521	RNF39	2097	LINC00704

370	EIF2S3L	946	LOC102467217	1522	RNF5	2098	LINC00705
371	EIF3B	947	LOC102723313	1523	RPAP1	2099	LINC00864
372	EIF3J	948	LOC102723335	1524	RPL13AP17	2100	LINC01005
373	EIF5A2	949	LOC102723373	1525	RPL22L1	2101	LINC01048
374	EIF6	950	LOC102723439	1526	RPL31	2102	LINC01102
375	ELAVL1	951	LOC102723544	1527	RPL6	2103	LINC01118
376	ELMOD2	952	LOC102723833	1528	RPS20	2104	LINC01220
377	ELOA2	953	LOC102724153	1529	RPS3A	2105	LINC01231
378	ELSPBP1	954	LOC102724264	1530	RPTN	2106	LINC01307
379	EMB	955	LOC102724297	1531	RRM1	2107	LINC01354
380	EML6	956	LOC102724601	1532	RRP15	2108	LINC01470
381	EMP1	957	LOC102725080	1533	RRP1B	2109	LINC01482
382	EMX1	958	LOC105372795	1534	RRP8	2110	LINC01555
383	ENG	959	LOC105373352	1535	RRP9	2111	LINC01611
384	ENOPH1	960	LOC105373876	1536	RRS1-AS1	2112	LINC01750
385	ENTPD3-AS1	961	LOC105376365	1537	RSPH6A	2113	LINC01910
386	ENTPD6	962	LOC105377342	1538	RTCA-AS1	2114	LINC01930
387	EPB41L4A-AS1	963	LOC105377651	1539	RTL9	2115	LINC02118
388	EPB41L4B	964	LOC105377967	1540	RUNX1-IT1	2116	LINC02214
389	EPB41L5	965	LOC105378098	1541	SIPR3	2117	LINC02219
390	EPCAM	966	LOC105378146	1542	SALL1	2118	LINC02274
391	EPHA10	967	LOC105378330	1543	SALL4	2119	LINC02280
392	EPN2	968	LOC105378385	1544	SALRNA2	2120	LINC02351
393	ERCC1	969	LOC105378853	1545	SAMD9L	2121	LOC100653233
394	ERGIC2	970	LOC106699570	1546	SAP30	2122	LOC101926933
395	ERICH2	971	LOC148709	1547	SASS6	2123	LOC101927278
396	ERICH3-AS1	972	LOC283177	1548	SATB2-AS1	2124	LOC101927365
397	ERN2	973	LOC283387	1549	SCAF8	2125	LOC101927822
398	ERVV-1	974	LOC283683	1550	SCARF1	2126	LOC101928087
399	ERVW-1	975	LOC284581	1551	SCART1	2127	LOC101928445
400	ESCO2	976	LOC286059	1552	SCG2	2128	LOC101929124
401	ESX1	977	LOC339529	1553	SCGB1B2P	2129	LOC102725126
402	ETFBKMT	978	LOC339666	1554	SCGB1D1	2130	LOC105370473
403	ETNPPL	979	LOC392364	1555	SCGB1D2	2131	LOC105375843
404	ETS2	980	LOC399716	1556	SCGB1D4	2132	LOC105376351
405	ETV4	981	LOC400655	1557	SCGB2B2	2133	LOC339685
406	EWSAT1	982	LOC401127	1558	SCGB3A2	2134	LOC399886
407	EXOC5	983	LOC401442	1559	SCML1	2135	LOC401176
408	EXOC6B	984	LOC403312	1560	SCO1	2136	LSP1P3
409	EXOSC7	985	LOC439933	1561	SCUBE3	2137	LTB4R
410	EXPH5	986	LOC440311	1562	SCYL3	2138	MAEL
411	EXTL1	987	LOC574538	1563	SDR39U1	2139	MAGEB5
412	EXTL3	988	LOC643201	1564	SDR9C7	2140	MAN2B1
413	EZR	989	LOC643406	1565	SECISBP2	2141	MAP6
414	FABP4	990	LOC643733	1566	SELENOP	2142	MARF1
415	FAM105A	991	LOC645513	1567	SELPLG	2143	MCHR2-AS1
416	FAM111A-DT	992	LOC645752	1568	SEMA4A	2144	MED13L
417	FAM117B	993	LOC646762	1569	SEPSECS-AS1	2145	METTL5

418	FAM124B	994	LOC728158	1570	SERBP1	2146	MGAT1
419	FAM131A	995	LOC728327	1571	SERPINA7	2147	MIATNB
420	FAM161B	996	LOC728392	1572	SERPINB4	2148	MIR1305
421	FAM162B	997	LOC729224	1573	SERTAD3	2149	MIR205HG
422	FAM183A	998	LOC729603	1574	SERTM2	2150	MIR3936
423	FAM196B	999	LOC729870	1575	SETD1B	2151	MIR3937
424	FAM199X	1000	LOC730202	1576	SGCD	2152	MIR4423
425	FAM19A3	1001	LOC730234	1577	SGO2	2153	MIR4477A
426	FAM200B	1002	LOC91548	1578	SH2D3C	2154	MIR548BA
427	FAM201A	1003	LOXL3	1579	SH2D5	2155	MIR576
428	FAM205C	1004	LPAR5	1580	SH3D21	2156	MIR585
429	FAM212B	1005	LPAR6	1581	SH3GLB1	2157	MIR6082
430	FAM217A	1006	LPP-AS2	1582	SHANK3	2158	MIR8074
431	FAM228B	1007	LRFN1	1583	SHB	2159	MIR8076
432	FAM238B	1008	LRT2	1584	SHCBP1	2160	MIXL1
433	FAM238C	1009	LRP8	1585	SHISA7	2161	MLX
434	FAM24A	1010	LRRCS2	1586	SHISA2A	2162	MOBP
435	FAM45BP	1011	LRRCS7	1587	SHOC2	2163	MPV17L
436	FAM47A	1012	LRRCS8	1588	SHOX2	2164	MRPS10
437	FAM69A	1013	LRRCS61	1589	SIAH1	2165	MRPS11
438	FAM76A	1014	LRRCS66	1590	SIKE1	2166	MSH2
439	FAM83C	1015	LRRCS75A	1591	SIRPA	2167	MYBPHL
440	FAM87B	1016	LRRQ1	1592	SLAIN2	2168	MYZAP
441	FAM90A1	1017	LRRQ4	1593	SLC10A5	2169	NAA10
442	FAM92A	1018	LSAMP-AS1	1594	SLC11A2	2170	NECAB2
443	FAM98B	1019	LSM14B	1595	SLC12A4	2171	NEDD1
444	FAM9C	1020	LSMEM1	1596	SLC16A10	2172	NELFE
445	FASTKD5	1021	LUC7L	1597	SLC16A12	2173	NEU2
446	FBXL14	1022	LURAP1	1598	SLC16A13	2174	NFKB1
447	FBXL2	1023	LUZP4	1599	SLC16A9	2175	NIPAL2
448	FBXL4	1024	LYPD5	1600	SLC17A2	2176	NNMT
449	FBXO15	1025	LYRM9	1601	SLC22A10	2177	NPL
450	FBXO4	1026	LYVE1	1602	SLC22A3	2178	NREP
451	FBXO40	1027	MAGEA11	1603	SLC22A4	2179	NTRK3
452	FBXO45	1028	MAGEA8	1604	SLC22A7	2180	ODC1
453	FBXW2	1029	MAGEA8-AS1	1605	SLC22A8	2181	OOEP
454	FCER1G	1030	MAGEB6	1606	SLC23A1	2182	OR4B1
455	FCN3	1031	MAGEE2	1607	SLC25A16	2183	OR4C11
456	FCRL2	1032	MAGEL2	1608	SLC25A19	2184	OSER1-AS1
457	FCRL3	1033	MAGIX	1609	SLC25A32	2185	OVCH2
458	FCRLA	1034	MAGOH2P	1610	SLC25A35	2186	PCAT19
459	FCRLB	1035	MALSU1	1611	SLC25A36	2187	PCDH9-AS4
460	FECH	1036	MAN1C1	1612	SLC25A4	2188	PCP4L1
461	FEZ2	1037	MAP1A	1613	SLC25A42	2189	PHOSPHO1
462	FEZF1-AS1	1038	MAP2K4P1	1614	SLC30A7	2190	PIGL
463	FGD3	1039	MAP3K21	1615	SLC30A8	2191	PIGW
464	FGF12-AS2	1040	MAP3K8	1616	SLC31A1	2192	PIP5K1C
465	FGF9	1041	MAPRE1	1617	SLC35B4	2193	PLA2G10

466	FGFBP3	1042	MAPT	1618	SLC35E4	2194	PMS2
467	FGFR1OP	1043	MAPT-IT1	1619	SLC35G1	2195	POLR2J4
468	FGG	1044	MASP1	1620	SLC35G3	2196	PPM1D
469	FGL2	1045	MATN3	1621	SLC36A1	2197	PPP2R2B
470	FHAD1	1046	MBL2	1622	SLC37A2	2198	PPP2R2C
471	FHL5	1047	MBLAC2	1623	SLC37A3	2199	PSG5
472	FILIP1L	1048	MCCC1	1624	SLC46A1	2200	PSMA6
473	FIX1	1049	MCF2L	1625	SLC51A	2201	PTEN
474	FLG2	1050	MCM6	1626	SLC52A3	2202	RALGDS
475	FLJ12825	1051	MCPH1-AS1	1627	SLC5A10	2203	RASSF5
476	FLJ31104	1052	MCRS1	1628	SLC5A3	2204	RBFADN
477	FLJ37201	1053	MDM2	1629	SLC5A6	2205	RBMI5
478	FLJ42393	1054	MEAT6	1630	SLC5A8	2206	RET
479	FOLR2	1055	MED13	1631	SLC6A11	2207	RGS10
480	FOXP1-AS1	1056	MED20	1632	SLC6A4	2208	RIMBP2
481	FRZB	1057	MED28	1633	SLC6A7	2209	RMS1
482	FSHB	1058	MED8	1634	SLC7A6OS	2210	RNF207
483	FSTL5	1059	MEDAG	1635	SLC7A9	2211	RNR1
484	FTSJ3	1060	METTL11B	1636	SLC8B1	2212	RNR2
485	FTX	1061	METTL25	1637	SLFN1	2213	RUBCNL
486	FUNDC2P2	1062	MFAP4	1638	SLITRK5	2214	RXFP2
487	FUT8-AS1	1063	MFGE8	1639	SMAD1-AS2	2215	RYR3
488	FUT9	1064	MFN2	1640	SMAD5-AS1	2216	S100A2
489	FXN	1065	MGAT4D	1641	SMIM10L2B	2217	SAMD7
490	FZD3	1066	MGC15885	1642	SMIM12	2218	SCARNA3
491	FZD4	1067	MGC16275	1643	SMIM18	2219	SEMA4F
492	FZD7	1068	MGC34796	1644	SMIM27	2220	SERP2
493	GABARAPL3	1069	MGEA5	1645	SMILR1	2221	SERPINB11
494	GABRA5	1070	MGME1	1646	SMO	2222	SFMBT2
495	GART	1071	MGST3	1647	SNAI2	2223	SFPQ
496	GAS1	1072	MICALCL	1648	SNAPC5	2224	SHISA6
497	GATA1	1073	MICU2	1649	SNAR-1	2225	SIM1
498	GATA6	1074	MICU3	1650	SNHG6	2226	SIRT5
499	GBA2	1075	MIER2	1651	SNIP1	2227	SKIV2L
500	GBP1	1076	MINDY1	1652	SNORA20B	2228	SLAMF8
501	GBP6	1077	MIR1243	1653	SNORA32	2229	SLC17A4
502	GCH1	1078	MIR1298	1654	SNORA8	2230	SLC22A5
503	GDPD2	1079	MIR1343	1655	SNORA80A	2231	SLC23A3
504	GEN1	1080	MIR23C	1656	SNORA87	2232	SLC5A4
505	GGPS1	1081	MIR2681	1657	SNORD114-29	2233	SLC6A16
506	GGT7	1082	MIR302D	1658	SNORD114-30	2234	SMIM15-AS1
507	GHRLOS	1083	MIR3121	1659	SNORD115-45	2235	SMUG1
508	GID8	1084	MIR3146	1660	SNORD38D	2236	SNORD114-14
509	GIMAP1	1085	MIR4306	1661	SNORD3G	2237	SNORD114-15
510	GIMAP7	1086	MIR4439	1662	SNORD89	2238	SNORD114-19
511	GIMAP8	1087	MIR4447	1663	SNORD91B	2239	SPACA6P-AS
512	GINMI	1088	MIR4464	1664	SNRNP27	2240	SPANXN5
513	GIPC1	1089	MIR4501	1665	SNX10	2241	SPIRE2

