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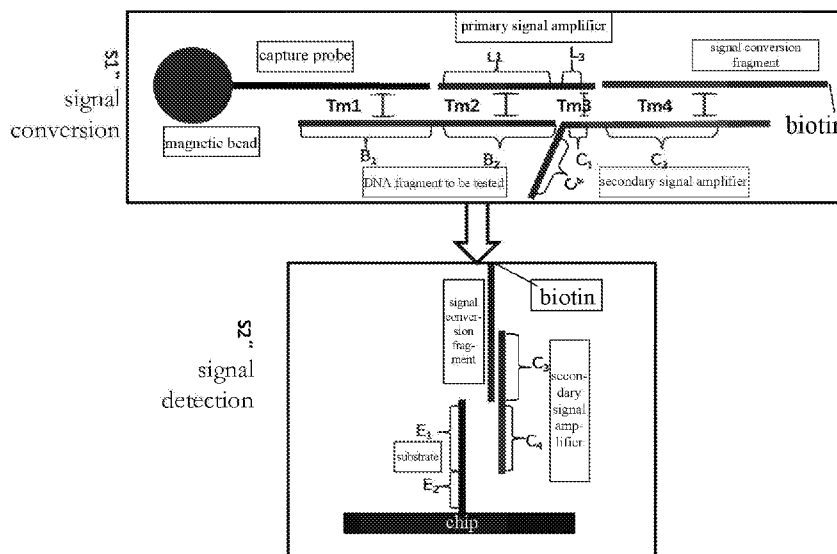


Fig. 3

(57) **Abstract:** The present disclosure provides a kit and method for detecting DNA. In particular, the present disclosure relates to a gene chip-based kit for detecting a DNA sample, a signal amplification system for use in the kit, and a method for using the kit. The present disclosure involves first unifying the substances to be tested by a biotransformation process performed outside the test system in the determination of DNA fragments to be tested, so that the substrate of the chip only corresponds to a unified standard. It can be achieved therefrom that a unified chip and substrate can be used to detect a plurality of objects, while the preparation of the chip is simplified, and cross-contamination caused by different substrates is avoided. Moreover, the kit contains a signal amplification system, which leads to a higher detection sensitivity when same is used for detecting DNA.



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Kit and Method for Detecting DNA

Technical Field

The present disclosure relates to a kit for detecting a DNA sample, a signal amplification system for use in the kit for detecting, and a method for detecting a DNA sample. In particular, the present disclosure relates to a gene chip-based kit for detecting a DNA sample, a signal amplification system for use in the kit, and a method for using the kit.

Background Art

DNA chips, also known as gene chips or microarrays, oligonucleotide chips, or DNA microarrays, involve attaching high-density DNA fragment arrays to materials such as glass and nylon in a certain arrangement by microarray technology. DNA chip technology comprises in-situ synthesis of oligonucleotides on a solid support or direct immobilizing a large number of DNA probes on the surface of the support by means of micro-printing, and then hybridizing with the labeled sample to obtain the genetic information of the sample by detecting and analyzing the hybridization signal. In layman's terms, the gene chip is made by regularly arranging tens of thousands or even millions of DNA fragments (gene probes) of specific sequences on 2 cm² supports such as silicon wafers and glass wafers through a micro-processing technology so as to form a two-dimensional array of DNA probe, which is very similar to the preparation for an electronic chip on an electronic computer, and thus the gene chip got its name.

The use of immobilized carriers to capture and detect disease markers is a common method used in modern diagnostic assays. However, the existing solution mainly detects a plurality of disease objects by a corresponding detection chip with specific binding ability, wherein the chip and the substrate must correspond to the detection object. This will result in that a plurality of substrates must be implanted

on a single chip when a plurality of objects are detected simultaneously. The disadvantage is that the preparation process of the chip is cumbersome and not easy to control, and the substrates in the adjacent chips may be contaminated with each other, resulting in inaccurate or unstable detection results.

5 In the process of DNA detection, the prior art mainly involves the following two ways: (1) template amplification technology, which increases sensitivity by amplifying target sequences; (2) a signal amplification system, which amplifies the signal intensity to increase sensitivity, and which is quick on reaction, easy to perform, and has no interference factor for template amplification.

10 However, many disease-related life substances (such as specific proteins, enzymes, sugars and other molecules or molecular groups, called markers in disease detection) are not easily detected in living organisms.

Furthermore, considering that almost all drug metabolizing enzymes have genetic polymorphisms, and the polymorphism of most of drug metabolizing enzymes is a single-nucleotide polymorphism (SNP) caused by multiple alleles present at the same gene locus. Metabolic enzymes encoded by alleles have different drug metabolism abilities, which have an important impact on the individual response and drug side effects of a treatment. Therefore, predicting the therapeutic effect of the drug by detecting whether there is a mutation in the drug metabolizing enzyme is also significantly important for personalized medicine.

20 Therefore, many methods for amplifying have been proposed to increase the sensitivity of DNA mutations or SNP detection. However, the existing methods are mainly for the amplification effect in the test system, provide no front-end signal mediation system, and have relatively low amplification efficiency.

25 Summary of the Invention

Problems to be solved by the present invention

30 An object of the present disclosure is to provide a new kit for detecting a DNA sample for overcoming the deficiencies of the prior art described above, wherein the kit can detect a plurality of objects using a unified chip and substrate, thereby avoiding cross-contamination of different substrates.

In an embodiment, the present disclosure also provides a method for detecting a DNA sample using the kit described above.

Another object of the present disclosure is to provide a kit and method for detecting a DNA sample for overcoming the deficiencies of the prior art described above, wherein the kit contains a signal amplification system, which leads to a higher detection sensitivity when the signal amplification system is used for detecting DNA.

Solutions to solve the problems

In order to achieve the objects described above, the technical solutions used in the present disclosure are as follows.

In a technical solution, the present disclosure relates to a kit for detecting a DNA sample, which comprises a capture probe, a mediator and a terminal signal, an unmodified solid phase carrier and magnetic beads modified with an affinity substance M5; wherein,

the capture probe is a DNA fragment containing a DNA fragment complementary to and paired with a partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B_1 contains a site to be tested;

the mediator and the terminal signal are both DNA fragments, in which the nucleotide sequence L_1 at one end of the mediator is complementary to and paired with a partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L_2 at the other end of the mediator is complementary to and paired with a partial nucleotide sequence C_1 of the terminal signal; and

the kit further comprises a chip with a substrate which is a DNA fragment, and part or all of the fragment of the substrate is complementary to and paired with a partial nucleotide sequence C_2 of the terminal signal; and/or the kit further comprises a signal conversion fragment which is a DNA fragment, and part or all of the fragment of the signal conversion fragment is complementary to and paired with a partial nucleotide sequence C_3 of the terminal signal;

wherein, the terminal signal or signal conversion fragment is labeled with an

affinity substance M6 which has an affinity with an affinity substance M5.

In a specific embodiment of the solution described above, Tm1 is a temperature at which binding base pairs of the capture probe and the partial nucleotide sequence B₁ of the DNA fragment to be tested are separated; Tm2 is a temperature at which binding base pairs of the nucleotide sequence L₁ of the mediator and the partial nucleotide sequence B₂ of the DNA fragment to be tested are separated; Tm3 is a temperature at which binding base pairs of the nucleotide sequence L₂ of the mediator and the partial nucleotide sequence C₁ of the terminal signal are separated; Tm4 is a temperature at which binding base pairs of the partial nucleotide sequence C₃ of the terminal signal and the signal conversion fragment are separated; characterized in that the numerical value of Tm3 among Tm1, Tm2, Tm3 and Tm4 is the lowest.

In a specific embodiment of the solution described above, when the kit comprises a chip with a substrate, the length of the partial nucleotide sequence C₂ of the terminal signal is longer than that of the partial nucleotide sequence C₁ of the terminal signal; when the kit comprises a signal conversion fragment, the length of the partial nucleotide sequence C₃ of the terminal signal is longer than that of the partial nucleotide sequence C₁ of the terminal signal.

In a specific embodiment of the solution described above, the capture probe is a DNA fragment, and the DNA fragment in the capture probe complementary to and paired with the partial nucleotide sequence B₁ is 6 to 20 bp in length.

In a specific embodiment of the solution described above, the DNA fragment in the capture probe complementary to and paired with the partial nucleotide sequence B₁ is 10 to 15 bp in length.

In a specific embodiment of the solution described above, the capture probe is provided with a modification capable of enhancing the binding of base pairing.

In a specific embodiment of the solution described above, the modification is selected from at least one of PNA, LNA, MNA, ANA, TNA, CeNA, GNA, XNA, HNA, INA and BNA.

In a specific embodiment of the solution described above, the unmodified solid phase carrier is a magnetic bead or a microparticle comprised of glass or nylon.

In a specific embodiment of the solution described above, the affinity substance M5 is selected from an amino group, a polylysine, a thiol group, a bovine serum albumin, an avidin, an agarose gel or a polyacrylamide gel.

In a preferred embodiment, the affinity substance M5 is a streptavidin, and the affinity substance M6 is a biotin.

In a specific embodiment of the solution described above, the solid carrier in the chip includes at least one which is selected from the group of Al_2O_3 , glass, polymer, and nylon.

In a specific embodiment of the solution described above, the partial nucleotide sequence C_1 of the terminal signal is 10 to 15 bp in length; the partial nucleotide sequence C_2 of the terminal signal is 16 to 60 bp in length; the partial nucleotide sequence C_3 of the terminal signal is 16 to 60 bp in length.

In a specific embodiment of the solution described above, the partial nucleotide sequence C_2 of the terminal signal is 25 to 60 bp in length; the partial nucleotide sequence C_3 of the terminal signal is 25 to 60 bp in length.

In a specific embodiment of the solution described above, when the kit comprises a chip with a substrate, the length of the nucleotide sequence in the substrate complementary to and paired with the nucleotide sequence C_2 is longer than that of the remaining nucleotide sequence in the substrate.

In a specific embodiment of the solution described above, the DNA sample is derived from the blood of a human body.

In a specific embodiment of the solution described above, the DNA fragment to be tested in the DNA sample is a DNA fragment having a mutation site.

In a specific embodiment of the solution described above, the DNA fragment to be tested in the DNA sample is a FGFR3 gene fragment containing a G380R mutation site.

In a specific embodiment of the solution described above, the nucleotide sequence of the DNA fragment to be tested in the DNA sample is as shown in SEQ ID NO: 1; the nucleotide sequence of the capture probe is as shown in SEQ ID NO: 2; the nucleotide sequence of the mediator is as shown in SEQ ID NO: 3; the nucleotide sequence of the terminal signal is as shown in SEQ ID NO: 4; the

nucleotide sequence of the signal conversion fragment is as shown in SEQ ID NO: 5; the nucleotide sequence of the substrate is as shown in SEQ ID NO: 6.

In a specific embodiment of the solution described above, the DNA fragment to be tested in the DNA sample is a CYP2C19 gene fragment containing a G681A site.

In a specific embodiment of the solution described above, the nucleotide sequence of the DNA fragment to be tested in the DNA sample is as shown in SEQ ID NO: 7; the nucleotide sequence of the capture probe is as shown in SEQ ID NO: 8; the nucleotide sequence of the mediator is as shown in SEQ ID NO: 9; the nucleotide sequence of the terminal signal is as shown in SEQ ID NO: 10; the nucleotide sequence of the signal conversion fragment is as shown in SEQ ID NO: 11; the nucleotide sequence of the substrate is as shown in SEQ ID NO: 12.

In a specific embodiment of the solution described above, a standard solution of the DNA fragment to be tested is further comprised.

In a preferred embodiment, the kit for detecting further comprises a hybridization solution and a cleaning solution.

In a specific embodiment of the solution described above, the eluate contains deionized formamide, 2 x SSC, 5 x Denhard's, SDS and deionized water; the cleaning solution contains Tris-Citric, NaCl, and Tween 20.

In another technical solution, the present disclosure also relates to a method for detecting a DNA sample, the method comprises the following steps:

(1) coupling the capture probe to the unmodified solid phase carrier;

(2) adding the solid phase carrier coupled with the capture probe and the DNA fragment to be tested in the DNA sample to the hybridization solution, so that the capture probe is hybridized with the partial nucleotide sequence B₁ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

(3) adding the solid phase carrier obtained from the isolation in step (2) and the mediator to the hybridization solution, so that the nucleotide sequence L₁ at one end of the mediator is hybridized with the partial nucleotide sequence B₂ of the DNA

fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is isolated;

(4) adding the solid phase carrier obtained from the isolation in step (3) and the terminal signal to the hybridization solution, so that the nucleotide sequence L_2 at the other end of the mediator is hybridized with the partial nucleotide sequence C_1 of the terminal signal; after the reaction is completed, the solid phase carrier coupled with the terminal signal, the mediator, the DNA fragment to be tested and the capture probe is isolated;

(5) adding the solid phase carrier obtained from the isolation in step (4) and the signal conversion fragment to the hybridization solution, so that the signal conversion fragment is hybridized with the partial nucleotide sequence C_3 of the terminal signal to form a complex of the terminal signal and the signal conversion fragment, and the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is isolated;

(6) coupling the obtained complex to the magnetic beads modified with the affinity substance M5 via the affinity substance M6 labeled on the terminal signal or the signal conversion fragment to form a signal magnetic bead;

(7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample; wherein

the capture probe is a DNA fragment, and contains a DNA fragment complementarily paired with the partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample;

the partial nucleotide sequence B_1 contains a site to be tested;

the mediator and the terminal signal are both DNA fragments, in which the nucleotide sequence L_1 at one end of the mediator is complementarily paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample;

the nucleotide sequence L_2 at the other end of the mediator is complementarily paired with the partial nucleotide sequence C_1 of the terminal signal;

the signal conversion is a DNA fragment, and part or all of the fragment of the

signal conversion is complementarily paired with the partial nucleotide sequence C_3 of the terminal signal.

In a specific embodiment of the solution described above, step (5a) is further comprised between step (5) and step (6), and involves: reacting the complex of the terminal signal and the signal conversion fragment with the chip with a substrate, so that the substrate is hybridized with the partial nucleotide sequence C_2 of the terminal signal to obtain a complex of the chip, the substrate, the terminal signal and the signal conversion; wherein

the substrate is a DNA fragment, and part or all of the fragment of the substrate is complementarily paired with a partial nucleotide sequence C_2 of the terminal signal;

the chip further contains a magnetic sensor.

In a specific embodiment of the solution described above, in step (2), step (3) and step (4), after isolation, the solid phase carrier is respectively washed with a cleaning solution.

In another technical solution, the present disclosure also relates to a method for detecting a DNA sample, the method comprises the following steps:

(1) coupling the capture probe to the unmodified solid phase carrier;

(2) adding the solid phase carrier coupled with the capture probe and the DNA fragment to be tested in the DNA sample to the hybridization solution, so that the capture probe is hybridized with the partial nucleotide sequence B_1 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

(3) adding the solid phase carrier obtained from the isolation in step (2) and the mediator to the hybridization solution, so that the nucleotide sequence L_1 at one end of the mediator is hybridized with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is isolated;

(4) adding the solid phase carrier obtained from the isolation in step (3) and the

terminal signal to the hybridization solution, so that the nucleotide sequence L_2 at the other end of the mediator is hybridized with the partial nucleotide sequence C_1 of the terminal signal; after the reaction is completed, the solid phase carrier coupled with the terminal signal, the mediator, the DNA fragment to be tested and the capture probe is isolated; after the unbound terminal signal is washed, the solid phase carrier is subjected to an appropriate temperature to detach the terminal signal from the solid phase carrier into the solution, and the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is removed;

(5) reacting the solution containing the terminal signal obtained in step (4) with the chip with a substrate, and the substrate is hybridized with the partial nucleotide sequence C_2 of the terminal signal to form a complex of the chip, the substrate and the terminal signal;

(6) coupling the obtained complex to the magnetic beads modified with the affinity substance M5 via the affinity substance M6 labeled on the terminal signal to form a signal magnetic bead;

(7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample; wherein

the capture probe is a DNA fragment, and contains a DNA fragment complementarily paired with the partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample;

the partial nucleotide sequence B_1 contains a site to be tested;

the mediator and the terminal signal are both DNA fragments, in which the nucleotide sequence L_1 at one end of the mediator is complementarily paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample;

the nucleotide sequence L_2 at the other end of the mediator is complementarily paired with the partial nucleotide sequence C_1 of the terminal signal;

the signal conversion is a DNA fragment, and part or all of the fragment of the signal conversion is complementarily paired with the partial nucleotide sequence C_3 of the terminal signal;

the substrate is a DNA fragment, and part or all of the fragment of the substrate

is complementarily paired with a partial nucleotide sequence C_2 of the terminal signal;

the chip further contains a magnetic sensor.

In a specific embodiment of the solution described above, in step (2) and step (3), after isolation, the solid phase carrier is respectively washed with a cleaning solution.

In a specific embodiment of the solution described above, in step (2), the reaction is carried out for 20 minutes at a temperature of 45°C; in step (3) and step (4), the reactions are both carried out for 20 minute at a temperature of 25°C.

In a specific embodiment of the solution described above, in step (7), the signal amount of the signal bead is detected by a device for detecting magnetic signals; wherein the device is at least one which may be selected from the group consisting of a Hall element, a magneto resistive effect element (Magneto Resistive Sensor); wherein the magneto resistive effect element may be selected from a GMR sensor (Giant Magneto Resistive Sensor) and a TMR sensor (Tunnel Magneto Resistive Sensor).

