

URINE MIRNA MARKER, DIAGNOSTIC REAGENT AND KIT FOR DIAGNOSIS OF PROSTATE CANCER

FIELD OF THE INVENTION

The invention relates to the technical field of molecular diagnosis, in particular to a urine
5 miRNA marker, a diagnostic reagent and a kit for diagnosis of prostate cancer.

BACKGROUND OF THE INVENTION

According to data from the National Cancer Center, prostate cancer has been the tumor with
the highest incidence in male urinary system since 2008, with its incidence reaching 9.8 per
100,000 in 2014, ranking sixth in male malignant tumors. The mortality of prostate cancer is 4.22
10 per 100,000. Prostate cancer (PCa) is one of the top ten leading causes of cancer-related death
among men in China. Moreover, prostate cancer in China shows a trend of increasing year by year.
It is noteworthy that there is a large difference in the incidence of prostate cancer between urban
and rural areas in China, especially the incidence is higher in large cities. Worldwide, with respect
to the incidence of prostate cancer, the United States ranks second and the Europe ranks third.

15 Although prostate cancer is a disease with a high incidence, its pathogenesis is currently poorly
understood. Established risk factors are limited to age, family history of malignancy, and certain
genetic mutations (e.g., BRCA1 and BRCA2) and predisposing factors (Lynch syndrome). Also,
the incidence of prostate cancer is related to race. Among different races, the incidence of prostate
cancer in black men is the highest. In addition, the currently identified risk factors of prostate
20 cancer also include lifestyle and environmental factors. For example, smoking, overweight and
nutritional factors may increase the risk of advanced prostate cancer in general.

PCa is a slow-growing tumor, and up to 40% of men diagnosed with PCa do not have any
clinical symptoms at all. However, the patient's five-year survival rate is greater than 99% if PCa
is diagnosed early (i.e., locally), but the patient's survival rate is only about 30% if it is diagnosed
25 in the advanced, high-grade stage of metastasis. Therefore, early diagnostic PCa screening has the
potential to fundamentally reduce the health care burden of this cancer.

Prostate-specific antigen (PSA) is a 34-kDa, single-chain glycoprotein composed of 237 amino

acids, which is produced almost exclusively by prostate epithelial cells. In a statement issued by the U.S. Preventive Services Task Force in 2018, PSA screening is not recommended for people over 70 years old, and for people aged 55-69 years old, regular PSA screening depends on individual situation. PSA-based prostate cancer screening has been widely used for a long time, but the false-positive rate of PSA results is high, which easily leads to over diagnosis and over treatment.

Prostate needle aspiration biopsy is a gold standard test for screening and diagnosis of prostate cancer. However, the major limitations of transrectal or transperineal biopsy of the prostate system are false negativity, missed diagnosis of high-risk prostate cancer, and over-diagnosis. How to improve the positive rate of biopsy while avoiding over-diagnosis is a great challenge in the early diagnosis of prostate cancer. Therefore, it is urgent to develop safer, more efficient and specific detection methods for prostate cancer and/or high-risk prostate cancer.

Many studies have shown that urine contains many different kinds of RNAs, including mRNA, microRNA (miRNA) and other non-coding protein (nc) RNAs. Because it is easier to detect than blood, urine is a particularly attractive source of biomarkers for urinary cancer screening and can capture diseases at an early stage.

miRNA is a short non-coding RNA with a length of 18-25 nucleotides. It can prevent protein expression by decomposing a specific target mRNA or inhibiting its translation, and participates in the regulation of vital activities such as individual development, apoptosis, proliferation and differentiation. It plays a role similar to that of oncogene or anti-oncogene in the occurrence and development of tumors. The expression profiles of miRNAs have significant tissue specificity and specific expression patterns in different tumors. All these characteristics have made miRNAs potentially become a new biological marker for tumor diagnosis and a therapeutic target. Studies have shown that miRNAs can exist in free form in the urine. Recently, many studies have proved that miRNAs in patients' urine can be used as biomarkers for early diagnosis of urinary cancer.

Although some miRNA markers for early diagnosis of prostate cancer have been reported in the existing studies, these markers have more or less limitations in detection. Urine miRNA

biomarkers and biomarker combination markers that can eventually be used for prostate cancer screening remain to be determined, for example:

Patent EP3256602B1 discloses a novel in vitro method for assessing the risk of prostate cancer in a subject, comprising measuring the expression levels of at least two miRNAs from a urine sample of a patient, preferably at least two miRNAs selected from hsa-miR-30c-5p, hsa-miR-222-3p, and hsa-miR-24-3p.

In summary, in view of the fact that there is no clear conclusion in the prior art as to which single miRNA marker or miRNA marker combination can be selected to accurately and effectively predict or diagnose prostate cancer, and which methods among various methods for collecting samples of miRNA markers from urine, plasma or tissue should be selected remains to be determined, therefore, those skilled in the art urgently need to develop a urine miRNA marker combination which is suitable for diagnosis of prostate cancer and has high population specificity.

SUMMARY OF THE INVENTION

The technical problem to be solved by the invention is to provide a urine miRNA marker, a diagnostic reagent and a kit for diagnosis of prostate cancer, which have higher population specificity compared to other miRNA markers reported internationally. The disclosed miRNA diagnostic markers and the combinations thereof are proposed for the first time, are more reliable compared with other miRNA molecular markers, and provide a brand new optional approach for diagnosis of prostate cancer.

In order to solve the above technical problem, the technical solution provided by the invention is specifically as follows:

The invention provides a urine miRNA marker for diagnosis of prostate cancer, which is selected from any one or more of the following 10 miRNAs: hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

In some embodiments, the urine miRNA marker is obtained by screening as follows: removing any one of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-

9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, and then selecting any one or more of the remaining 9 miRNAs.

Specifically, when selecting any one or more of the 9 miRNAs in the above manner, the urine miRNA marker comprises, but is not limited to, the following combinations:

5 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

10 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

In some embodiments, the urine miRNA marker is obtained by screening as follows: removing any two of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, and then selecting any one or more of the remaining 8 miRNAs.