514	GJA10	1090	MIR548F3	1666	SNX18	2242	SRRM1
515	GJA3	1091	MIR548X	1667	SNX21	2243	STK3
516	GJD3	1092	MIR562	1668	SOST	2244	STK35
517	GKN2	1093	MIR5689HG	1669	SOWAHB	2245	STT3B
518	GLI3	1094	MIR571	1670	SOWAHC	2246	STX4
519	GLS	1095	MIR6130	1671	SP1	2247	SYAP1
520	GLT8D1	1096	MIR620	1672	SP2	2248	SYT17
521	GLYR1	1097	MIR624	1673	SPACA1	2249	TARDBP
522	GMFB	1098	MIR8062	1674	SPACA3	2250	TAS2R16
523	GMPPA	1099	MKRN2	1675	SPATA3-AS1	2251	TBC1D26
524	GNAI3	1100	MKX-AS1	1676	SPATA3 ID1	2252	TBL2
525	GNPDA1	1101	MLLT3	1677	SPATA3 IE1	2253	TCFL5
526	GOLGA8J	1102	MMAA	1678	SPATA33	2254	THRA
527	GOLIM4	1103	MMP1	1679	SPATA41	2255	THY1
528	GOLPH3L	1104	MMP12	1680	SPC25	2256	TLR8
529	GON7	1105	MNAT1	1681	SPEN	2257	TMBIM1
530	GORASP2	1106	MOB1B	1682	SPINK7	2258	TMCC1
531	GOT2	1107	MORF4L1	1683	SPINK9	2259	TMEM139
532	GP1BA	1108	MORF4L2	1684	SPINT4	2260	TMEM42
533	GP6	1109	MORN1	1685	SPRR1A	2261	TMEM5-AS1
534	GPALPP1	1110	MPZ	1686	SPRR2E	2262	TMEM98
535	GPR1-AS	1111	MRAS	1687	SPRY2	2263	TNFRSF1A
536	GPR141	1112	MRFAP1	1688	SPRYD4	2264	TOX4
537	GPR142	1113	MRGPRX1	1689	SRP14-AS1	2265	TRNP1
538	GPR149	1114	MRGPRX3	1690	SRR	2266	TTC32
539	GPR52	1115	MRPL17	1691	SRSF4	2267	TXN2
540	GPR6	1116	MRPL19	1692	SRSF7	2268	TXNL4A
541	GPR75	1117	MRPL30	1693	SSFA2	2269	TYR
542	GPR82	1118	MRPL37	1694	SSR1	2270	UBE4B
543	GRHL1	1119	MRPL45P2	1695	SSR3	2271	UBXN10-AS1
544	GRIN2D	1120	MRPL52	1696	SSX9	2272	UGT2A1
545	GRIPAP1	1121	MRPL9	1697	ST13P4	2273	UTP4
546	GSK3B	1122	MRPS18C	1698	ST8SLA6	2274	VIM
547	GSN	1123	MRPS22	1699	STARD5	2275	VTRNA1-1
548	GSN-AS1	1124	MRS2P2	1700	STEAP4	2276	VWC2L
549	GTF3C3	1125	MS4A14	1701	STK4-AS1	2277	WDR11-AS1
550	GTSE1-AS1	1126	MS4A4E	1702	STN1	2278	WDR61
551	GUCA1C	1127	MSANTD4	1703	STOM	2279	WDR91
552	GYPE	1128	MSC-AS1	1704	STON1	2280	WLS
553	GZF1	1129	MSH6	1705	STPG1	2281	YEATS2
554	H1FNT	1130	MSL3	1706	STX16	2282	YEATS2-AS1
555	H3F3B	1131	MSL3P1	1707	STX1B	2283	ZC3H12C
556	HAAO	1132	MSRB2	1708	STXBP3	2284	ZDHHC11B
557	HAND1	1133	MTERF2	1709	STYK1	2285	ZFP2
558	HAS3	1134	MTRNR2L3	1710	SUGT1	2286	ZMYND10
559	HBH	1135	MTRNR2L4	1711	SUMF1	2287	ZNF14
560	HBP1	1136	MTRNR2L5	1712	SUMO1P3	2288	ZNF142
561	HCCS	1137	MTX3	1713	SUPT20HL2	2289	ZNF157

562	HCFC2	1138	MUC21	1714	SUPT3H	2290	ZNF160
563	HCG24	1139	MUM1L1	1715	SURF4	2291	ZNF182
564	HCG9	1140	MUTYH	1716	SUV39H1	2292	ZNF230
565	HCRTR2	1141	MXD4	1717	SVBP	2293	ZNF346
566	HEATR1	1142	MXRA5	1718	SYCE1L	2294	ZNF441
567	HEYL	1143	MYC	1719	SYDE2	2295	ZNF462
568	HHAT	1144	MYF5	1720	SYNE1-AS1	2296	ZNF534
569	HIF1A-AS1	1145	MYH16	1721	SYPL1	2297	ZNF544
570	HIGD1B	1146	MYL1	1722	SYT4	2298	ZNF56
571	HIKESHI	1147	MYLK2	1723	TAAR9	2299	ZNF569
572	HINT2	1148	MYSM1	1724	TAB2-AS1	2300	ZNF613
573	HINT3	1149	NAA30	1725	TACSTD2	2301	ZNF627
574	HIST1H2AL	1150	NAA35	1726	TAF1L	2302	ZNF684
575	HIST1H2BA	1151	NAALAD2	1727	TASIR1	2303	ZNF707
576	HIST1H2BD	1152	NAGA	1728	TAS2R1	2304	ZNF883
						2305	ZNF98
						2306	ZRSR2

[00332] **Example 7: Construction of a classifier for typing cervical cancer patients using erythrocyte micronuclei DNA**

[00333] In this example, there were 8 subjects, including 2 patients diagnosed as cervical adenocarcinoma and 5 patients diagnosed as cervical squamous cell carcinoma by other methods.

[00334] The peripheral blood samples from patients with cervical cancer were expressed in the form of “P” plus patient number. For example, “P1” represents a peripheral blood sample from the first cervical cancer patient (“Patient 1”), “P2” represents a peripheral blood sample from the second cervical cancer patient (“Patient 2”), and so on.

[00335] The basic information of 7 patients with cervical cancer is shown in Table 3. “Cervical cancer type” refers to the type of cervical cancer diagnosed by other methods.

Table 3

Patient number	Age	HPV Detected value	Maximum diameter of tumor (cm)	Type of cervical cancer	Existence of lymph node metastasis or not
1	44	68.36	2.1	Medium-high differentiated adenocarcinoma	No
2	36	50.13	4.2	Low-medium differentiated adenocarcinoma	No
3	71	1212.8	2.6	Medium differentiated	Yes (without surgery)

				squamous cell carcinoma	
4	43	240.09	4.1	Low differentiated squamous cell carcinoma	Yes (without surgery)
5	65	25.55	3.3	Medium differentiated squamous cell carcinoma	No
6	56	503.61	3.2	Low-medium differentiated squamous cell carcinoma	No
7*	37	No	5.4	Low-medium differentiated squamous cell carcinoma	Yes

*: Patient 7 is an HPV negative patient.

[00336] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and bioinformatics analysis was carried out as described in Example 5.

- 5 [00337] Specifically, 2 adenocarcinoma samples and 6 squamous cell carcinoma (including one HPV-negative sample) in primary cervical cancer samples were selected for the reads counting, and 360 differential genes were screened out by ANOVA test to distinguish the two classes of samples. Then, according to Pearson correlation, the two classes of samples were clustered in unsupervised hierarchy, showing that there were
- 10 significant differences between the two classes of samples.

[00338] As shown in Figure 6, 360 differential genes (forming a classifier for distinguishing cervical adenocarcinoma from cervical squamous cell carcinoma patients) were clustered. In Figure 6, each row represents a differential gene, and each column represents a patient.

- 15 [00339] The list of 360 differential genes is shown in Table 4. Each gene corresponds to each row from top to bottom in Figure 6.

Table 4

1	ACAD10	91	ZNF718	181	LCE1E	271	NKD1
2	ACOD1	92	ADAM30	182	LINC00312	272	NMNAT1
3	ACTR1A	93	AMER3	183	LINC00449	273	NUDT16
4	ANP32A-IT1	94	ANKRD62P1-PARP4P3	184	LINC00469	274	NUS1
5	ARL5C	95	ANP32A	185	LINC00547	275	OR13H1
6	ATP13A2	96	APOF	186	LINC00561	276	OR2B6

7	B2M	97	APOOP5	187	LINC00656	277	OR4K2
8	C11orf70	98	ARHGEF25	188	LINC00680	278	OR51D1
9	C16orf70	99	ASAP1-IT2	189	LINC00851	279	OR5M11
10	C19orf66	100	ASF1B	190	LINC00857	280	OTOF
11	C1orf194	101	ASIC2	191	LINC00887	281	OTUB2
12	CABYR	102	ASNS	192	LINC01058	282	P3H2-AS1
13	CADM2-AS2	103	ATP6V0B	193	LINC01118	283	PACERR
14	CALB1	104	BANCR	194	LINC01496	284	PADI4
15	CAPSL	105	BMS1P21	195	LINC01565	285	PCDHGA12
16	CCT8	106	BOD1L2	196	LINC01579	286	PDLIM2
17	CNFN	107	BRWD1-AS2	197	LINC01621	287	PGAM4
18	CROCC	108	BTNL8	198	LINC01658	288	PHGR1
19	CYP27C1	109	C12orf71	199	LINC01674	289	PIGW
20	DNAAF1	110	C2orf38	200	LINC01737	290	PIP5KL1
21	DYNLRB2	111	C6orf222	201	LINC01752	291	PLEC
22	ESCO1	112	C9orf3	202	LINC01833	292	PMCHL1
23	ESYT3	113	CAHM	203	LINC01872	293	POLH
24	FAM199X	114	CD24	204	LINC01894	294	POU5F1
25	FAM19A1	115	CDKN2AIP	205	LINC01953	295	PRAMEF12
26	FGFBP3	116	CEP170	206	LINC01964	296	PRAMEF2
27	FITM2	117	CERS3	207	LINC01982	297	PRSS16
28	FLJ37201	118	CFL2	208	LINC02145	298	PSG9
29	GLA	119	CHAMP1	209	LINC02214	299	PSMA6
30	GPN1	120	CHERP	210	LINC02226	300	PTPN12
31	GTF2H1	121	CHRNE	211	LINC02283	301	RBM15
32	HAS2-AS1	122	CLLU1	212	LINC02313	302	RGS1
33	HOXD1	123	CNOT8	213	LINC02468	303	RPUSD2
34	IP6K3	124	COL23A1	214	LOC100129129	304	SALL3
35	KCNH3	125	COL4A1	215	LOC100130872	305	SCG5
36	KRTAP22-2	126	CREB3L3	216	LOC100190986	306	SELL
37	LINC00605	127	CTAGE9	217	LOC100505824	307	SERPINE1
38	LINC01393	128	CTRC	218	LOC100506585	308	SEZ6L2
39	LINC01602	129	CYB5R1	219	LOC100996415	309	SHISA2
40	LINC01803	130	CYMP-AS1	220	LOC101593348	310	SIGLEC14
41	LINC01845	131	CYP4F29P	221	LOC101927342	311	SLC25A21-AS1
42	LINC01991	132	DCAF8L1	222	LOC101927497	312	SLC25A3P1
43	LOC101928211	133	DCK	223	LOC101927588	313	SLC39A1
44	LOC101929130	134	DEFA11P	224	LOC101927762	314	SLC5A2
45	LOC101929524	135	DGCR11	225	LOC101928307	315	SMAD5-AS1
46	LOC102467081	136	DGCR9	226	LOC101928489	316	SMARCD2
47	LOC107133515	137	DICER1-AS1	227	LOC103611081	317	SNHG20
48	LRRC17	138	DLGAP3	228	LOC105369632	318	SNORA120
49	LUC7L2	139	DPPA3	229	LOC105371880	319	SNORA70I
50	LYPLA1	140	DPY19L1P2	230	LOC105372493	320	SNORD113-5
51	MARCH4	141	DSCR9	231	LOC105373876	321	SOX4
52	MGAT4EP	142	EDAR	232	LOC105377967	322	SPATA19
53	MINDY4B	143	EIF3IP1	233	LOC105378385	323	SPON2
54	MIR3663HG	144	EIF5A	234	LOC105378663	324	SPSB4