In a specific embodiment of the solution described above, the method for obtaining the DNA fragment to be tested in the DNA sample comprises: collecting the blood of a human body, extracting the DNA in the blood and then fragmenting the extracted DNA.

In a specific embodiment of the protocol described above, the extracted DNA is fragmented by an enzyme digestion method or an ultrasonication method.

In another technical solution, the present disclosure relates to a kit for detecting a DNA sample, which comprises a capture probe, a primary signal amplifier and a secondary signal amplifier, an unmodified solid phase carrier, magnetic beads modified with an affinity substance M5 and a signal conversion fragment; wherein

the capture probe is a DNA fragment containing a DNA fragment complementary to and paired with a partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B_1 contains a site to be tested;

the primary signal amplifier comprises a first mediator, the nucleotide

sequence L_1 at one end of which is complementary to and paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L_2 at the other end of which contains m_1 nucleotide fragment (s) L_3 ;

5 the secondary signal amplifier comprises a first terminal signal containing a nucleotide sequence C_1 and a nucleotide sequence C_2 , and the nucleotide sequence C_2 contains n_1 nucleotide sequence (s) C_3 complementary to and paired with part or all of the DNA fragment of the signal conversion fragment;

10 the signal conversion fragment is a DNA fragment, and part or all of the fragment of the signal conversion fragment is complementary to and paired with the partial nucleotide sequence C_3 of the terminal signal;

the terminal signal or signal conversion fragment is labeled with an affinity substance M_6 which has an affinity with an affinity substance M_5 ;

15 the nucleotide fragment L_3 is complementary to and paired with the nucleotide sequence C_1 ;

m_1 and n_1 are both positive integers.

In a specific embodiment of the solution described above, optionally, m_1 and n_1 are not 1 at the same time.

20 In a specific embodiment of the solution described above, the end of the nucleotide sequence L_2 of the first mediator is modified with the affinity substance M_2 ; the primary signal amplifier further comprises an affinity substance M_1 and a second mediator ; the second mediator contains m_2 nucleotide fragment (s) L_3 , and m_2 is a positive integer; the 5' end and/or the 3' end of the second mediator is modified with the affinity substance M_2 ; the affinity substance M_2 has an affinity
25 with the affinity substance M_1 ; the first mediator and the second mediator are linked to the affinity substance M_1 via the affinity substance M_2 .

30 In a specific embodiment of the solution described above, the end of the first terminal signal is modified with the affinity substance M_4 ; the secondary signal amplifier further comprises an affinity substance M_3 and a second terminal signal containing n_2 nucleotide sequences C_3 , and n_2 is a positive integer; the 5' end and/or 3' end of the second terminal signal is modified with the affinity substance M_4 ; the

affinity substance M3 has an affinity with the affinity substance M4; the first terminal signal and the second terminal signal are linked to the affinity substance M3 via the affinity substance M4.

In a specific embodiment of the solution described above, $m_1 = 5$, and/or $n_1 = 2$.

In another technical solution, the present disclosure relates to a kit for detecting a DNA sample, characterized in that the kit for detecting a DNA sample comprises a capture probe, a primary signal amplifier and a secondary signal amplifier, an unmodified solid phase carrier, magnetic beads modified with an affinity substance M5, and a signal conversion fragment; wherein

the capture probe is a DNA fragment containing a DNA fragment complementary to and paired with a partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B_1 contains a site to be tested;

the primary signal amplifier comprises a first mediator, the nucleotide sequence L_1 at one end of which is complementary to and paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L_2 at the other end of which contains m_1 nucleotide fragment (s) L_3 ;

the secondary signal amplifier comprises a first terminal signal containing a nucleotide sequence C_1 and a nucleotide sequence C_2 , and the nucleotide sequence C_2 contains n_1 nucleotide sequence (s) C_3 complementary to and paired with part or all of the DNA fragment of the signal conversion fragment;

the signal conversion fragment is a DNA fragment, and part or all of the fragment of the signal conversion fragment is complementary to and paired with the partial nucleotide sequence C_3 of the terminal signal; and

the end of the nucleotide sequence L_2 of the first mediator is modified with the affinity substance M2; the primary signal amplifier further comprises an affinity substance M1 and a second mediator ; the second mediator contains m_2 nucleotide fragment (s) L_3 ; the 5' end and/or the 3' end of the second mediator is modified with the affinity substance M2; the affinity substance M2 has an affinity with the affinity

substance M1, the first mediator and the second mediator are linked to the affinity substance M1 via the affinity substance M2; or, the end of the first terminal signal is modified with the affinity substance M4; the secondary signal amplifier further comprises the affinity substance M3 and the second terminal signal containing n_2 nucleotide sequences C_3 ; the 5' end and/or 3' end of the second terminal signal is modified with the affinity substance M4; the affinity substance M3 has an affinity with the affinity substance M4, the first terminal signal and the second terminal signal are linked to the affinity substance M3 via the affinity substance M4;

the terminal signal or signal conversion fragment is labeled with an affinity substance M6 which has an affinity with an affinity substance M5;

the nucleotide fragment L_3 is complementary to and paired with the nucleotide sequence C_1 ;

m_1 and n_1 are both positive integers; m_2 and n_2 are both positive integers;

optionally, m_1 and n_1 are both 1.

In a specific embodiment of the aforementioned technical solution, T_{m1} is a temperature at which binding base pairs of the capture probe and the nucleotide sequence B_1 of the DNA fragment to be tested are separated; T_{m2} is a temperature at which binding base pairs of the nucleotide sequence L_1 of the primary signal amplifier and the nucleotide sequence B_2 of the DNA fragment to be tested are separated; T_{m3} is a temperature at which binding base pairs of the nucleotide sequence L_3 of the primary signal amplifier and the nucleotide sequence C_1 of the secondary signal amplifier are separated; T_{m4} is a temperature at which binding base pairs of the nucleotide sequence C_3 of the secondary signal amplifier and the signal conversion fragment are separated; characterized in that the numerical value of T_{m3} among T_{m1} , T_{m2} , T_{m3} and T_{m4} is the lowest.

In a specific embodiment of the aforementioned technical solution, the kit further comprises a chip with a substrate which is a DNA fragment, and the first terminal signal further comprises the nucleotide sequence C_4 ; part or all of the fragment of the substrate is complementary to and paired with nucleotide sequence C_4 .

In a specific embodiment of the aforementioned technical solution, the

nucleotide sequence of the second mediator is the same as the nucleotide sequence L_2 at the other end of the first mediator.

In a specific embodiment of the aforementioned technical solution, the nucleotide sequence of the second terminal signal is the same as the nucleotide sequence C_2 of the first terminal signal.

In a specific embodiment of the aforementioned technical solution, the affinity substance M1 or M3 or M5 is selected from an amino group, a polylysine, a thiol group, a bovine serum albumin, an avidin, an agarose gel or a polyacrylamide gel.

In a preferred embodiment, the affinity substance M1 is a streptavidin, and the affinity substance M2 is a biotin; or, the affinity substance M3 is a streptavidin, and the affinity substance M4 is a biotin; or, the affinity substance M5 is a streptavidin, and the affinity substance M6 is a biotin.

In a specific embodiment of the aforementioned technical solution, the length of the nucleotide sequence C_3 is longer than that of the nucleotide sequence C_1 .

In a specific embodiment of the aforementioned technical solution, the nucleotide sequence C_3 is 16 to 60 bp in length, and the nucleotide sequence C_1 is 10 to 15 bp in length.

In a preferred embodiment, the nucleotide sequence C_3 is 25 to 60 bp in length.

In a specific embodiment of the solution described above, the length of the nucleotide sequence C_4 is longer than that of the nucleotide sequence C_1 .

In a specific embodiment of the solution described above, the nucleotide sequence C_4 is 16 to 60 bp in length, and the nucleotide sequence C_1 is 10 to 15 bp in length.

In a preferred embodiment, the nucleotide sequence C_4 is 25 to 60 bp in length.

In a specific embodiment of the solution described above, the length of the nucleotide sequence in the substrate complementary to and paired with the nucleotide sequence C_4 is longer than that of the remaining nucleotide sequence in the substrate.

In a specific embodiment of the aforementioned technical solution, the solid carrier in the chip includes at least one which is selected from a group of Al_2O_3 , glass, polymer, and nylon.

In a specific embodiment of the aforementioned technical solution, the DNA sample is derived from the blood of a human body.

In a specific embodiment of the aforementioned technical solution, the DNA fragment to be tested in the DNA sample is a DNA fragment having a mutation site.

5 In a specific embodiment of the solution described above, the DNA fragment to be tested in the DNA sample is a FGFR3 gene fragment containing a G380R mutation site.

In a specific embodiment of the solution described above, the nucleotide sequence of the DNA fragment to be tested in the DNA sample is as shown in SEQ ID NO: 13; the nucleotide sequence of the capture probe is as shown in SEQ ID NO: 14; the nucleotide sequence of the mediator is as shown in SEQ ID NO: 15; the nucleotide sequence of the terminal signal is as shown in SEQ ID NO: 16; the nucleotide sequence of the signal conversion fragment is as shown in SEQ ID NO: 17; the nucleotide sequence of the substrate is as shown in SEQ ID NO: 18.

15 In a specific embodiment of the solution described above, the DNA fragment to be tested in the DNA sample is a CYP2C19 gene fragment containing a G681A site.

In a specific embodiment of the solution described above, the nucleotide sequence of the DNA fragment to be tested in the DNA sample is as shown in SEQ ID NO: 19; the nucleotide sequence of the capture probe is as shown in SEQ ID NO: 20; the nucleotide sequence of the mediator is as shown in SEQ ID NO: 21; the nucleotide sequence of the terminal signal is as shown in SEQ ID NO: 22; the nucleotide sequence of the signal conversion fragment is as shown in SEQ ID NO: 23; the nucleotide sequence of the substrate is as shown in SEQ ID NO: 24.

25 In a specific embodiment of the solution described above, a standard solution of the DNA fragment to be tested is further comprised; preferably, the kit for detecting further comprises a hybridization solution and a cleaning solution.

In a specific embodiment of the solution described above, the eluate contains deionized formamide, 2 x SSC, 5 x Denhard's, SDS and deionized water; the cleaning solution contains Tris-Citric, NaCl, and Tween 20.

30 In another technical solution, the present disclosure relates to a method for

detecting DNA using a kit for detecting a DNA sample, characterized in that the method comprises the following steps:

(1) coupling the capture probe to an unmodified solid phase carrier;

(2) adding the solid phase carrier coupled with the capture probe and the DNA fragment to be tested in the DNA sample to the hybridization solution, so that the capture probe is hybridized with the partial nucleotide B_1 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

(3) adding the solid phase carrier obtained from the isolation in step (2) and the primary signal amplifier to the hybridization solution, so that the nucleotide sequence L_1 at one end of the first mediator is hybridized with the partial nucleotide B_2 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(4) adding the solid phase carrier obtained from the isolation in step (3) and the secondary signal amplifier to the hybridization solution, so that the nucleotide sequence L_3 in the primary signal amplifier is hybridized with the nucleotide sequence C_1 of the secondary signal amplifier; after the reaction is completed, the solid phase carrier coupled with the secondary signal amplifier, the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(5) adding the solid phase carrier obtained from the isolation in step (4) and the signal conversion fragment to the hybridization solution, so that the signal conversion fragment is hybridized with the nucleotide sequence C_3 of the secondary signal amplifier to form a complex of the secondary signal amplifier and the signal conversion fragment and the solid phase carrier coupled with the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(6) coupling the obtained complex to the magnetic beads modified with the affinity substance M5 via the affinity substance M6 labeled on the signal conversion fragment to form a signal magnetic bead;

(7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample.

In a specific embodiment of the solution described above, step (5a) is further comprised between step (5) and step (6), and involves: reacting the complex of the secondary signal amplifier and the signal conversion fragment with the chip with a substrate, so that the substrate is hybridized with the nucleotide sequence C_4 of the first terminal signal to obtain a complex of the chip, the substrate, the secondary signal amplifier and the signal conversion fragment.

In another technical solution, the present disclosure also provides the use of the aforementioned kit in the preparation of a kit for detecting disease markers, and predicting therapeutic effects of drugs and/or side effects of treatment with drugs.

Effects of the present invention

In an embodiment, the present disclosure involves first unifying the substances to be tested by a biotransformation process performed outside the test system in the determination of DNA fragments to be tested, so that the substrate of the chip only corresponds to a unified standard. It can be achieved therefrom that a unified chip and substrate can be used to detect a plurality of objects, while the preparation of the chip is simplified, and cross-contamination caused by different substrates is avoided.

In another embodiment, the present disclosure involves hybridizing a plurality of secondary signal amplifiers to a primary signal amplifier by designing at least one sequence complementary to and paired with the nucleotide sequence C_1 in the secondary signal amplifier on the primary signal amplifier; further hybridizing a plurality of signal conversion fragments to the secondary signal amplifier by designing at least one nucleotide sequence complementary to and paired with the signal conversion fragment on the secondary signal amplifier. It can be achieved therefrom that in the case of the same amount of DNA fragments to be tested, the obtained complex of the secondary signal amplifier and the signal conversion fragment is amplified many times, and the final test signal is multiplied, so that the sensitivity of the determination is multiplied.

In another embodiment, the present disclosure involves modifying the end of the mediator (the first mediator and the second mediator) or the terminal signal (the first terminal signal and the second terminal signal) with affinity substances, one

molecule of the DNA fragment to be tested can indirectly bind to a plurality of secondary signal amplifiers by using the high affinity between the affinity substances, so that the complex of the secondary signal amplifier and the signal conversion fragment is multiplied, and the final test signal is multiplied, thereby further improving the sensitivity.

Brief Description of the Drawings

Figure 1 shows a schematic diagram of a method for detecting a DNA sample in example 1 of the present disclosure.

Figure 2 shows a diagram of the result of nucleotide sequence detected at the G380R mutation site of the FGFR3 gene in example 1 of the present disclosure.

Figure 3 shows a schematic diagram of a method for detecting a DNA sample in example 3 of the present disclosure.

Figure 4 shows a structural diagram of the sequence in example 3 of the present disclosure.

Figure 5 shows a schematic diagram of detecting DNA using the signal amplification system in example 3 of the present disclosure.

Figure 6 shows a structural diagram of the sequence in example 4 of the present disclosure.

Figure 7 shows a structural diagram of the sequence in example 5 of the present disclosure.

Figure 8 shows a structural diagram of the primary signal amplifier in example 5 of the present disclosure.

Detailed Description of Embodiments

Definitions

In the claims and/or the description of the present disclosure, the words "a" or "an" or "the" refer to "one", but may also refer to "one or more", "at least one" and "one or more than one".

As used in the claims and the description, the words "comprising", "having", "including" or "containing" refer to an inclusive and open-ended expression, which

does not exclude additional, unquoted elements or method steps. Moreover, "comprising", "having", "including" or "containing" may also refer to a closed expression, which excludes additional, unquoted elements or method steps.

In the application document, the term "about" means a value comprising the standard deviation in the error caused by the device or method used to determine the value.

Although the content disclosed support the term "or" only refers to a substitute and "and/or", the term "or" in the claims refers to "and/or" unless it is specifically indicated that the term only refers to a substitute or the substitutes are mutually exclusive.

In the prior art, a plurality of disease objects are detected mainly using a corresponding detection chip with specific binding ability, wherein the chip and the substrate must correspond to the detection object. Thus, the preparation process of the chip is cumbersome and not easy to control, and the substrates in the adjacent chips may be contaminated with each other, resulting in inaccurate or unstable detection results.

In order to overcome these disadvantages, in a technical solution, the present disclosure provides a kit for detecting a DNA sample, which comprises a capture probe, a mediator and a terminal signal, an unmodified solid phase carrier and magnetic beads modified with an affinity substance M5; the capture probe is a DNA fragment containing a DNA fragment complementary to and paired with a partial nucleotide sequence B₁ of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B₁ contains a site to be tested; the mediator and the terminal signal are both DNA fragments, in which the nucleotide sequence L₁ at one end of the mediator is complementary to and paired with a partial nucleotide sequence B₂ of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L₂ at the other end of the mediator is complementary to and paired with a partial nucleotide sequence C₁ of the terminal signal;

the kit further comprises a chip with a substrate which is a DNA fragment, and part or all of the fragment E₁ of the substrate is complementary to and paired with a partial nucleotide sequence C₂ of the terminal signal; and/or the kit further

comprises a signal conversion fragment which is a DNA fragment, and part or all of the fragment of the signal conversion fragment is complementary to and paired with a partial nucleotide sequence C_3 of the terminal signal;

the terminal signal or signal conversion fragment is labeled with an affinity substance M6 which has an affinity with an affinity substance M5.

