

CLAIMS

What is claimed is:

1. A combination of gene markers, characterized in that the genetic markers comprise HOXB4 and SRCIN1.

2. Use of a multi-gene combined methylation detection reagent in preparation of a lung cancer detection reagent or a kit, characterized in that the genes comprise HOXB4 and SRCIN1.

3. A multi-gene combined methylation detection reagent/kit, comprising a methylation detection reagent for HOXB4 and SRCIN1 genes.

4. The use according to claim 2 or the reagent/kit according to claim 3, characterized in that, the methylation detection reagent comprises a primer and/or a probe for methylation detection of each gene,

optionally comprises a primer and/or a probe obtained from CpG islands of each gene, and optionally comprises a primer and/or a probe obtained from CpG islands of a genome, an intergenic region, a promoter region or a region near the promoter region of each gene.

5. The use according to claim 2 or the reagent/kit according to claim 3, characterized in that a forward primer in the primer for methylation detection of the HOXB4 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 1, SEQ ID NO: 16 or SEQ ID NO: 19, or a complementary sequence thereof; and/or

a reverse primer in the primer for methylation detection of the HOXB4 gene comprises a

nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 2, SEQ ID NO: 17 or SEQ ID NO: 20, or a complementary sequence thereof; and/or

a forward primer in the primer for methylation detection of the SRCIN1 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 4 or SEQ ID NO: 22, or a complementary sequence thereof; and/or

a reverse primer in the primer for methylation detection of the SRCIN1 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 5, or a complementary sequence thereof.

6. The use according to claim 2 or 5 or the reagent/kit according to claim 3 or 5, characterized in that

a primer pair for methylation detection of the HOXB4 gene is shown as SEQ ID NO: 1 and SEQ ID NO: 2; and/or

a primer pair for methylation detection of the SRCIN1 gene is shown as SEQ ID NO: 4 and SEQ ID NO: 5.

7. The use according to any one of claim 2 and 5–6 or the reagent/kit according to any one of claims 3 and 5–6, characterized in that

a probe for methylation detection of the HOXB4 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93%

or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 3, SEQ ID NO: 18 or SEQ ID NO: 21, or a complementary sequence thereof; and/or

a probe for methylation detection of the SRCIN1 gene comprise a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 6, or a complementary sequence thereof.

8. The use according to claim 2, characterized in that the lung cancer is selected from small cell lung cancer and non-small cell lung cancer.

9. The use according to claim 8, characterized in that, the non-small cell lung cancer is selected from squamous-cell carcinoma or adenomatous carcinoma.

10. The use according to claim 2, characterized in that a sample to be detected for the detection reagent is selected from at least one of pulmonary alveoli lavage fluid, tissues, hydrothorax, sputum, blood, serum, plasma, urine, prostatic fluid or excrements.

11. The use according to claim 10, characterized in that, the sample is selected from at least one of pulmonary alveoli lavage fluid, tissues or sputum.

12. The use according to claim 10, characterized in that, the sample is selected from at least one of pulmonary alveoli lavage fluid or sputum.

13. A lung cancer detection system, characterized by comprising the following components:

(1) a combined methylation detection component of HOXB4 and SRCIN1 genes;

(2) a data processing component; and

(3) a result output component;

preferably, the methylation detection component comprises a methylation detection instrument;

preferably, the methylation detection instrument comprises one or more of a fluorescent quantitative PCR instrument, a PCR instrument and a sequencer;

preferably, the data processing component comprises a data processing machine;

preferably, the data processing machine comprises one or more of calculators and computers;

preferably, the computers comprise a computer loaded with one or more of software in SPSS, SAS and Excel;

preferably, the result output component comprises a result output unit;

preferably, the result output unit comprises one or more of screens and paper reports;

preferably, the methylation detection component further comprises the multi-gene combined methylation detection reagent of any one of claims 3-5;

preferably, the data processing component is configured to: a. receive test data of sample to be detected and normal control sample; b. store the test data of the sample to be detected and the normal control sample; c. compare the test data of the sample to be detected and the normal control sample of the same type; d. respond to the probability or possibility of the subject suffering from the lung cancer according to comparison results;

preferably, the result output component is used for outputting the probability or possibility of the subject suffering from the lung cancer;

preferably, a criterion of the data processing component is: methylation results of the sample to be detected and the normal sample are compared through the results; and when the methylation of the sample to be detected and the normal sample has significant differences or extremely significant differences, it is determined from the results that, the sample to be detected have high disease risk.

