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(54) **SERUM/PLASMA MICRONAS AND USES THEREOF**

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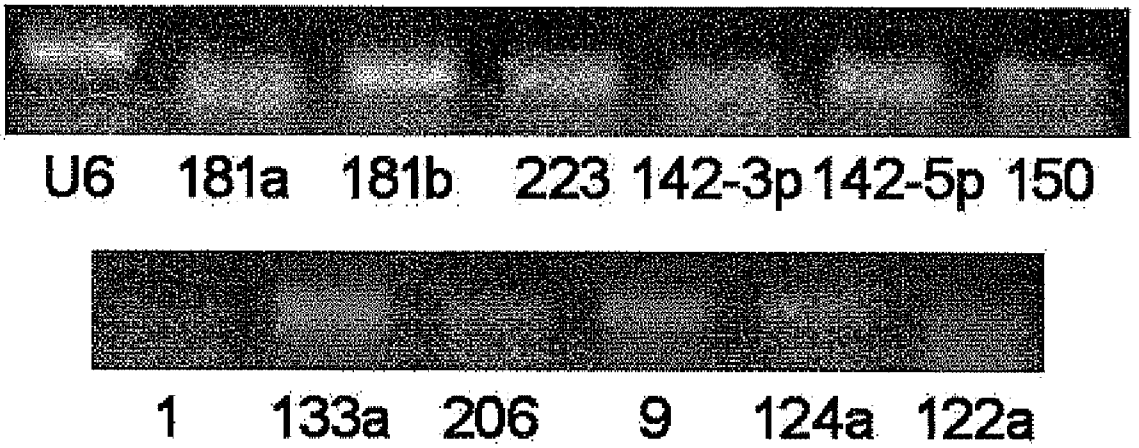
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(57) **ABSTRACT**

This invention provides a combination of microRNAs for evaluating the physiological and/or pathological condition of a subject, wherein the combination comprises all detectable microRNAs stably existing in the serum/plasma of a subject; and a method for evaluating the physiological and/or pathological condition of a subject, wherein the method includes determining all detectable microRNAs stably existing in the serum/plasma of a subject; and a kit for evaluating the physiological and/or pathological condition of a subject, wherein the kit contains the tools for determining all detectable microRNAs that stably existing in the serum/plasma of a subject; and a biochip for evaluating the physiological and/or pathological condition of a subject, wherein the biochip contains the components for determining all detectable microRNAs stably existing in the serum/plasma of a subject. The aforementioned combination, method, kit and biochip can be used for diagnosis as well as differentially diagnosis of diseases including various tumors; various acute/chronic infectious diseases, e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute/chronic infectious diseases caused by various pathogenic microorganisms; other acute/chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardiocerebrovascular diseases, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system, prediction of complications occurrence and malignant diseases relapse, evaluation of therapeutic effects, screening of pharmaceutical active ingredients, assessment of drug efficacy as well as forensic authentication and prohibited drug inspection and the like, possessing a number of advantages such as extensive detection spectrum, high sensitivity, low cost, convenience for sampling, ease for sample preservation, etc. The said method can be widely used in work related to general survey of diseases and so on, improve the low-specificity and low-sensitivity caused by individual differences which single markers are difficult to overcome, significantly increasing the clinical detection rate of diseases, all of which make it become an effective means for diagnosing diseases in an early phase.