55	MIR553	145	ESRP2	235	LOC285556	325	SSX8
56	MKRN3	146	FABP3	236	LOC400682	326	SUCLG1
57	MRPS18C	147	FAM129A	237	LOC441666	327	SYNJ2BP
58	NBPF9	148	FAM49A	238	LOC643802	328	TARP
59	NDUFV2	149	FBXL19-AS1	239	LOC646903	329	TAS2R14
60	NKRF	150	FFAR2	240	LOC729681	330	TCEAL1
61	NUDT5	151	FKBP1A	241	LRRC3-AS1	331	TIGIT
62	OMA1	152	FRMD1	242	LRRC4B	332	TMEM134
63	OR10J1	153	GABPB1-AS1	243	LY6K	333	TMEM14A
64	OR10Z1	154	GAGE1	244	LYZL2	334	TMEM45B
65	OR1S1	155	GALNT16	245	MAGEB6	335	TMEM80
66	OR4C11	156	GCK	246	MECOM	336	TNK1
67	OR4X1	157	GNAI3	247	MESTIT1	337	TRAPPC2
68	OR51B5	158	GPR83	248	MGAT1	338	TRIM6
69	OR5AN1	159	GRIK1-AS1	249	MIR205HG	339	TRIP13
70	P4HA1	160	GSLX1	250	MIR30B	340	TSTD3
71	PCED1B	161	GTF2H5	251	MIR4677	341	TTPAL
72	SLC22A7	162	GTPBP2	252	MIR502	342	TUBA3FP
73	SLC35B2	163	H19	253	MIR503HG	343	TUSC8
74	SLC35F3	164	HLA-DQB1	254	MIR513A1	344	UBE2E1
75	SMS	165	HOXC4	255	MIR7852	345	UBE2G2
76	SNORD3K	166	HRAT5	256	MIRLET7F2	346	UBE2V2
77	SVIP	167	HSD17B1	257	MKLN1-AS	347	UBXN1
78	TAF6	168	IFTM10	258	MN1	348	UG0898H09
79	TAS2R3	169	IFNA1	259	MRGPRX2	349	UPK3A
80	TCAMIP	170	IGLL1	260	MRPL33	350	VAMP5
81	TGMI	171	INSL4	261	MTIP	351	WDR83
82	TGOLN2	172	KAZN-AS1	262	MTCH1	352	ZCCHC10
83	TMCO2	173	KCNG1	263	MXRA5	353	ZFH4-AS1
84	TMEM132A	174	KCNIP3	264	NANOGP8	354	ZFP57
85	TMEM14EP	175	KCNJ9	265	NCF4	355	ZFP69B
86	TREML4	176	KCNQ4	266	NDRG4	356	ZNF217
87	TSPAN31	177	KIRREL2	267	NDST1-AS1	357	ZNF319
88	UBE2N	178	KLF4	268	NDUFC2	358	ZNF76
89	VAMP4	179	KRTAP20-2	269	NECAP1	359	ZNF792
90	XIAP	180	LAMB2P1	270	NEIL1	360	ZWINT

[00340] Example 8: Construction of a classifier for staging cervical cancer patients using erythrocyte micronuclei DNA

[00341] In this example, there were 5 subjects, including 2 patients diagnosed as medium differentiated cervical squamous cell carcinoma by other methods and 3 patients diagnosed as low differentiated and low-medium differentiated cervical squamous cell carcinoma.

[00342] The peripheral blood samples from patients with cervical cancer were expressed in the form of “P” plus patient number. For example, “P1” represents a peripheral blood

sample from the first cervical cancer patient ("Patient 1"), "P2" represents a peripheral blood sample from the second cervical cancer patient ("Patient 2"), and so on.

[00343] The basic information of 5 patients with cervical cancer is shown in Table 5. "Cervical cancer type" refers to the type of cervical cancer diagnosed by other methods.

5

Table 5

Patient number	Age	HPV Detected value	Maximum diameter of tumor (cm)	Type of cervical cancer	Existence of lymph node metastasis or not
1'	65	25.55	3.3	Medium differentiated squamous cell carcinoma	No
2'	71	1212.8	2.6	Medium differentiated squamous cell carcinoma	Yes (without surgery)
3'	43	240.09	4.1	low differentiated squamous cell carcinoma	Yes (without surgery)
4'*	37	No	5.4	Low-medium differentiated squamous cell carcinoma	Yes
5'	56	503.61	3.2	Low-medium differentiated squamous cell carcinoma	No

*. Patient 4 was HPV negative.

[00344] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and bioinformatics analysis was carried out as described in Example 5.

- 10 [00345] Specifically, 2 medium differentiated cervical squamous cell carcinoma samples and 3 low differentiated and low-medium differentiated squamous cell carcinoma samples in primary cervical squamous cell carcinoma samples were selected for the reads counting, and 466 differential genes were screened out by ANOVA test to distinguish the two classes of samples. Then, according to Pearson correlation, the two classes of samples were clustered in
- 15 unsupervised hierarchy, showing that there were significant differences between the two classes of samples.

[00346] As shown in Figure 7, 466 differential genes (forming a classifier for distinguishing medium differentiated cervical squamous cell carcinoma from cervical low

differentiated and low-medium differentiated squamous cell carcinoma patients) were clustered. In Figure 7, each row represents a differential gene, and each column represents a patient.

[00347] The list of 466 differential genes is shown in Table 6. Each gene corresponds to each row from top to bottom in Figure 7.

Table 6

1	ACPI	118	LOC100505715	235	ZMYM3	351	LINC00552
2	ADAD1	119	LOC101927402	236	ZNF212	352	LINC00578
3	AGAP1-IT1	120	LOC101927523	237	ZNF329	353	LINC01143
4	AKAP7	121	LOC101927884	238	ZNF492	354	LINC01204
5	AKR7A2	122	LOC101929718	239	ZNF550	355	LINC01502
6	ANKRD27	123	LOC102723883	240	ZNF70	356	LINC01732
7	ANXA9	124	LOC105370954	241	ZNF740	357	LINC01782
8	ARMC12	125	LOC105373064	242	ZNF770	358	LINC01833
9	ASCL4	126	LOC105377348	243	ZNF827	359	LINC01855
10	AZIN1	127	LOC105378052	244	ZBP2	360	LINC01867
11	B2M	128	LOC643406	245	ZSCAN29	361	LINC01884
12	BACE1	129	LOC646548	246	AARD	362	LINC01960
13	BANF2	130	LOC727896	247	ABC8	363	LINC02043
14	C12orf74	131	LPCAT3	248	ACOXL-AS1	364	LINC02441
15	C15orf48	132	LRR17	249	ACTN1-AS1	365	LINC02530
16	C15orf59-AS1	133	LRR42	250	ADAMTS2	366	LIPE-AS1
17	C18orf61	134	LY9	251	AHSG	367	LMO1
18	C19orf66	135	MAGEA11	252	AP2A1	368	LNX1-AS2
19	C21orf62	136	MBIP	253	ARHGEF7-AS2	369	LOC100128361
20	C2orf69	137	MCM3	254	ARMS2	370	LOC100130476
21	C2orf81	138	MED28	255	BAALC-AS2	371	LOC100131303
22	C3orf56	139	METTL18	256	BMP4	372	LOC100421746
23	C8orf34-AS1	140	MIR1322	257	BOLA1	373	LOC100506178
24	C9orf66	141	MIR3663HG	258	C11orf42	374	LOC100507507
25	CARD18	142	MIR4303	259	C16orf52	375	LOC101926963
26	CASC11	143	MIR4699	260	C17orf102	376	LOC101927138
27	CASP14	144	MIR570HG	261	C17orf62	377	LOC101927143
28	CCR5	145	MLIP-AS1	262	C19orf48	378	LOC101928177
29	CDIP1	146	MORF4L1	263	C19orf57	379	LOC101928725
30	CDK9	147	MSANTD2	264	C5orf66-AS2	380	LOC101928791
31	CENPVL3	148	MYH2	265	C6orf120	381	LOC101928887
32	CERNA2	149	MYO9B	266	CAPNS2	382	LOC101929549
33	CFTR-AS1	150	MYOZ3	267	CASC17	383	LOC101929721
34	CIITA	151	NAA30	268	CATIP-AS2	384	LOC105371083
35	CLEC2B	152	NDNF	269	CCL3L1	385	LOC105373609
36	CLRN1-AS1	153	NEU2	270	CD274	386	LOC105374952
37	COA5	154	NFYC-AS1	271	CDC42EP2	387	LOC105377621
38	CPHXL	155	NHS-AS1	272	CDKN2A-AS1	388	LOC105378397
39	CPNE6	156	NKILA	273	CELSR3	389	LOC105379393

40	CPXCR1	157	NKX3-1	274	CEP19	390	LOC107984703
41	CRYBG3	158	NPTN-IT1	275	CEP85	391	LOC148413
42	CYP4V2	159	NRG1-IT1	276	CLLU1	392	LOC257396
43	DBF4B	160	NRN1	277	COX16	393	LOC387810
44	DBIL5P	161	NTM-IT	278	CSEIL	394	LOC401320
45	DDX39B-AS1	162	ORI0Q1	279	CSPG4	395	LOC644936
46	DPYS	163	ORI0Z1	280	CSRP2	396	LOC646903
47	DUSP1	164	ORI3C3	281	CSTA	397	LOC729224
48	EBNA1BP2	165	ORIQ1	282	DCP2	398	LOC729305
49	EDEM1	166	OR2K2	283	DCUN1D1	399	LRG1
50	EEF1E1	167	OR8K3	284	DDIT4L	400	LRF12
51	ELOA2	168	OR9G4	285	DDX21	401	LSR
52	EMC1	169	OTUD7B	286	DGCR9	402	LUM
53	FAM111A-DT	170	OTX2	287	DHH	403	LUNAR1
54	FAM185A	171	PCDHA10	288	DOHH	404	MAGEB2
55	FAM201B	172	PCDHB14	289	DPYD-AS2	405	MAGEF1
56	FAM219A	173	PCDHGA1	290	DSCAM-AS1	406	MAGIX
57	FAM237A	174	PCDHGC4	291	EAF1	407	MALL
58	FAM24A	175	PCDHGC5	292	EGFLAM-AS4	408	MAPK13
59	FAR2P2	176	PCED1B	293	EHBP1L1	409	MAPKAPK5-AS1
60	FGFBP3	177	PFAS	294	EPOR	410	MESP1
61	FLJ36000	178	PICART1	295	FAM189B	411	METTL3
62	FLJ37201	179	PLP1	296	FAM52A	412	MGC4859
63	FOXPI-AS1	180	PNISR	297	FAM43A	413	MIP
64	FTSJ1	181	PRKAB2	298	FAM90A27P	414	MIR1255A
65	GASAL1	182	PRKAR1A	299	FBR5	415	MIR1265
66	GKN2	183	PROKR2	300	FBXL15	416	MIR1291
67	GNG5	184	PSMD3	301	FLNB-AS1	417	MIR17HG
68	GOLM1	185	PSTK	302	FOXDI	418	MIR2682
69	GPR55	186	PTTG2	303	FOXO4	419	MIR3145
70	GSN-AS1	187	QARS	304	FPR1	420	MIR31HG
71	HACD4	188	RAB11FIP4	305	FPR2	421	MIR4255
72	HHEX	189	RAB31	306	FRY-AS1	422	MIR548U
73	HIP1	190	RBMXL2	307	FSCN3	423	MIR5692C2
74	HIST1H3H	191	RBPMS-AS1	308	GCSAM	424	MIR643
75	HIST4H4	192	RMDN3	309	GDF6	425	MIR7-3
76	HNRNPA1P10	193	RNF152	310	GFRA4	426	MIR891B
77	HSPA1L	194	RPS17	311	GJB4	427	MOB3A
78	IKZF4	195	RRAS	312	GPR179	428	MRAP
79	IL10RB-AS1	196	SAMD9L	313	GPRC5C	429	MRPL32
80	IL1R1	197	SCARNA17	314	GPSM3	430	MRPS30-DT
81	IL26	198	SCARNA6	315	GRM7-AS1	431	MSGN1
82	INAFM1	199	SCRT2	316	GSG1L	432	MUC22
83	ITPK1	200	SET	317	H3F3AP4	433	NDUFAB1
84	KAZN	201	SLC39A2	318	HCG4B	434	NELFA
85	KCNAB1-AS2	202	SLC3A2	319	HCP5	435	NHLRC1
86	KDM4A-AS1	203	SLC9A9-AS1	320	HHATL	436	OLFML3
87	KDM8	204	SMARCA5-AS1	321	HIST1H2AB	437	OR12D2