Specifically, when screening any one or more of the 8 miRNAs in the above manner, the urine miRNA marker comprises, but is not limited to, the following combinations:

20 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

25 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-

31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

5 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

10 hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

In some embodiments, the urine miRNA marker is obtained by screening as follows: removing any three of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, and then selecting any one or more of the remaining 7 miRNAs.

Specifically, when screening any one or more of the 7 miRNAs in the above manner, the urine miRNA marker comprises, but is not limited to, the following combinations:

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

20 hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

25 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

5 hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306; or

10 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306.

In some embodiments, the urine miRNA marker is obtained by screening as follows: removing any four of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, and then selecting any one or more of the remaining 6 miRNAs.

Specifically, when screening any one or more of the 6 miRNAs in the above manner, the urine miRNA marker comprises, but is not limited to, the following combinations:

20 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p; or

25 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-

miR-4306; or

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306.

In some preferred embodiments, the urine miRNA marker may be selected from any one or more of the following 6 miRNAs: hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306.

In the invention, the urine miRNA markers that consist of at least two miRNAs selected from the 6 miRNAs including hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, and hsa-miR-4306, and have AUCs higher than that of a single miRNA (such as hsa-miR-199b-5p or hsa-miR-518e-5p), including but not limited to the following combinations, can all be taken as preferred embodiments:

1) Preferably, the urine miRNA marker may be selected from at least 2 of the following miRNAs:

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; and

hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306.

2) Preferably, the urine miRNA marker may be selected from at least 2 of the following miRNAs:

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-31-3p;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-518e-5p, hsa-miR-31-3p;
 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p;
 hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p;
 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-4306;
 5 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-518e-5p, hsa-miR-4306;
 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-4306;
 hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-4306;
 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-31-3p, hsa-miR-4306;
 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-31-3p, hsa-miR-4306;
 10 hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-31-3p, hsa-miR-4306;
 hsa-miR-375, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;
 hsa-miR-520d-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; and
 hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306.

3) Preferably, the urine miRNA marker is obtained by screening as follows: removing any three
 15 of the 6 miRNAs including hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p,
 hsa-miR-31-3p and hsa-miR-4306, and then selecting any two of the remaining 3 miRNAs.

Under these conditions, the inventors found through high-throughput screening that the AUCs
 of all the miRNA combinations are higher than the AUC of hsa-miR-199b-5p alone or hsa-miR-
 518e-5p alone.

4) Preferably, the urine miRNA markers are obtained by screening in the following way:
 removing any four of the 6 miRNAs including hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p,
 hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306 to have 2 miRNAs remained.

Under these conditions, the inventors found through high-throughput screening that the AUCs
 of all the miRNA combination are higher than the AUC of hsa-miR-199b-5p alone or hsa-miR-
 25 518e-5p alone.

5) Preferably, the urine miRNA marker may be selected from one miRNA as follows:
 hsa-miR-199b-5p (AUC=0.565);

hsa-miR-518e-5p (AUC=0.583).

In some preferred embodiments, the invention further provides urine miRNA markers containing specific miRNAs, wherein:

when the urine miRNA marker can be selected from at least 5 of the 10 miRNAs including
5 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, or

when the urine miRNA marker is obtained by screening as follows: removing any one of the
10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306,
10 and then selecting at least 5 of the remaining 9 miRNAs, or

when the urine miRNA marker is obtained by screening as follows: removing any two of the
10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306,
and then selecting at least 5 of the remaining 8 miRNA markers, or

15 when the urine miRNA marker is obtained by screening as follows: removing any three of the
10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306,
and then selecting at least 5 of the remaining 7 miRNAs, or

when the urine miRNA marker is obtained by screening as follows: removing any four of the
20 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306,
and then selecting at least 5 of the remaining 6 miRNAs,

one of the miRNAs must be hsa-miR-199b-5p.

Similarly, when the urine miRNA marker can be selected from at least 5 of the 10 miRNAs
25 including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, or

when the urine miRNA marker is obtained by screening as follows: removing any one of the

10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, and then selecting at least 5 of the remaining 9 miRNAs, or

when the urine miRNA marker is obtained by screening as follows: removing any two of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, and then selecting at least 5 of the remaining 8 miRNAs, or

when the urine miRNA marker is obtained by screening as follows: removing any three of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, and then selecting at least 5 of the remaining 7 miRNAs, or

when the urine miRNA marker is obtained by screening as follows: removing any four of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, and hsa-miR-4306, and then selecting at least 5 of the remaining 6 miRNAs,

one of the miRNAs must be hsa-miR-518e-5p.

In the invention, the urine miRNA markers that consist of at least 5 miRNAs selected from the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306, must comprise one specific miRNA (hsa-miR-199b-5p or hsa-miR-518e-5p), and have AUCs of not less than 0.738, including but not limited to the following combinations, can be taken as preferred embodiments:

1) Preferably, the urine miRNA marker may be selected from at least 5 of the following 10 miRNAs:

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

2) Preferably, the urine miRNA marker may be selected from at least 5 of the following 9

miRNAs:

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

5 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; and

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

10 3) Preferably, the urine miRNA marker may be selected from at least 5 of the following 8 miRNAs:

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

15 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

20 hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

25 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; and

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

5 4) Preferably, the urine miRNA marker may be selected from at least 5 of the following 7 miRNAs:

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

15 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306;

25 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; and

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-

31-3p, hsa-miR-4306.

5) Preferably, the urine miRNA marker may be selected from at least 5 of the following 6 miRNAs:

5 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p;

10 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-4306;

15 hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; and

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306.

20 6) Preferably, the urine miRNA marker may be selected from the following 5 miRNAs:

hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306.

The following urine miRNA markers that have AUCs ≥ 0.738 and consist of 5-10 miRNAs have the common characteristic that they all comprise at least hsa-miR-199b-5p and hsa-miR-518e-5p.

25 In some preferred embodiments, the urine miRNA marker may consist of 10 miRNAs as follows:

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

In some preferred embodiments, the urine miRNA marker may consist of 9 miRNAs as follows:

5 hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-10 518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; and

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

In some preferred embodiments, the urine miRNA marker may consist of 8 miRNAs as follows:

15 hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-20 518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

25 hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-

miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; and

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

In some preferred embodiments, the urine miRNA marker may consist of 7 miRNAs as follows:

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; and

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306.