In the technical solution as described above, the kit for detecting a DNA sample of the present disclosure has the detection principle as shown in figure 1. In figure 1, T_m indicates a temperature at which binding base pairs are separated, wherein the higher the T_m , the more stable the binding force of the 2 bound sequences. T_{m1} is a temperature at which binding base pairs of the capture probe and the nucleotide sequence B_1 of the DNA fragment to be tested are separated; T_{m2} is a temperature at which binding base pairs of the nucleotide sequence L_1 of the mediator and the nucleotide sequence B_2 of the DNA fragment to be tested are separated; T_{m3} is a temperature at which binding base pairs of the nucleotide sequence L_2 of the mediator and the nucleotide sequence $C1$ of the terminal signal are separated; T_{m4} is a temperature at which binding base pairs of the nucleotide sequence C_3 of the terminal signal and the signal conversion fragment are separated; the numerical value of T_{m3} among T_{m1} , T_{m2} , T_{m3} and T_{m4} is the lowest (for example: $T_{m4} \approx T_{m1} \approx T_{m2} > T_{m3}$), so that at a certain temperature (for example, 65°C), only separating the binding bases of the nucleotide sequence L_2 and the nucleotide sequence $C1$ of the terminal signal can be achieved.

In a specific embodiment of the technical solution described above, the detection principle of the disclosure specifically involves: hybridizing the DNA fragment to be tested in a DNA sample with a specifically-designed capture probe, so as to capture the DNA fragment to be tested; converting the hybrid of the capture probe and the DNA fragment to be tested into a uniformly-designed detection signal via the mediator and the terminal signal; the detection signal is detached from the complex at the set conditions, entering the test system; finally, binding the detection signal to the magnetic beads by the high affinity between the affinity substance M5 and the affinity substance M6, and detecting the signal of the magnetic beads.

In a specific embodiment of the technical solution described above, the kit for

detecting of the present disclosure solves the problem of cumbersome preparation process of the chip and contamination of the substrates in the adjacent chips; and also facilitates the controllability of the process.

In a specific embodiment of the technical solution described above, the kit of the present disclosure can be used for the detection of a DNA sample.

In a technical solution, the present disclosure provides a signal amplification system for detecting DNA, the amplification system comprises: a capture probe, a primary signal amplifier, and a secondary signal amplifier; the capture probe, primary signal amplifier, and secondary signal amplifier are all DNA fragments.

In another technical solution, the present disclosure provides a kit for detecting a DNA sample, which comprises a capture probe, a primary signal amplifier and a secondary signal amplifier, an unmodified solid phase carrier, magnetic beads modified with an affinity substance M5 and a signal conversion fragment; wherein

the capture probe is a DNA fragment containing a DNA fragment complementary to and paired with a partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B_1 contains a site to be tested; the primary signal amplifier comprises a first mediator, the nucleotide sequence L_1 at one end of which is complementary to and paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L_2 at the other end of which contains m_1 nucleotide fragment (s) L_3 ; the secondary signal amplifier comprises a first terminal signal containing a nucleotide sequence C_1 and a nucleotide sequence C_2 , and the nucleotide sequence C_2 contains n_1 nucleotide sequence (s) C_3 complementary to and paired with part or all of the DNA fragment of the signal conversion fragment; the signal conversion fragment is a DNA fragment, and part or all of the fragment of the signal conversion fragment is complementary to and paired with the partial nucleotide sequence C_3 of the terminal signal; the terminal signal or signal conversion fragment is labeled with an affinity substance M6 which has an affinity with an affinity substance M5; the nucleotide fragment L_3 is complementary to and paired with the nucleotide sequence C_1 ; m_1 and n_1 are both positive integers.

In a preferred embodiment of the technical solution described above, m_1 and

n1 are not both 1.

In another technical solution, the present disclosure provides a kit for detecting a DNA sample, which comprises a capture probe, a primary signal amplifier and a secondary signal amplifier, an unmodified solid phase carrier, magnetic beads modified with an affinity substance M5 and a signal conversion fragment; wherein, the capture probe is a DNA fragment containing a DNA fragment complementary to and paired with a partial nucleotide sequence B₁ of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B₁ contains a site to be tested; the primary signal amplifier comprises a first mediator, the nucleotide sequence L₁ at one end of which is complementary to and paired with the partial nucleotide sequence B₂ of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L₂ at the other end of which contains m₁ nucleotide fragment (s) L₃; the secondary signal amplifier comprises a first terminal signal containing a nucleotide sequence C₁ and a nucleotide sequence C₂, and the nucleotide sequence C₂ contains n₁ nucleotide sequence (s) C₃ complementary to and paired with part or all of the DNA fragment of the signal conversion fragment; the signal conversion fragment is a DNA fragment, and part or all of the fragment of the signal conversion fragment is complementary to and paired with the partial nucleotide sequence C₃ of the terminal signal.

In a specific embodiment of the technical solution described above, the end of the nucleotide sequence L₂ of the first mediator is modified with the affinity substance M2; the primary signal amplifier further comprises an affinity substance M1 and a second mediator ; the second mediator contains m₂ nucleotide fragment (s) L₃; the 5' end and/or the 3' end of the second mediator is modified with the affinity substance M2; the affinity substance M2 has an affinity with the affinity substance M1, the first mediator and the second mediator are linked to the affinity substance M1 via the affinity substance M2.

In another specific embodiment of the technical solution described above, the end of the first terminal signal is modified with the affinity substance M4; the secondary signal amplifier further comprises the affinity substance M3 and the second terminal signal containing n₂ nucleotide sequences C₃; the 5' end and/or 3'

end of the second terminal signal is modified with the affinity substance M4; the affinity substance M3 has an affinity with the affinity substance M4, the first terminal signal and the second terminal signal are linked to the affinity substance M3 via the affinity substance M4; the terminal signal or signal conversion fragment is labeled with an affinity substance M6 which has an affinity with an affinity substance M5; the nucleotide fragment L_3 is complementary to and paired with the nucleotide sequence C_1 ; m_1 and n_1 are both positive integers; m_2 and n_2 are both positive integers.

In a preferred embodiment of the technical solution described above, m_1 and n_1 are both 1.

In a specific embodiment of the technical solution described above, by labeling the affinity substances M2 at the end of the sequence and linking all the affinity substances M2 to M1, a primary signal amplifier can be linked to a first mediator and at least one second mediator, the nucleotide fragment L_3 linked to the primary signal amplifier will increase, thereby linking more first terminal signals. For example, when the affinity substance M1 is a streptavidin and the affinity substance M2 is a biotin, one molecule of the streptavidin can be linked to 4 molecules of the biotin, so that when one end of the second mediator is modified with the affinity substance M2, the primary signal amplifier contains 1 first mediator and 3 second mediators, thus the primary signal amplifier contain $(m_1 + 3 m_2)$ nucleotide fragments L_3 .

In a specific embodiment of the technical solution described above, the signal conversion fragment is a nucleotide fragment linked to a detection signal (for example, a magnetic bead). The capture probe is complementary to and paired with the nucleotide sequence B_1 in the DNA fragment to be tested, thereby capturing the DNA fragment to be tested; the first mediator is complementary to and paired with the nucleotide sequence B_2 in the DNA fragment to be tested via the nucleotide fragment L_1 , and is complementary to and paired with the nucleotide sequence C_1 of the first terminal signal via the nucleotide fragment L_3 ; the nucleotide sequence C_3 of the first terminal signal is complementary to and paired with the signal conversion fragment. When the first mediator contains two or more nucleotide

fragments L_3 , one molecule of the DNA fragment to be tested will link two or more first terminal signals, so that the linked signal conversion fragments are also multiplied.

In another specific embodiment of the technical solution described above, when the first terminal signal contains two or more nucleotide sequences C_3 , the linked signal conversion fragments are also multiplied. It can be seen therefrom that when the first mediator contains m_1 nucleotide fragment (s) L_3 and the first terminal signal contains only 1 nucleotide sequence C_3 , the signal is amplified by m_1 time (s); when the first mediator only contains 1 nucleotide fragment L_3 , and the first terminal signal only contains n_1 nucleotide sequence (s) C_3 , the signal is amplified by n_1 time (s); when the first mediator contains m_1 nucleotide fragment (s) L_3 and the first terminal signal only contains n_1 nucleotide sequence (s) C_3 , the signal is amplified by $m_1 \times n_1$ time (s).

In a specific embodiment of the technical solution described above, in the nucleotide sequence L_2 of the other end of the first mediator, if m_1 is greater than 1, no nucleotides or several nucleotides, for example, 1, 2, 3 ... nucleotides may be contained between any two adjacent nucleotide fragments L_3 ; in the nucleotide sequence C_2 of the first terminal signal, if n_1 is greater than 1, no nucleotides or several nucleotides, for example, 1, 2, 3 ... nucleotides may be contained between any two adjacent nucleotide fragments C_3 .

In a preferred embodiment of the technical solution described above, the end of the nucleotide sequence L_2 of the first mediator is modified with the affinity substance M2; the primary signal amplifier further comprises an affinity substance M1 and a second mediator; the second mediator contains m_2 nucleotide fragment (s) L_3 , and m_2 is a positive integer; the 5' end and/or the 3' end of the second mediator is modified with the affinity substance M2; the affinity substance M2 has an affinity with the affinity substance M1; the first mediator and the second mediator are linked to the affinity substance M1 via the affinity substance M2.

In another specific embodiment of the technical solution described above, by labeling the affinity substances M2 at the end of the sequence and linking all the affinity substances M2 to M1, a primary signal amplifier can be linked to a first

mediator and at least one second mediator, the nucleotide fragment L_3 linked to the primary signal amplifier will increase, thereby linking more first terminal signals. For example, when the affinity substance M1 is a streptavidin and the affinity substance M2 is a biotin, one molecule of the streptavidin can be linked to 4 molecules of the biotin, so that when one end of the second mediator is modified with the affinity substance M2, the primary signal amplifier contains 1 first mediator and 3 second mediators, thus the primary signal amplifier contains $(m_1 + 3m_2)$ nucleotide fragments L_3 .

In a specific embodiment of the technical solution described above, the nucleotide sequence of the second mediator is the same as the nucleotide sequence L_2 at the other end of the first mediator. In this case, when one end of the second mediator is modified with the affinity substance M2, the nucleotide fragment L_3 linked on the primary signal amplifier is amplified by 4 times compared with no modification with the affinity substance, so that the total signal is amplified by $4m_1n_1$ times; when the two ends of the second mediator are modified with the affinity substance M2, the total signal is amplified by $(3^X - 1)m_1n_1/2$ times, wherein X represents the number of levels of the linking.

In a specific embodiment of the technical solution described above, the end of the first terminal signal is modified with the affinity substance M4; the secondary signal amplifier further comprises an affinity substance M3 and a second terminal signal containing n_2 nucleotide sequences C_3 , and n_2 is a positive integer; the 5' end and/or 3' end of the second terminal signal is modified with the affinity substance M4; the affinity substance M3 has an affinity with the affinity substance M4; the first terminal signal and the second terminal signal are linked to the affinity substance M3 via the affinity substance M4.

In another specific embodiment of the technical solution described above, by modifying the end of the first terminal signal and the second terminal signal with the affinity substances M4 and linking all the affinity substances M4 to M3, the nucleotide sequence C_3 on the first terminal signal is increased, thereby linking more signal conversion fragments. For example, when the affinity substance M3 is a streptavidin and the affinity substance M4 is a biotin, one molecule of the

streptavidin can be linked to 4 molecules of the biotin, so that when one end of the second terminal signal is modified with the affinity substance M4, the secondary signal amplifier contains 1 first terminal signal and 3 second terminal signals, thus the secondary signal amplifier contain $(n_1 + 3n_2)$ nucleotide fragments L_3 . In this case, the nucleotide fragment C_3 linked on the secondary signal amplifier is amplified by 4 times compared with no modification with the affinity substance, so that the total signal is amplified by $4m_1n_1$ times.

In a specific embodiment of the technical solution described above, the nucleotide sequence of the second terminal signal is the same as the nucleotide sequence C_2 of the first terminal signal. In this case, when one end of the second terminal signal is modified with the affinity substance M4, the signal is amplified by 4 times compared with no modification with the affinity substance.

In a specific embodiment of the technical solution described above, $m_1 = 5$, and/or $n_1 = 2$. When $m_1 = 5$, the signal is amplified by 5 time; when $n_1 = 2$, the signal is amplified by 2 time; when $m_1 = 5$ and $n_1 = 2$, the signal is amplified by 10 times.

In a technical solution, the present disclosure involves modifying the end of the mediator (the first mediator and the second mediator) or the terminal signal (the first terminal signal and the second terminal signal) with affinity substances, one molecule of the DNA fragment to be tested can indirectly bind to a plurality of secondary signal amplifiers by using the high affinity between the affinity substances, so that the complex of the secondary signal amplifier and the signal conversion fragment is multiplied, and the final test signal is multiplied, thereby further improving the sensitivity.

In a specific embodiment of the technical solution described above, the nucleotide sequence of the second mediator is the same as the nucleotide sequence L_2 at the other end of the first mediator.

In a specific embodiment of the technical solution described above, the nucleotide sequence of the second terminal signal is the same as the nucleotide sequence C_2 of the first terminal signal.

In a specific embodiment of the technical solution described above, if the

length of the DNA fragment in the capture probe complementary to and paired with the partial nucleotide B₁ is too short, then the stability of the combination thereof is poor; Although the longer length of the DNA fragment leads to the higher stability of binding to partial nucleotide B₁, if the length is too long, it is difficult to distinguish between wild-type genes and mutant-type genes.

In a specific embodiment of the technical solution described above, the DNA fragment in the capture probe complementary to and paired with the partial nucleotide sequence B₁ is 6 to 20 bp in length. For example, the length of the DNA fragment in the capture probe complementary to and paired with the partial nucleotide B₁ is 6 bp, 8 bp, 10 bp, 12 bp, 16 bp, 18 bp, 20 bp, etc.

In a preferred embodiment of the technical solution described above, the DNA fragment in the capture probe complementary to and paired with the partial nucleotide B₁ is 10 to 15 bp in length.

In a specific embodiment of the technical solution described above, in order to improve the stability of the hybridization of the capture probe and the DNA fragment to be tested, the capture probe is provided with a modification capable of enhancing the binding ability of base pairing. The modification is more advantageous to distinguish between wild-type genes and mutant-type genes.

In a preferred embodiment of the technical solution described above, the modification is selected from at least one of PNA, LNA, MNA, ANA, TNA, CeNA, GNA, XNA, HNA, INA and BNA. Obviously, the modification is not limited to the substance, and a person skilled in the art would have been able to select other modifications that enhance the binding ability of base pairing.

In addition, the present disclosure further provides a kit for detecting DNA, wherein the kit comprises the signal amplification system described above, and further comprises an unmodified solid phase carrier, magnetic beads modified with an affinity substance M5, and a signal conversion fragment; the signal conversion fragment is a DNA fragment, and part or all of the DNA fragment of the signal conversion fragment is complementary to and paired with the nucleotide sequence C₃, wherein the signal conversion fragment is modified with the affinity substance M6, which has an affinity with the affinity substance M5.

In a technical solution, the detection principle of the kit of the present disclosure is as shown in figure 3. In figure 3, T_m indicates a temperature at which binding base pairs are separated, wherein the higher the T_m , the more stable the binding force of the 2 bound sequences. T_{m1} is a temperature at which binding base pairs of the capture probe and the nucleotide sequence B_1 of the DNA fragment to be tested are separated; T_{m2} is a temperature at which binding base pairs of the nucleotide sequence L_1 of the primary signal amplifier and the nucleotide sequence B_2 of the DNA fragment to be tested are separated; T_{m3} is a temperature at which binding base pairs of the nucleotide sequence L_3 of the primary signal amplifier and the nucleotide sequence C_1 of the secondary signal amplifier are separated; T_{m4} is a temperature at which binding base pairs of the nucleotide sequence C_3 of the secondary signal amplifier and the signal conversion fragment are separated. the numerical value of T_{m3} among T_{m1} , T_{m2} , T_{m3} and T_{m4} is the lowest (for example: $T_{m4} \approx T_{m1} \approx T_{m2} > T_{m3}$), so that at a certain temperature (for example, 65°C), only separating the binding bases of the nucleotide sequence L_3 and the nucleotide sequence $C1$ of the secondary signal amplifier can be achieved.

In a technical solution, the detection principle of the disclosure specifically involves: hybridizing the DNA fragment to be tested in a DNA sample with a specifically-designed capture probe, so as to capture the DNA fragment to be tested; then converting the hybrid of the capture probe and the DNA fragment to be tested into a uniformly-designed detection signal via the primary signal amplifier and the secondary signal amplifier; the detection signal is detached from the complex at the set conditions, entering the test system; finally, binding the detection signal to the magnetic beads by the high affinity between the affinity substances, and detecting the signal of the magnetic beads. More importantly, the kit for detecting of the present disclosure uses the signal amplification system of the present disclosure, which achieves a greatly-improved sensitivity.

In a specific embodiment of the technical solution described above, the affinity substance $M1$ and the affinity substance $M2$ are a pair of substances having specific binding (i.e., affinity interaction), and when the affinity substance $M1$ is determined, the corresponding affinity substance $M2$ can be selected according to the affinity

interaction; when the affinity substance M2 is determined, the corresponding affinity substance M1 can be selected according to the affinity interaction. For example, the affinity substance M1 is preferably, but not limited to, an amino group, a polylysine, a thiol group, a bovine serum albumin, an avidin, an agarose gel or a polyacrylamide gel. More preferably, the affinity substance M1 is a streptavidin, and the affinity substance M2 is a biotin.

In a specific embodiment of the technical solution described above, the affinity substance M3 and the affinity substance M4 are a pair of substances having specific binding (i.e., affinity interaction), and when the affinity substance M3 is determined, the corresponding affinity substance M4 can be selected according to the affinity interaction; when the affinity substance M4 is determined, the corresponding affinity substance M3 can also be selected according to the affinity interaction. For example, the affinity substance M3 is preferably, but not limited to, an amino group, a polylysine, a thiol group, a bovine serum albumin, an avidin, an agarose gel or a polyacrylamide gel. More preferably, the affinity substance M3 is a streptavidin, and the affinity substance M4 is a biotin.