88	KIAA1456	205	SNAP25-AS1	322	HLA-DMB	438	OR2AK2
89	KLF12	206	SNORD3K	323	HLA-F	439	OR2H1
90	KLHL15	207	SNRNP35	324	HLA-H	440	OR51F1
91	KRR1	208	SPATA7	325	HNRNPUL2	441	OR51I2
92	KRT77	209	SPRR4	326	HULC	442	OR5AP2
93	KRTAP16-1	210	SRGAP3-AS2	327	HYAL4	443	OR6C65
94	KRTAP29-1	211	SYNPR-AS1	328	IER2	444	OR6J1
95	KRTAP6-1	212	TBCEL	329	IFIT5	445	OR6V1
96	LGALS12	213	TENM4	330	IGLL1	446	OR8K1
97	LHFPL6	214	TEX53	331	ING4	447	OSCAR
98	LHX4-AS1	215	TIGD2	332	INTS5	448	OXA1L
99	LINC00299	216	TMEM140	333	IQCE	449	PAX6-AS1
100	LINC00317	217	TMEM214	334	IRX6	450	PCDHA11
101	LINC00415	218	TRIM3	335	KCNN1	451	PLAC9
102	LINC00485	219	TXNDC2	336	KCNS2	452	PLCH1-AS2
103	LINC00563	220	TXNL1	337	KIAA1147	453	PLVAP
104	LINC00676	221	TYW5	338	KLHL11	454	PPP1R3A
105	LINC00919	222	UBN1	339	KLK1	455	PPP1R3D
106	LINC01114	223	UBQLN2	340	KRT7	456	PRC1-AS1
107	LINC01203	224	UCK2	341	LAMTOR5	457	PRR15
108	LINC01644	225	UPP1	342	LCE1E	458	PRR23C
109	LINC01876	226	UQCRR	343	LGI4	459	PRRT2
110	LINC02041	227	VPS35	344	LILRB1	460	PRSS41
111	LINC02120	228	VPS37A	345	LINC00174	461	PRSS57
112	LINC02210	229	VPS45	346	LINC00459	462	PTGS2
113	LINC02308	230	VTG1A	347	LINC00513	463	RAB6C-AS1
114	LINC02310	231	XAF1	348	LINC00526	464	RAI2
115	LOC100128288	232	XPC	349	LINC00547	465	RASGRP4
116	LOC100131496	233	ZBTB33	350	LINC00550	466	RBMXL3
117	LOC100505635	234	ZFP37				

[00348] Example 9. Classification of healthy individuals and cervical cancer patients using erythrocyte micronuclei DNA

[00349] Using the classifier (2,306 genes) constructed in Example 6 for clustering healthy individuals and cervical cancer patients, 8 unknown samples from 8 subjects were predicted.

[00350] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and bioinformatics analysis was carried out as described in Example 5.

[00351] After testing, it was found that 5 of the 8 samples were at high risk of cervical cancer (the risk probabilities were all over 85%), and 3 were at low risk of cervical cancer (the risk probabilities were all less than 5%). Tracing back the sample sources of subjects predicted to be high risk and subjects predicted to be low risk, it was found that 5 samples with high risk of cervical cancer were obtained from patients who were diagnosed as cervical

cancer by other diagnostic methods. Three samples with low risk of cervical cancer were obtained from healthy individuals detected by other diagnostic methods.

[00352] The result is shown in Figure 8. In Figure 8, P1, P2, P3, P4 and P5 were 5 cervical cancer patients, P3, P4 and P5 were 3 of 9 cervical cancer samples in training set, and P1 and P2 were cervical cancer samples not in model training set; H1, H2 and H3 were all samples of non-cervical cancer healthy individuals.

[00353] Therefore, the method and the gene classifier of the present disclosure can effectively distinguish cervical cancer patients from healthy individuals.

10 [00354] **Example 10: Typing of cervical cancer patients using erythrocyte micronuclei DNA**

[00355] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and bioinformatics analysis was carried out as described in Example 5.

15 [00356] Using the classifier (360 genes) constructed in Example 7 for clustering patients with cervical squamous cell carcinoma and cervical adenocarcinoma, three cervical cancer samples with unknown classification were predicted.

20 [00357] After testing, it was found that two of the three samples were of high risk (the risk probabilities were all over 85%) and one was of low risk (the risk probability was less than 5%). Tracing back the sample sources of subjects with high risk of cervical squamous cell carcinoma and subjects with low risk of cervical squamous cell carcinoma, it was found that two samples with high risk of cervical squamous cell carcinoma were obtained from patients with cervical squamous cell carcinoma detected by other diagnostic methods, and one sample with low risk of cervical squamous cell carcinoma was obtained from healthy individuals as detected by other diagnostic methods.

25 [00358] The result is shown in Figure 9. In Figure 9, P1 was a patient with cervical adenocarcinoma, and P2 and P3 were patients with cervical squamous cell carcinoma.

[00359] Therefore, the method and gene classifier of the present disclosure can effectively classify cervical cancer patients and distinguish cervical squamous cell carcinoma from cervical adenocarcinoma.

30 [00360] **Example 11: Construction of a classifier for classifying healthy individuals and colorectal cancer patients using erythrocyte micronuclei DNA**

[00361] In this Example, there were 17 subjects, including:

Experimental group: 4 patients diagnosed as colorectal cancer by other methods

Control group: 13 healthy individuals (non-colorectal cancer individuals).

35 [00362] The peripheral blood samples from patients with colorectal cancer were expressed in the form of "P" plus patient number. For example, "P1" represented a peripheral blood

sample from the first colorectal cancer patient ("Patient 1"), "P2" represented a peripheral blood sample from the second colorectal cancer patient ("Patient 2"), and so on. In addition, peripheral blood samples from healthy individuals were expressed in the form of "H" plus individual number. For example, "H1" represents the peripheral blood sample from the first healthy individual, "H2" represents the peripheral blood sample from the second healthy individual, and so on.

[00363] The basic information of 4 patients with colorectal cancer is shown in Table 7. Colorectal cancer type, e.g., "adenocarcinoma", refers to the type of colorectal cancer diagnosed by other methods.

10

Table 7

Patient number	Gender	Complaint	Enteroscopy pathology
1	Female	Increased stool frequency with bloody stool for more than 2 months	(sigmoid colon) low differentiated adenocarcinoma
2	Female	Black stool for more than 2 months	Adenocarcinoma (65cm from anus)
3	Male	Rectal cancer was diagnosed for more than 1 month	Adenocarcinoma (rectum)
4	Male	Latent pain in the left lower abdomen for 1 week	(40cm from anus) villous adenoma, high-grade intraepithelial neoplasia, and focal Cancerization cannot be excluded

[00364] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and bioinformatics analysis was carried out as described in Example 5.

15 [00365] Specifically, the reads counts of the gene regions of 4 primary colorectal cancer samples and 13 healthy female samples were selected, and 903 differential genes were screened out by ANOVA test to distinguish the two classes of samples. Then, the unsupervised hierarchical clustering of the two classes of samples was carried out according to Pearson correlation, and it was found that there were significant differences between the two classes of samples.

20

[00366] As shown in Figure 10, red cell micronuclei DNA from peripheral blood samples of colorectal cancer patients and red cell micronuclei DNA from peripheral blood samples of healthy individuals were clustered to obtain 903 differential genes (forming a classifier for

distinguishing healthy individuals from colorectal cancer patients). In Figure 10, each row represents a differential gene, and each column represents a patient.

[00367] The list of 903 differential genes is shown in Table 8. Each gene corresponds to each row from top to bottom in Figure 10.

5

Table 8

1	FXVD3	227	ABCA4	453	RPLP1	679	TXN
2	CD9	228	RAB3B	454	CYP2E1	680	TAGLN2
3	DYNLL1	229	ZNF732	455	IFITM3	681	LRG1
4	CTSA	230	CTNND2	456	SERPINC1	682	MYL12B
5	PDCD6IP	231	TTC24	457	ALDOB	683	RPL6
6	IDH2	232	PPM1E	458	GAPDH	684	HRSP12
7	RBMX	233	GPC5	459	FTH1	685	DDT
8	CORO1B	234	FBXO15	460	MT2A	686	COX5B
9	SLC12A2	235	AR	461	RPL8	687	HSD17B6
10	RCC2	236	LY6G6F	462	HPX	688	COX7B
11	SETD3	237	GRIK4	463	ORM2	689	COX8A
12	PAPOLA	238	CTNNA3	464	EPHX1	690	CHI3L1
13	PLEC	239	DDX53	465	CLU	691	GSTK1
14	DRG1	240	TECTA	466	SAA2	692	TAT
15	DTYMK	241	CCL16	467	ACTB	693	RPS3
16	RBM47	242	TMC3	468	H19	694	MAT1A
17	NFU1	243	EPO	469	GC	695	SULT2A1
18	DNAJB6	244	PTPR	470	CES1	696	AFP
19	CDC42SE2	245	KCTD8	471	RPL30	697	CTSB
20	CCND2	246	CDH12	472	SERPINC1	698	ALDOA
21	EPHA2	247	KCNQ3	473	RPL37A	699	PAH
22	STT3B	248	CADM2	474	PLG	700	RPL15
23	PITRM1	249	DAZL	475	RPS11	701	GAMT
24	NARF	250	GRM1	476	FGL1	702	ATOX1
25	WDR45	251	SLC12A5	477	HRG	703	XBP1
26	EXT2	252	LRP1B	478	RPL41	704	BTF3
27	LTBP4	253	MED12L	479	RPL19	705	GRHPR
28	HSPG2	254	OR14I1	480	SEPP1	706	PRDX5
29	OTUD5	255	CDH10	481	CRP	707	CYP2C9
30	COG4	256	CNTN5	482	AGT	708	GABARAP
31	NEK6	257	TMC1	483	RARRES2	709	DBI
32	CCNK	258	TRDN	484	RPS12	710	C8G
33	UBE3C	259	ZNF716	485	KNG1	711	TPI1
34	VDR	260	MYT1L	486	RPS16	712	KRTCAP2
35	IGF2R	261	GSX1	487	RPS6	713	GPX1
36	FOXA3	262	CSMD3	488	CFH	714	SHFM1
37	SH3BP4	263	UNC13C	489	RPS24	715	ADI1
38	LAMC1	264	C20orf173	490	RPL3	716	SDC1
39	SPPL2A	265	KCNU1	491	TMSB10	717	CYP27A1
40	GBP2	266	DKFZp434L192	492	P4HB	718	TFR2

41	KREMEN1	267	VSX2	493	APCS	719	APOA4
42	PIP5K1B	268	C4orf22	494	RPLP2	720	RPL36AL
43	FAF1	269	KCNK16	495	RPS20	721	F12
44	RAB9A	270	FSD2	496	UBB	722	HSPD1
45	ALAD	271	CPXCR1	497	A1BG	723	NDUFA1
46	PDE9A	272	GRID2	498	MT1G	724	RPL10
47	CORO7	273	ATP13A5	499	TMEM176B	725	MASP2
48	TFE3	274	H1FOO	500	CTSD	726	LDHA
49	NEDD9	275	SPINK8	501	PEBP1	727	ARG1
50	CCDC107	276	LEUTX	502	RPS8	728	TUBA1B
51	TMEM181	277	GFRA4	503	RPS9	729	ARF1
52	SLC1A4	278	IQCJ	504	RPLP0	730	TM4SF5
53	HDAC7	279	KRTAP19-8	505	ADH1B	731	CD14
54	MEMO1	280	HTN3	506	RPS4X	732	BSG
55	ATRN	281	TTC24	507	RPL27	733	SSR2
56	VWF	282	PPP4R3C	508	CD74	734	CYP2D6
57	MXI1	283	SYNDIG1	509	RPL11	735	GSTO1
58	FOXK2	284	TRAPPC2	510	RPS19	736	COX6C
59	MCCC1	285	OTUD5	511	DCXR	737	DEFB1
60	IPO8	286	RBMX	512	ACTG1	738	ATP5J2
61	EPS15	287	PDE10A	513	RPL23	739	POR
62	CCDC80	288	LINC00871	514	RPL35	740	SAT1
63	ACOT1	289	DNAJB6	515	AGXT	741	PNKD
64	ABCB7	290	LOC101928254	516	C1S	742	GJB1
65	RANBP2	291	GMNC	517	AZGP1	743	GRINA
66	HS6ST1	292	CSGALNACT1	518	RPL38	744	LGALS4
67	PIP5K1C	293	LINC01581	519	RPL36	745	SLC25A5
68	PLXND1	294	AGFG1	520	CALR	746	PSMB3
69	MED27	295	PLS3-AS1	521	RPS14	747	HSPA5
70	SLC25A29	296	LINC00972	522	RPS21	748	HSD11B1
71	XIAP	297	FAF1	523	GLUL	749	HAMP
72	CCDC85C	298	PPP4R3C	524	IL32	750	CHCHD2
73	ATP11A	299	DMD	525	ITIH2	751	RHOB
74	AGFG1	300	DAZL	526	GNB2L1	752	GNAS
75	BNIP2	301	TNNI3	527	F2	753	TFPI
76	LRRC32	302	DMD	528	MGST1	754	ITM2B
77	WDR4	303	TNNI3	529	PPIB	755	C4BPB
78	ISY1	304	PIP5K1B	530	ITIH4	756	OST4
79	RBM19	305	MEMO1	531	EEF1A1	757	SLC38A3
80	CYBB	306	MZF1	532	GSTA1	758	BAAT
81	PARVA	307	PPM1E	533	PRAP1	759	LCN2
82	UPF2	308	ADAMTS5	534	RPL18	760	REEP6
83	RECQL	309	MED27	535	TMEM176A	761	PTGR1
84	UBIAD1	310	WDR45	536	HPD	762	SERPINA5
85	MICAL1	311	PPP4R3C	537	RPS5	763	CFB
86	CRTC3	312	MAP3K9	538	RPL24	764	SEPHS2
87	PEX1	313	CDH12	539	A2M	765	SLC25A3
88	PPP1R13B	314	DYNC111	540	SOD1	766	GPC3