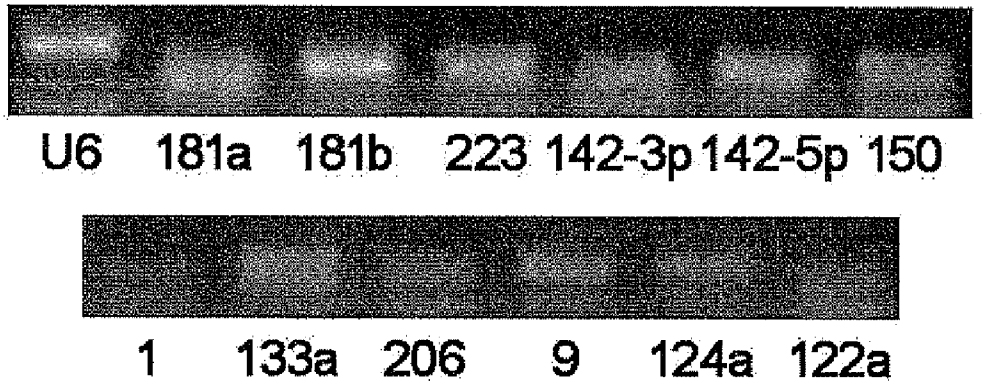

U6 181a 181b 223 142-3p 142-5p 150
1 133a 206 9 124a 122a

Fig. 1

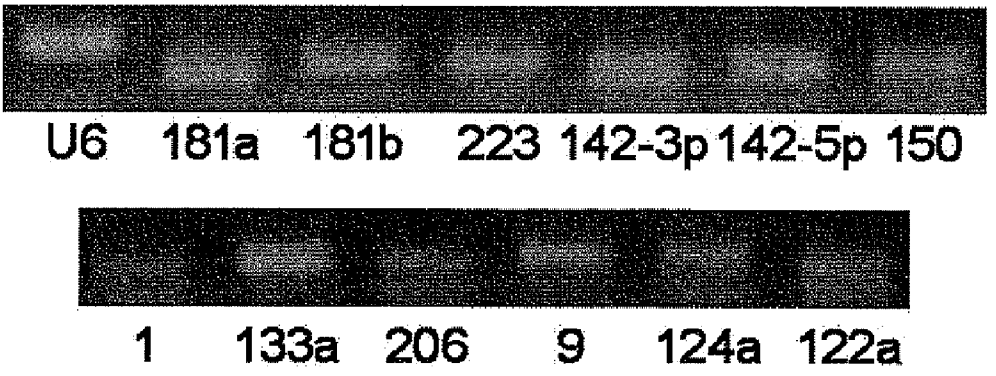

U6 181a 181b 223 142-3p 142-5p 150
1 133a 206 9 124a 122a

Fig. 2

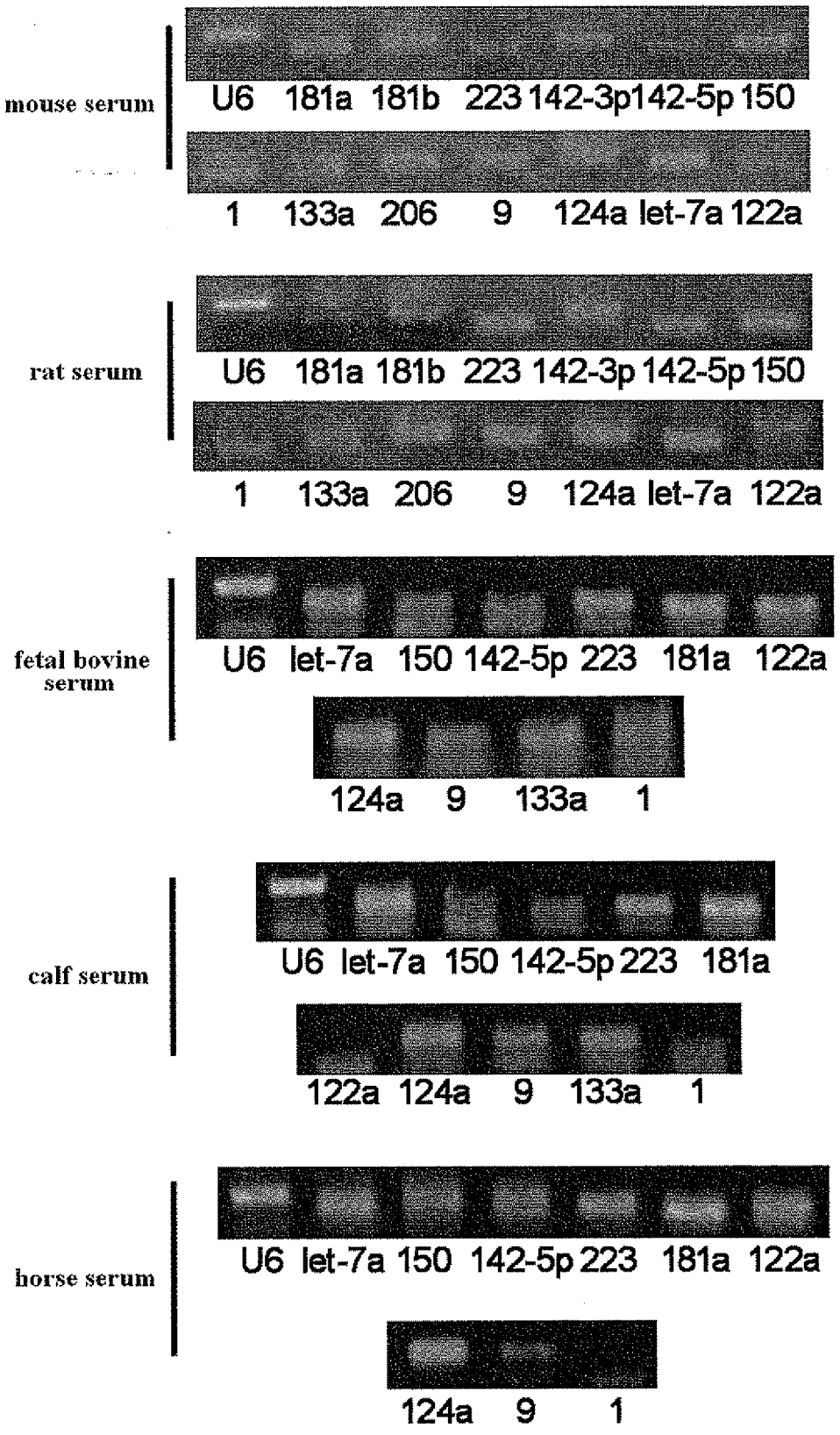

mouse serum
U6 181a 181b 223 142-3p 142-5p 150
1 133a 206 9 124a let-7a 122a
rat serum
U6 181a 181b 223 142-3p 142-5p 150
1 133a 206 9 124a let-7a 122a
fetal bovine serum