5 In some preferred embodiments, the urine miRNA marker may consist of 6 miRNAs as follows:

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306;

hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-
10 miR-4306;

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-4306;

15 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p;

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-
20 miR-4306; and

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306.

In some preferred embodiments, the urine miRNA marker may consist of 5 miRNAs as follows: hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, and hsa-miR-
25 4306.

In addition, the invention further provides a diagnostic reagent for prostate cancer, which

comprises a reagent for specifically detecting the aforementioned urine miRNA markers.

In addition, the invention further provides a kit for diagnosis of prostate cancer, which comprises the aforementioned urine miRNA markers as a standard and corresponding primers for detecting the aforementioned urine miRNA markers.

5 In addition, the invention further provides use of the aforementioned urine miRNA markers in preparing a product for early diagnosis and/or recurrence monitoring.

The product includes, but is not limited to, devices (e.g., oligonucleotide probes or assemblies thereof, high-throughput miRNA detection chips on chip substrates or detection substrates, and microfluidic detection chips), kits, and equipment.

10 In some preferred embodiments, the prepared product for early diagnosis and/or recurrence monitoring has one or more of the following functions:

- 1) identification or auxiliary identification of prostate cancer;
- 2) diagnosis or auxiliary diagnosis of primary prostate cancer; and
- 3) diagnosis or auxiliary diagnosis of recurrent prostate cancer.

15 In addition, the invention also provides a method for early diagnosis and/or recurrence monitoring of prostate cancer by using the aforementioned urine miRNA markers, comprising the following steps:

step 1, detecting the presence of miRNAs in a urine sample obtained from a subject;

20 step 2, measuring the expression level of at least one miRNA in the miRNA markers in the urine sample; and

step 3, comparing the scores based on the previously measured expression level of miRNAs with a reference to determine whether there is a significant difference between the subject sample and the reference sample for early diagnosis and/or recurrence monitoring of the possibility of prostate cancer in the subject.

25 Further provided herein is a method of determining whether a subject has prostate cancer or determining whether a subject is at risk for prostate cancer, comprising detecting the expression level of at least one miRNA in a urine sample obtained from the subject,

wherein the at least one miRNA is selected from hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p and hsa-miR-4306; and wherein the differential expression level of the miRNA compared to the control indicates that the subject has or is at risk for prostate cancer.

5 In some embodiments, in the method as described, the at least one miRNA comprises the combinations of urine miRNA markers as described above.

In some embodiments, the expression level of the miRNA is up-regulated or down-regulated in a subject having or at risk for prostate cancer compared to a control. In some embodiments, the control is selected from subjects without cancer, subjects without prostate cancer, subjects without
10 or not at risk for prostate cancer.

In some embodiments, the method provided herein further comprises performing a clinical procedure and/or measuring additional biomarkers in a biological sample (i.e., urine, tissue blood, plasma, serum samples). Other biomarkers may include, but are not limited to, for example, prostate specific antigen (PSA), prostate cancer antigen 3 (PSA3), and other cell cycle and
15 proliferation markers. Clinical procedures may include biopsy, fine needle aspiration, prostate needle aspiration biopsy, and diagnostic imaging tests, including but not limited to ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET) imaging.

In some embodiments, the present disclosure provides a method for treating a subject diagnosed
20 with prostate cancer using the method disclosed herein. A subject diagnosed with prostate cancer will be subjected to referral to a medical practitioner and, where determined to be appropriate, treated with a suitable anticancer compound, surgery, targeted therapy, immunotherapy or radiation therapy, or a combination of these therapies.

Compared with the prior art, the invention has the following beneficial effects:

25 1. The invention found a plurality of miRNAs capable of detecting prostate cancer, such as hsa-miR-9-5p, hsa-miR-199b-5p, and hsa-miR-518e-5p for the first time, and no existing literature or patents have reported that these miRNAs are capable of detecting prostate cancer.

2. According to the invention, 18 miRNA markers which can be used for detecting prostate cancer are initially found from urine samples of subjects with cancer and healthy control subjects, and creative discovery made after further research is that when the urine miRNA markers are selected from one or more of the following 10 miRNAs, prostate cancer can be detected with significant difference ($p < 0.05$): hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306.

3. The invention also surprisingly found that as long as the urine miRNA marker is composed of at least 2 of the 6 miRNAs including hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306, its AUC will be higher than that of a single miRNA (such as hsa-miR-199b-5p and hsa-miR-518e-5p).

4. Based on the study that the AUC of the urine miRNA marker composed of 10 miRNAs (hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p and hsa-miR-4306) is 0.738, the invention accidentally found urine miRNA markers with $AUC \geq 0.738$ through optimization and screening, and the common characteristic of these urine miRNA markers is that the urine miRNA markers are selected from at least 5 of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306 and must comprise one specific miRNA (hsa-miR-199b-5p or hsa-miR-518e-5p). In particular, hsa-miR-518e-5p is capable of up-regulation and hsa-miR-199b-5p is capable of down-regulation.

Therefore, the invention tried to take hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306 as novel prostate cancer marker combinations and carried out clinical sample collection and validation investigation based on this. The results show that the marker combination is of great value in early detection and diagnosis of prostate cancer.

In conclusion, the invention provides a plurality of urine miRNA markers in combination. The

invention features simple and convenient detection procedure, strong feasibility, high sensitivity and specificity and is of significance in clinical diagnosis and guiding. Moreover, the invention can detect the miRNA expression level through urine, thereby achieving the non-invasive purpose.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a heat map showing the expression levels of 10 differentially expressed ($P < 0.05$) miRNA diagnostic markers for prostate cancer. Expression levels (copies/mL) of miRNAs are presented on a log2 scale and normalized relative to the zero mean. The color of dots indicates the concentration. Hierarchical clustering is performed for two dimensions (miRNA and sample) based on the Euclidean distance. For the horizontal dimension, case-control subjects are indicated by colors.

Fig. 2 shows the AUCs of the prostate cancer miRNA diagnostic marker combinations in the cohorts; where Fig. 2-A shows the AUC of the combinations of 10 miRNA diagnostic markers for prostate cancer in the cohorts, and Fig. 2-B shows the AUC of the combinations of 6 miRNA diagnostic markers for prostate cancer in the cohorts.

