

CLAIMS

1. A method of detection or early diagnosis of cancer in a subject in need thereof, the method comprising:
 - a) providing a peripheral blood sample that has been previously obtained from the subject;
 - b) isolating mononuclear cells and red blood cells from the peripheral blood sample;
 - c) collecting red blood cells;
 - d) treating collected blood cells with a red blood cell lysis buffer;
 - e) extracting micronuclei DNA from the lysed red blood cells;
 - f) 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).
2. The method according to claim 1, wherein in step (b) the peripheral blood sample is subjected to density gradient centrifugation to obtain a mononuclear cell layer and a red blood cell layer; and wherein the red blood cells are collected from the bottom of said red blood cell layer, and the collected red blood cells are subjected to two or more sequential filtrations through 10 μ m cell strainers.
3. 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).

5. The method according to any one of claims 1-4, wherein the sequencing is high-throughput sequencing.
6. The method according to any one of claims 1-5, further comprising:
the step of performing whole genome sequencing on the genomic DNA of the peripheral blood mononuclear cells of the subject; and
before step (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 specific cleavage fragments of the peripheral red blood cell of the subject to be used for comparison in step (g).
7. The method according to any one of claims 1-6, wherein the gene classifier comprises the genes shown in Tables 2, 4, 6, 8 and 10.
8. The method according to any one of claims 1-7, wherein the cancer is selected from the group consisting of lung cancer, cervical cancer, hepatocellular carcinoma, and colorectal cancer.
9. The method according to any one of claims 1-8, further comprising determining type and/or stage of cancer by analyzing the gene classifier.
10. A method for constructing a gene classifier to be used in a system for detection or early diagnosis of cancer, the method comprising:
 - a) providing a peripheral 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;
 - c) extracting micronuclei DNA from the isolated red blood cells;
 - d) sequencing the whole genome of the micronuclei DNA to obtain fragment sequence information of the micronuclei DNA;

- e) comparing the fragment sequence information of micronuclei DNA from peripheral red blood cells in 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;
 - g) thereby obtaining a gene classifier for cancer detection or diagnosis.
11. The method according to claim 10, further comprising:
the step of performing whole genome sequencing on the genomic DNA of peripheral blood mononuclear cells of each subject; and
before step (e), obtaining an enriched fragment in a specific chromosome region of the peripheral red blood cell micronuclei DNA of the subject relative to sequenced reads of the peripheral blood mononuclear cell genome DNA of the subject, thereby obtaining specific cleavage fragments of the peripheral red blood cell of each subject to be used for comparison in step (e).
12. The method according to claim 10 or 11, wherein in step (f) the signature DNA fragment set for a specific cancer is classified through hierarchical cluster analysis.
13. The method according to any one of claims 10-12, wherein the cancer is selected from the group consisting of lung cancer, cervical cancer, hepatocellular carcinoma, and colorectal cancer.
14. The method according to any one of claims 10-13, wherein the fragment sequence information of micronuclei DNA are further classified for type or stage of cancer.