U6 let-7a 150 142-5p 223 181a 122a
124a 9 133a 1
calf serum
U6 let-7a 150 142-5p 223 181a
122a 124a 9 133a 1
horse serum
U6 let-7a 150 142-5p 223 181a 122a
124a 9 1

Fig. 3

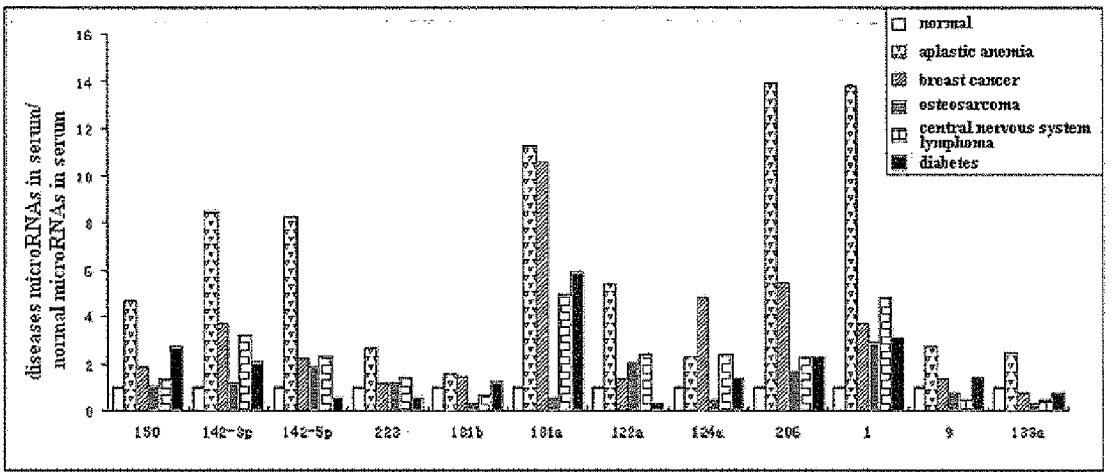

diseases microRNAs in serum/
normal microRNAs in serum
16
14
12
10
8
6
4
2
0
150
142-3p
142-5p
223
181b
181a
122a
124a
206
1
9
133a
normal
aplastic anemia
breast cancer
osteosarcoma
central nervous system lymphoma
diabetes