14. The lung cancer detection system according to claim 13, characterized in that the lung cancer is selected from small cell lung cancer and non-small cell lung cancer; and more preferably, the non-small cell lung cancer is selected from squamous-cell carcinoma or adenomatous carcinoma.

15. The lung cancer detection system according to claim 13, characterized in that the sample to be detected for the detection system is selected from at least one of pulmonary alveoli lavage fluid, tissues, hydrothorax, sputum, blood, serum, plasma, urine, prostatic fluid or excrements;

preferably, the sample is selected from at least one of pulmonary alveoli lavage fluid, tissues or sputum;

more preferably, the sample is selected from at least one of pulmonary alveoli lavage fluid or sputum.

16. A lung cancer diagnosis method, characterized by comprising the following steps:

(1) detecting methylation levels of HOXB4 and SRCIN1 genes in a sample to be detected derived from a subject;

(2) comparing methylation levels of HOXB4 and SRCIN1 genes of the sample to be detected and a normal control sample; and

(3) diagnosing the lung cancer based on deviation of the methylation levels of the sample to be detected and the normal control sample;

optionally, the methylation levels of HOXB4 and SRCIN1 genes are detected by using quantitative methylation-specific PCR (qMSP);

optionally, in the step (1), the detection comprises contact between the sample to be detected of the subject and a detection reagent for detecting the methylation levels of the HOXB4 and SRCIN1 genes;

optionally, the methylation results of the sample to be detected and the normal sample are compared through the results; and when the methylation of the sample to be detected and the normal sample has significant differences or extremely significant differences, it is determined from the results that, the sample to be detected have high disease risk.

17. The method of claim 16, characterized in that, the sample to be detected are selected from at least one of pulmonary alveoli lavage fluid, tissues, hydrothorax, sputum, blood, serum, plasma, urine, prostatic fluid or excrements;

optionally, the sample is selected from at least one of pulmonary alveoli lavage fluid, sputum or tissues;

optionally, the sample is selected from at least one of pulmonary alveoli lavage fluid or sputum.

18. The method of claim 16 or 17, characterized in that, the lung cancer is selected from small cell lung cancer and non-small cell lung cancer;

optionally, the non-small cell lung cancer is selected from squamous-cell carcinoma or adenomatous carcinoma.

19. The method of any one of claims 16–18, characterized in that, in the step (1), the methylation levels of HOXB4 and SRCIN1 genes are detected by the multi-gene combined methylation detection reagent, and the combined detection reagent comprises a primer and/or a probe for methylation detection of each gene;

optionally, the combined detection reagent comprises a primer and/or a probe obtained from CpG islands of each gene, and

optionally, the combined detection reagent comprises a primer and/or a probe obtained from CpG islands of a genome, an intergenic region, a promoter region or a region near the promoter

region of each gene.

20. The method of any one of claims 16–19, characterized in that,

a forward primer in the primer for methylation detection of the HOXB4 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 1, SEQ ID NO: 16 or SEQ ID NO: 19, or a complementary sequence thereof; and/or

a reverse primer in the primer for methylation detection of the HOXB4 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 2, SEQ ID NO: 17 or SEQ ID NO: 20, or a complementary sequence thereof; and/or

a forward primer in the primer for methylation detection of the SRCIN1 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 4 or SEQ ID NO: 22, or a complementary sequence thereof; and/or

a reverse primer in the primer for methylation detection of the SRCIN1 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 5, or a complementary sequence thereof; and/or

a probe for methylation detection of the HOXB4 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or

100% to a nucleotide sequences shown as SEQ ID NO: 3, SEQ ID NO: 18 or SEQ ID NO: 21, or a complementary sequence thereof; and/or

a probe for methylation detection of the SRCIN1 gene comprises a nucleotide sequence having an identity of at least 85% or at least 90% or at least 91% or at least 92% or at least 93% or at least 94% or at least 95% or at least 96% or at least 97% or at least 98% or at least 99% or 100% to a nucleotide sequence shown as SEQ ID NO: 6, or a complementary sequence thereof.