

CLAIMS

1. A primer pair, wherein the upstream primer has a nucleotide sequence shown in SEQ ID NO: 2, and the downstream primer has a nucleotide sequence shown in SEQ ID NO: 3.
2. A nucleic acid fragment having a nucleotide sequence shown in SEQ ID NO: 1.
3. A probe having a nucleotide sequence shown in SEQ ID NO: 4.
4. A methylation detection reagent of COL4A1 gene,
comprising a nucleic acid fragment, a primer and/or a probe for detection of methylation of COL4A1 gene,
the nucleic acid fragment has a nucleotide sequence shown in SEQ ID NO: 1,
the upstream primer in the primer has a nucleotide sequence shown in SEQ ID NO: 2, and
the upstream primer in the primer has a nucleotide sequence shown in SEQ ID NO: 3, and
the probe has a nucleotide sequence shown in SEQ ID NO: 4.
5. A kit comprising nucleic acid fragment of claim 2, or the primer pair of claim 1, or the probe of claim 3, or the methylation detection reagent of claim 4.
6. The kit according to claim 5, wherein the kit comprising: a first container comprising the nucleic acid fragment ; a second container comprising the primer pair for amplification; and a third container comprising the probe.
7. Use of the nucleic acid fragment of claim 2, or the primer pair of claim 1, or the probe of claim 3, or the methylation detection reagent of claim 4, in the preparation of a kit in detecting tumors;
the tumor is colorectal tumor.
8. Use of the nucleic acid fragment of claim 2, or the primer pair of claim 1, or the probe of claim 3, or the methylation detection reagent of claim 4, or the kit of claim 5 in detecting tumors;
the tumor is colorectal tumor.
9. The use of claim 7 or claim 8, wherein the tumor is colorectal cancer or adenoma.

10. The use of claim 7 or claim 8, wherein the specimen to be detected is tissue, body fluid or excreta.

11. The use of claim 10, wherein the tissue is intestinal tissue.

12. The use of claim 10, wherein the body fluid is blood, serum, plasma, extracellular fluid, tissue fluid, lymph fluid, cerebrospinal fluid or aqueous humor.

13. The use of claim 10, wherein the excreta is sputum, urine, saliva or feces.

14. A method for determining the risk of a tumor *in vitro*, comprising:

(1) using the nucleic acid fragment of claim 2, or the primer pair of claim 1, or the probe of claim 3, or the methylation detection reagent of claim 4, or the kit of claim 5 to detect the methylation level of COL4A1 gene of a sample obtained from a subject;

(2) comparing the methylation level of COL4A1 gene of the subject with the methylation level of a normal control specimen; and

(3) indicating that the subject has or is at a risk of developing a tumor so as to distinguish a normal specimen from a tumor specimen, according to the increase in the methylation level of COL4A1 gene of the subject compared with the methylation level of the normal control specimen; and

wherein the tumor is colorectal tumor.

15. The method of claim 14, wherein, in step (1), detecting the methylation level of COL4A1 gene of the subject comprising:

step a) of extracting DNA of a specimen to be detected by adopting a magnetic bead capture method;

step b) of converting the DNA of the specimen to be detected by hydrosulphite, bisulfite or hydrazine salt; and

step c) of performing methylation-specific quantitative PCR detection.

16. The method of claim 15, wherein, in the step a), extracting the DNA of the specimen to be detected by adopting a magnetic bead capture method comprising the following steps of:

mixing and grinding a specimen to be detected in a protective liquid, followed by centrifuging, and taking supernatant;

centrifuging the supernatant, taking the supernatant, adding a lysis solution and magnetic beads with specific complementary oligonucleotide nucleotide acid fragments into the supernatant for incubation;

discarding part of the supernatant, washing off the magnetic beads and transferring to a clean centrifuge tube, adding washing liquid, performing incubation under 100-2000 rpm at room temperature for 0.5-5 min, placing on a magnetic frame and pipetting the supernatant, and repeating 3 times; and

eluting the target gene DNA with a buffer.

17. A tumor detection system, comprising:

(1) a methylation detection component of COL4A1 gene;

(2) a data processing component; and

(3) a result output component;

wherein the methylation detection component further comprises the nucleic acid fragment of claim 2, or the primer pair of claim 1, or the probe of claim 3, or the methylation detection reagent of claim 4, or the kit of claim 5; and

the tumor is colorectal tumor.

18. The tumor detection system of claim 17, wherein the methylation detection component comprises one or more of a fluorescence quantitative PCR instrument, a PCR instrument and a sequencer.

19. The tumor detection system of claim 17, wherein the data processing component is configured to

a. receive test data of a specimen to be tested and a normal control specimen;

b. store test data of a specimen to be tested and a normal control specimen;

c. compare test data of a specimen to be tested of the same type and a normal control specimen;

and

d. respond to the probability or possibility that a subject suffers from a tumor according to the comparison results.

20. The tumor detection system of claim 17, wherein the result output component is used for outputting the probability or possibility that a subject suffers from a tumor.

21. The method of any one of claims 14 to 16, wherein the tumor is colorectal tumor.

22. The method of any one of claims 14 to 16, wherein the method detects the specimen type as tissue, body fluid or excreta.

23. The method of claim 22, wherein the tissue is intestinal tissue.

24. The method of claim 22, wherein the body fluid is blood, serum, plasma, extracellular fluid, tissue fluid, lymph fluid, cerebrospinal fluid or aqueous humor.

25. The method of claim 22, wherein the excreta is sputum, urine, saliva or feces.