In a specific embodiment of the technical solution described above, when the kit comprises a signal conversion fragment, the affinity substance M6 may be labeled on the terminal signal or may be labeled on the signal conversion fragment; when the kit does not comprise a signal conversion fragment, the affinity substance M6 is labeled on the terminal signal. Wherein the affinity substance M5 and the affinity substance M6 are a pair of substances having specific binding (i.e., affinity interaction), and when the affinity substance M5 is determined, the corresponding affinity substance M6 can be selected according to the affinity interaction; when the affinity substance M6 is determined, the corresponding affinity substance M5 can also be selected according to the affinity interaction. For example, the affinity substance M5 is preferably, but not limited to, an amino group, a polylysine, a thiol group, a bovine serum albumin, an avidin, an agarose gel or a polyacrylamide gel. When the affinity substance M5 is an avidin, the affinity substance M6 is a biotin. More preferably, the affinity substance M5 is a streptavidin, and the affinity substance M6 is a biotin.

As a preferred embodiment of the kit for detecting of the present disclosure, the unmodified solid phase carrier is selected from a magnetic bead or a microparticle comprised of glass or nylon. More preferably, the unmodified solid phase carrier is a magnetic bead.

5 As a preferred embodiment of the kit for detecting of the present disclosure, the carrier in the chip is selected from inorganic material or polymer material (e.g. glass, nylon). It should be noted that the solid carrier in the chip cannot be selected from magnetic beads. This is because when using the kit of the present disclosure, the chip is linked to the terminal signal, which is directly or indirectly linked to the
10 signal magnetic beads (the magnetic beads modified with the affinity substance M5), and if the solid carrier in the chip is a magnetic bead, the signal of the signal bead will be interfered, resulting in inaccurate determination of the DNA sample.

In a specific embodiment, the solid carrier in the chip, such as inorganic material or polymer material has one or more than one layers, such as two layers,
15 three layers, or four layers.

In a specific embodiment, the solid carrier in the chip has three layers. To be specific, the upper layer linked to the substrate comprising at least one of the following compositions: phosphonic acid, silane coupling agent, or any other composition(s) which can be linked to the substrate; the medium layer of the solid
20 carrier is coated by Al_2O_3 ; and the lower layer of the solid carrier contains a GMR sensor.

In a specific embodiment, in order to make the binding between the terminal signal and the substrate or the signal conversion fragment more stable than the that between the terminal signal and the mediator to facilitate the detachment of the
25 terminal signal from the mediator, when the kit comprises a chip with a substrate, the length of the partial nucleotide sequence C_2 of the terminal signal is longer than that of the partial nucleotide sequence C_1 of the terminal signal; when the kit comprises a signal conversion fragment, the length of the partial nucleotide sequence C_3 of the terminal signal is longer than that of the partial nucleotide
30 sequence C_1 of the terminal signal. In the manner described above, the fragment L_2 and the fragment C_1 can be detached, and the other fragments are not detached,

thereby separating the target fragment for detection from other fragments.

As a preferred embodiment of the kit for detecting DNA of the present disclosure, the partial nucleotide sequence C_1 of the terminal signal is 10 to 15 bp in length; the partial nucleotide sequence C_2 of the terminal signal is 16 to 60 bp in length; the partial nucleotide sequence C_3 of the terminal signal is 16 to 60 bp in length.

As a more preferred embodiment of the kit for detecting of the present disclosure, the partial nucleotide sequence C_2 of the terminal signal is 25 to 60 bp in length; the partial nucleotide sequence C_3 of the terminal signal is 25 to 60 bp in length.

Considering the stability of direct binding of bases, when the kit comprises a chip with a substrate, the length of the nucleotide sequence (nucleotide sequence E_1 in figure 1) in the substrate complementary to and paired with the nucleotide sequence C_2 is longer than that of the remaining nucleotide sequence (nucleotide sequence E_2 in figure 1) in the substrate.

As a preferred embodiment of the kit for detecting of the present disclosure, the DNA sample is derived from blood of a human body.

In a preferred embodiment of the technical solution described above, the present disclosure is applicable to the detection of disease markers, such as genetic mutations.

As a preferred embodiment of the kit for detecting of the present disclosure, the DNA fragment to be tested in the DNA sample is a DNA fragment having a mutation site.

In a specific embodiment of the technical solution described above, it is found in the study that fibroblast growth factor receptor 3 (short for FGFR3) transmembrane region mutation causes achondroplasia (ACH). Currently, more than 97% of the mutations reported in foreign countries and Taiwan are located in the 1138th nucleotide of exon 10 of the FGFR3 gene. Among them, 95% are mutations with G to A base conversion, and the rest are mutations with G to C transversion. Both mutations result in the substitution of Glycine at position 380 of FGFR3 for arginine (G380R).

As a specific embodiment of the kit for detecting of the present disclosure, the DNA fragment to be tested in the DNA sample is a FGFR3 gene fragment containing a G380R mutation site.

In the aforementioned specific embodiment, the nucleotide sequence of the DNA fragment to be tested in the DNA sample is the following sequence:

the nucleotide sequence of the DNA fragment to be tested in the DNA sample is as shown in SEQ ID NO: 1; the nucleotide sequence of the capture probe is as shown in SEQ ID NO: 2; the nucleotide sequence of the mediator is as shown in SEQ ID NO: 3; the nucleotide sequence of the terminal signal is as shown in SEQ ID NO: 4; the nucleotide sequence of the signal conversion fragment is as shown in SEQ ID NO: 5; the nucleotide sequence of the substrate is as shown in SEQ ID NO: 6.

In another preferred embodiment, the disclosure is applicable to the detection of SNP sites, such as the detection of CYP2C19*2 (G681A).

In a specific embodiment of the technical solution described above, it is found in the study that CYP2C19 is involved in the formation of clopidogrel active metabolites and intermediate metabolites. CYP2C19 gene has genetic diversity, resulting in three phenotypes in human population, i.e., extensive metabolizer (EM), intermediate metabolizer (IM) and poor metabolizer (PM). The pharmacokinetics and antiplatelet effects of clopidogrel active metabolites vary with the genotype of CYP2C19. CYP2C19*2 (G681A) is the most common type of genetic variation in the Chinese population that leads to the splicing mutation of transcriptional proteins, which then causes loss of enzyme activity and results in a poor metabolizer (PM) phenotype in the population. In patients with CYP2C19 poor metabolizer (PM), the production of active metabolites and the inhibition of platelets are both reduced after the conventional dose of clopidogrel. That is to say, patients with a poor metabolizer (PM) phenotype have a higher rate of cardiovascular events than patients with an intermediate metabolizer (IM) phenotype after treatment with conventional doses, so other antiplatelet agents may be considered or the dose of clopidogrel may be adjusted.

Furthermore, commonly-used drugs metabolized via the CYP2C19 enzyme

comprise at least: valproic acid, diazepam, phenytoin, phenobarbital, fluoxetine, amitriptyline, trimipramine, phosphoric acid amide, progesterone, proguanil, rifampicin, lansoprazole and nelfinavir. Therefore, as long as the drug is metabolized via the CYP2C19 enzyme, it is necessary to detect the CYP2C19 gene to improve therapeutic effects and reduce the side effects.

As a specific embodiment of the kit for detecting of the present disclosure, the DNA fragment to be tested in the DNA sample is a CYP2C19 gene fragment containing a G681A mutation site.

Thus, in a technical solution, the method and product of the present disclosure can be used to predict the therapeutic effect and/or side effects of a drug.

In another technical solution, the method and product involved in the present disclosure may provide a corresponding reference for the selection of a treatment schedule.

In a specific embodiment, if the patient is detected by the kit described above as having CYP2C19*2 (G681A), the physician is prompted to consider the use of other antiplatelet agents or to adjust the dose of clopidogrel.

In the aforementioned specific embodiment, the nucleotide sequence of the DNA fragment to be tested in the DNA sample is the following sequence:

the nucleotide sequence of the DNA fragment to be tested in the DNA sample is as shown in SEQ ID NO: 7; the nucleotide sequence of the capture probe is as shown in SEQ ID NO: 8; the nucleotide sequence of the mediator is as shown in SEQ ID NO: 9; the nucleotide sequence of the terminal signal is as shown in SEQ ID NO: 10; the nucleotide sequence of the signal conversion fragment is as shown in SEQ ID NO: 11; the nucleotide sequence of the substrate is as shown in SEQ ID NO: 12.

In a specific embodiment of the solution described above, the kit further comprises a standard solution of the DNA fragment to be tested. From the standard solution of the DNA fragment to be tested, a standard curve about the signal amount of the signal magnetic bead and the DNA fragment to be tested can be obtained, thereby quantitatively analyzing the DNA fragment to be tested in the DNA sample.

In a specific embodiment of the solution described above, the kit further

comprises a hybridization solution and a cleaning solution.

In a specific embodiment of the solution described above, the eluate contains deionized formamide, 2 x SSC, 5 x Denhard's, SDS and deionized water; the cleaning solution contains Tris-Citric, NaCl, and Tween 20.

5 In a technical solution, the present disclosure also provides a method for detecting a DNA sample using the kit described above, the method comprising the following steps:

(1) coupling the capture probe to an unmodified solid phase carrier;

(2) adding the solid phase carrier coupled with the capture probe and the DNA
10 fragment to be tested in the DNA sample to the hybridization solution, so that the capture probe is hybridized with the partial nucleotide sequence B₁ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

15 (3) adding the solid phase carrier obtained from the isolation in step (2) and the mediator to the hybridization solution, so that the nucleotide sequence L₁ at one end of the mediator is hybridized with the partial nucleotide sequence B₂ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the
20 capture probe is isolated;

(4) adding the solid phase carrier obtained from the isolation in step (3) and the terminal signal to the hybridization solution, so that the nucleotide sequence L₂ at the other end of the mediator is hybridized with the partial nucleotide sequence C₁ of the terminal signal; after the reaction is completed, the solid phase carrier
25 coupled with the terminal signal, the mediator, the DNA fragment to be tested and the capture probe is isolated;

(5) adding the solid phase carrier obtained from the isolation in step (4) and the signal conversion fragment to the hybridization solution, so that the signal conversion fragment is hybridized with the partial nucleotide sequence C₃ of the
30 terminal signal to form a complex of the terminal signal and the signal conversion fragment, and the solid phase carrier coupled with the mediator, the DNA fragment

to be tested and the capture probe is isolated;

(6) coupling the obtained complex to the magnetic beads modified with the affinity substance M5 via the affinity substance M6 labeled on the terminal signal or the signal conversion fragment to form a signal magnetic bead;

5 (7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample.

When the kit of the present disclosure has a signal conversion fragment, the DNA sample can be assayed using the method described above. It should be noted that, the method for detecting of the present disclosure comprises using a magnetic
10 sensor, a GMR sensor, or a TMR sensor for the detection.

As a specific embodiment of the method for detecting a DNA sample of the present disclosure, step (5a) is further comprised between step (5) and step (6), and involves: reacting the complex of the terminal signal and the signal conversion fragment with the chip with a substrate, so that the substrate is hybridized with the
15 partial nucleotide sequence C₂ of the terminal signal to obtain a complex of the chip, the substrate, the terminal signal and the signal conversion fragment.

As a specific embodiment of the method for detecting a DNA sample of the present disclosure, in step (2), step (3) and step (4), after isolation, the solid phase carrier is respectively washed with a cleaning solution.

20 In another technical solution, the present disclosure also provides a method for detecting a DNA sample using the kit described above, the method comprising the following steps:

(1) coupling the capture probe to an unmodified solid phase carrier;

(2) adding the solid phase carrier coupled with the capture probe and the DNA
25 fragment to be tested in the DNA sample to the hybridization solution, so that the capture probe is hybridized with the partial nucleotide sequence B1 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

30 (3) adding the solid phase carrier obtained from the isolation in step (2) and the mediator to the hybridization solution, so that the nucleotide sequence L₁ at one end

of the mediator is hybridized with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is isolated;

5 (4) adding the solid phase carrier obtained from the isolation in step (3) and the terminal signal to the hybridization solution, so that the nucleotide sequence L_2 at the other end of the mediator is hybridized with the partial nucleotide sequence C_1 of the terminal signal; after the reaction is completed, the solid phase carrier coupled with the terminal signal, the mediator, the DNA fragment to be tested and
10 the capture probe is isolated; after the unbound terminal signal is washed, the solid phase carrier is subjected to an appropriate temperature to detach the terminal signal from the solid phase carrier into the solution, and the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is removed;

(5) reacting the solution containing the terminal signal obtained in step (4) with
15 the chip with a substrate, and the substrate is hybridized with the partial nucleotide sequence C_2 of the terminal signal to form a complex of the chip, the substrate and the terminal signal;

(6) coupling the obtained complex to the magnetic beads modified with the affinity substance M5 via the affinity substance M6 labeled on the terminal signal to
20 form a signal magnetic bead;

(7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample.

When the kit of the present disclosure has no signal conversion fragment, the DNA sample can be assayed using the method described above. When there is no
25 signal conversion fragment, the appropriate temperature treatment is required to separate the terminal signal and react same with the substrate. Wherein, the appropriate temperature is required to ensure that the terminal signal is separated from the binding base pair of the mediator, while the binding base pair between the DNA fragment to be tested and the mediator and the capture probe is not separated,
30 for example, an appropriate temperature can be selected as 65°C.

As a preferred embodiment of the method for detecting a DNA sample of the

present disclosure, in step (2) and step (3), after isolation, the solid phase carrier is respectively washed with a cleaning solution.

As a preferred embodiment of the method for detecting a DNA sample of the present disclosure, in step (2), the reaction is carried out for 20 minutes at a temperature of 45°C; in step (3) and step (4), the reactions are both carried out for 20 minute at a temperature of 25°C.

In another technical solution, the present disclosure also provides a method for detecting DNA using the kit described above, the method comprising the following steps:

(1) coupling the capture probe to an unmodified solid phase carrier;

(2) adding the solid phase carrier coupled with the capture probe and the DNA fragment to be tested in the DNA sample to the hybridization solution, so that the capture probe is hybridized with the partial nucleotide B₁ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

(3) adding the solid phase carrier obtained from the isolation in step (2) and the primary signal amplifier to the hybridization solution, so that the nucleotide sequence L₁ at one end of the first mediator is hybridized with the partial nucleotide B₂ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(4) adding the solid phase carrier obtained from the isolation in step (3) and the secondary signal amplifier to the hybridization solution, so that the nucleotide sequence L₃ in the primary signal amplifier is hybridized with the nucleotide sequence C₁ of the secondary signal amplifier; after the reaction is completed, the solid phase carrier coupled with the secondary signal amplifier, the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(5) adding the solid phase carrier obtained from the isolation in step (4) and the signal conversion fragment to the hybridization solution, so that the signal conversion fragment is hybridized with the nucleotide sequence C₃ of the secondary signal amplifier to form a complex of the secondary signal amplifier and the signal

conversion fragment and the solid phase carrier coupled with the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(6) coupling the obtained complex to the magnetic beads modified with the affinity substance M5 via the affinity substance M6 labeled on the signal conversion
5 fragment to form a signal magnetic bead;

(7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample.

It should be noted that, the method for detecting of the present disclosure comprises using a device for detecting magnetic signals. The device is at least one
10 which may be selected from the group consisting of a Hall element, a magneto resistive effect element (Magneto Resistive Sensor), the magneto resistive effect element may be selected from a GMR sensor (Giant Magneto Resistive Sensor) and a TMR sensor (Tunnel Magneto Resistive Sensor).

As a preferred embodiment of the method for detecting DNA of the present
15 disclosure, step (5a) is further comprised between step (5) and step (6), and involves: reacting the complex of the secondary signal amplifier and the signal conversion fragment with the chip with a substrate, so that the substrate is hybridized with the nucleotide sequence C_4 of the first terminal signal to obtain a complex of the chip, the substrate, the secondary signal amplifier and the signal conversion fragment.

As a preferred embodiment of the method for detecting DNA of the present
20 disclosure, in step (2), step (3) and step (4), after isolation, the solid phase carrier is respectively washed with a cleaning solution.

As a preferred embodiment of the method for detecting DNA of the present
25 disclosure, in step (7), the signal amount of the signal bead is detected by a device for detecting magnetic signals.

As a preferred embodiment of the method for detecting DNA of the present
disclosure, the method for obtaining the DNA fragment to be tested in the DNA sample comprises: collecting the blood of a human body, extracting the DNA in the blood and then fragmenting the extracted DNA.

As a more preferred embodiment with respect to the method for detecting
30 DNA of the present disclosure, the extracted DNA is fragmented by an enzyme

digestion method or an ultrasonication method.

Examples

Other objects, features, and advantages of the present disclosure will be apparent from the following detailed description. However, it should be understood that the detailed description and particular example (while indicating the specific implementation manners of the disclosure) are given for the purpose of explanation only, because various changes and modifications made within the spirit and scope of the present disclosure will become apparent to a person skilled in the art.

All reagents used in the examples are all commercially available unless otherwise emphasized.