Fig. 4

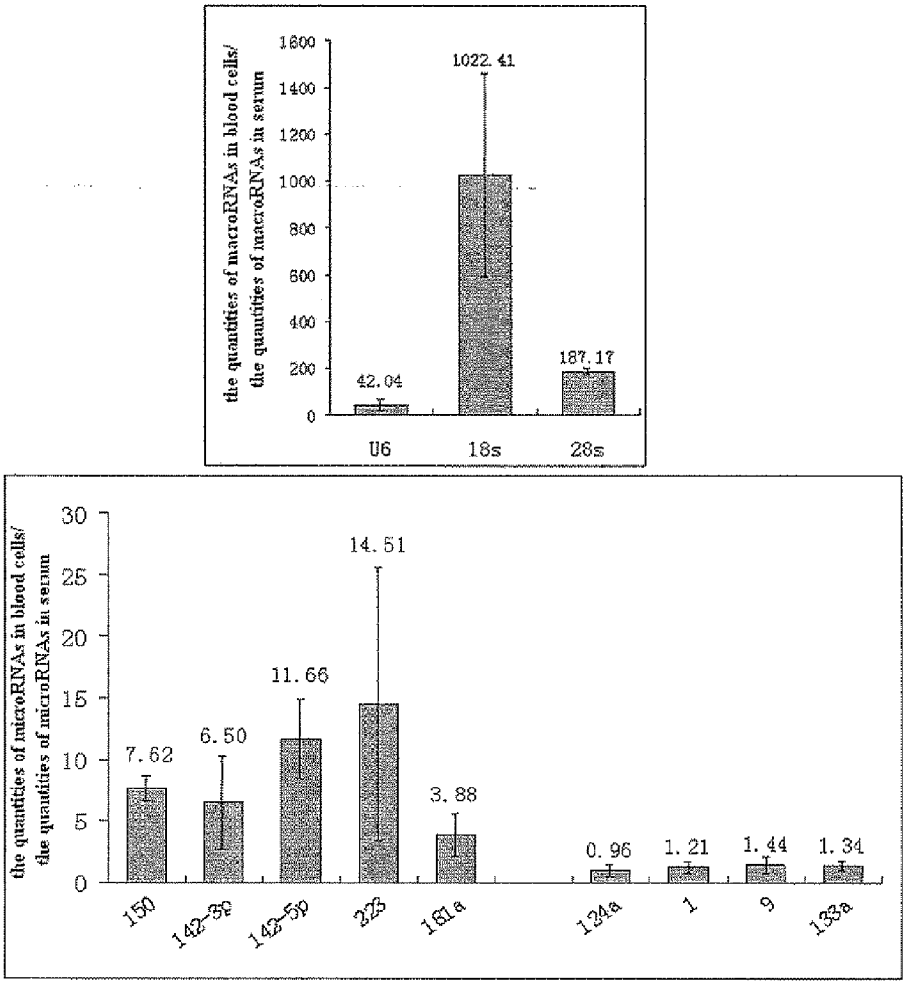

the quantities of macroRNAs in blood cells/ the quantities of macroRNAs in serum
1600
1400
1200
1000
800
600
400
200
0
42.04
1022.41
187.17
U6
18s
28s
the quantities of microRNAs in blood cells/ the quantities of microRNAs in serum
30
25
20
15
10
5
0
7.62
6.50
11.66
14.51
3.88
0.96
1.21
1.44
1.34
150
142-3p
142-5p
223
181a
124a
1
9
133a

Fig. 5

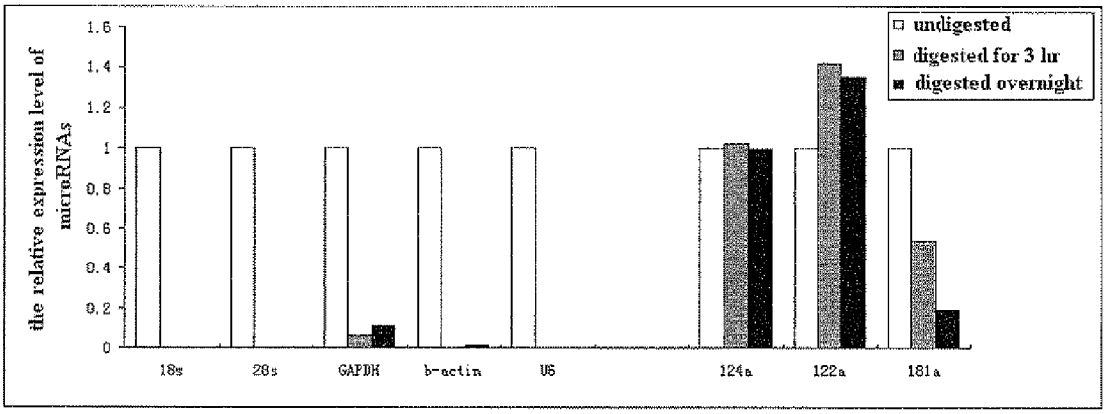

the relative expression level of microRNAs
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
undigested
digested for 3 hr
digested overnight
18s
28s
GAPDH
b-actin
U6
124a
122a
181a