Fig. 3 is a box plot of the combinations of 6 miRNA diagnostic markers for prostate cancer in control and cancer predictive values.

DETAILED DESCRIPTION OF THE INVENTION

The technical solution of the invention is further specifically described below through specific examples.

In the invention, unless otherwise specified, the raw materials and equipment used are commercially available or commonly used in the art. The methods in the following examples, unless otherwise specified, are conventional methods in the art.

Definitions

The term "miRNA" refers to microRNAs, i.e., small non-coding RNA molecules that in some example comprise about 22 nucleotides and are present in plants, animals, and some viruses. miRNAs are known to function in RNA silencing and post-transcriptional regulation of gene expression. These highly conserved RNAs regulate gene expression through binding to the 3'-

untranslated region (3'-UTR) of a specific mRNA. For example, each miRNA is thought to regulate multiple genes, and hundreds of miRNA genes are predicted to exist in higher eukaryotes. miRNAs tend to be transcribed from several different loci in the genome. These genes encode long RNAs with hairpin structures that, when processed by a series of RNase III enzymes including Drosha and Dicer, form miRNA duplexes of typically about 22 nucleotides in length with two nucleotide overhangs at the 3' end.

The term "regulation" refers to the process in which a cell increases or decreases the amount of a cellular component such as RNA or protein in response to an external variable. Increase of a cell component is referred to as up-regulation, while decrease of a cell component is referred to as down-regulation. The term "deregulation" or "mis-regulation" as used herein means up- or down-regulation. An example of down-regulation is a decrease in the number of receptors for molecules such as hormones or neurotransmitters in a cell, which reduces the sensitivity of the cell to the molecules. This phenomenon is an example of the negative feedback mechanism of local action. An example of up-regulation is an increase in the number of cytochrome P450 enzymes in hepatocytes when heterotypic biomass molecules such as dioxene are administered, causing greater degradation of these molecules. Up-regulation and down-regulation may also occur in response to a toxin or hormone. An example of up-regulation during pregnancy is a hormone that causes cells in the uterus to become more sensitive to oxytocin.

The term "differential expression" refers to measuring a cellular component for comparison to a control or another sample to determine, for example, a difference in concentration, presence, or intensity of the cellular component. The result of such a comparison may be given as an absolute value, i.e., the component is present in the sample and not in the control; or as a relative value, i.e., an increase or decrease in the expression or concentration of the component as compared to the control. In this case, the terms "increase" and "up-regulation" are the same and interchangeable, and the terms "decrease" and "down-regulation" are the same and interchangeable.

The term "miRNA combination" relates to combinations of miRNAs of the invention. The amount of miRNAs may be determined in a sample of a subject by techniques well known in the

art. Depending on the nature of the sample, the amount may be determined by PCR-based techniques for quantifying the amount of polynucleotide or by other methods (e.g., mass spectrometry or sequencing).

The term "probe" refers to a single-stranded oligonucleotide commonly used to detect a target RNA and/or RNA sequence complementary to the sequence of the probe. The probe and the nucleotide sequence allow hybridization of single-stranded nucleic acids (DNAs or RNAs) with paired nucleotides because of the complementarity between the probe and the target sequence. The length of the probe depends on the intended use and the desired probe specificity. Typically, the length of the probe is 20 to 500 (i.e., 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500) nucleotides, preferably 20 to 100, more preferably 20 to 50 nucleotides. For the detection of microRNAs, the probe consists of 12 to 30 nucleotides. The probes are used in a variety of experimental settings such as, but not limited to, Southern and Northern blotting, real-time PCR and in situ hybridization (ISH), and microarray experiments. The probe may be unlabeled, directly labeled or indirectly labeled, e.g., with biotin to which a streptavidin complex may subsequently bind. The label may be a molecule detectable by spectroscopic, photochemical, biochemical, immunochemical, chemical or other physical means. For example, suitable labels include ^{32}P , fluorescent dyes, electron dense reagents, enzymes (e.g., commonly used in ELISA), biotin, digoxigenin, or haptens, and other entities that is detectable or that can become detectable. The label may be introduced at any location in the nucleic acid, such as the 3' end, the 5' end, or internally. The term "probe" also encompasses nucleic acids having different backbone compositions, such as, but not limited to, peptide nucleic acids (PNAs), locked nucleic acids (LNAs), diol nucleic acids (GNAs), and threose nucleic acids (TNAs).

The terms "indicator" and "marker" are used interchangeably in the invention and refer to signs or signals of a disorder or for monitoring a disorder. Such a "disorder" refers to a biological state of a cell, tissue, or organ, or to a healthy and/or diseased state of an individual. The indicator may be the presence or absence of molecules including, but not limited to, peptides, proteins, and nucleic acids, or may be a change in expression level or pattern of such molecules in a cell, or

tissue, organ, or individual. The indicator may be a sign of the occurrence, development or presence of a disease or further progression of such a disease in an individual. The indicator may also be a sign of the risk of a disease occurring in an individual.

5 The term "score" refers to an integer or number that may be determined mathematically, such as by using a computational model known in the art (which may include, but is not limited to, SMV as an example), and calculated using any of a number of mathematical equations and/or algorithms known in the art for statistical classification purposes. Such a score is used to enumerate one result over a range of possible results. The relevance and statistical significance of such scores depends on the size and quality of the underlying data set used to establish the result range. For example, 10 blind samples may be input into an algorithm to calculate a score based on information provided by analysis of the blind samples. This results in a score for the blind sample. Based on the score, a decision can be made, such as how likely it is that the patient who produces the blind sample has cancer or does not have cancer. The end values of the ranges may be logically defined based on the data provided or may be arbitrarily defined as desired by the experimenter. In both cases, the 15 range needs to be defined before the blind samples are tested.