Example 1

This example is for detecting the 1138th nucleotide G to C mutation (G380R) of the 10th exon of the FGFR3 gene, and the kit for detecting of the present example comprises: a capture probe, a mediator, a terminal signal, a signal conversion fragment, unmodified magnetic beads, magnetic beads modified with affinity substance M5, a glass chip with a substrate, a standard solution of the DNA fragment to be tested, a hybridization solution and a cleaning solution.

In this example, the nucleotide sequence of the DNA fragment to be tested is: 5'-CTTTGCAGCCGAGGAGGAGCTGGTGGAGGCTGACGAGGCGGGCAGTG TGTATGCAGGCATCCTCAGCTACCGGGTGGGCTTCTTCCTGTTTCATCCTGG TGGTGGCGGCTGTGACGCTCTGCCGCCTGCGCAGCCCCCCCCAAGAAAGG CCT-3' (as shown in SEQ ID NO: 1); the nucleotide sequence of the capture probe is: 5'-AGCCACCCGGTAGCTGAGG-3' (as shown in SEQ ID NO: 2); the nucleotide sequence of the mediator is: 5'-ACACACTGCCCCGCCTCGTCAGCCTC GTC TCT GTG TCG TGC-3' (as shown in SEQ ID NO: 3); the nucleotide sequence of the terminal signal is: 5'-CACACACGAGACGCAGACGCCGAGCACAGACGAGCAGCACGACACA GAGACGCAGCACCGAC-3' (as shown in SEQ ID NO: 4); the nucleotide sequence of the signal conversion fragment is: 5'-GTCTGTGCTCGGCGTCTGCGTCTCGTGT GTGCTGCGTGGCGT GTG TGT GC-3' (as shown in SEQ ID NO: 5); the nucleotide sequence of the substrate

is: 5'-GT CGGTGCTGCGTCTCTGTGTCGTGCTGCT-3' (as shown in SEQ ID NO: 6).

The sequence of the nucleotide described above is as shown in figure 2, wherein the 11th nucleotide of the partial nucleotide sequence B1 of the DNA fragment to be tested is a mutation site, and the capture probe is complementary to and paired with the partial nucleotide sequence B1 of the DNA fragment to be tested; the nucleotide sequence L_1 at one end of the mediator is complementary to and paired with a partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L_2 at the other end of the mediator is complementary to and paired with a partial nucleotide sequence C_1 of the terminal signal; partial fragment D_1 of the signal conversion fragment is complementary to and paired with the partial nucleotide sequence C_3 of the terminal signal; the substrate is complementary to and paired with a partial nucleotide sequence C_2 of the terminal signal.

Wherein, the capture probe is modified with LNA, and the terminal signal is labeled with biotin; the affinity substance M5 is a streptavidin; the hybridization solution contains the following components: 50% deionized formamide, 2 x SSC, 5 x Denhard's, 2% SDS and deionized water; the cleaning solution contains the following components: 50 mM Tris-Citric acid (pH 6.0), 0.5 M NaCl and 0.01% Tween 20.

The method for detecting G380R mutation of the FGFR3 gene using the kit described in this example comprises:

(1) collecting the blood of a human body, extracting the DNA in the blood, and then fragmenting the extracted DNA by an enzyme digestion to obtain a DNA fragment to be tested;

(2) coupling the capture probe to unmodified magnetic beads (for transfer), then adsorbing the magnetic beads with a magnet, discarding the waste liquid, and washing the unabsorbed capture probe to obtain magnetic beads coupled with the capture probe;

(3) adding 10 pmol of the magnetic beads coupled with the capture probe and the DNA fragment to be tested in the DNA sample to 50 μ l of the hybridization

solution and reacting same at 45°C for 20 minutes, so that the capture probe is hybridized with the partial nucleotide sequence B₁ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the magnetic beads are adsorbed by the magnet to obtain magnetic beads coupled with the DNA fragment to be tested and the capture probe, and the magnetic beads are washed in 100 µl of the cleaning solution;

(4) adding the magnetic beads obtained in step (3) and 10 pmol of the mediator to 50 µl of the hybridization solution, and reacting same at 25°C for 20 minutes, so that the nucleotide sequence L₁ at one end of the mediator is hybridized with the partial nucleotide sequence B₂ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the magnetic beads are adsorbed by the magnet to obtain magnetic beads coupled with the mediator, the DNA fragment to be tested and the capture probe, and the magnetic beads are washed in 100 µl of the cleaning solution;

(5) adding the magnetic beads obtained in step (4) and 10 pmol of the terminal signal to 50 µl of the hybridization solution, and reacting same at 25°C for 20 minutes, so that the nucleotide sequence L₂ at the other end of the mediator is hybridized with the partial nucleotide sequence C₁ of the terminal signal; after the reaction is completed, the magnetic beads are adsorbed by the magnet to obtain magnetic beads coupled with the terminal signal, the mediator, the DNA fragment to be tested and the capture probe, and the magnetic beads are washed in 100 µl of the cleaning solution;

(6) adding the magnetic beads obtained from the isolation in step (5) and the signal conversion fragment to the hybridization solution, so that the signal conversion fragment is hybridized with the partial nucleotide sequence C₃ of the terminal signal to form a complex of the terminal signal and the signal conversion fragment into the solution, and the magnetic beads coupled with the mediator, the DNA fragment to be tested and the capture probe are isolated by adsorbing with a magnet; carefully pipetting the solution containing the complex for use;

(7) reacting a solution containing the complex of the terminal signal and the signal conversion fragment with a glass chip with a substrate, so that the substrate is

hybridized with the partial nucleotide sequence C₂ of the terminal signal to obtain a solution containing the complex of the chip, the substrate, the terminal signal and the signal conversion fragment;

(8) coupling the obtained complex to the streptavidin-modified magnetic beads via the biotin labeled on the terminal signal to form a signal magnetic bead;

(9) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample.

It should be noted that in the above method for detecting the G380R mutation of the FGFR3 gene described above, biotin may also be labeled on the signal conversion fragment rather than the terminal signal. Furthermore, the G380R mutation of the FGFR3 gene can also be detected if the above step (7) is omitted; or, according to this example, the biotin is labeled on the terminal signal, step (6) is omitted and after step (5), the terminal signal is separated from the magnetic beads, and operation is conducted on the solution containing the terminal signal obtained after the separation and the glass chip with the substrate according to steps (7) to (9), the G380R mutation of the FGFR3 gene can still be detected. Of course, when the corresponding operational steps are omitted, the kit can omit the corresponding substance.

Example 2

This example is used to detect whether the CYP2C19 gene fragment in the DNA sample contain the G681A mutation. The kit for detecting of this example comprises: a capture probe, a mediator, a terminal signal, a signal conversion fragment, unmodified magnetic beads, magnetic beads modified with affinity substance M5, a glass chip with a substrate, a standard solution of the DNA fragment to be tested, a hybridization solution and a cleaning solution.

In this example, the nucleotide sequence of the DNA fragment to be tested is: 5'-

AATTACAACCAGAGCTTGGCATATTGTATCTATACCTTTATTAAATGCTTTT
AATTTAATAAATTATTGTTTTCTCTTAGATATGCAATAATTTCCCACTATCA
TTGATTATTTCCCGGGAACCCATAACAAATTACTTAAAAACCTTGCTTTTA
TGGAAAGTGATATTTTGGAGAAAG-3' (as shown in SEQ ID NO: 7); the

nucleotide sequence of the capture probe is: 5'-TATGGGTTCCCGGGAAATAAT-3' (as shown in SEQ ID NO: 8); the nucleotide sequence of the mediator is: 5'-GGTATAGATAACAATATGCCAAGCTCTGGTTGGTCTCTGTGTCGTGC-3' (as shown in SEQ ID NO: 9); the nucleotide sequence of the terminal signal is: 5'-CACACACGAGACGCAGACGCCGAGCACAGACGAGCAGCACGACACAGAGACGCAGCACCGAC-3' (as shown in SEQ ID NO: 10); the nucleotide sequence of the signal conversion fragment is: 5'-GTCTGTGCTCGGCGTCTGCGTCTCGTGTGTGCTGCGTGGCGTGTGTGTGC-3' (as shown in SEQ ID NO: 11); the nucleotide sequence of the substrate is: 5'-GTCGGTGCTGCGTCTCTGTGTCGTGCTGCT-3' (as shown in SEQ ID NO: 12).

The experimental procedure of example 2 is identical to that of example 1, except that the sequence of the specific DNA used is partially different, while the other experimental schemes and experimental conditions are identical to those in example 1.

Example 3

This example is for detecting the 1138th nucleotide G to C mutation (G380R) of the 10th exon of the FGFR3 gene, and the kit for detecting of the present example comprises a signal amplification system which comprises a capture probe, a primary signal amplifier, and a secondary signal amplifier.

Wherein, the nucleotide sequence of the DNA fragment to be tested is: 5'-CTTTGCAGCCGAGGAGGAGCTGGTGGAGGCTGACGAGGCGGGCAGTG TGTATGCAGGCATCCTCAGCTACCGGGTGGGCTTCTTCCTGTTCATCCTGG TGGTGGCGGCTGTGACGCTCTGCCGCCTGCGCAGCCCCCCCCAAGAAAGG CCT-3' (as shown in SEQ ID NO: 13); the nucleotide sequence of the capture probe is: 5'-AGCCCACCCGGTAGCTGAGG-3' (as shown in SEQ ID NO: 14), wherein the 10th base in the capture probe binds to the mutation site to be tested; the primary signal amplifier is the first mediator, the nucleotide sequence of which is: 5'-ACACACTGCCCCGCCTCGTCAGCCTC GTC TCT GTG TCG TGCATCG GTC TCT GTG TCG TGCATCGGTC TCT GTG TCG TGCATCG GTC TCT GTG TCG TGC-3' (as shown in SEQ ID NO: 15); the

secondary signal amplifier is the first terminal signal, the nucleotide sequence of which is: 5'-CACACACGAGACGCAGACGCCGAGCACAGACATCGCACACACGAGACGCAGACGCCGAGCACAGACGAGCAGCACGACACAGAGACGCAGCACCGAC-3' (as shown in SEQ ID NO: 16).

As shown in figure 4, the capture probe is complementary to and paired with the nucleotide sequence B₁ in the DNA fragment to be tested, wherein the 11th base in the sequence of the nucleotide B₁ is the site to be tested; the nucleotide sequence L₁ at one end of the first mediator is complementary to and paired with the nucleotide sequence B₂ of the DNA fragment to be tested; the nucleotide sequence at the other end of the first mediator (a nucleotide sequence other than the nucleotide sequence L₁ in the first mediator) contains 5 nucleotide fragments L₃; the first terminal signal contains a nucleotide sequence C₁ and a nucleotide sequence C₂, and the nucleotide sequence C₂ contains 2 nucleotide sequences C₃ complementary to and paired with partial DNA fragment of the signal conversion fragment; the nucleotide fragment L₃ is complementary to and paired with the nucleotide sequence C₁.

With the signal amplification system described above, the signal is amplified by $5 \times 2 = 10$ times.

The kit for detecting G380R of the FGFR3 gene of this example comprises the signal amplification system described above, and further comprises unmodified magnetic beads, streptavidin-modified magnetic beads, signal conversion fragments and a glass chip with a substrate; the signal conversion fragment and the substrate are both DNA fragments, and the nucleotide sequence of the signal conversion fragment is: 5'-GTCTGTGCTCGGCGTCTGCGTCTCGTGTGTGCTGCGTGCGGT GTG TGT GC-3' (as set forth in SEQ ID NO: 17), wherein the signal conversion fragment is labeled with biotin; the nucleotide sequence of the substrate is: 5'-GTCGGTGCTGCGTCTCTGTGTGCTGCTGCT-3' (as set forth in SEQ ID NO: 18); as set forth in figure 4, partial DNA fragment D₁ of the signal conversion fragment is complementary to and paired with the nucleotide sequence C₃ of the first terminal signal, and the substrate is complementary to and paired with the nucleotide sequence C₄ of the first terminal signal.

The method for detecting the G380R of the FGFR3 gene in this example comprises:

(1) collecting the blood of a human body, extracting the DNA in the blood, and then fragmenting the extracted DNA by an enzyme digestion to obtain a DNA
5 fragment to be tested;

(2) coupling the capture probe to unmodified magnetic beads (for transfer), then adsorbing the magnetic beads with a magnet, discarding the waste liquid, and washing the unabsorbed capture probe to obtain magnetic beads coupled with the
capture probe;

(3) adding of the magnetic beads coupled with the capture probe and the DNA
10 fragment to be tested in the DNA sample to 50 μ l of the hybridization solution and reacting same at 45°C for 20 minutes, so that the capture probe is hybridized with the partial nucleotide sequence B₁ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the magnetic beads are adsorbed by the
15 magnet to obtain magnetic beads coupled with the DNA fragment to be tested and the capture probe, and the magnetic beads are washed in 100 μ l of the cleaning solution;

(4) adding the magnetic beads obtained in step (3) and the primary signal
amplifier to 50 μ l of the hybridization solution, and reacting same at 25°C for 20
20 minutes, so that the nucleotide sequence L₁ of the primary signal amplifier is hybridized with the partial nucleotide B₂ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the magnetic beads are adsorbed by the magnet to obtain magnetic beads coupled with the primary signal amplifier, the DNA fragment to be tested and the capture probe, and the magnetic beads are
25 washed in 100 μ l of the cleaning solution;

(5) adding the magnetic beads obtained in step (4) and the secondary signal
amplifier to 50 μ l of the hybridization solution, and reacting same at 25°C for 20
minutes, so that the nucleotide sequence L₃ of the primary signal amplifier is
hybridized with the nucleotide sequence C₁ of the secondary signal amplifier; after
30 the reaction is completed, the magnetic beads are adsorbed by the magnet to obtain magnetic beads coupled with the secondary signal amplifier, the primary signal

amplifier, the DNA fragment to be tested and the capture probe, and the magnetic beads are washed in 100 µl of the cleaning solution;

(6) adding the magnetic beads obtained from the isolation in step (5) and the signal conversion fragment to the hybridization solution, so that the signal conversion fragment is hybridized with the nucleotide sequence C₃ of the secondary signal amplifier to form a complex of the secondary signal amplifier and the signal conversion fragment into the solution, and the magnetic beads coupled with the primary signal amplifier, the DNA fragment to be tested and the capture probe are isolated by adsorbing with a magnet; carefully pipetting the solution containing the complex for use;

(7) reacting a solution containing the complex of the secondary signal amplifier and the signal conversion fragment with a glass chip with a substrate, so that the substrate is hybridized with the nucleotide sequence C₄ of the secondary signal amplifier to obtain a solution containing the complex of the glass chip, the substrate, the secondary signal amplifier and the signal conversion fragment;

(8) coupling the obtained complex to the streptavidin-modified magnetic beads via the biotin labeled on the signal conversion fragment to form a signal magnetic bead;

(9) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample.

The schematic diagram of detecting DNA using the signal amplification system of this example is as set forth in figure 5. In figure 5, A represents the capture probe; B represents the DNA fragment to be tested; L represents the primary signal amplifier; C represents the secondary signal amplifier; D represents the signal conversion fragment; and S represents the signal (e.g., streptavidin-modified magnetic beads).

Example 4 (Comparative example)

This example is for detecting the 1138th nucleotide G to C mutation (G380R) of the 10th exon of the FGFR3 gene, and the kit for detecting of the present example comprises a signal amplification system which comprises a capture probe, a primary signal amplifier, and a secondary signal amplifier.

Wherein, the DNA fragment to be tested and the capture probe are identical to those in example 3; the primary signal amplifier consists of a first mediator, a second mediator, and streptavidin, and the nucleotide sequence of the first mediator is: 5'-ACACACTGCCCGCCTCGTCAGCCTC GTC TCT GTG TCG TGC-biotin (as set forth in SEQ ID NO: 19), wherein the 3' end of the first mediator is modified with the biotin; the nucleotide sequence of the second mediator is: 5'-GTC TCT GTG TCG TGC-biotin (as set forth in SEQ ID NO: 20), wherein the 3' end of the second mediator is modified with the biotin; in the primary signal amplifier, 1 molecule of the first mediator and 3 molecules of the second mediator are linked to the streptavidin via the biotin; the secondary signal amplifier is the first terminal signal, the nucleotide sequence of which is: 5'-CACACACGAGACGCGAGACGCCGAGCACAGACGAGCAGCACGACACA GAGACGCAGCACCGAC-3' (as set forth in SEQ ID NO: 21).

As set forth in figure 6: the nucleotide sequence L_1 at one end of the first mediator is complementary to and paired with the nucleotide sequence B_2 of the DNA fragment to be tested; the first terminal signal contains a nucleotide sequence C_1 and a nucleotide sequence C_3 , and the nucleotide sequence C_3 is complementary to and paired with partial DNA fragment of the signal conversion fragment; in the first mediator and the second mediator, the nucleotide fragment L_3 is complementary to and paired with the nucleotide sequence C_1 .

With the signal amplification system described above, the signal is amplified by 4 times.

The kit for detecting G380R of the FGFR3 gene of this example comprises the signal amplification system described above, and further comprises unmodified magnetic beads, streptavidin-modified magnetic beads, signal conversion fragments and a glass chip with a substrate; The signal conversion fragment and substrate are both identical to those in example 3.

The method for detecting the G380R of the FGFR3 gene in this example is identical to that in example 3.