Fig. 6

# SERUM/PLASMA MICRONAS AND USES THEREOF

## TECHNICAL FIELD

**[0001]** The present invention relates to microRNAs and uses thereof, more specifically, to serum/plasma microRNAs and the uses of serum/plasma microRNAs for diagnosis and differential diagnosis of diseases, prediction of complication occurrence and malignant disease relapse, evaluation of therapeutic effects, screening of pharmaceutical active ingredients, assessment of drug efficacy, forensic authentication and prohibited drug inspection and the like.

## BACKGROUND ART

**[0002]** To locate and precisely detect disease markers has already been the important precondition for the diagnosis and treatment of various clinical diseases including various tumors; various acute/chronic infectious diseases, e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute/chronic infectious diseases caused by various pathogenic microorganisms; other acute/chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular diseases, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system. Although more and more disease markers have been found and utilized in general survey and diagnosis of clinical diseases as well as monitoring and controlling of therapeutic effects, their clinical application effects are obviously insufficient. For instance, tumor marker, e.g. alphafetoprotein, lactic dehydrogenase and carcinoembryonic antigen have been widely used in clinic. But these disease markers are far from meeting the needs of early diagnosis for cancer for the following two main reasons: (1) the sensitivity and specificity for the above-mentioned disease markers are relatively low, thus their detection results cannot be used as a diagnostic indicator of disease; (2) the early diagnosis rate of disease shall be positively correlative with the therapeutic effects. However, it is difficult for any of the aforesaid disease markers to meet such requirements for early diagnosis. Take cancer for example, the specificity of tumor differentiation is too high, the integrated sensitivity of tumor is relatively low, the samples sent to be detected are difficult to be repeatedly taken and the conditions to meet the preservation requirements for samples are too exacting, meanwhile, the cost is very high, thus under existing technology the spreading and use of the tumor markers available are hard to realize. The inherent defects of some traditional medical means such as biopsy, for example, incorrect material-extraction position, the inadequacy of sample materials for histocytes and human inexperience, etc., will all lead to misdiagnosis. Although other techniques such as imaging technique have been widely used for examination and diagnosis of diseases, there exists considerable limitation on the determination for disease degree. Consequently, it is very necessary to find out a maker for disease detection which is novel, sensitive and convenient to use and can also overcome the defects of existing markers as mentioned above.

**[0003]** MicroRNAs are defined as a kind of non-coding single-stranded small RNA moleculars of approximately from 19 to 23 nucleotides in length. They are highly conservative in evolution; and are closely related to many normal physiological activities of animals such as development process, tissue differentiation, cell apoptosis and energy metabolism; in addition, bear close relation with the occurrence and development of many diseases. Recent studies show that the expression levels of several microRNAs in chronic lymphocytic leukemia and Burkitt lymphoma are on average down-regulated to various extents; and that by analyzing and comparing the expressions of microRNAs in tissues of human lung cancer and human breast cancer, the expression levels of several tissue specific microRNAs have changed relative to normal tissues. Some studies demonstrate that microRNAs affect the occurrence and development of cardio-cerebrovascular diseases such as myocardial hypertrophy, heart failure, atherosclerosis, and are closely relative to metabolic diseases such as Diabetes II. These experimental results indicate that there exists inevitable connection between the expression and specificity changes of microRNAs and the occurrence and development of diseases.