89	GBP4	315	MAP3K9	541	SOD2	767	UBL5
90	MCF2L	316	LINC01019	542	RPL4	768	PTGDS
91	DCUN1D1	317	GBP2	543	IGF2	769	PGRMC1
92	CBWD6	318	CADM2	544	MYL6	770	CTSZ
93	IFNAR2	319	SLA	545	RPL35A	771	ACADVL
94	HAUS2	320	SPTLC3	546	RPS15A	772	CYP2C8
95	IGSF9	321	KCTD8	547	AKR1C1	773	EIF5A
96	PTPRE	322	SEMA3A	548	RPS25	774	EEF1B2
97	CCDC120	323	UNC13C	549	RPL31	775	FBP1
98	JMJD1C	324	TFE3	550	RPL29	776	AKR1C4
99	RLIM	325	XRCC6P5	551	FAU	777	PARK7
100	TRAPPC2	326	RALGAPA1	552	HSPB1	778	EFNA1
101	MSRA	327	CPXCR1	553	MT1X	779	CALM2
102	TRAF3IP1	328	ALPK2	554	CYP2A6	780	FDPS
103	MZF1	329	XRCC6P5	555	SERPINF2	781	SEC61B
104	RAI1	330	LOC102724908	556	RPL5	782	LGALS1
105	C11orf80	331	CCDC80	557	MIF	783	MPST
106	SHF	332	ALPK2	558	RPL7A	784	RPSAP58
107	LZTFL1	333	KCTD8	559	HMGCS2	785	SLPI
108	ASH1L	334	DTNA	560	CYP3A4	786	INSIG1
109	PKD1	335	FAM129A	561	ATP5E	787	SLC27A5
110	NFAT5	336	PDE9A	562	CFHR1	788	NDUFB7
111	POFUT2	337	GRM1	563	ITIH1	789	IGFBP7
112	CD300A	338	CYBB	564	COX7C	790	PRDX2
113	CEMP1	339	ZNF91	565	REG3A	791	TCEB2
114	SDR16C5	340	SYNDIG1	566	RPL13A	792	MYL12A
115	PTPRD	341	CPXCR1	567	SERPINF1	793	ATP5A1
116	ENOX2	342	CPXCR1	568	ASS1	794	NPM1
117	ZSWIM7	343	PLCB1-IT1	569	CD63	795	ALDH1L1
118	CABLES1	344	RNA5-8SN5	570	C4BPA	796	LGALS3BP
119	ZNF74	345	GRM1	571	RPL26	797	HSPE1
120	MYPOP	346	PNMA5	572	C1R	798	RPSA
121	ZNF91	347	SYNDIG1	573	RPS13	799	PSME1
122	CREB1	348	CADM2	574	ITIH3	800	PCBP1
123	CHRD1	349	SYNDIG1	575	EEF2	801	ATP5G2
124	LIMS1	350	LINC00548	576	FN1	802	EBP
125	NOX4	351	LOC101928012	577	SQSTM1	803	ANXA2
126	CELSR1	352	LINC00972	578	ALDH2	804	HPN
127	SLA	353	KLHL13	579	KRT18	805	PSME2
128	PRKCH	354	KCTD8	580	HINT1	806	PCK2
129	ARID2	355	MIR5011	581	ALDH1A1	807	GUK1
130	PKD2	356	EPS15	582	RPL12	808	HSP90AA1
131	INVS	357	GBP4	583	RPL10A	809	PLIN2
132	PASK	358	LEUTX	584	UBC	810	PSMD4
133	MSX1	359	CCDC120	585	TMBIM6	811	CYP4A11
134	FAM129A	360	DKFZp434L192	586	CYB5A	812	TGM2
135	TCF4	361	MAP3K21	587	IGFBP1	813	NDUFS5
136	ABTB2	362	NFU1	588	PSAP	814	PDIA3

137	ZAP70	363	DMD	589	SPP1	815	BLVRB
138	RALGAPA1	364	MAP3K9	590	PLA2G2A	816	GSTA2
139	HIVEP2	365	LOC728739	591	TPT1	817	ATP5C1
140	RIC8B	366	CPXCR1	592	GPX3	818	ACAA2
141	ORAI2	367	PHACTR1	593	PTMS	819	CFI
142	DUSP28	368	PPM1E	594	IFI27	820	IFI6
143	CSGALNACT1	369	LINC00871	595	UGT2B4	821	LMAN2
144	CDADC1	370	LOC101928012	596	SPINK1	822	TMED9
145	SEMA3A	371	PDE10A	597	ENO1	823	PON1
146	ZBP1	372	SLC16A2	598	IGFBP4	824	NUCB1
147	ABI3BP	373	XRCC6P5	599	AKR1B10	825	PHYH
148	GAMT	374	SDR16C5	600	SERPIND1	826	ADAM6
149	KLHL13	375	CSGALNACT1	601	RPS10	827	FMO3
150	EME1	376	LINC01317	602	ECHS1	828	ADH6
151	ANO7	377	GBP4	603	ADH4	829	ERBB3
152	PNMA5	378	RALGAPA1	604	ANG	830	UGT2B7
153	ZFH3	379	GBP2	605	RPS2	831	ATPIF1
154	SLC13A3	380	LINC00871	606	ASGR2	832	HIST1H1C
155	SLC16A2	381	CPXCR1	607	HSP90B1	833	BCAP31
156	BDKRB1	382	ALPK2	608	COX4I1	834	SSR4
157	TOR1AIP2	383	PPM1E	609	CFL1	835	HGD
158	USP45	384	ATP13A5	610	RPL14	836	UQCRCQ
159	LDLRAD2	385	CYBB	611	EDF1	837	AADAC
160	ACTL8	386	DMD	612	COX6B1	838	OS9
161	TNNI3	387	GRIP1	613	EIF1	839	NDUFB2
162	SLIT3	388	RLIM	614	GATM	840	ATP5O
163	TFAP2A	389	LINC01467	615	ADH1A	841	IFI30
164	TNRC6C	390	AR	616	HULC	842	EIF4A1
165	SPTLC3	391	ANKS1B	617	HSP90AB1	843	DAD1
166	ITPR1	392	LINC00871	618	LBP	844	TALDO1
167	ZNF222	393	SDR16C5	619	RPL37	845	CHCHD10
168	EPHB1	394	MIR4675	620	MT1E	846	ANGPTL4
169	PRICKLE2	395	ZFPM2-AS1	621	SAA4	847	EIF3K
170	DMD	396	LINC00972	622	RPS7	848	FIS1
171	RASGEF1A	397	RAB9A	623	IGFBP2	849	UQCRC1
172	DTNA	398	ZFPM2-AS1	624	CFHR2	850	BST2
173	ATAD3C	399	UNC13C	625	KRT8	851	PROC
174	HHIPL1	400	DMD	626	RPS27A	852	RPL23A
175	ADAM33	401	RALGAPA1	627	APOC4	853	DECR1
176	CHAD	402	CBWD6	628	PRDX1	854	CAT
177	WDR31	403	ANKS1B	629	RPS15	855	UGT1A6
178	NRXN3	404	NFU1	630	ASGR1	856	ATP6V0E1
179	GPM6A	405	PDE10A	631	NNMT	857	COL18A1
180	C22orf23	406	CBWD6	632	RPL32	858	CANX
181	TBXA2R	407	SYNDIG1	633	PTMA	859	ANPEP
182	SETBP1	408	NFU1	634	AKR1C3	860	C1orf43
183	CREB5	409	CSGALNACT1	635	SCD	861	DYNLL1
184	SIRPB2	410	PDE10A	636	APOB	862	YBX1

185	HECW2	411	NXPE4	637	RPS17	863	AOX1
186	GDPD1	412	SLC12A2	638	ATP5B	864	SCP2
187	ADAMTS5	413	CPXCR1	639	CD81	865	PDIA6
188	TIAM1	414	MAP3K21	640	EEF1G	866	BHMT
189	PII5	415	TCEANC	641	NME2	867	CES2
190	TCEANC	416	SLC16A2	642	CPS1	868	PSMB1
191	PDE10A	417	MAP3K21	643	LASS2	869	AQP9
192	DYNC1I1	418	GBP2	644	OAZ1	870	HDGF
193	SNAP25	419	ZFPM2-AS1	645	PCK1	871	ACSL1
194	FASLG	420	ABI3BP	646	HPR	872	CYC1
195	GRIP1	421	ANKS1B	647	SDS	873	FTCD
196	MAP3K9	422	TECTA	648	TM4SF4	874	EIF6
197	LSAMP	423	ALPK2	649	TIMP1	875	PCBD1
198	SOX6	424	NRXN3	650	NDUFB9	876	NDUFS6
199	SCG3	425	ABI3BP	651	TST	877	NACA
200	CDKL3	426	JAKMIP1	652	IFITM2	878	STARD10
201	CACNA2D1	427	ALB	653	DHCR24	879	RPN1
202	CCDC157	428	APOA2	654	SERPINA6	880	LAMP1
203	FAM155A	429	HP	655	LYZ	881	BRI3
204	ADAMTS17	430	FTL	656	PFN1	882	C11orf10
205	OPRL1	431	APOC1	657	EEF1D	883	RHOA
206	FUNDC2P2	432	APOA1	658	CPB2	884	CD151
207	ACSM1	433	APOC3	659	PRDX6	885	TMEM59
208	ALPK2	434	ORM1	660	NDUFA13	886	RNASE4
209	SEMA5B	435	SERPINA1	661	ATP5H	887	CCT3
210	PHACTR1	436	APOE	662	PSMB4	888	NCRNA00188
211	KRT12	437	RBP4	663	CP	889	HLA-B
212	UNC5A	438	FGA	664	COX6A1	890	ACAA1
213	JAKMIP1	439	AMBP	665	ADH1C	891	CAPNS1
214	DGKB	440	FGG	666	ETFB	892	APLP2
215	GRIN2B	441	VTN	667	NUPR1	893	SDC4
216	CCDC17	442	APOC2	668	ATP5I	894	HIST1H2AC
217	C8orf48	443	APOH	669	HSPA8	895	RPS29
218	FAM163A	444	TF	670	ATF5	896	ATP5D
219	MYLK4	445	TTR	671	RPS18	897	SELENBP1
220	UGT2B10	446	B2M	672	ECH1	898	RPN2
221	ANKRD53	447	FGB	673	GPX4	899	GLUD1
222	SH2D5	448	C3	674	RPL17	900	PSMA7
223	KCNA2	449	SAA1	675	RPL34	901	ST6GAL1
224	CFC1B	450	FABP1	676	PFDN5	902	SNRPD2
225	OPCML	451	AHSG	677	GPX2	903	MLF2
226	ANKS1B	452	SERPINA3	678	PABPC1		

[00368] **Example 12: Construction of a classifier for the typing of colorectal cancer patients using erythrocyte micronuclei DNA**

[00369] In this Example, there were 10 patients with colorectal cancer, including 5 patients diagnosed with colon cancer and 5 patients diagnosed with rectal cancer by other methods.