Example 5

This example is for detecting the 1138th nucleotide G to C mutation (G380R)

of the 10th exon of the FGFR3 gene, and the kit for detecting of the present example comprises a signal amplification system which comprises a capture probe, a primary signal amplifier, and a secondary signal amplifier.

The difference between this example and example 4 lies merely in that the nucleotide sequence of the first mediator is:
 5'-ACACACTGCCCCGCCTCGTCAGCCTCGTCTCTGTGTCGTGCATCGGTC
 TCTGTGTCGTGCATCGGTCTCTGTGTCGTGCATCGGTCTCTGTGTGTCG
 TGCATCGGTCTCTGTGTCGTGC-biotin (as set forth in SEQ ID NO: 15); the nucleotide sequence of the second mediator is:
 5'-GTCTCTGTGTCGTGCATCGGTCTCTGTGTCGTGCATCGGTCTCTGTGTC
 GTGCATCGGTCTCTGTGTCGTGCATCGGTC TCTGTGTCGTGC-biotin (as set forth in SEQ ID NO: 22). As set forth in figure 7, the nucleotide sequence L_1 at one end of the first mediator is complementary to and paired with the nucleotide sequence B_2 of the DNA fragment to be tested; the nucleotide sequence L_2 at the other end of the first mediator (a nucleotide sequence other than the nucleotide sequence L_1 in the first mediator) contains 5 nucleotide fragments L_3 ; and the second mediator contains 5 nucleotide fragments L_3 . A structural diagram of the primary signal amplifier of this example is as set forth in figure 8.

With the signal amplification system described above, the signal is amplified by $4 \times 5 = 20$ times.

The kit for detecting the G380R of the FGFR3 gene in this example is identical to that in example 4 except for the first mediator and the second mediator. The method for detecting the G380R of the FGFR3 gene in this example is identical to that in example 3.

Example 6

The difference between the signal amplification system for detecting G380R of the FGFR3 gene in this example and that in example 5 lies merely in that both ends of the second mediator are modified with the biotin. The number of levels of the linking is represented by X, and if only the first mediator is present, X is 1; if the first mediator is linked to 3 second mediator s via 1 streptavidin, then X is 2; if each second mediator is linked to 3 second mediator s via 1 streptavidin fraction, then X

is 3, and so on, in this case, in the primary signal amplifier, 1 molecule of the first mediator and $(3^X-3)/2$ molecules of the second mediator s are linked to streptavidin via biotin, wherein the amount of streptavidin is 1/3 of the second mediator. With the signal amplification system of this example, the signal can be amplified
 5 $(3^X-1)*5*1/2$ times.

The kit for detecting the G380R of the FGFR3 gene in this example is identical to that in example 5 except for the second mediator. The method for detecting the G380R of the FGFR3 gene in this example is identical to that in example 3.

Example 7

10 This example is used to detect whether the CYP2C19 gene fragment in the DNA sample contain the G681A mutation. The kit for detecting of this example comprises a signal amplification system which comprises a capture probe, a primary signal amplifier, and a secondary signal amplifier.

15 This example differs from example 3 merely in the DNA fragment to be tested and the specific DNA capture probe used, and the specific sequences of primary signal amplifier and secondary signal amplifier, while the other experimental conditions are identical.

In the specific reagents used in this example, the nucleotide sequence of the DNA fragment to be tested is:
 20 5'-AATTACAACCAGAGCTTGGCATATTGTATCTATACCTTTATTAAATGCTT
 TTAATTTAATAAATTATTGT TTTCTCTTAG ATATGCAATA ATTTTCCCAC
 TATCATTGATTATTTCCCGGGAACCCATAACAAATTACTTAAAAACCTTG
 CTTTTATGGA AAGTGATATT TTGGAGAAAG-3' (as set forth in SEQ ID NO:
 23); the nucleotide sequence of the capture probe is: 5'-TATGGGTTC
 25 CCGGGAAATA AT-3' (as set forth in SEQ ID NO: 24), wherein the 10th base in the capture probe binds to the mutation site to be tested; the primary signal amplifier is the first mediator, the nucleotide sequence of which is: 5'-GGTATA
 GATACAATAT GCCAAGCTCT GGTTG GTC TCT GTG TCG TGCATCG GTC
 TCT GTG TCG TGCATCG GTC TCT GTG TCG TGCATCG GTC TCT GTG
 30 TCG TGCATCG GTC TCT GTG TCG TGC-3' (as set forth in SEQ ID NO: 25); the secondary signal amplifier is the first terminal signal, the nucleotide sequence of

which is: 5'-CACACACGAGACGCAGACGCCGAGCACAGAC
ATCGCACACACGAGACGCAGACGCCGAGCACAGACGAGCAGCACGACA
CAGAGACGCAGCACCG AC-3' (as set forth in SEQ ID NO: 26).

This example relates to the kit for detecting whether the G681A mutation is
contained in the CYP2C19 gene fragment of the DNA sample, the kit comprises the
signal amplification system described above, and further comprises unmodified
magnetic beads, streptavidin-modified magnetic beads, signal conversion fragments
and a glass chip with a substrate. Wherein, the signal conversion fragment and the
substrate are both DNA fragments, and the nucleotide sequence of the signal
conversion fragment is: 5'-GTC TGT GCT CGG CGT CTG CGT CTC GTG TGT
GCT GCG TGG CGT GTG TGT GC-3' (as set forth in SEQ ID NO: 27), the signal
conversion fragment is labeled with biotin; the nucleotide sequence of the substrate
is: 5'-GTCGGTGCTGCGTCTCTGTGTCGTGCTGCT-3' (as set forth in SEQ ID
NO: 28).

Example 8 (Effect example)

This effect exemplifies the experimental results of example 1, and compares
the sensitivity of the experimental kit and the comparative kit.

Wherein, the experimental kit differs from the kit of example 3 merely in that
the nucleotide sequence of the first terminal signal is:
5'-CACACACGAGACGCAGACGCCGAGCACAGACGAGCAGCACGACACA
GAGACGCAGCACCGAC-3' (as set forth in SEQ ID NO: 21); and in this effect
example, the first terminal signal is labeled with biotin. That is to say, the
comparative kit should theoretically have an amplification factor of $5 \times 1 = 5$ times
as compared with the kit used in example 3 of the present disclosure.

The comparative kit differs from the experimental kit merely in that the
nucleotide sequence of the first mediator is:
5'-ACACACTGCCCGCCTCGTCAGCCTC GTC TCT GTG TCG TGC-3' (as set
forth in SEQ ID NO: 19). That is to say, the comparative kit is identical to the kit
employed in example 1 of the present disclosure.

This effect example comprises first using a comparative kit to make a standard
curve, which comprises the following specific steps:

1. 5 tube experimental group: 10 pmol of the capture probe bound to magnetic beads (Bead-AM1) are respectively reacted with 1 pmol, 5 pmol, 10 pmol, 50 pmol, 100 pmol of the DNA fragments to be tested (BM1) in a hybridization solution to obtain unequal amounts of Bead-AM1-BM1 (a complex of the capture probe bound to magnetic beads and DNA fragments to be tested);

50 pmol of the first mediator (normal P0(L)) in the comparative kit for detecting are respectively added to Bead-AM1-BM1, and reacted in the hybridization solution to obtain Bead-AM1-BM1-L (a complex of the capture probe bound to magnetic beads, DNA fragments to be tested and the first mediator);

50 pmol of the first terminal signal bound to biotin (C/biotin) are respectively added to Bead-AM1-BM1-L, and reacted in the hybridization solution to obtain Bead-AM1-BM1-L-C/biotin (a complex of the capture probe bound to magnetic beads, DNA fragments to be tested, the first mediator, and the first terminal signal);

the amount of C/biotin on the beads (magnetic beads) are tested to output signal gradients of different BM1 concentration gradients as a standard curve; the test results are as set forth in table 2.

Then, use the experimental kit for comparison, which comprises the following specific steps:

1. 1 tube experimental group, 10 pmol of Bead-AM1 and 1pmol BM1 are reacted in the hybridization solution to obtain Bead-AM1-BM1;

50 pmol of the first mediator (new P0(L1)) in the kit for detecting of the present disclosure are added to Bead-AM1-BM1, and reacted in the hybridization solution to obtain Bead-AM1-BM1-L1;

50 pmol of C/biotin are added to Bead-AM1-BM1-L1, and reacted in the hybridization solution to obtain Bead-AM1-BM1-L1-C/biotin, and the amount of C/biotin on the beads are tested to output the BM1 signal amplified by L1, wherein the test results are as set forth in table 2.

In the present effect example, the amount of the substance involved in making the standard curve described above and the comparison process using the experimental kit are as set forth in table 1. The test result of this effect example is as set forth in table 2.

Table 1

Normal P0						New P0
	1	2	3	4	5	6
Bead-AM1	10 pmol	10 pmol	10 pmol	10 pmol	10 pmol	10 pmol
BM1	1 pmol	5 pmol	10 pmol	50 pmol	100 pmol	1 pmol
Normal P0	50 pmol	50 pmol	50 pmol	50 pmol	50 pmol	
New P0						50 pmol
C/b	50 pmol	50 pmol	50 pmol	50 pmol	50 pmol	50 pmol

Table 2

	1	2	3	4	5	6
BM1	1 P	5	10	50	100	1
OD	16.87	19.23	21.09	21.36	22.59	19.25

Wherein, tubes 1-5 are used as a control group for making a standard curve. Tube 6 is an experimental group for comparison of experimental effects.

5 It can be seen from table 2 that tubes 1-5 can exhibit a good linear relationship, demonstrating that the kits involved in example 1 can be used for the detection of genetic mutations.

Moreover, it can be seen from table 2 that since the OD value of tube 6 and the OD value of tube 2 are almost the same, the two can be directly used for comparison. That is to say, the detection results in which the experimental kit is used and the concentration of the DNA fragment (BM1) to be tested is 1 pmol are consistent with those in which the comparative kit is used and the concentration of the DNA fragment (BM1) to be tested is 5 pmol. Therefore, when using the experimental kit, the sensitivity is increased by 5 times.

15 The examples described above of the present disclosure are merely examples used for clearly describing the present disclosure, instead of limiting the implementation modes of the present disclosure. For one skilled in the art, other forms of changes or variations may also be made on the basis of the above description. There is no need and no way to exhaust all implementation modes here.

20 Within the spirit and principle of the present disclosure, any modifications, equivalent replacements, improvements, etc., shall be comprised within the scope of protection of the present disclosure.

Claims

1. A kit for detecting a DNA sample, characterized in that the kit for detecting a DNA sample comprises a capture probe, a mediator and a terminal signal, an unmodified solid phase carrier and magnetic beads modified with an affinity substance M5; wherein,

the capture probe is a DNA fragment, and contains a DNA fragment complementarily paired with a partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B_1 contains a site to be tested;

the mediator and the terminal signal are both DNA fragments, in which the nucleotide sequence L_1 at one end of the mediator is complementarily paired with a partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L_2 at the other end of the mediator is complementarily paired with a partial nucleotide sequence C_1 of the terminal signal; and

the kit further comprises a chip with a substrate which is a DNA fragment, and part or all of the fragment of the substrate is complementarily paired with a partial nucleotide sequence C_2 of the terminal signal; and/or the kit further comprises a signal conversion which is a DNA fragment, and part or all of the fragment of the signal conversion is complementarily paired with a partial nucleotide sequence C_3 of the terminal signal;

wherein the terminal signal or the signal conversion is labeled with an affinity substance M6, which has an affinity with the affinity substance M5.

2. The kit for detecting a DNA sample of claim 1, wherein T_{m1} is a temperature at which binding base pairs of the capture probe and the partial nucleotide sequence B_1 of the DNA fragment to be tested are separated; T_{m2} is a temperature at which binding base pairs of the nucleotide sequence L_1 of the mediator and the partial nucleotide sequence B_2 of the DNA fragment to be tested are separated; T_{m3} is a temperature at which binding base pairs of the nucleotide

sequence L_2 of the mediator and the partial nucleotide sequence C_1 of the terminal signal are separated; Tm_4 is a temperature at which binding base pairs of the partial nucleotide sequence C_3 of the terminal signal and the signal conversion are separated; characterized in that the numerical value of Tm_3 , among Tm_1 , Tm_2 , Tm_3 and Tm_4 , is the lowest.

3. The kit for detecting a DNA sample of claim 1, characterized in that when the kit comprises the chip with the substrate, the length of the partial nucleotide sequence C_2 of the terminal signal is longer than that of the partial nucleotide sequence C_1 of the terminal signal; and/or when the kit comprises the signal conversion, the length of the partial nucleotide sequence C_3 of the terminal signal is longer than that of the partial nucleotide sequence C_1 of the terminal signal.

4. The kit for detecting a DNA sample of claim 1, characterized in that the capture probe is a DNA fragment, and the DNA fragment in the capture probe complementarily paired with the partial nucleotide sequence B_1 is 6 to 20 bp in length.

5. The kit for detecting a DNA sample of claim 4, characterized in that the DNA fragment in the capture probe complementarily paired with the partial nucleotide sequence B_1 is 10 to 15 bp in length.

6. The kit for detecting a DNA sample of any one of claims 1 to 5, characterized in that the capture probe is provided with a modification capable of enhancing the binding of base pairing.

7. The kit for detecting a DNA sample of claim 6, characterized in that the modification is at least one of PNA, LNA, MNA, ANA, TNA, CeNA, GNA, XNA, HNA, INA and BNA.

8. The kit for detecting a DNA sample of claim 1, characterized in that the

unmodified solid phase carrier is a magnetic bead or a microparticle comprised of glass or nylon.

9. The kit for detecting a DNA sample of claim 1, characterized in that the
5 affinity substance M5 is an amino group, a polylysine, a thiol group, a bovine serum albumin, an avidin, an agarose gel or a polyacrylamide gel; preferably, the affinity substance M5 is a streptavidin, and the affinity substance M6 is a biotin.

10. The kit for detecting a DNA sample of claim 1, characterized in that the
10 solid carrier in the chip includes at least one which is selected from the group of Al_2O_3 , glass, polymer and nylon.

11. The kit for detecting a DNA sample of claim 1, characterized in that the
15 partial nucleotide sequence C_1 of the terminal signal is 10 to 15 bp in length; the partial nucleotide sequence C_2 of the terminal signal is 16 to 60 bp in length; the partial nucleotide sequence C_3 of the terminal signal is 16 to 60 bp in length.

12. The kit for detecting a DNA sample of claim 1, characterized in that the
20 partial nucleotide sequence C_2 of the terminal signal is 25 to 60 bp in length; the partial nucleotide sequence C_3 of the terminal signal is 25 to 60 bp in length.

13. The kit for detecting a DNA sample of claim 1, characterized in that when
25 the kit comprises the chip with the substrate, the length of the nucleotide sequence in the substrate complementarily paired with the nucleotide sequence C_2 is longer than that of the remaining nucleotide sequence in the substrate.

14. The kit for detecting a DNA sample of claim 1, characterized in that the
DNA sample is derived from the blood of a human body.

15. The kit for detecting a DNA sample of claim 1 or 14, characterized in that
30 the DNA fragment to be tested in the DNA sample is a DNA fragment having a

mutation site.

16. The kit for detecting a DNA sample of claim 15, characterized in that the DNA fragment to be tested in the DNA sample is a FGFR3 gene fragment
5 containing a G380R mutation site.

17. The kit for detecting a DNA sample of claim 16, characterized in that the nucleotide sequence of the DNA fragment to be tested in the DNA sample is set forth in SEQ ID NO: 1; the nucleotide sequence of the capture probe is set forth in
10 SEQ ID NO: 2; the nucleotide sequence of the mediator is set forth in SEQ ID NO: 3; the nucleotide sequence of the terminal signal is set forth in SEQ ID NO: 4; the nucleotide sequence of the signal conversion is set forth in SEQ ID NO: 5; and the nucleotide sequence of the substrate is set forth in SEQ ID NO: 6.

18. The kit for detecting a DNA sample of claim 15, characterized in that the DNA fragment to be tested in the DNA sample is a CYP2C19 gene fragment
15 containing a G681A site.

19. The kit for detecting a DNA sample of claim 18, characterized in that the nucleotide sequence of the DNA fragment to be tested in the DNA sample is set forth in SEQ ID NO: 7; the nucleotide sequence of the capture probe is set forth in
20 SEQ ID NO: 8; the nucleotide sequence of the mediator is set forth in SEQ ID NO: 9; the nucleotide sequence of the terminal signal is set forth in SEQ ID NO: 10; the nucleotide sequence of the signal conversion is set forth in SEQ ID NO: 11; and the
25 nucleotide sequence of the substrate is set forth in SEQ ID NO: 12.

20. The kit for detecting a DNA sample of claim 1, characterized in that the kit further comprises a standard solution of the DNA fragment to be tested; preferably, the kit for detecting further comprises a hybridization solution and a
30 cleaning solution.

21. The kit for detecting a DNA sample of claim 19, characterized in that the hybridization solution contains deionized formamide, 2 x SSC, 5 x Denhard's, SDS and deionized water; the cleaning solution contains Tris-Citric, NaCl, and Tween 20.