The term "expression level" refers to the amount of a gene product present in vivo or in a sample at a particular time point. The expression level may be measured/quantified/detected, for example, by proteins or mRNAs expressed by the gene. The expression level may be quantified, for example, by normalizing the amount of the gene product of interest present in the sample with 20 the total amount (total proteins or mRNAs) of the gene products of the same type in the same sample or reference sample (e.g., a sample obtained from the same individual at the same time or portions of the same size (weight, volume) of the same sample, or determining the amount of the gene product of interest/defined sample size (weight, volume, etc.). The expression level may be measured or detected by any method known in the art, such as a method for direct detection and 25 quantification of a gene product of interest (e.g., mass spectrometry), or a method for indirect detection and measurement of a gene product of interest, typically operating by binding of the gene product of interest to one or more different molecules or detection devices specific for the gene

product of interest (e.g., primers, probes, antibodies, protein scaffolds). It is also known to the skilled artisan to determine the level of gene copies, which further comprises determining the absence or presence of one or more fragments (e.g., by nucleic acid probes or primers, e.g., quantitative PCR, multiplex ligation-dependent probe amplification PCR).

5 The detection method for prostate cancer comprises the following steps:

a) determining expression levels of hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306 in a test sample and a reference sample of human origin;

b) comparing the expression levels of hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-
10 miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306 in the test sample with those in the reference sample; wherein the expression level of any one of the above miRNAs in the test sample is significantly different compared with the expression level of the corresponding miRNA in the reference sample, indicating that the individual may have prostate cancer.

Specifically, when the expression level of any one of the miRNAs in prostate cancer to be
15 tested is significantly different from that in the reference sample, the sample provider may have prostate cancer.

In a preferred embodiment, when the expression levels of any 10 miRNAs in the test urine sample are significantly different from the expression levels of miRNAs in the reference sample, the sample provider may have prostate cancer.

20 In a preferred embodiment, when the expression levels of 6 miRNAs in the test urine sample are significantly different from the expression levels of the miRNAs in the reference sample, the sample provider may have prostate cancer.

In the invention, the reference sample is a urine sample from a healthy human.

In another aspect, the invention further provides a method for detecting prostate cancer
25 microRNAs. Specifically, the method comprises reverse transcription and real-time fluorescent quantitative polymerase chain reaction (RT-qPCR). More specifically, in the real-time fluorescent quantitative polymerase chain reaction, primer combinations used are reverse transcription primers

and PCR primer combinations for detecting hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, and hsa-miR-4306.

The primer sequences of the invention can be appropriately adjusted and modified according to the sequences of related miRNAs, and the modified primer sequences can still be used for detecting the miRNA markers. The invention also includes these equivalent technical solutions.

Compared with a chip based on hundreds of molecules, the invention uses the RT-qPCR method, which has more reliable clinical feasibility and practicability, is low in cost, and is easier to popularize. In addition, the method for detecting miRNAs as described above is not limited to the RT-qPCR method provided by the invention, and other methods such as sequencing, microarray, RNA imprinting, bioluminescence, and probe method can also be used for detecting the miRNA markers.

The applicant has found in the research that by using combinations of any one miRNA marker that can be used to diagnose prostate cancer and combinations of any 2-5 miRNA markers that can be used to diagnose prostate cancer, prostate cancer can be reliably identified using the above markers.

From 18 miRNA markers capable of detecting prostate cancer, the invention found through further study that 10 of these miRNA markers can detect prostate cancer with significant difference ($p < 0.05$): hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p and hsa-miR-4306. In addition, through further optimization and screening, combinations with AUCs higher than those of the 10 miRNA markers are obtained, namely combinations of prostate cancer markers including the combination of hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306. hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306 are composed of multiple nucleic acid molecules, and each nucleic acid molecule encodes at least one miRNA sequence. The specific sequence information is shown in Table 1 below.

All miRNA sequences disclosed by the invention have been stored in the miRBase database

(<http://www.mirbase.org/>).

Table 1. Sequence Listing of 10 miRNAs

miRNA	Accession number	Sequence (5'→3')
hsa-miR-375	MIMAT0037313	gcgacgagccccucgcacaaacc (SEQ ID No.1)
hsa-miR-520d-5p	MIMAT0002855	cuacaaagggaagcccuuc (SEQ ID No.2)
hsa-miR-199b-5p	MIMAT0000263	cccaguguuuagacuaucuguuc (SEQ ID No.3)
hsa-miR-518e-5p	MIMAT0005450	cucuagagggaagcgcuucug (SEQ ID No.4)
hsa-miR-31-3p	MIMAT0004504	ugcuauGCCAACAUAUUGCCAU (SEQ ID No.5)
hsa-miR-4306	MIMAT0016858	uggagagaaaggcagua (SEQ ID No.6)
hsa-miR-183-5p	MIMAT0000261	uauggcacugguagaauucacu (SEQ ID No.7)
hsa-miR-9-5p	MIMAT0000441	ucuuugguuauacuagcuguauga (SEQ ID No.8)
hsa-miR-338-3p	MIMAT0000763	uccagcaucagugauuuuguug (SEQ ID No.9)
hsa-miR-338-5p	MIMAT0004701	aacaauauccuggugcugagug (SEQ ID No.10)

The invention discloses a prostate cancer diagnostic marker combination, which comprises multiple nucleic acid molecules encoding hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306. The expression of any one or any two or any three or any four or any five or any six nucleic acid molecules encoding hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, and hsa-miR-4306 in urine of a patient diagnosed with prostate cancer is significantly different compared to the expression in the control. In an optimal embodiment for diagnosis of prostate cancer, the above multiple nucleic acid molecules comprise combinations of six nucleic acid molecules encoding hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, and hsa-miR-4306.

The miRNA and methods disclosed herein are used to diagnose prostate cancer. Thus, as a result of the determination based on the method provided herein, a subject who has been diagnosed with prostate cancer using the method described herein may receive treatment with a necessary and related drug (e.g., chemotherapeutic agent) or be scheduled with a necessary treatment regimen such as radiation therapy, surgical resection, targeted therapy, and immunotherapy due to the diagnosis. Therefore, the disclosed methods enable the treatment of a subject diagnosed with prostate cancer with compounds and compositions known in the art to be effective for the treatment of prostate cancer. Thus, in an embodiment, a subject is diagnosed with prostate cancer through the method as disclosed herein, the subject is then administered a treatment for prostate cancer as known in the art. The method as disclosed herein thus enables the treatment of prostate cancer.