- 5 [00370] The peripheral blood samples from the above patients are expressed in the form of “P” plus patient number. For example, “P1” represents a peripheral blood sample from the first colorectal cancer patient (“Patient 1”), “P2” represents a peripheral blood sample from the second colorectal cancer patient (“Patient 2”), and so on.

[00371] The basic information of 10 colorectal cancer patients is shown in Table 9.

- 10 Colorectal cancer type, e.g., “adenocarcinoma”, refers to the type of colorectal cancer diagnosed by other methods.

Table 9

Patient number	Gender	Complaint	Enteroscopy pathology
1	Male	Rectal cancer was diagnosed for more than 1 month	Adenocarcinoma (rectum)
2	Female	The stool habit changed for more than half a year	(rectal) villous adenoma with low-grade intraepithelial neoplasia, local high-grade intraepithelial neoplasia, the lesion was confined to the lamina propria, and no submucosal infiltration was found. It was recommended to biopsy again to exclude Cancerization.
3	Male	Changes in stool characteristics with hematochezia for more than 3 months	Medium differentiated adenocarcinoma (rectum)
4	Female	Rectal cancer was diagnosed for more than 3 months	Medium differentiated adenocarcinoma (rectum)
5	Male	CEA gradually increased for more than 9 months	Adenocarcinoma (rectum)
6	Male	After more than 1 month of hematochezia, colon cancer was diagnosed for 4 days.	Medium differentiated adenocarcinoma (sigmoid colon)
7	Male	Blood in stool for 1 week	(sigmoid colon) The broken mucosa showed high-grade intraepithelial neoplasia. Cancerization cannot be excluded.

8	Female	Repeated abdominal pain for more than 2 months	Adenocarcinoma of colon
9	Female	Diarrhea and hematochezia for three months, and fatigue for one month	(colon biopsy) low differentiated adenocarcinoma
10	Female	Colonic tumor was found for 1 week	Considered as adenocarcinoma (liver curvature of colon).

[00372] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and bioinformatics analysis was carried out as described in Example 5.

[00373] Specifically, 97 different genes were screened out by ANOVA test from the reads count of gene regions of 5 colon cancer samples and 5 rectal cancer samples, and then unsupervised hierarchical clustering of the two types of samples according to Pearson correlation showed that there were significant differences between the two classes of samples.

[00374] As shown in Figure 11, a total of 97 genes from colon cancer and rectal cancer samples were clustered. Each row represents a differential gene, and each column represents a patient.

[00375] The list of 97 differential genes is shown in Table 10. Each gene corresponds to each row from top to bottom in Figure 12.

Table 10

1	FXYD3	26	PITRM1	51	IDH2	76	LRRC32
2	CD9	27	LTBP4	52	RBMX	77	WDR4
3	DYNLL1	28	HSPG2	53	CORO1B	78	ISY1
4	CTSA	29	OTUD5	54	SLC12A2	79	RBM19
5	CCDC107	30	COG4	55	RCC2	80	CYBB
6	TMEM181	31	NEK6	56	SETD3	81	PARVA
7	SLC1A4	32	CCNK	57	NARF	82	UPF2
8	HDAC7	33	UBE3C	58	WDR45	83	RECQL
9	MEMO1	34	VDR	59	EXT2	84	UBIAD1
10	ATRN	35	IGF2R	60	IPO8	85	MICALL1
11	VWF	36	FOXA3	61	EPS15	86	CREB1
12	MXI1	37	SH3BP4	62	CCDC80	87	CHRD1
13	FOCK2	38	LAMC1	63	ACOT1	88	LIMS1
14	MCCC1	39	SPPL2A	64	ABCB7	89	NOX4
15	PAPOLA	40	GBP2	65	RANBP2	90	CELSR1
16	PLEC	41	KREMEN1	66	HS6ST1	91	SLA
17	DRG1	42	PIP5K1B	67	PIP5K1C	92	PRKCH
18	DTYMK	43	FAF1	68	PLXND1	93	ARID2
19	RBM47	44	RAB9A	69	MED27	94	PKD2
20	NFU1	45	ALAD	70	SLC25A29	95	INVS
21	DNAJB6	46	PDE9A	71	XIAP	96	PASK

22	CDC42SE2	47	CORO7	72	CCDC85C	97	MSX1
23	CCND2	48	TFE3	73	ATP11A		
24	EPHA2	49	NEDD9	74	AGFG1		
25	STT3B	50	PDCD6IP	75	BNIP2		

[00376] Example 13. Classification of healthy individuals and colorectal cancer patients using erythrocyte micronuclei DNA

[00377] Using the classifier (903 genes) constructed in Example 11 for clustering healthy individuals and colorectal cancer patients, four unknown samples from four subjects were predicted.

[00378] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and bioinformatics analysis was carried out as described in Example 5.

[00379] After testing, it was found that two of the four samples were at high risk of colorectal cancer (the risk probabilities were all over 90%) and two were at low risk of colorectal cancer (the risk probabilities were all less than 5%). Tracing back the sample sources of subjects predicted to be high risk and subjects predicted to be low risk, it was found that the two samples with high risk of colorectal cancer were obtained from patients who were diagnosed as colorectal cancer by other diagnostic methods, and the two samples with low risk of colorectal cancer were obtained from healthy individuals detected by other diagnostic methods.

[00380] The result is shown in Figure 12. In Figure 12, P1 and P2 were two patients with colorectal cancer, and H1 and H2 were samples of non-colorectal cancer healthy individuals.

[00381] Therefore, the method and gene classifier of the present disclosure can effectively distinguish colorectal cancer patients from healthy individuals.

[00382] Example 14. Typing of colorectal cancer patients using erythrocyte micronuclei DNA

[00383] Erythrocyte micronuclei DNA and genomic DNA of peripheral blood mononuclear cells of each subject were obtained as described in Examples 1-4, and bioinformatics analysis was carried out as described in Example 5.

[00384] Using the classifier (97 genes) constructed in Example 12 for clustering colon cancer and rectal cancer patients, four colorectal cancer samples with unknown classification were predicted.

[00385] After testing, it was found that two of the four samples were at high risk of colon cancer (the risk probabilities were all over 85%) and two were at low risk of colon cancer (the risk probabilities were all less than 5%). Tracing back the sample sources of subjects with high risk of colon cancer and subjects with low risk of colon cancer, it was found that

the two samples with high risk of colon cancer were obtained from patients with colon cancer detected by other diagnostic methods, and the two samples with low risk of colon cancer came from subjects who were diagnosed as rectal cancer by other diagnostic methods.

[00386] The result is shown in Figure 13. In Figure 13, P1 and P2 are colon cancer patients, and P2 and P3 are rectal cancer patients.

[00387] Therefore, the method and gene classifier of the present disclosure can effectively classify colorectal cancer patients and distinguish colon cancer from rectal cancer.

[00388] **Example 15. Discriminative performance of the rbcDNA signature in cancer patients**

[00389] We randomly assigned HD and cancer samples to a training set (70%, n=236) for model development, a validation set (10%, n=34) for hyper-parameter selection and a test set (20%, n=68) for model validation. Our results showed that 91% (95% confidence interval 84-100%) of cancer patients including 85% LC, 100% CRC and 90% HCC were detected with 99% specificity. This includes 86% patients with stage I, 92% of patients with stage II and 100% of patients with stage III cancers (Table 14). This data suggests the presence of specific rbcDNA signatures that can differentiate between healthy donors and cancer patients. We next tested the efficacy of rbcDNA in differentiating among specific cancer types. rbcDNA signatures exhibit high discriminatory performance in pairwise comparisons of healthy and cancer groups, our results showed that 90% (95% confidence interval 68-100%) of HCC patients, 100% (95% confidence interval 100-100%) of CRC patients and 85% (95% confidence interval 70-100%) of LC patients, all were detected with 95% specificity (Table 15). Moreover, pairwise and multiclass tests showed overall high accuracy in detecting specific cancers indicating significant discriminative power of rbcDNA profiles (Figure 14).

[00390] The result is shown in Table 14, the list of differential rbcDNA signature is shown in Table 16.

[00391] The result is shown in Table 15, the list of differential rbcDNA signature is shown in Table 17 for HD vs. LC, Table 18 for HD vs. CRC, Table 19 for HD vs. HCC.

[00392] The result is shown in Figure 15, the list of differential rbcDNA signature is shown in Table 20.

Table 14

Pan-Cancer analysed		99% specificity		
	Test (Total)	Individual detected	Sensitivity (%)	95% CI (%)
HD	21 (106)	0		
Cancer	47 (232)	43	91	84-100
	HCC 9 (44)	8	90	68-100
Type	CRC 18 (99)	18	100	100-100
	LC 20 (89)	17	85	69-100

Stage	I	22 (106)	19	86	72-100
	II	12 (61)	11	92	76-100
	III	13 (65)	13	100	100-100

Table 15

Cancer Type	95% specificity			
	Test (Total)	Individual detected	Sensitivity	95% CI
HCC	9	8	90	68-100
CRC	18	18	100	100-100
LC	20	17	85	70-100

[00393] Table 14 shows accuracy ratios of pan-cancer deep neural network classification in the test set for each cancer type, including corresponding sensitivity with 99% specificity (CI, Confidence Interval).

[00394] Table 15 shows accuracy ratios of each cancer type deep neural network classification in the test set for each cancer type, including corresponding sensitivity with 95% specificity (CI, Confidence Interval).

