

**What is claimed is:****1. Micronuclei DNA isolated CLAIMS**

1. A method of detection or purified from early diagnosis of cancer in a subject in need thereof, the method comprising:
  - a) providing a peripheral red blood cell blood sample that has been previously obtained from the subject;
2. ~~The micronuclei DNA according to claim 1, wherein the peripheral red blood cells is a human peripheral red blood cell.~~
3. ~~The micronuclei DNA according to claim 1 or 2, wherein the micronuclei DNA is used for cancer detection, for example, screening, diagnosis, typing and/or staging of cancer.~~
4. ~~The micronuclei DNA according to claim 3, wherein the cancer is cervical cancer, colorectal cancer lung cancer, or hepatocellular cancer.~~
5. ~~The micronuclei DNA according to claim 4 wherein the cancer is cervical cancer.~~
6. ~~The micronuclei DNA according to claim 5, wherein the micronuclei DNA comprises a gene classifier shown in Table 2, 4 or 6.~~
7. ~~The micronuclei DNA according to claim 4, wherein the cancer is colorectal cancer.~~
8. ~~The micronuclei DNA according to claim 7, wherein the micronuclei DNA comprises a gene classifier shown in Table 8 or 10.~~
9. ~~The method for isolating or purifying micronuclei DNA from peripheral red blood cells comprises the following steps:~~
  - a) ~~providing peripheral blood samples;~~
  - b) ~~b)-isolating mononuclear cells and red blood cells from the peripheral blood samples; sample;~~
  - c) ~~e)-collecting red blood cells;~~
  - d) ~~d)-treating collected red blood cells with a red blood cell lysis buffer; and~~
  - e) ~~e)-extracting micronuclei DNA from the lysed red blood cells;~~
10. ~~The method according to claim 9, wherein the red blood cell lysis buffer specifically lyses red blood cells by changing osmotic pressure of cell suspension without lysing nucleated cells.~~

- f) 11. sequencing the whole genome of the micronuclei DNA to obtain fragment sequence information of the micronuclei DNA;
- g) comparing the fragment sequence information of the micronuclei DNA with a gene classifier to, thereby, classify the subject into one or more of the different classes underlying the gene classifier;
- h) diagnosing or detecting cancer in the subject based on the result of the classification in step (g).

The method according to claim 9~~1~~, wherein ~~the red blood cell lysis buffer comprises NH<sub>4</sub>Cl, NaHCO<sub>3</sub>, EDTA or a combination thereof.~~

~~12. The method according to claim 11, wherein the micronuclei DNA is extracted by a DNA extraction reagent in step e), and the DNA extraction reagent contains protease, such as protease K.~~

~~13. The method according to any one of claims 9-12, wherein before step (b), the peripheral blood sample is diluted, preferably, by phosphate buffer solution, and more preferably, by phosphate buffer solution in equal volume.~~

~~14. The method according to claim 9, wherein 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.~~

~~1.2. 15. The method of claim 14,; and wherein the red blood cells are collected from the bottom of the red blood cell layersaid red blood cell layer, and the collected red blood cells are subjected to two or more sequential filtrations through 10μm cell strainers.~~

3. 16. The method according to claim 1, wherein in step (e) the lysed red blood cells are treated with an extraction reagent comprising protease K.

4. The method according to claim 1, further comprising amplifying the micronuclei DNA prior to performing said sequencing, wherein the amplification method is selected from the group consisting of multiple displacement amplification (MDA), Multiple Annealing

and Looping-based Amplification Cycles (MALBAC) and degenerate oligonucleotide primer PCR (DOP-PCR).

~~2.5. The method according to any one of claims 9-12, wherein the lysed red blood cells are centrifuged, supernatant is taken, and erythrocyte micronuclei DNA is extracted from the supernatant~~1-4, wherein the sequencing is high-throughput sequencing.

~~3.6.17. The method according to any one of claims 9-12, wherein the lysed red blood cells are centrifuged, supernatant is taken, and erythrocyte micronuclei DNA is extracted from the supernatant.~~1-5, further comprising:

~~18. The method according to any one of claims 9-12, wherein the collected red blood cells are subjected to two or more sequential filtrations, e.g., filtrations by cell strainers, e.g., filtrations by 10µm cell strainers.~~

~~19. The micronuclei DNA from peripheral red blood cells obtained by the method of any one of claims 9-18.~~

~~20. A method for constructing a gene classifier for cancer detection by a peripheral red blood cell micronuclei DNA, which comprises the following steps:~~

- ~~a) providing more than one different classes, wherein each class represents a group of subjects with common characteristics;~~
- ~~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 peripheral red blood cells in different classes of subjects;~~
- ~~e) training the characteristic DNA fragment set for a specific cancer 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.~~

~~21. The method of claim 20, wherein the different classes include:~~

- ~~—cancer subjects and non-cancer subjects;~~
- ~~—subjects with different types of the same cancer; or~~
- ~~—subjects at different stages of the same type of cancer.~~

~~22. The method of claim 20 or 21, wherein the cancer is cervical cancer, colorectal cancer lung cancer, or hepatocellular cancer.~~