5

22. A method for detecting a DNA sample, characterized in that the method comprises the following steps:

(1) coupling a capture probe to an unmodified solid phase carrier;

10 (2) adding the solid phase carrier coupled with the capture probe and a DNA fragment to be tested in the DNA sample to a hybridization solution, so that the capture probe is hybridized with a partial nucleotide sequence B_1 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

15 (3) adding the solid phase carrier obtained from the isolation in step (2) and a mediator to the hybridization solution, so that a nucleotide sequence L_1 at one end of the mediator is hybridized with a partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is isolated;

20 (4) adding the solid phase carrier obtained from the isolation in step (3) and the terminal signal to the hybridization solution, so that a nucleotide sequence L_2 at the other end of the mediator is hybridized with a partial nucleotide sequence C_1 of the terminal signal; after the reaction is completed, the solid phase carrier coupled with the terminal signal, the mediator, the DNA fragment to be tested and the capture probe is isolated;

25 (5) adding the solid phase carrier obtained from the isolation in step (4) and the signal conversion to the hybridization solution, so that the signal conversion is hybridized with a partial nucleotide sequence C_3 of the terminal signal to form a complex of the terminal signal and the signal conversion, and the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is

30

isolated;

(6) coupling the obtained complex to the magnetic beads modified with an affinity substance M5 via an affinity substance M6 labeled on the terminal signal or the signal conversion to form a signal magnetic bead; and

5 (7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample; wherein

the capture probe is a DNA fragment, and contains a DNA fragment complementarily paired with the partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample;

10 the partial nucleotide sequence B_1 contains a site to be tested;

the mediator and the terminal signal are both DNA fragments, in which the nucleotide sequence L_1 at one end of the mediator is complementarily paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample;

15 the nucleotide sequence L_2 at the other end of the mediator is complementarily paired with the partial nucleotide sequence C_1 of the terminal signal;

the signal conversion is a DNA fragment, and part or all of the fragment of the signal conversion is complementarily paired with the partial nucleotide sequence C_3 of the terminal signal.

20 23. The method for detecting a DNA sample of claim 22, characterized in that step (5a) is further comprised between step (5) and step (6), and involves: reacting the complex of the terminal signal and the signal conversion with a chip with a substrate, so that the substrate is hybridized with the partial nucleotide sequence C_2 of the terminal signal to obtain a complex of the chip, the substrate, the terminal signal and the signal conversion; wherein

the substrate is a DNA fragment, and part or all of the fragment of the substrate is complementarily paired with a partial nucleotide sequence C_2 of the terminal signal;

30 the chip further contains a magnetic sensor.

24. The method for detecting a DNA sample of claim 22, characterized in that in step (2), step (3) and step (4), after isolation, the solid phase carrier is respectively washed with a cleaning solution.

5 25. A method for detecting a DNA sample, characterized in that the method comprises the following steps:

(1) coupling a capture probe to an unmodified solid phase carrier;

(2) adding the solid phase carrier coupled with the capture probe and a DNA fragment to be tested in the DNA sample to a hybridization solution, so that the capture probe is hybridized with a partial nucleotide sequence B_1 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

(3) adding the solid phase carrier obtained from the isolation in step (2) and a mediator to the hybridization solution, so that a nucleotide sequence L_1 at one end of the mediator is hybridized with a partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is isolated;

(4) adding the solid phase carrier obtained from the isolation in step (3) and the terminal signal to the hybridization solution, so that a nucleotide sequence L_2 at the other end of the mediator is hybridized with a partial nucleotide sequence C_1 of the terminal signal; after the reaction is completed, the solid phase carrier coupled with the terminal signal, the mediator, the DNA fragment to be tested and the capture probe is isolated; after the unbound terminal signal is washed, the solid phase carrier is subjected to a treatment at an appropriate temperature to detach the terminal signal from the solid phase carrier into the solution, and the solid phase carrier coupled with the mediator, the DNA fragment to be tested and the capture probe is removed;

(5) reacting the solution containing the terminal signal obtained in step (4) with a chip with the substrate, and the substrate is hybridized with a partial nucleotide

sequence C_2 of the terminal signal to form a complex of the chip, the substrate and the terminal signal;

(6) coupling the obtained complex to the magnetic beads modified with an affinity substance M5 via an affinity substance M6 labeled on the terminal signal to form a signal magnetic bead; and

(7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample; wherein

the capture probe is a DNA fragment, and contains a DNA fragment complementarily paired with the partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample;

the partial nucleotide sequence B_1 contains a site to be tested;

the mediator and the terminal signal are both DNA fragments, in which the nucleotide sequence L_1 at one end of the mediator is complementarily paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample;

the nucleotide sequence L_2 at the other end of the mediator is complementarily paired with the partial nucleotide sequence C_1 of the terminal signal;

the signal conversion is a DNA fragment, and part or all of the fragment of the signal conversion is complementarily paired with the partial nucleotide sequence C_3 of the terminal signal;

the substrate is a DNA fragment, and part or all of the fragment of the substrate is complementarily paired with a partial nucleotide sequence C_2 of the terminal signal;

the chip further contains a magnetic sensor.

26. The method for detecting a DNA sample of claim 25, characterized in that in step (2) and step (3), after isolation, the solid phase carrier is respectively washed with the cleaning solution.

27. The method for detecting a DNA sample of claim 22 or 25, characterized in that in step (2), the reaction is carried out for 20 minutes at a temperature of 45°C ;

in step (3) and step (4), the reactions are both carried out for 20 minutes at a temperature of 25°C.

28. The method for detecting a DNA sample of claim 22 or 25, characterized in that in step (7), the signal amount of the signal bead is detected by a device for detecting magnetic signals; wherein the device is at least one which may be selected from the group consisting of a Hall element, a magneto resistive effect element (Magneto Resistive Sensor); wherein the magneto resistive effect element may be selected from a GMR sensor (Giant Magneto Resistive Sensor) and a TMR sensor (Tunnel Magneto Resistive Sensor).

29. The method for detecting a DNA sample of claim 22 or 25, characterized in that the method for obtaining the DNA fragment to be tested in the DNA sample comprises collecting the blood of a human body, extracting the DNA in the blood and then fragmenting the extracted DNA.

30. The method for detecting a DNA sample of claim 29, characterized in that the extracted DNA is fragmented by an enzyme digestion method or an ultrasonication method.

31. A kit for detecting a DNA sample, characterized in that the kit for detecting a DNA sample comprises a capture probe, a primary signal amplifier and a secondary signal amplifier, an unmodified solid phase carrier, magnetic beads modified with an affinity substance M5, and a signal conversion; wherein,

the capture probe is a DNA fragment containing a DNA fragment complementarily paired with a partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B_1 contains a site to be tested;

the primary signal amplifier comprises a first mediator, the nucleotide sequence L_1 at one end of the first mediator is complementarily paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample,

and the nucleotide sequence L_2 at the other end of the first mediator contains m_1 nucleotide fragment (s) L_3 ;

the secondary signal amplifier comprises a first terminal signal containing a nucleotide sequence C_1 and a nucleotide sequence C_2 , and the nucleotide sequence C_2 contains n_1 nucleotide sequence (s) C_3 complementarily paired with part or all of the DNA fragment of the signal conversion;

the signal conversion is a DNA fragment, and part or all of the fragment of the signal conversion is complementarily paired with the partial nucleotide sequence C_3 of the terminal signal;

the terminal signal or signal conversion is labeled with an affinity substance M6 which has an affinity with an affinity substance M5;

the nucleotide fragment L_3 is complementarily paired with the nucleotide sequence C_1 ;

m_1 and n_1 are both positive integers;

optionally, m_1 and n_1 are not 1 at the same time.

32. The kit for detecting a DNA sample of claim 31, wherein the end of the nucleotide sequence L_2 of the first mediator is modified with an affinity substance M2; the primary signal amplifier further comprises an affinity substance M1 and a second mediator containing m_2 nucleotide fragment (s) L_3 , and m_2 is a positive integer; the 5' end and/or the 3' end of the second mediator is modified with the affinity substance M2; the affinity substance M2 has an affinity with the affinity substance M1; and the first mediator and the second mediator are linked to the affinity substance M1 via the affinity substance M2.

33. The kit for detecting a DNA sample of claim 31, wherein the end of the first terminal signal is modified with an affinity substance M4; the secondary signal amplifier further comprises an affinity substance M3 and a second terminal signal containing n_2 nucleotide sequences C_3 , and n_2 is a positive integer; the 5' end and/or 3' end of the second terminal signal is modified with the affinity substance M4; the affinity substance M3 has an affinity with the affinity substance M4; and the first

terminal signal and the second terminal signal are linked to the affinity substance M3 via the affinity substance M4.

34. The kit for detecting a DNA sample of claim 31, wherein $m_1 = 5$, and/or $n_1 = 2$.

35. A kit for detecting a DNA sample, characterized in that the kit for detecting a DNA sample comprises a capture probe, a primary signal amplifier and a secondary signal amplifier, an unmodified solid phase carrier, magnetic beads modified with an affinity substance M5, and a signal conversion; wherein,

the capture probe is a DNA fragment containing a DNA fragment complementarily paired with a partial nucleotide sequence B_1 of a DNA fragment to be tested in the DNA sample, and the partial nucleotide sequence B_1 contains a site to be tested;

the primary signal amplifier comprises a first mediator, the nucleotide sequence L_1 at one end of which is complementarily paired with the partial nucleotide sequence B_2 of the DNA fragment to be tested in the DNA sample, and the nucleotide sequence L_2 at the other end of which contains m_1 nucleotide fragment (s) L_3 ;

the secondary signal amplifier comprises a first terminal signal containing a nucleotide sequence C_1 and a nucleotide sequence C_2 , and the nucleotide sequence C_2 contains n_1 nucleotide sequence (s) C_3 complementarily paired with part or all of the DNA fragment of the signal conversion;

the signal conversion is a DNA fragment, and part or all of the fragment of the signal conversion is complementarily paired with the partial nucleotide sequence C_3 of the terminal signal; and

the end of the nucleotide sequence L_2 of the first mediator is modified with the affinity substance M2; the primary signal amplifier further comprises an affinity substance M1 and a second mediator ; the second mediator contains m_2 nucleotide fragment (s) L_3 ; the 5' end and/or the 3' end of the second mediator is modified with the affinity substance M2; the affinity substance M2 has an affinity with the affinity

substance M1, the first mediator and the second mediator are linked to the affinity substance M1 via the affinity substance M2; or, the end of the first terminal signal is modified with the affinity substance M4; the secondary signal amplifier further comprises the affinity substance M3 and the second terminal signal containing n_2 nucleotide sequences C_3 ; the 5' end and/or 3' end of the second terminal signal is modified with the affinity substance M4; the affinity substance M3 has an affinity with the affinity substance M4, the first terminal signal and the second terminal signal are linked to the affinity substance M3 via the affinity substance M4;

the terminal signal or signal conversion is labeled with an affinity substance M6 which has an affinity with an affinity substance M5;

the nucleotide fragment L_3 is complementarily paired with the nucleotide sequence C_1 ;

m_1 and n_1 are both positive integers; m_2 and n_2 are both positive integers;

optionally, m_1 and n_1 are both 1.

36. The kit for detecting a DNA sample of claim 31 or 35, wherein T_{m1} is a temperature at which binding base pairs of the capture probe and the nucleotide sequence B_1 of the DNA fragment to be tested are separated; T_{m2} is a temperature at which binding base pairs of the nucleotide sequence L_1 of the primary signal amplifier and the nucleotide sequence B_2 of the DNA fragment to be tested are separated; T_{m3} is a temperature at which binding base pairs of the nucleotide sequence L_3 of the primary signal amplifier and the nucleotide sequence C_1 of the secondary signal amplifier are separated; and T_{m4} is a temperature at which binding base pairs of the nucleotide sequence C_3 of the secondary signal amplifier and the signal conversion are separated; characterized in that the numerical value of T_{m3} , among T_{m1} , T_{m2} , T_{m3} and T_{m4} , is the lowest.

37. The kit for detecting a DNA sample of claim 31 or 35, wherein the kit further comprises a chip with a substrate which is a DNA fragment, the first terminal signal further comprises the nucleotide sequence C_4 , and part or all of the fragment of the substrate is complementarily paired with nucleotide sequence C_4 .

38. The kit for detecting a DNA sample of claim 31 or 35, wherein the nucleotide sequence of the second mediator is the same as the nucleotide sequence L_2 at the other end of the first mediator.

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39. The kit for detecting a DNA sample of claim 31 or 35, wherein the nucleotide sequence of the second terminal signal is the same as the nucleotide sequence C_2 of the first terminal signal.

10

40. The kit for detecting a DNA sample of any one of claims 32 to 33 and 35, wherein the affinity substance M1 or M3 or M5 is selected from an amino group, a polylysine, a thiol group, a bovine serum albumin, an avidin, an agarose gel or a polyacrylamide gel; preferably, the affinity substance M1 is a streptavidin, and the affinity substance M2 is a biotin; or, the affinity substance M3 is a streptavidin, and the affinity substance M4 is a biotin; or, the affinity substance M5 is a streptavidin, and the affinity substance M6 is a biotin.

15

41. The kit for detecting a DNA sample of claim 31 or 35, wherein the length of the nucleotide sequence C_3 is longer than that of the nucleotide sequence C_1 .

20

42. The kit for detecting a DNA sample of claim 31 or 35, wherein the nucleotide sequence C_3 is 16 to 60 bp in length, and the nucleotide sequence C_1 is 10 to 15 bp in length; preferably, the nucleotide sequence C_3 is 25 to 60 bp in length.

25

43. The kit for detecting a DNA sample of claim 37, wherein the length of the nucleotide sequence C_4 is longer than that of the nucleotide sequence C_1 .

44. The kit for detecting a DNA sample of claim 37, wherein the nucleotide sequence C_4 is 16 to 60 bp in length, and the nucleotide sequence C_1 is 10 to 15 bp in length; preferably, the nucleotide sequence C_4 is 25 to 60 bp in length.

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45. The kit for detecting a DNA sample of claim 37, wherein the length of the nucleotide sequence in the substrate complementarily paired with the nucleotide sequence C₄ is longer than that of the remaining nucleotide sequence in the substrate.

46. The kit for detecting a DNA sample of claim 31 or 35, characterized in that the solid carrier in the chip includes at least one which is selected from a group of Al₂O₃, glass, polymer, and nylon.

47. The kit for detecting a DNA sample of claim 31 or 35, characterized in that the DNA sample is derived from the blood of a human body.

48. The kit for detecting a DNA sample of claim 31 or 35, characterized in that the DNA fragment to be tested in the DNA sample is a DNA fragment having a mutation site.

49. The kit for detecting a DNA sample of claim 48, characterized in that the DNA fragment to be tested in the DNA sample is a FGFR3 gene fragment containing a G380R mutation site.

50. The kit for detecting a DNA sample of claim 49, characterized in that the nucleotide sequence of the DNA fragment to be tested in the DNA sample is set forth in SEQ ID NO: 13; the nucleotide sequence of the capture probe is set forth in SEQ ID NO: 14; the nucleotide sequence of the mediator is set forth in SEQ ID NO: 15; the nucleotide sequence of the terminal signal is set forth in SEQ ID NO: 16; the nucleotide sequence of the signal conversion is set forth in SEQ ID NO: 17; and the nucleotide sequence of the substrate is set forth in SEQ ID NO: 18.

51. The kit for detecting a DNA sample of claim 48, characterized in that the DNA fragment to be tested in the DNA sample is a CYP2C19 gene fragment

containing a G681A site.

52. The kit for detecting a DNA sample of claim 51, characterized in that the nucleotide sequence of the DNA fragment to be tested in the DNA sample is set forth in SEQ ID NO: 19; the nucleotide sequence of the capture probe is set forth in SEQ ID NO: 20; the nucleotide sequence of the mediator is set forth in SEQ ID NO: 21; the nucleotide sequence of the terminal signal is set forth in SEQ ID NO: 22; the nucleotide sequence of the signal conversion is set forth in SEQ ID NO: 23; and the nucleotide sequence of the substrate is set forth in SEQ ID NO: 24.

53. The kit for detecting a DNA sample of claim 48, characterized in that the kit further comprises a standard solution of the DNA fragment to be tested; preferably, the kit for detecting further comprises a hybridization solution and a cleaning solution.

54. The kit for detecting a DNA sample of claim 53, characterized in that the hybridization solution contains deionized formamide, 2 x SSC, 5 x Denhard's, SDS and deionized water; the cleaning solution contains Tris-Citric, NaCl, and Tween 20.

55. A method for detecting DNA using the kit for detecting a DNA sample of claim 31 or 35, characterized in that the method comprises the following steps:

(1) coupling the capture probe to the unmodified solid phase carrier;

(2) adding the solid phase carrier coupled with the capture probe and the DNA fragment to be tested in the DNA sample to the hybridization solution, so that the capture probe is hybridized with the partial nucleotide B₁ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the DNA fragment to be tested and the capture probe is isolated;

(3) adding the solid phase carrier obtained from the isolation in step (2) and the primary signal amplifier to the hybridization solution, so that the nucleotide sequence L₁ at one end of the first mediator is hybridized with the partial nucleotide

B₂ of the DNA fragment to be tested in the DNA sample; after the reaction is completed, the solid phase carrier coupled with the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(4) adding the solid phase carrier obtained from the isolation in step (3) and the secondary signal amplifier to the hybridization solution, so that the nucleotide sequence L₃ in the primary signal amplifier is hybridized with the nucleotide sequence C₁ of the secondary signal amplifier; after the reaction is completed, the solid phase carrier coupled with the secondary signal amplifier, the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(5) adding the solid phase carrier obtained from the isolation in step (4) and the signal conversion to the hybridization solution, so that the signal conversion is hybridized with the nucleotide sequence C₃ of the secondary signal amplifier to form a complex of the secondary signal amplifier and the signal conversion and the solid phase carrier coupled with the primary signal amplifier, the DNA fragment to be tested and the capture probe is isolated;

(6) coupling the obtained complex to the magnetic beads modified with the affinity substance M5 via the affinity substance M6 labeled on the signal conversion to form a signal magnetic bead; and

(7) detecting the signal amount of the signal magnetic bead, and calculating the content of the DNA fragment to be tested in the DNA sample.