Table 16

1	chr1:500000-1000000, chr1:1500000-2000000, chr1:2500000-3000000, chr1:8000000-8500000, chr1:8500000-9000000, chr1:9500000-10000000, chr1:10000000-10500000, chr1:11500000-12000000, chr1:12000000-12500000, chr1:13000000-13500000, chr1:15500000-16000000, chr1:16000000-16500000, chr1:16500000-17000000, chr1:19000000-19500000, chr1:19500000-20000000, chr1:20000000-20500000, chr1:20500000-21000000, chr1:21000000-21500000, chr1:23000000-23500000, chr1:24500000-25000000, chr1:25500000-26000000, chr1:26000000-26500000, chr1:26500000-27000000, chr1:27000000-27500000, chr1:27500000-28000000, chr1:28000000-28500000, chr1:28500000-29000000, chr1:30500000-31000000, chr1:31500000-32000000, chr1:32000000-32500000, chr1:32500000-33000000, chr1:33000000-33500000, chr1:33500000-34000000, chr1:35500000-36000000, chr1:36000000-36500000, chr1:36500000-37000000, chr1:37500000-38000000, chr1:38500000-39000000, chr1:39000000-39500000, chr1:40000000-40500000, chr1:40500000-41000000, chr1:42000000-42500000, chr1:44500000-45000000, chr1:45500000-46000000, chr1:51500000-52000000, chr1:52500000-53000000, chr1:53500000-54000000, chr1:54000000-54500000, chr1:58500000-59000000, chr1:59000000-59500000, chr1:61000000-61500000, chr1:63500000-64000000, chr1:64500000-65000000, chr1:65000000-65500000, chr1:66000000-66500000, chr1:77000000-77500000, chr1:77500000-78000000, chr1:83000000-83500000, chr1:88500000-89000000, chr1:91500000-92000000, chr1:94500000-95000000, chr1:98500000-99000000, chr1:101000000-101500000, chr1:107500000-108000000, chr1:108500000-109000000, chr1:109000000-109500000, chr1:109500000-110000000, chr1:110500000-111000000, chr1:111500000-112000000, chr1:112500000-113000000, chr1:114000000-114500000, chr1:116000000-116500000, chr1:116500000-117000000, chr1:120000000-120500000, chr1:120500000-121000000, chr1:121000000-121500000, chr1:125000000-125500000, chr1:143000000-143500000, chr1:143500000-144000000, chr1:144000000-144500000, chr1:144500000-145000000, chr1:145000000-145500000, chr1:146000000-146500000, chr1:146500000-147000000, chr1:147500000-148000000, chr1:148000000-148500000, chr1:148500000-149000000, chr1:149000000-149500000, chr1:149500000-150000000, chr1:150000000-150500000, chr1:150500000-151000000, chr1:151000000-151500000, chr1:151500000-152000000, chr1:153500000-154000000, chr1:154000000-154500000, chr1:154500000-155000000, chr1:155000000-155500000, chr1:155500000-156000000, chr1:156000000-156500000, chr1:156500000-157000000, chr1:158000000-158500000, chr1:159000000-159500000, chr1:160000000-160500000, chr1:160500000-161000000, chr1:161000000-161500000, chr1:162000000-162500000, chr1:163500000-164000000, chr1:165500000-166000000, chr1:167000000-167500000, chr1:169500000-170000000, chr1:171000000-171500000, chr1:171500000-172000000, chr1:172500000-173000000, chr1:173500000-174000000, chr1:177500000-178000000, chr1:181000000-181500000, chr1:182000000-182500000, chr1:182500000-183000000, chr1:183000000-183500000, chr1:193000000-193500000, chr1:197000000-197500000, chr1:198500000-199000000, chr1:199000000-199500000, chr1:200000000-200500000, chr1:200500000-201000000, chr1:202500000-203000000, chr1:203000000-203500000, chr1:203500000-204000000, chr1:204000000-204500000, chr1:205000000-205500000, chr1:206500000-207000000, chr1:208000000-208500000, chr1:211500000-212000000, chr1:212500000-213000000, chr1:214000000-214500000, chr1:221500000-222000000, chr1:222500000-223000000, chr1:225500000-226000000, chr1:226000000-226500000, chr1:230500000-231000000, chr1:235000000-235500000, chr1:235500000-236000000, chr1:242000000-242500000, chr1:242500000-243000000, chr1:243500000-244000000, chr1:246500000-247000000, chr1:247000000-247500000
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15	chr15:17000000-17500000, chr15:19500000-20000000, chr15:20000000-20500000, chr15:20500000-21000000, chr15:21000000-21500000, chr15:21500000-22000000, chr15:22000000-22500000, chr15:23000000-23500000, chr15:24000000-24500000, chr15:24500000-25000000, chr15:26500000-27000000, chr15:28500000-29000000, chr15:30000000-30500000, chr15:30500000-31000000, chr15:32000000-32500000, chr15:34000000-34500000, chr15:35000000-35500000, chr15:36000000-36500000, chr15:41000000-41500000, chr15:41500000-42000000, chr15:43000000-43500000, chr15:46000000-46500000, chr15:47000000-47500000, chr15:48500000-49000000, chr15:50000000-50500000, chr15:53000000-53500000, chr15:55000000-55500000, chr15:57000000-57500000, chr15:58000000-58500000, chr15:60000000-60500000, chr15:63000000-63500000, chr15:63500000-64000000, chr15:64000000-64500000, chr15:64500000-65000000, chr15:65500000-66000000, chr15:69000000-69500000, chr15:70000000-70500000, chr15:74500000-75000000, chr15:81000000-81500000, chr15:81500000-82000000, chr15:82000000-82500000, chr15:84000000-84500000, chr15:85500000-86000000, chr15:90000000-90500000, chr15:90500000-91000000, chr15:91500000-92000000, chr15:94000000-94500000
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18	chr18:3000000-3500000, chr18:5500000-6000000, chr18:6500000-7000000, chr18:11000000-11500000, chr18:14000000-14500000, chr18:14500000-15000000, chr18:15000000-15500000, chr18:21000000-21500000, chr18:23500000-24000000, chr18:27500000-28000000, chr18:35000000-35500000, chr18:46000000-46500000, chr18:51000000-51500000, chr18:61000000-61500000, chr18:78000000-78500000
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21	chr21:5000000-5500000, chr21:5500000-6000000, chr21:6000000-6500000, chr21:6500000-7000000, chr21:7000000-7500000, chr21:7500000-8000000, chr21:8000000-8500000, chr21:8500000-9000000, chr21:9000000-9500000, chr21:9500000-10000000, chr21:10000000-10500000, chr21:10500000-11000000, chr21:13000000-13500000, chr21:13500000-14000000, chr21:17000000-17500000, chr21:25500000-26000000, chr21:29000000-29500000, chr21:31000000-31500000, chr21:33000000-33500000, chr21:34500000-35000000, chr21:36000000-36500000, chr21:40000000-40500000, chr21:42000000-42500000

22	chr22:10500000-11000000, chr22:11000000-11500000, chr22:11500000-12000000, chr22:12000000-12500000, chr22:12500000-13000000, chr22:15000000-15500000, chr22:15500000-16000000, chr22:16000000-16500000, chr22:16500000-17000000, chr22:18000000-18500000, chr22:18500000-19000000, chr22:21000000-21500000, chr22:21500000-22000000, chr22:27000000-27500000, chr22:29500000-30000000, chr22:30000000-30500000, chr22:31000000-31500000, chr22:31500000-32000000, chr22:35000000-35500000, chr22:35500000-36000000, chr22:36000000-36500000, chr22:36500000-37000000, chr22:37000000-37500000, chr22:37500000-38000000, chr22:38000000-38500000, chr22:40000000-40500000, chr22:40500000-41000000, chr22:41000000-41500000, chr22:41500000-42000000, chr22:48500000-49000000
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Table 17

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18	chr18:3000000-3500000, chr18:5500000-6000000, chr18:7000000-7500000, chr18:11000000-11500000, chr18:12500000-13000000, chr18:14000000-14500000, chr18:15000000-15500000, chr18:21000000-21500000, chr18:24000000-24500000, chr18:31500000-32000000, chr18:35000000-35500000, chr18:42000000-42500000, chr18:48500000-49000000, chr18:73000000-73500000, chr18:78000000-78500000
19	chr19:0-500000, chr19:1000000-1500000, chr19:2000000-2500000, chr19:3000000-3500000, chr19:4000000-4500000, chr19:4500000-5000000, chr19:6500000-7000000, chr19:7000000-7500000, chr19:7500000-8000000, chr19:10000000-10500000, chr19:10500000-11000000, chr19:11000000-11500000, chr19:12500000-13000000, chr19:13000000-13500000, chr19:13500000-14000000, chr19:14000000-14500000, chr19:14500000-15000000, chr19:15000000-15500000, chr19:15500000-16000000, chr19:16500000-17000000, chr19:17000000-17500000, chr19:18000000-18500000, chr19:18500000-19000000, chr19:19000000-19500000, chr19:20500000-21000000, chr19:21000000-21500000, chr19:21500000-22000000, chr19:22000000-22500000, chr19:22500000-23000000, chr19:28000000-28500000, chr19:34000000-34500000, chr19:35000000-35500000, chr19:38000000-38500000, chr19:38500000-39000000, chr19:39000000-39500000, chr19:40500000-41000000, chr19:41000000-41500000, chr19:42000000-42500000, chr19:42500000-43000000, chr19:43500000-44000000, chr19:44500000-45000000, chr19:45000000-45500000, chr19:45500000-46000000, chr19:46500000-47000000, chr19:47000000-47500000, chr19:48500000-49000000, chr19:49500000-50000000, chr19:51000000-51500000, chr19:54000000-54500000, chr19:55000000-55500000, chr19:58500000-58617616

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22	chr22:10500000-11000000, chr22:11000000-11500000, chr22:11500000-12000000, chr22:12000000-12500000, chr22:12500000-13000000, chr22:15000000-15500000, chr22:15500000-16000000, chr22:16000000-16500000, chr22:16500000-17000000, chr22:18000000-18500000, chr22:18500000-19000000, chr22:21000000-21500000, chr22:23000000-23500000, chr22:31000000-31500000, chr22:31500000-32000000, chr22:35500000-36000000, chr22:36000000-36500000, chr22:36500000-37000000, chr22:39500000-40000000, chr22:41500000-42000000

Table 18

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Table 19

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19	chr19:0-500000, chr19:500000-1000000, chr19:1000000-1500000, chr19:1500000-2000000, chr19:2500000-3000000, chr19:3000000-3500000, chr19:4500000-5000000, chr19:5500000-6000000, chr19:6000000-6500000, chr19:6500000-7000000, chr19:7500000-8000000, chr19:8000000-8500000, chr19:8500000-9000000, chr19:9500000-10000000, chr19:10000000-10500000, chr19:10500000-11000000, chr19:11000000-11500000, chr19:12500000-13000000, chr19:14500000-15000000, chr19:15500000-16000000, chr19:16000000-16500000, chr19:16500000-17000000, chr19:17000000-17500000, chr19:17500000-18000000, chr19:18000000-18500000, chr19:19000000-19500000, chr19:27500000-28000000, chr19:35000000-35500000, chr19:35500000-36000000, chr19:38000000-38500000, chr19:38500000-39000000, chr19:39000000-39500000, chr19:39500000-40000000, chr19:40000000-40500000, chr19:40500000-41000000, chr19:41000000-41500000, chr19:41500000-42000000, chr19:42000000-42500000, chr19:43000000-43500000, chr19:44500000-45000000, chr19:45000000-45500000, chr19:45500000-46000000, chr19:46500000-47000000, chr19:47000000-47500000, chr19:48000000-48500000, chr19:49500000-50000000, chr19:50500000-51000000, chr19:51000000-51500000, chr19:52000000-52500000, chr19:55500000-56000000, chr19:56000000-56500000
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Table 20