~~23. The method of claim 21, wherein the different classes include:~~

- ~~—cervical cancer subjects and non-cervical cancer subjects;~~
- ~~—cervical squamous cell carcinoma subjects and cervical adenocarcinoma subjects; or~~
- ~~—subjects in the stage of low differentiation, low-medium differentiation, medium differentiation or high differentiation stages of cervical squamous cell carcinoma.~~

~~24. The method of claim 21, wherein the different classes include:~~

- ~~—colorectal cancer subjects and non-colorectal cancer subjects; or~~
- ~~—colon cancer subjects and rectal cancer subjects.~~

~~25. The method according to any one of claims 20-24, wherein in step e), the characteristic DNA fragment set for a specific cancer is trained through hierarchical cluster analysis.~~

~~26. The method according to any one of claims 20-24, wherein the method further comprises the step of performing whole genome sequencing on the genomic DNA of the peripheral blood mononuclear cells of eachthe subject in each class; and~~

~~27. The method of claim 26, wherein the method further comprises: before step d), (g), obtaining an enriched fragment in a specific chromosome region of the peripheral red blood cell micronuclei DNA of the subject relative to the sequenced reads of the peripheral blood mononuclear cell genome DNA, thereby obtaining a-specific cleavage fragments of the peripheral red blood cell of the subject, which is to be used for comparison in step d)-(g).~~

7. 28. A ~~The method according to any one of claims 1-6, wherein the gene classifier constructed by~~comprises the genes shown in Tables 2, 4, 6, 8 and 10.

4.8. The method of~~according to any one of claims 20-27~~1-7, wherein the cancer is selected from the group consisting of lung cancer, cervical cancer, hepatocellular carcinoma, and colorectal cancer.

9. ~~29.~~ The gene classifier method according to claim 28, which comprises or consists any one of claims 1-8, further comprising determining type and/or stage of cancer by analyzing the genes shown gene classifier.

A method for constructing a gene classifier to be used in Table 2, 4, 6, 8 or 10.

10. ~~30.~~ A system for cancer detection or early diagnosis of a test subject, cancer, the method comprising a comparison means for comparing:
- a) providing a peripheral red blood cell blood sample from healthy subjects and subjects diagnosed with cancer that has been previously obtained from said subjects;
  - b) isolating red blood cells from the peripheral blood sample from each subject;

extracting micronuclei DNA from the test subject with the gene classifier of claim 28.

31. ~~The system of claim 30, further comprising~~  
~~—an isolation means for isolating peripheral red blood cell micronuclei DNA from the test subject;~~  
~~—a sequencing means for sequencing peripheral red blood cell micronuclei DNA from the test subject.~~

32. ~~The system of claim 31, wherein the sequencing is high-throughput sequencing.~~

33. ~~The system according to claim 30, wherein the system performs cancer detection by a method comprising the steps of:~~

- a)c) ~~a) isolating or purifying micronuclei DNA in peripheral~~ a) isolating or purifying micronuclei DNA in peripheral isolated red blood cells of the test subject;
- b)d) ~~b) sequencing the whole genome of the micronuclei DNA to obtain fragment sequence information of the micronuclei DNA in peripheral red blood cells of the test subject;~~
- e) ~~e) comparing the fragment sequence information of the micronuclei DNA obtained from peripheral red blood cells in step b) said healthy subjects and said subjects diagnosed with cancer;~~

- f) classifying the signature DNA fragment set for a specific cancer according to the differences in the distribution of the fragment in said healthy subjects and said subjects diagnosed with cancer;
- e)g) thereby obtaining a gene classifier according to claim 28, thereby classifying the test subjects into one for cancer detection or more of the classesdiagnosis.

11. ~~34.~~ The systemmethod according to claim 33, wherein the method 10, further comprises comprising:

the step of performing whole genome sequencing on the genomic DNA of the peripheral blood mononuclear cells of the testeach subject; and

~~35. The system according to claim 34, wherein the method further comprises, before step e)-(e), obtaining an enriched fragment in a specific chromosome region of the peripheral red blood cell micronuclei DNA of the subject relative to the sequenced reads of the peripheral blood mononuclear cell genome DNA of the subject, thereby obtaining a specific cleavage fragments of the peripheral red blood cell of theeach subject, which is to be used for comparison in step e)-(e).~~

12. ~~36.~~ The system ofmethod according to claim 3010 or 11, wherein in step (f) the signature DNA fragment set for a specific cancer detection comprises screening, diagnosis, typing and/or stagingis classified through hierarchical cluster analysis.

The method according to any one of cancer.

5.13. ~~37.~~ The system of claim 36claims 10-12, wherein the cancer is selected from the group consisting of lung cancer, cervical cancer or, hepatocellular carcinoma, and colorectal cancer.

~~38. Use~~ The method according to any one of an agent for analyzing claims 10-13, wherein the fragment sequence information of micronuclei DNA of peripheral red blood cells in the preparationare further classified for type or stage of a detection device or a detection kit for cancer screening, diagnosing, typing and/or staging.

6.14. \_\_\_\_\_.