The invention illustratively described herein may be suitably practiced in the absence of any one or more elements, and one or more limitations not specifically disclosed herein. Thus, for example, the terms "comprising," "including," "containing," and the like should be interpreted broadly without limitation. Furthermore, the terms and expressions used herein have been used as terms of description and not of limitation, and it is not intended to use such terms and expressions excluding any equivalents of the features shown and described or portions thereof, but it is to be recognized that various modifications are possible within the claimed scope of the invention. Thus, it is to be understood that while the invention has been specifically disclosed with respect to preferred embodiments and optional features, modifications and variations of the invention disclosed and embodied herein may be resorted to by those skilled in the art, and such modifications and variations are considered to fall in the scope of the invention.

Example 1: Validation of combinations of 10 miRNAs for diagnosis of prostate cancer using validation cohort

I. Urine sample requirements, collection and extraction in the research and development cohort of prostate cancer diagnostic marker combinations

In the study of prostate cancer diagnostic markers, 2 groups of samples were used, including a case group comprising 83 prostate cancer samples and a control group comprising 88 healthy human urine samples. Biomarkers and biomarker combinations for the detection of early-stage prostate cancer were found and validated. Prostate cancer cases and healthy samples in the research and development cohort were all from the Second Affiliated Hospital of Zhejiang University School of Medicine.

II. Procedure and results of reverse transcription - real-time fluorescent PCR

Extraction was performed according to the operation steps of the Zymo serum plasma cfDNA rapid extraction kit. After extraction, reverse transcription was performed according to the operation steps of the MIRXE reverse transcription kit. Quality inspection was performed by qPCR amplification after reverse transcription.

After the quality inspection was completed, the sample was subjected to qPCR amplification,

the expression levels of 312 miRNAs were detected, the expression levels of all the 312 miRNAs in the cancer group and the healthy control group were not significantly graded, and 18 miRNA markers which can be used for detecting prostate cancer were initially found. 10 miRNA biomarkers for detecting prostate cancer with significant difference were found through further research, and p values of the 10 miRNAs were found to be less than 0.05 after correction; 4 of these were up-regulated and 6 were down-regulated in subjects with prostate cancer.

Up-regulated miRNAs include: hsa-miR-183-5p, hsa-miR-520d-5p, hsa-miR-518e-5p and hsa-miR-31-3p; and

Down-regulated miRNAs include: hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-338-5p and hsa-miR-4306.

In the research and development cohort, the 10 miRNAs were extracted to plot a heat map, and significant difference in miRNA expression levels between the subjects with cancer and the control subjects was observed, as shown in Fig. 1.

III. Validation of the above 10 miRNAs using validation cohort

In the case-control cohort, the 10 urine miRNA biomarkers were tested using leave-one-out cross-validation. The validation cohort consisted of 171 samples and the diagnostic efficacy AUC was 0.738, as shown in Fig. 2-A.

Example 2: Validation of combinations of optimal 6 miRNAs for diagnosis of prostate cancer using validation cohort

The operation process and results of urine sample requirements, collection and extraction, reverse transcription real-time fluorescent PCR of the research and development cohort were the same as those describe in Example 1. Based on the previously screened 10 miRNAs, the optimization of miRNA combinations was carried out by using algorithms such as Lasso, Stepwise and exhaust algorithm respectively, and the combinations of 6 optimal miRNAs were finally determined. The combinations of the 6 urine biomarkers, including hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306 were further validated using leave-one-out cross-validation. The validation cohort consisted of 171 samples, and the

diagnostic efficacy AUC of the combinations of the 6 miRNAs in the validation cohort is 0.75, as shown in Fig. 2-B.

The results of model interpretation are shown in Fig. 3. It can be seen that the model output probability was significantly higher for cancer patients than for control subjects, indicating that the model can realize predictive classification of cancer patients and control subjects well.

Based on the above comparison, it is confirmed that the combination of the 6 miRNAs is one of the preferred combinations for diagnosis of prostate cancer.

Example 3: Optimization of miRNA combinations

In this example, the AUC of multivariate miRNA combinations was detected by leave-one-out cross-validation, and the detection method was the same as that in Example 1.

1. Calculating AUC of multi-miRNA panel in validation cohort

For the 10 miRNAs (hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306, hsa-miR-183-5p, hsa-miR-9-5p, hsa-miR-338-3p, hsa-miR-338-5p) used in the clinical trial, any one of the miRNAs was selected as a non-variable, and then based on the non-variable miRNA, the mean AUCs of the multivariate combinations of 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9- or 10-miRNAs were detected, the difference of the AUCs was analyzed, and the results are shown in Table 2.

The mean AUC from training and testing was calculated for each miRNA based on the results of the cross-validation experiment involving 2 to 10 miRNAs (Table 2). When the 810 miRNAs we identified were used alone, subjects with or at risk for developing prostate cancer could be identified. Combining two or more of these miRNAs further increased AUC.

As shown in Table 2, when three or more miRNAs were combined, the AUCs of most multivariate miRNA combinations could reach high levels.

Table 2. Mean AUC for either miRNA alone or combinations of up to 10 miRNAs

	Mean AUC									
	The number of miRNAs combined in the panel									
	1	2	3	4	5	6	7	8	9	10
hsa-miR-375	0.601	0.649	0.678	0.696	0.709	0.719	0.725	0.730	0.735	0.738

	Mean AUC									
	The number of miRNAs combined in the panel									
	1	2	3	4	5	6	7	8	9	10
hsa-miR-520d-5p	0.594	0.618	0.646	0.668	0.686	0.701	0.713	0.723	0.732	0.738
hsa-miR-4306	0.593	0.631	0.655	0.676	0.693	0.706	0.718	0.726	0.733	0.738
hsa-miR-9-5p	0.586	0.617	0.644	0.666	0.683	0.698	0.711	0.721	0.731	0.738
hsa-miR-338-3p	0.585	0.624	0.648	0.669	0.685	0.699	0.711	0.722	0.731	0.738
hsa-miR-518e-5p	0.584	0.637	0.665	0.685	0.700	0.711	0.720	0.727	0.733	0.738
hsa-miR-183-5p	0.573	0.617	0.647	0.669	0.687	0.701	0.713	0.723	0.732	0.738
hsa-miR-199b-5p	0.565	0.610	0.642	0.666	0.686	0.701	0.714	0.724	0.732	0.738
hsa-miR-31-3p	0.556	0.617	0.653	0.677	0.695	0.708	0.718	0.726	0.733	0.738
hsa-miR-338-5p	0.460	0.606	0.641	0.665	0.684	0.700	0.713	0.722	0.731	0.738