**[0004]** For the unimaginable important role microRNAs played in the regulation of expression after gene transcription, microRNAs have some associations with diseases. First of all, the changes of microRNAs may be the cause of diseases. This is because both the inhibitor and the promoter of diseases may be target sites for microRNAs. If the expression of microRNA itself is disturbed, e.g., the expression level of microRNA which is originally to inhibit disease promoters decreases or the expression level of microRNA which is to inhibit disease inhibitor increases, its end results will both lead to changes in the expression of downstream genes and the overall disorder of some pathways, further inducing the occurrence of diseases. Secondly, the changes of microRNAs may also result from diseases. This is because, when a kind of disease such as cancer occurs, it will lead to the loss of chromosome segments, gene mutation or rapid amplification of chromosome segments; moreover, if the microRNAs happen to locate in the changing segment, then their expression level will extremely significantly change. Therefore, in theory, microRNAs can be completely regarded as a kind of new disease markers, the specificity changes of which inevitably correlate with the occurrence and development of diseases. Meanwhile, microRNA can also be used as a potential drug target, and it may greatly alleviate the occurrence and development of diseases by inhibiting the up-regulated microRNAs and overexpressedly down-regulated microRNAs in the course of a disease.

**[0005]** The inventor has carried out the research in the relevant fields of using microRNAs as disease markers, for instance, choosing colonic carcinoma which ranks forth in the incidence of malignant tumor as the research object. The research suggests that, during the process of colon benign polyps developing into malignant tumor, some microRNAs exhibit specificity changes, thereby a more sensitive and accurate method for the early diagnosis of colonic carcinoma having been set up through detecting the specific changes in microRNAs. However, since the sampling for tissue specimen is not easy, the wide application of this method in clinics is limited.

## DETAILED DESCRIPTION OF THE INVENTION

**[0006]** The inventor focuses the research on the blood which is relatively easy to obtain and even can be collected via routine physical examination. Blood will circulate to all

tissues in body and convey nutrients to cells whilst scavenging waste materials; therefore, blood is able to reflect the physiological pathology of the whole organism and its detection results is an indicator of human health. It is known that in serum/plasma there are many kinds of proteins such as total protein, albumin and globulin, many kinds of lipids such as HDL cholesterol and triglycerides, many kinds of carbohydrates, pigments, electrolytes, inorganic salts, and many kinds of enzymes such as amylase, alkaline phosphatase, acid phosphatase, cholinesterase and aldolase; moreover, there also exist many kinds of signaling molecules such as cytokines and hormone from tissues and organs in whole body. Currently, disease diagnosis is only limited to the above-mentioned biochemical indexes in serum/plasma, and no report is available regarding microRNAs in serum/plasma. It traditionally believed that there is no microRNA in serum/plasma, and that, if any, it will be rapidly degraded by RNase into small molecule segments and hence cannot be detected. However, microRNAs, consisting of from 19 to 23 nucleotides, possess specificity and relative stability in structure and hence are very likely present in serum/plasma. Meanwhile, since microRNAs are a new type of disease markers, it is anticipated that by studying whether or not microRNAs are present in serum/plasma, whether or not they can be detected and the connection between microRNAs and diseases, a new technology is established for the early disease diagnosis, disease identification as well as monitoring and controlling of course of diseases, prediction of malignant disease relapse and prognosis and complication occurrence, assessment of drug efficacy, guide of medication, individualized treatment, screening of active ingredients of Chinese Traditional Medicines, population taxonomy, etc., by use of the microRNAs stably existing in serum/plasma as well as their specificity changes.

**[0007]** The present invention provides a combination of microRNAs for evaluating physiological and/or pathological condition in a subject, wherein the combination comprises all detectable microRNAs stably existing in the serum/plasma of the subject.

**[0008]** The present invention further provides a method for evaluating physiological and/or pathological condition in a subject, wherein the method comprises determining all detectable microRNAs stably existing in the serum/plasma of the subject.