56. The method for detecting DNA of claim 55, characterized in that step (5a) is further comprised between step (5) and step (6), and involves: reacting the complex of the secondary signal amplifier and the signal conversion with the chip with the substrate, so that the substrate is hybridized with the nucleotide sequence C₄ of the first terminal signal to obtain a complex of the chip, the substrate, the secondary signal amplifier and the signal conversion.

57. Use of the kit of any one of claims 1 to 21 or 31 to 54 in the preparation of a kit for detecting disease markers, predicting therapeutic effects of drugs and/or side effects of treatment with drugs.

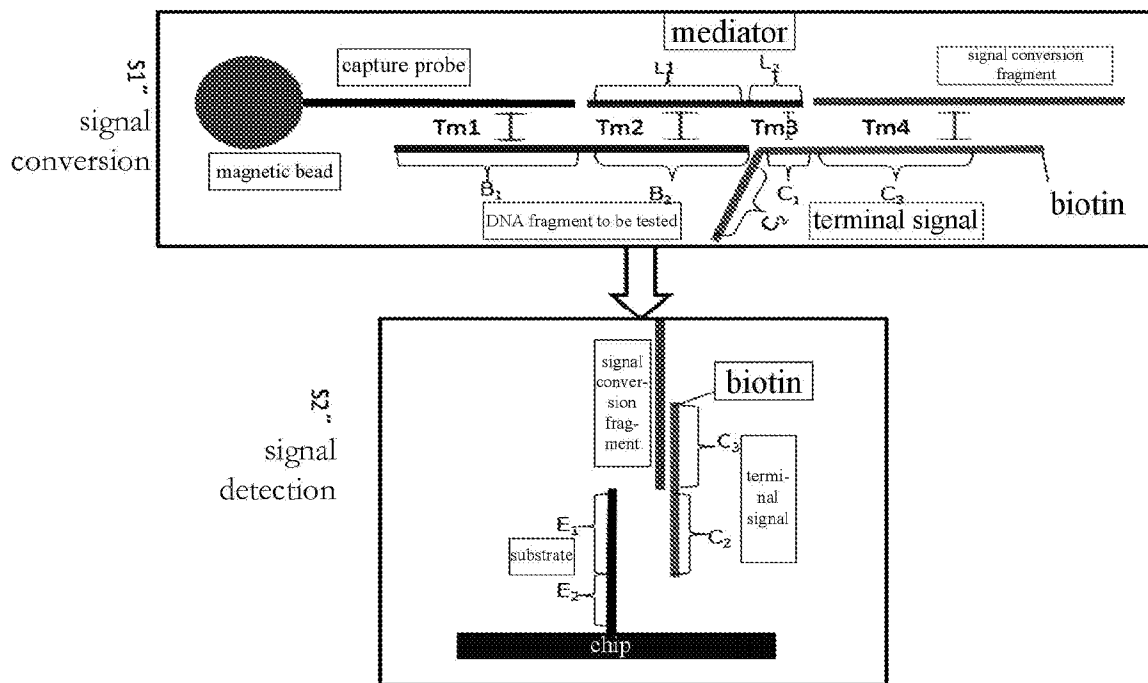


Fig. 1

capture probe 5'-AGCCACCCGGTAGCTGAGG-3'

DNA fragment to be tested 5'-CTTGCAGCC GAGGAGGAGC TGGTG GAGGCTGACGAGGCG GGCAGTGTGT
 ATGCAGGCAT CCTCAGCTAC CGGGTGGGCT TCTTCCTGTT CATCCTGGTG
 GTGGCGGCTG TGACGCTCTG CCGCCTGCGC AGCCCCCCCCA AGAAAGGCCT-3'
 mediator 5'-ACACACTGCCCGCCTCGTCAGCCTC ATC TCT GTG TCG TGC-3'
 terminal signal 5'-CACACACGAG ACGCAGACGC CGAGCACAGA C
 GAGCA GCAC GACACAGAGA GCAGCACCG AC-3'
 signal conversion fragment 5'-ATC TGT GCT CGG CGT CTG CGT CTC GTG TGT GCT GCG TGG CGT
 GTG TGT GC-3' D₁
 substrate 5'- GTCGGTGCTGCGTCTCTGTGTCGTGCTGCT -3'

Fig. 2

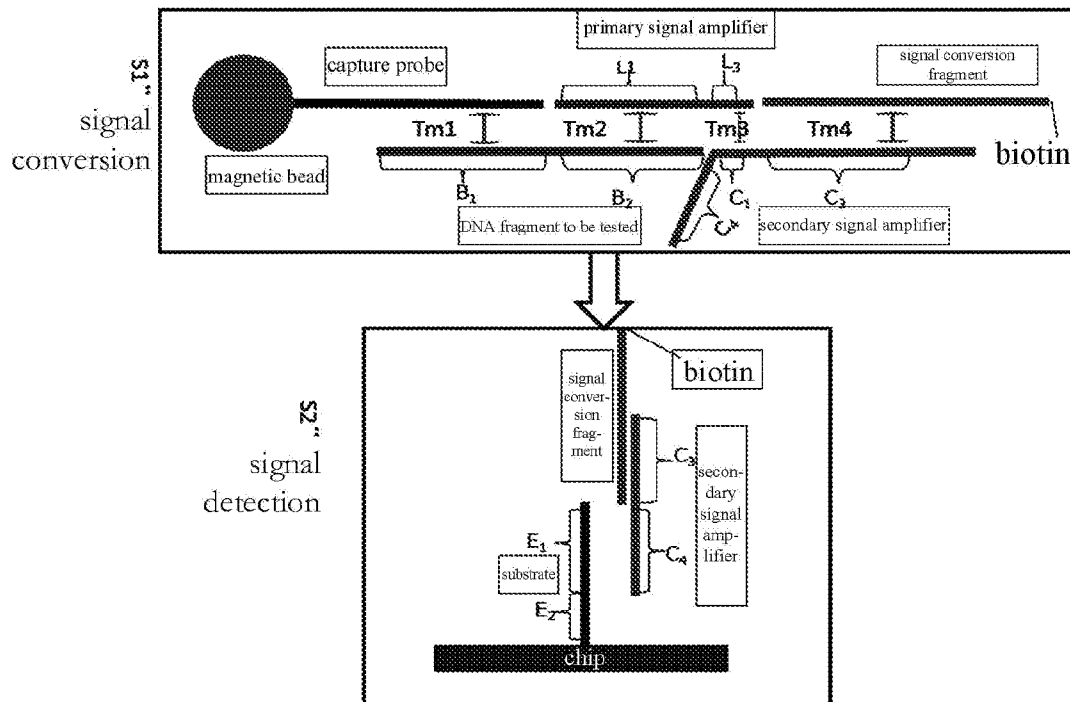


Fig. 3

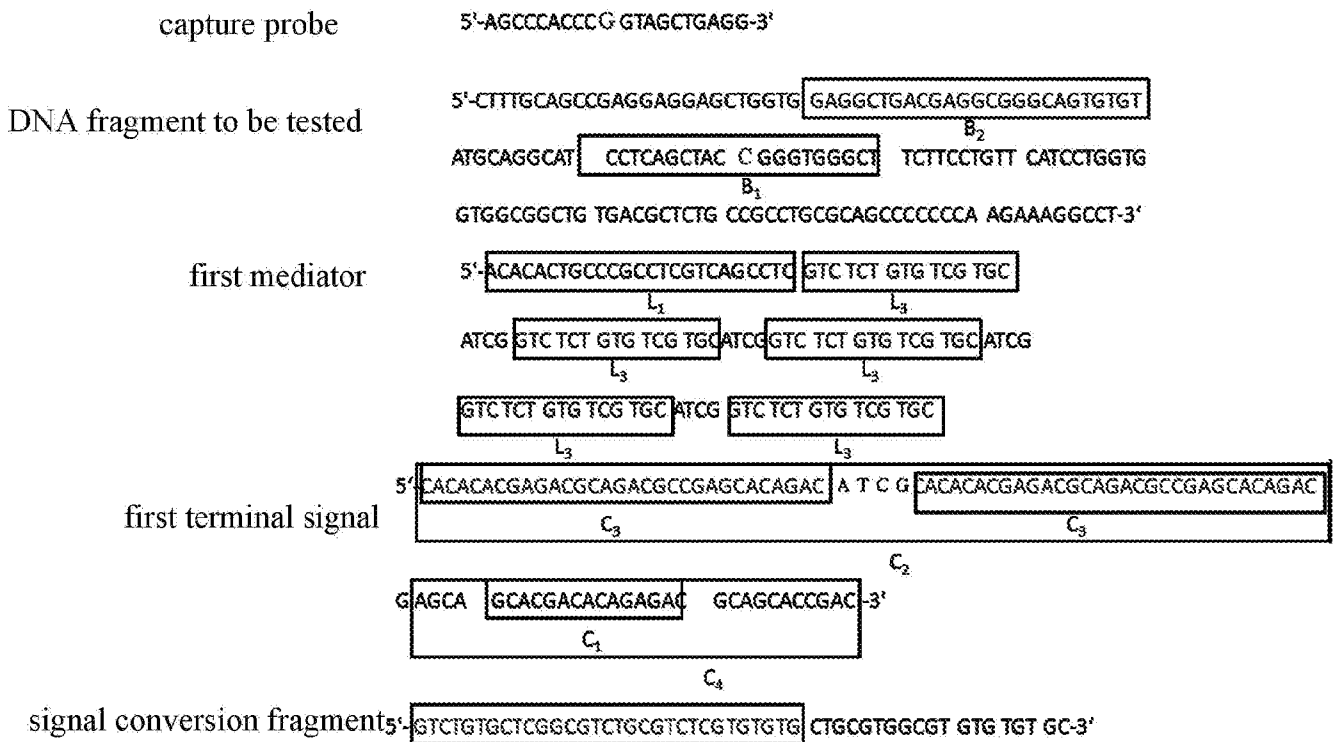


Fig. 4

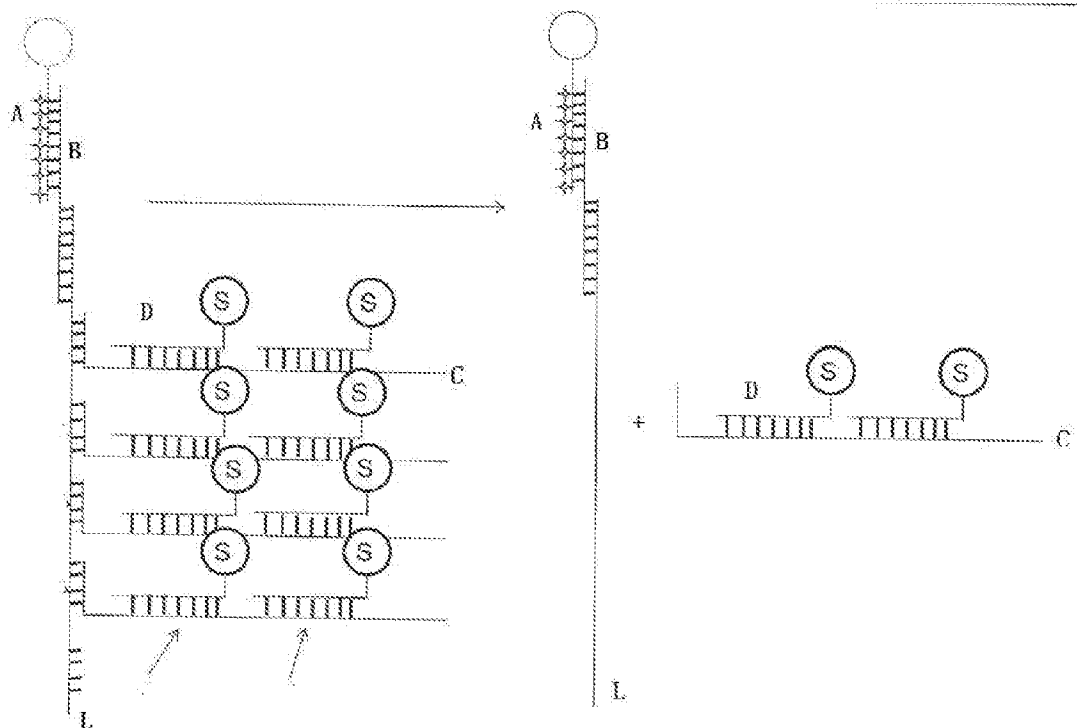


Fig. 5

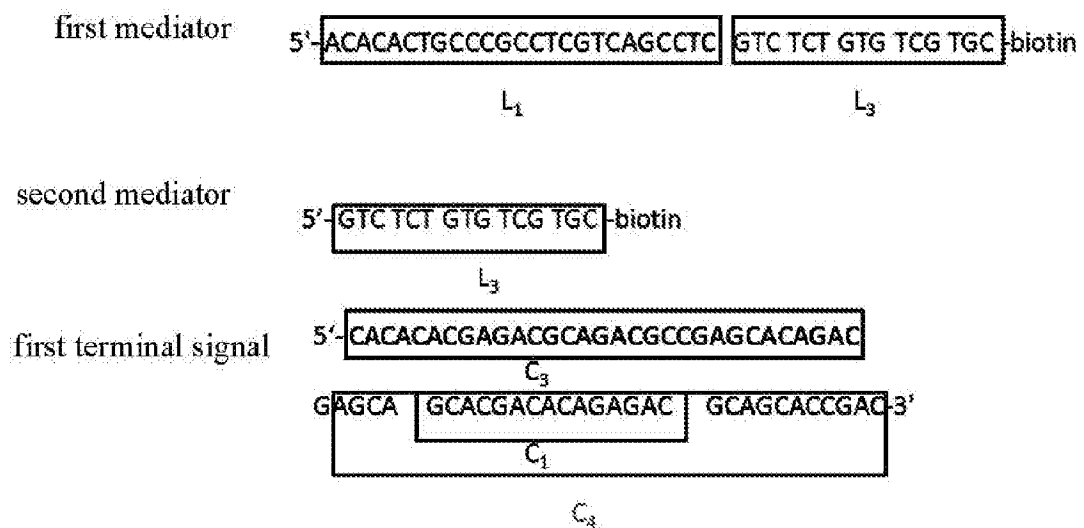


Fig. 6



Fig. 7

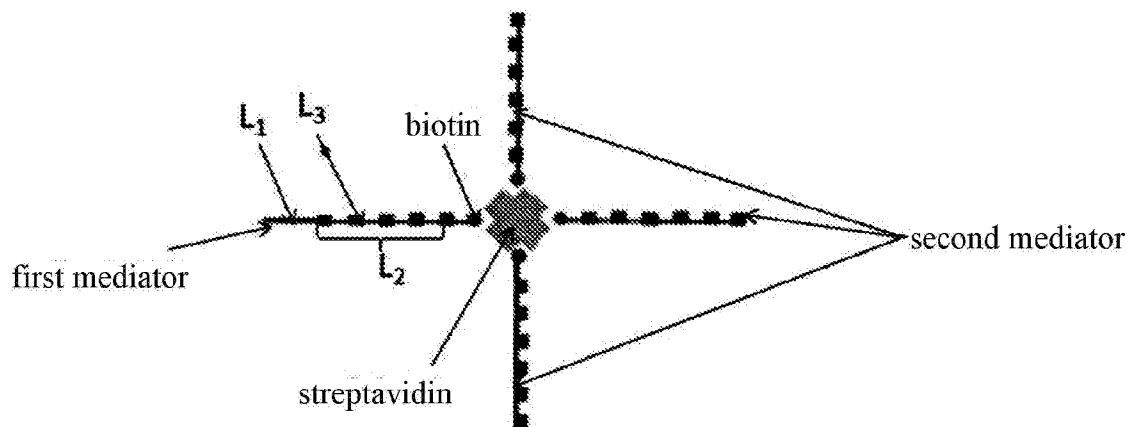


Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2019/055286

A. CLASSIFICATION OF SUBJECT MATTER		
Int.Cl. C12N15/09(2006.01)i, C12M1/00(2006.01)i, C12Q1/6813(2018.01)i, G01N33/53(2006.01)i, G01N37/00(2006.01)i		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Int.Cl. C12N15/09, C12M1/00, C12Q1/6813, G01N33/53, G01N37/00		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Published examined utility model applications of Japan 1922-1996 Published unexamined utility model applications of Japan 1971-2019 Registered utility model specifications of Japan 1996-2019 Published registered utility model applications of Japan 1994-2019		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0068696 A1 (CORNELL RESEARCH FOUNDATION, INC.) 2010.03.18, claims, Fig. 2B, examples & WO 2003/102541 A2 & EP 1512010 A1 & EP 1939627 A1	1-57
A	US 2018/0127803 A1 (QUANDX INC.) 2018.05.10, claims, FIG. 1, examples & WO 2003/102541 A2 & EP 1512010 A1 & EP 1939627 A1	1-57
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
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03.09.2019		01.10.2019
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