2. Combinations comprising miRNAs for diagnosis of prostate cancer

To further validate the effectiveness of combinations of miRNAs for diagnosis of prostate cancer, exemplary combinations comprising the above miRNAs listed in Table 2 were analyzed. The mean AUC, mean sensitivity, mean specificity, mean positive predictive value (PPV), and mean negative predictive value (NPV), respectively, in the validation cohort are as shown in Table 3. The exemplary combinations comprising 1-10 miRNA features can be used for predictive classification of cancer patients and control subjects, with their mean AUCs being 0.570 to 0.738.

Table 3. Mean AUC, sensitivity, specificity, positive predictive value and negative predictive value for combinations of 1 to 10 features

Number of features	Mean AUC	Mean sensitivity	Mean specificity	Mean positive predictive value	Mean negative predictive value
1 feature	0.570	0.575	0.605	0.589	0.613
2 features	0.623	0.610	0.636	0.621	0.639
3 features	0.652	0.627	0.663	0.646	0.660
4 features	0.674	0.641	0.681	0.661	0.673
5 features	0.691	0.663	0.682	0.667	0.687
6 features	0.704	0.686	0.677	0.672	0.701
7 features	0.716	0.698	0.679	0.676	0.709
8 features	0.724	0.712	0.678	0.679	0.717
9 features	0.732	0.714	0.690	0.687	0.721
10 features	0.738	0.663	0.739	0.705	0.699

3. Selecting miRNAs with optimal performance from the study cohort, and then adding other miRNAs to improve the AUC of the combination

As an example, these miRNAs performed well in both testing and validation cohorts. One or more of these miRNAs were then selected and combined with other miRNAs. The exemplary combination with hsa-miR-199b-5p as the fixed miRNA had significantly increased AUC after further increasing the number of miRNAs (other miRNAs listed in Table 2) (Table 4). However, AUC began to decrease slightly with the addition of more than 6 miRNAs. Exemplary combinations with hsa-miR-518e-5p and hsa-miR-9-5p are shown in Tables 5 and 6, respectively. AUC increased with the increasing number of miRNAs in the combination.

Taking hsa-miR-199b-5p as an example, hsa-miR-199b-5p alone, and multivariate miRNA combinations consisting of any one, two, three, four, five, six, seven, eight or nine miRNAs selected from the remaining 9 miRNAs and hsa-miR-199b-5p were compared, mean AUC was detected, and difference in AUCs was analyzed. The results are shown in Table 4.

Table 4. Analysis of difference in parameters of interest for combinations of hsa-miR-199b-5p with the above miRNA features

Mean AUC, sensitivity, specificity, positive predictive value and negative predictive value

Feature	Mean AUC	Mean sensitivity	Mean specificity	Mean positive predictive value	Mean negative predictive value
hsa-miR-199b-5p only	0.565	0.398	0.784	0.635	0.580
+ 1 feature	0.610	0.584	0.643	0.609	0.624
+ 2 features	0.642	0.632	0.650	0.638	0.659
+ 3 features	0.666	0.638	0.678	0.658	0.670
+ 4 features	0.686	0.662	0.676	0.663	0.685
+ 5 features	0.701	0.685	0.674	0.669	0.700
+ 6 features	0.714	0.693	0.681	0.676	0.706
+ 7 features	0.724	0.710	0.679	0.678	0.716
+ 8 features	0.732	0.710	0.698	0.691	0.720
+ 9 features	0.738	0.663	0.739	0.705	0.699

Taking hsa-miR-518e-5p as an example, hsa-miR-518e-5p alone, and multivariate miRNA combinations consisting of any one, two, three, four, five, six, seven, eight or nine miRNAs selected from the remaining nine miRNAs and hsa-miR-518e-5p were compared, mean AUC was detected, and difference in AUCs was analyzed. The results are shown in Table 5.

Table 5. Analysis of difference in parameters of interest for combinations of hsa-miR-518e-5p with the above miRNA features

Mean AUC, sensitivity, specificity, positive predictive value and negative predictive value

Feature	Mean AUC	Mean sensitivity	Mean specificity	Mean positive predictive value	Mean negative predictive value
hsa-miR-518e-5p only	0.584	0.892	0.318	0.552	0.757
+ 1 feature	0.637	0.668	0.593	0.613	0.661
+ 2 features	0.665	0.662	0.635	0.639	0.674
+ 3 features	0.685	0.664	0.666	0.659	0.683
+ 4 features	0.700	0.679	0.675	0.669	0.696
+ 5 features	0.711	0.696	0.673	0.672	0.707
+ 6 features	0.720	0.704	0.678	0.677	0.712
+ 7 features	0.727	0.719	0.674	0.678	0.721
+ 8 features	0.733	0.718	0.687	0.686	0.722
+ 9 features	0.738	0.663	0.739	0.705	0.699

Taking hsa-miR-9-5p as an example, hsa-miR-9-5p alone and multivariate miRNA

5 combinations consisting of any one, two, three, four, five, six, seven, eight or nine miRNAs selected from the remaining 9 miRNAs and hsa-miR-9-5p were compared, mean AUC was detected, and difference in AUCs was analyzed. The results are shown in Table 6.