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16	16_10500000_11000000,16_11500000_12000000,16_12000000_12500000,16_12500000_13000000,16_13000000_13500000,16_13500000_14000000,16_14000000_14500000,16_14500000_15000000,16_15000000_15500000,16_15500000_16000000,16_16000000_16500000,16_16500000_17000000,16_17000000_17500000,16_17500000_18000000,16_18000000_18500000,16_18500000_19000000,16_19000000_20000000,16_20000000_21500000,16_21500000_22000000,16_22000000_22500000,16_22500000_23000000,16_23000000_23500000,16_23500000_24000000,16_24000000_24500000,16_24500000_25000000,16_25000000_26000000,16_26000000_26500000,16_26500000_27000000,16_27000000_27500000,16_27500000_28000000,16_28000000_28500000,16_28500000_29000000,16_29000000_29500000,16_29500000_30000000,16_30000000_30500000,16_30500000_31000000,16_31000000_31500000,16_31500000_32000000,16_32000000_32500000,16_32500000_33000000,16_33000000_33500000,16_33500000_34000000,16_34000000_34500000,16_34500000_35000000,16_35000000_35500000,16_35500000_36000000,16_36000000_36500000,16_36500000_37000000,16_37000000_37500000,16_37500000_38000000,16_38000000_38500000,16_38500000_39000000,16_39000000_39500000,16_39500000_40000000,16_40000000_40500000,16_40500000_41000000,16_41000000_41500000,16_41500000_42000000,16_42000000_42500000,16_42500000_43000000,16_43000000_43500000,16_43500000_44000000,16_44000000_44500000,16_44500000_45000000,16_45000000_45500000,16_45500000_46000000,16_46000000_46500000,16_46500000_47000000,16_47000000_47500000,16_47500000_48000000,16_48000000_48500000,16_48500000_49000000,16_49000000_49500000,16_49500000_50000000,16_50000000_50500000,16_50500000_51000000,16_51000000_51500000,16_51500000_52000000,16_52000000_52500000,16_52500000_53000000,16_53000000_53500000,16_53500000_54000000,16_54000000_54500000,16_54500000_55000000,16_55000000_55500000,16_55500000_56000000,16_56000000_56500000,16_56500000_57000000,16_57000000_57500000,16_57500000_58000000,16_58000000_58500000,16_58500000_59000000,16_59000000_59500000,16_59500000_60000000,16_60000000_60500000,16_60500000_61000000,16_61000000_61500000,16_61500000_62000000,16_62000000_62500000,16_62500000_63000000,16_63000000_63500000,16_63500000_64000000,16_64000000_64500000,16_64500000_65000000,16_65000000_65500000,16_65500000_66000000,16_66000000_66500000,16_66500000_67000000,16_67000000_67500000,16_67500000_68000000,16_68000000_68500000,16_68500000_69000000,16_69000000_69500000,16_69500000_70000000,16_70000000_70500000,16_70500000_71000000,16_71000000_71500000,16_71500000_72000000,16_72000000_72500000,16_72500000_73000000,16_73000000_73500000,16_73500000_74000000,16_74000000_74500000,16_74500000_75000000,16_75000000_75500000,16_75500000_76000000,16_76000000_76500000,16_76500000_77000000,16_77000000_77500000,16_77500000_78000000,16_78000000_78500000,16_78500000_79000000,16_79000000_79500000,16_79500000_80000000,16_80000000_80500000,16_80500000_81000000,16_81000000_81500000,16_81500000_82000000,16_82000000_82500000,16_82500000_83000000,16_83000000_83500000,16_83500000_84000000,16_84000000_84500000,16_84500000_85000000,16_85000000_85500000,16_85500000_86000000,16_86000000_86500000,16_86500000_87000000,16_87000000_87500000,16_87500000_88000000,16_88000000_88500000,16_88500000_89000000,16_89000000_89500000,16_89500000_90000000,16_90000000_90338345,
17	17_0_500000,17_1000000_1500000,17_1000000_10500000,17_10500000_11000000,17_11000000_11500000,17_11500000_12000000,17_12000000_13000000,17_13000000_13500000,17_13500000_14000000,17_14000000_14500000,17_14500000_15000000,17_15000000_20000000,17_16000000_16500000,17_16500000_17000000,17_17000000_18000000,17_18000000_19000000,17_19000000_19500000,17_19500000_20000000,17_20000000_20500000,17_20500000_21000000,17_21000000_21500000,17_21500000_22000000,17_22000000_22500000,17_22500000_23000000,17_23000000_23500000,17_23500000_24000000,17_24000000_24500000,17_24500000_25000000,17_25000000_25500000,17_25500000_26000000,17_26000000_26500000,17_26500000_27000000,17_27000000_27500000,17_27500000_28000000,17_28000000_28500000,17_28500000_29000000,17_29000000_29500000,17_29500000_30000000,17_30000000_30500000,17_30500000_31000000,17_31000000_31500000,17_31500000_32000000,17_32000000_32500000,17_32500000_33000000,17_33000000_33500000,17_33500000_34000000,17_34000000_34500000,17_34500000_35000000,17_35000000_35500000,17_35500000_36000000,17_36000000_36500000,17_36500000_37000000,17_37000000_37500000,17_37500000_38000000,17_38000000_38500000,17_38500000_39000000,17_39000000_39500000,17_39500000_40000000,17_40000000_40500000,17_40500000_41000000,17_41000000_41500000,17_41500000_42000000,17_42000000_42500000,17_42500000_43000000,17_43000000_43500000,17_43500000_44000000,17_44000000_44500000,17_44500000_45000000,17_45000000_45500000,17_45500000_46000000,17_46000000_46500000,17_46500000_47000000,17_47000000_47500000,17_47500000_48000000,17_48000000_48500000,17_48500000_49000000,17_49000000_49500000,17_49500000_50000000,17_50000000_50500000,17_50500000_51000000,17_51000000_51500000,17_51500000_52000000,17_52000000_52500000,17_52500000_53000000,17_53000000_53500000,17_53500000_54000000,17_54000000_54500000,17_54500000_55000000,17_55000000_55500000,17_55500000_56000000,17_56000000_56500000,17_56500000_57000000,17_57000000_57500000,17_57500000_58000000,17_58000000_58500000,17_58500000_59000000,17_59000000_59500000,17_59500000_60000000,17_60000000_60500000,17_60500000_61000000,17_61000000_61500000,17_61500000_62000000,17_62000000_62500000,17_62500000_63000000,17_63000000_63500000,17_63500000_64000000,17_64000000_64500000,17_64500000_65000000,17_65000000_65500000,17_65500000_66000000,17_66000000_66500000,17_66500000_67000000,17_67000000_67500000,17_67500000_68000000,17_68000000_68500000,17_68500000_69000000,17_69000000_69500000,17_69500000_70000000,17_70000000_70500000,17_70500000_71000000,17_71000000_71500000,17_71500000_72000000,17_72000000_72500000,17_72500000_73000000,17_73000000_73500000,17_73500000_74000000,17_74000000_74500000,17_74500000_75000000,17_75000000_75500000,17_75500000_76000000,17_76000000_76500000,17_76500000_77000000,17_77000000_77500000,17_77500000_78000000,17_78000000_78500000,17_78500000_79000000,17_79000000_79500000,17_79500000_80000000,17_80000000_80500000,17_80500000_81000000,17_81000000_81500000,17_81500000_82000000,17_82000000_82500000,17_82500000_83000000,17_83000000_83500000,17_83500000_84000000,17_84000000_84500000,17_84500000_85000000,17_85000000_85500000,17_85500000_86000000,17_86000000_86500000,17_86500000_87000000,17_87000000_87500000,17_87500000_88000000,17_88000000_88500000,17_88500000_89000000,17_89000000_89500000,17_89500000_90000000,

18	18_1000000_1500000,18_1000000_1050000,18_1100000_1150000,18_1200000_1250000,18_1250000_1300000,18_1400000_1450000,18_1450000_1500000,18_1500000_1550000,18_2050000_2100000,18_2100000_2150000,18_2350000_2400000,18_2400000_2450000,18_2500000_3000000,18_2500000_2550000,18_2700000_2750000,18_2750000_2800000,18_3000000_3500000,18_3050000_3100000,18_3150000_3200000,18_3500000_4000000,18_3500000_3550000,18_3900000_3950000,18_4200000_4250000,18_4250000_4300000,18_4600000_4650000,18_4700000_4750000,18_4850000_4900000,18_4900000_4950000,18_5000000_5500000,18_5050000_5100000,18_5100000_5150000,18_5300000_5350000,18_5350000_5400000,18_5500000_6000000,18_5950000_6000000,18_6100000_6150000,18_6300000_6350000,18_6450000_6500000,18_6500000_7000000,18_6500000_6550000,18_6700000_6750000,18_7000000_7500000,18_7300000_7350000,18_7350000_7400000,18_7450000_7500000,18_7600000_7650000,18_7800000_7850000,18_8000000_8500000,18_9000000_9500000,18_9500000_1000000
19	19_0_500000,19_1000000_1500000,19_1000000_1050000,19_1050000_1100000,19_1100000_1150000,19_1150000_1200000,19_1250000_1300000,19_1300000_1350000,19_1350000_1400000,19_1400000_1450000,19_1450000_1500000,19_1500000_2000000,19_1500000_1550000,19_1550000_1600000,19_1600000_1650000,19_1650000_1700000,19_1700000_1750000,19_1750000_1800000,19_1800000_1850000,19_1850000_1900000,19_1900000_1950000,19_2000000_2500000,19_2050000_2100000,19_2100000_2150000,19_2150000_2200000,19_2200000_2250000,19_2250000_2300000,19_2500000_3000000,19_2750000_2800000,19_2800000_2850000,19_3000000_3500000,19_3400000_3450000,19_3500000_3550000,19_3550000_3600000,19_3800000_3850000,19_3850000_3900000,19_3900000_3950000,19_3950000_4000000,19_4000000_4500000,19_4000000_4050000,19_4050000_4100000,19_4100000_4150000,19_4150000_4200000,19_4200000_4250000,19_4250000_4300000,19_4300000_4350000,19_4350000_4400000,19_4450000_4500000,19_4500000_5000000,19_4500000_4550000,19_4550000_4600000,19_4650000_4700000,19_4700000_4750000,19_4800000_4850000,19_4850000_4900000,19_4900000_4950000,19_4950000_5000000,19_500000_1000000,19_5050000_5100000,19_5100000_5150000,19_5150000_5200000,19_5200000_5250000,19_5350000_5400000,19_5400000_5450000,19_5500000_6000000,19_5500000_5550000,19_5550000_5600000,19_5600000_5650000,19_5850000_58617616,19_6000000_6500000,19_6500000_7000000,19_7000000_7500000,19_7500000_8000000,19_8000000_8500000,19_8500000_9000000,19_9500000_1000000
20	20_0_500000,20_1000000_1500000,20_1000000_1050000,20_1050000_1100000,20_1150000_1200000,20_1250000_1300000,20_1450000_1500000,20_1500000_2000000,20_1600000_1650000,20_1900000_1950000,20_2000000_2500000,20_2000000_2050000,20_2050000_2100000,20_2100000_2150000,20_2150000_2200000,20_2450000_2500000,20_2500000_3000000,20_2500000_2550000,20_2550000_2600000,20_2600000_2650000,20_2850000_2900000,20_2900000_2950000,20_2950000_3000000,20_3000000_3500000,20_3000000_3050000,20_3050000_3100000,20_3150000_3200000,20_3200000_3250000,20_3300000_3350000,20_3350000_3400000,20_3400000_3450000,20_3450000_3500000,20_3500000_4000000,20_3500000_3550000,20_3550000_3600000,20_3650000_3700000,20_3700000_3750000,20_3750000_3800000,20_3800000_3850000,20_3850000_3900000,20_3950000_4000000,20_4050000_4100000,20_4100000_4150000,20_4250000_4300000,20_4350000_4400000,20_4400000_4450000,20_4450000_4500000,20_4500000_5000000,20_4500000_4550000,20_4550000_4600000,20_4600000_4650000,20_4700000_4750000,20_4750000_4800000,20_4800000_4850000,20_4850000_4900000,20_4900000_4950000,20_4950000_5000000,20_5000000_5500000,20_5000000_5050000,20_5050000_5100000,20_5100000_5150000,20_5150000_5200000,20_5200000_5250000,20_5250000_5300000,20_5350000_5400000,20_5500000_6000000,20_5500000_5550000,20_5550000_5600000,20_5650000_5700000,20_5700000_5750000,20_5750000_5800000,20_5850000_5900000,20_5900000_5950000,20_6100000_6150000,20_6200000_6250000,20_6250000_6300000,20_6300000_6350000,20_8000000_8500000

21	21_10000000_10500000,21_10500000_11000000,21_13000000_13500000,21_13500000_14000000,21_14000000_14500000,21_14500000_15000000,21_15000000_15500000,21_15500000_16000000,21_16000000_16500000,21_17000000_17500000,21_17500000_18000000,21_18000000_18500000,21_23500000_24000000,21_24500000_25000000,21_25500000_26000000,21_29000000_29500000,21_31000000_31500000,21_31500000_32000000,21_32500000_33000000,21_33000000_33500000,21_33500000_34000000,21_34500000_35000000,21_35000000_35500000,21_35500000_36000000,21_36000000_36500000,21_37000000_37500000,21_38000000_38500000,21_39500000_40000000,21_40000000_40500000,21_40500000_41000000,21_42000000_42500000,21_44000000_44500000,21_44500000_45000000,21_50000000_55000000,21_55000000_60000000,21_60000000_65000000,21_65000000_70000000,21_70000000_75000000,21_75000000_80000000,21_80000000_85000000,21_85000000_90000000,21_90000000_95000000,21_95000000_10000000,
22	22_10500000_11000000,22_11000000_11500000,22_11500000_12000000,22_12000000_12500000,22_12500000_13000000,22_15000000_15500000,22_15500000_16000000,22_16000000_16500000,22_16500000_17000000,22_18000000_18500000,22_18500000_19000000,22_19000000_19500000,22_20500000_21000000,22_21000000_21500000,22_21500000_22000000,22_23000000_23500000,22_23500000_24000000,22_25000000_25500000,22_26000000_26500000,22_27000000_27500000,22_27500000_28000000,22_28500000_29000000,22_29500000_30000000,22_30000000_30500000,22_31000000_31500000,22_31500000_32000000,22_32000000_32500000,22_32500000_33000000,22_35000000_35500000,22_35500000_36000000,22_36000000_36500000,22_36500000_37000000,22_37000000_37500000,22_37500000_38000000,22_38000000_38500000,22_38500000_39000000,22_39000000_39500000,22_39500000_40000000,22_40000000_40500000,22_40500000_41000000,22_41000000_41500000,22_41500000_42000000,22_42500000_43000000,22_43000000_43500000,22_48000000_48500000,22_48500000_49000000

[00395] **Example 16. Characterization of the rbcDNA signature in healthy donors and cancer patients**

[00396] Genome-wide sequencing profiles revealed rbcDNA signals distribute across autosomal chromosomes with specific patterns distinct from those of the corresponding genomic DNA (gDNA) (Figure 15A). The mean genomic coverage of rbcDNA is higher in healthy donors compared to that of cancer patients, while no significant difference in coverage is observed among patients of different cancer types (Figure 15B and 15C). Nonetheless, genome-wide analysis showed pronounced signal enrichment of intergenic, intronic and exonic regions in rbcDNA of cancer patients versus healthy donors. A modest differential enrichment of rbcDNA signals was detected in intergenic and intronic regions of CRC patients compared to patients of other cancer types (Figure 15D).

[00397] It can be clearly seen from the above examples that the inventors have successfully isolated the micronuclei DNA of peripheral red blood cells, and constructed a classifier for cancer detection by using the micronuclei DNA of peripheral red blood cells, thus realizing the effective detection of cancer, which is of great significance for clinical screening, diagnosis, classification and staging of cancer.

[00398] Although the specific embodiments of the present invention have been described in detail, it will be understood by those skilled in the art that various modifications and variations can be made to the details in light of all the teachings disclosed, and these changes are within the scope of protection of the present invention. The full scope of the invention is
5 provided by the appended claims and any equivalents thereof.