Table 6. Analysis of difference in parameters of interest for combinations of hsa-miR-9-5p with the above miRNA features

10 Mean AUC, sensitivity, specificity, positive predictive value and negative predictive value

Feature	Mean AUC	Mean sensitivity	Mean specificity	Mean positive predictive value	Mean negative predictive value
hsa-miR-9-5p only	0.586	0.711	0.511	0.578	0.652
+ 1 feature	0.617	0.620	0.619	0.612	0.635
+ 2 features	0.644	0.581	0.698	0.653	0.642
+ 3 features	0.666	0.624	0.690	0.661	0.665
+ 4 features	0.683	0.653	0.684	0.666	0.681
+ 5 features	0.698	0.680	0.676	0.669	0.698
+ 6 features	0.711	0.695	0.674	0.672	0.706
+ 7 features	0.721	0.713	0.673	0.675	0.717
+ 8 features	0.731	0.712	0.688	0.685	0.719

+ 9 features	0.738	0.663	0.739	0.705	0.699
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In order to better explain that the various miRNA combinations provided by the invention have good AUCs, the invention lists several miRNA combinations with $AUC \geq 0.738$ as shown in Table 7, and their common characteristic is that both hsa-miR-199b-5p and hsa-miR-518e-5p are in the miRNA combinations as shown in No.1 to No.35, but it does not mean that the range of combinations of miRNAs to be protected by the invention is only limited to Table 7.

Table 7

No.	Features	AUC
1	hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.738
2	hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.740
3	hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.741
4	hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.745
5	hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.746
6	hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.738
7	hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.740
8	hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.741
9	hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.742
10	hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.742
11	hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.742
12	hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.744
13	hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.746
14	hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.751
15	hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.752
16	hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.738
17	hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p,	0.738

	hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	
18	hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306	0.738
19	hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306 ;	0.743
20	hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-35p, hsa-miR-4306 ;	0.743
21	hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.744
22	hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.746
23	hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.747
24	hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.749
25	hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.750
26	hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306	0.751
27	hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306	0.738
28	hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306	0.741
29	hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.741
30	hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-4306	0.741
31	hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.741
32	hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p	0.742
33	hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.747
34	hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.750
35	hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306	0.744

The invention establishes a complete working flow for detecting and validating urine miRNA biomarker combinations and successfully determines the biomarkers and the biomarker combinations for detecting prostate cancer.

It should be noted that the urine miRNA marker combinations provided by the invention are obtained by performing statistics, screening and analysis based on clinical trial data of human urine samples, so that they are miRNA combinations suitable for high-population-specific detection with AUC significant difference; but it does not mean that such miRNA combinations can be used

universally for the detection of plasma or tissue samples of human origin.

The above-described examples are only preferred solutions of the invention and are not intended to limit the invention in any way, and other variations and modifications are possible without departing from the technical solution as set forth in the claims.

SEQUENCE LISTING

<110> MIRXES (Hangzhou) Biotechnology Co., Ltd.; MIRXES Lab Pte. Ltd.

<120> Urine miRNA marker, diagnostic reagent and kit for diagnosis of prostate cancer

<130> 2021.12

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CLAIMS

1. A urine miRNA marker for diagnosis of prostate cancer, wherein the miRNA marker is selected from one or more of the following 10 miRNAs: hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p and hsa-miR-4306.

2. The urine miRNA marker for diagnosis of prostate cancer according to claim 1, wherein the miRNA marker is obtained by screening as follows: removing any one of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p and hsa-miR-4306, and then selecting any one or more of the remaining 9 miRNAs.

3. The urine miRNA marker for diagnosis of prostate cancer according to claim 1, wherein the miRNA marker is obtained by screening as follows: removing any two of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p and hsa-miR-4306, and then selecting any one or more of the remaining 8 miRNAs.

4. The urine miRNA marker for diagnosis of prostate cancer according to claim 1, wherein the miRNA marker is obtained by screening as follows: removing any three of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p and hsa-miR-4306, and then selecting any one or more of the remaining 7 miRNAs.

5. The urine miRNA marker for diagnosis of prostate cancer according to claim 1, wherein the miRNA marker is obtained by screening as follows: removing any four of the 10 miRNAs including hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p and hsa-miR-4306, and then selecting any one or more of the remaining 6 miRNAs.

6. The urine miRNA marker for diagnosis of prostate cancer according to claim 5, wherein the miRNA marker is selected from one or more of the following 6 miRNAs: hsa-miR-375, hsa-

miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p and hsa-miR-4306.

7. The urine miRNA marker for diagnosis of prostate cancer according to any one of claims 1 to 6, wherein when the number of the miRNA markers is at least 5, one of the miRNAs must be hsa-miR-199b-5p.

5 8. The urine miRNA marker for diagnosis of prostate cancer according to any one of claims 1 to 6, wherein when the number of the miRNA markers is at least 5, one of the miRNAs must be hsa-miR-518e-5p.

9. The urine miRNA marker for diagnosis of prostate cancer according to claim 1, wherein the miRNA marker includes any one or more of the following combinations:

10 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

15 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

20 hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

25 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

5 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

10 hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

15 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306; or

20 hsa-miR-183-5p, hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

25 hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-

miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

5 hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-338-5p, hsa-miR-4306; or

10 hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-9-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

15 hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-199b-5p, hsa-miR-338-3p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-338-5p; or

20 hsa-miR-183-5p, hsa-miR-375, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-375, hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306; or

hsa-miR-520d-5p, hsa-miR-199b-5p, hsa-miR-518e-5p, hsa-miR-31-3p, hsa-miR-4306.

25 9. A diagnostic reagent for prostate cancer, comprising a reagent for specifically detecting the urine miRNA marker according to any one of claims 1 to 7.

10. A kit for diagnosis of prostate cancer, comprising the urine miRNA marker according to

any one of claims 1 to 7 as a standard and a corresponding primer for detecting the urine miRNA marker according to any one of claims 1 to 7.

11. Use of the urine miRNA marker according to any one of claims 1 to 7 in preparing a product for early diagnosis and/or recurrence monitoring; preferably, the product has one or more

5 of the following functions:

- 1) identification or auxiliary identification of prostate cancer;
- 2) diagnosis or auxiliary diagnosis of primary prostate cancer; and
- 3) diagnosis or auxiliary diagnosis of recurrent prostate cancer.

12. A method for early diagnosis and/or recurrence monitoring of prostate cancer by using the
10 urine miRNA marker according to any one of claims 1 to 7, comprising the following steps:

step 1, detecting the presence of miRNAs in a urine sample obtained from a subject;

step 2, measuring the expression level of at least one miRNA of the miRNA markers in the
urine sample; and

step 3, by using scores based on the previously measured expression levels of miRNAs,
15 conducting early diagnosis and/or recurrence monitoring of the possibility of prostate cancer in the
subject.