**[0009]** In the above-mentioned combination or method, all detectable microRNAs stably existing in serum/plasma of a subject may be all mature microRNAs in human serum/plasma, specifically include let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-100, miR-101, miR-103, miR-105, miR-106a, miR-106b, miR-107, miR-10a, miR-10b, miR-122a, miR-124a, miR-125a, miR-125b, miR-126, miR-126*, miR-127, miR-128a, miR-128b, miR-129, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-134, miR-135a, miR-135b, miR-136, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-144, miR-145, miR-146a, miR-146b, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-153, miR-154, miR-154*, miR-155, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-181a, miR-181b, miR-181c, miR-181d, miR-182, miR-182*, miR-183, miR-184, miR-185, miR-186, miR-187, miR-188, miR-189, miR-18a, miR-18a*, miR-18b, miR-190, miR-191, miR-191*, miR-192, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a, miR-199a*, miR-199b, miR-19a, miR-19b, miR-200a, miR-200a*, miR-200b, miR-200c, miR-202, miR-202*, miR-203, miR-204, miR-205, miR-206, miR-208, miR-20a, miR-20b, miR-21, miR-210, miR-211, miR-212, miR-213, miR-214, miR-215 miR-216, miR-217, miR-218, miR-219, miR-22, miR-220, miR-221 miR-222, miR-223, miR-224, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-27b, miR-28, miR-296, miR-299-3p, miR-299-5p, miR-29a, miR-29b, miR-29c, miR-301, miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302c*, miR-302d, miR-30a-3p, miR-30a-5p, miR-30b, miR-30e, miR-30d, miR-30e-3p, miR-30e-5p, miR-32, miR-320, miR-323, miR-324-3p, miR-324-5p, miR-325, miR-326, miR-328, miR-329, miR-33, miR-330, miR-331, miR-335, miR-337, miR-338, miR-339, miR-33b, miR-340, miR-342, miR-345, miR-346, miR-34a, miR-34b, miR-34c, miR-361, miR-362, miR-363, miR-363*, miR-365, miR-367, miR-368, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-372, miR-373, miR-373*, miR-374, miR-375, miR-376a, miR-376a*, miR-376b, miR-377, miR-378, miR-379, miR-380-3p, miR-380-5p, miR-381, miR-382, miR-383, miR-384, miR-409-3p, miR-409-5p, miR-410, miR-411, miR-412, miR-421, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-425-5p, miR-429, miR-431, miR-432, miR-432*, miR-433, miR-448, miR-449, miR-450, miR-451, miR-452, miR-452*, miR-453, miR-455, miR-483, miR-484, miR-485-3p, miR-485-5p, miR-486, miR-487a, miR-487b, miR-488, miR-489, miR-490, miR-491, miR-492, miR-493, miR-493-3p, miR-494, miR-495, miR-496, miR-497, miR-498, miR-499, miR-500, miR-501, miR-502, miR-503, miR-504, miR-505, miR-506, miR-507, miR-508, miR-509, miR-510, miR-511, miR-512-3p, miR-512-5p, miR-513, miR-514, miR-515-3p, miR-515-5p, miR-516-3p, miR-516-5p, miR-517*, miR-517a, miR-517b, miR-517c, miR-518a, miR-518a-2*, miR-518b, miR-518c, miR-518c*, miR-518d, miR-518e, miR-518f, miR-518f*, miR-519a, miR-519b, miR-519c, miR-519d, miR-519e, miR-519e*, miR-520a, miR-520a*, miR-520b, miR-520c, miR-520d, miR-520d*, miR-520e, miR-520f, miR-520g, miR-520h, miR-521, miR-522, miR-523, miR-524, miR-524*, miR-525, miR-525*, miR-526a, miR-526b, miR-526b*, miR-526c, miR-527, miR-532, miR-542-3p, miR-542-5p, miR-544, miR-545, miR-548a, miR-548b, miR-548c, miR-548d, miR-549, miR-550, miR-551a, miR-552, miR-553, miR-554, miR-555, miR-556, miR-557, miR-558, miR-559, miR-560, miR-561, miR-562, miR-563, miR-564, miR-565, miR-566, miR-567, miR-568, miR-569, miR-570, miR-571, miR-572, miR-573, miR-574, miR-575, miR-576, miR-577, miR-578, miR-579, miR-580, miR-581, miR-582, miR-583, miR-584, miR-585, miR-586, miR-587, miR-588, miR-589, miR-590, miR-591, miR-592, miR-593, miR-594, miR-595, miR-596, miR-597, miR-598, miR-599, miR-600, miR-601, miR-602, miR-603, miR-604, miR-605, miR-606, miR-607, miR-608, miR-609, miR-610, miR-611, miR-612, miR-613, miR-614, miR-615, miR-616, miR-617, miR-618, miR-619, miR-620, miR-621, miR-622, miR-623, miR-624, miR-625, miR-626, miR-627, miR-628, miR-629, miR-630, miR-631, miR-632, miR-633, miR-634, miR-635, miR-636, miR-637, miR-638, miR-639, miR-640, miR-641, miR-642, miR-643, miR-644, miR-645, miR-646, miR-647, miR-648, miR-649, miR-650, miR-651, miR-652, miR-653, miR-654, miR-655, miR-656, miR-657, miR-658, miR-659, miR-660, miR-661, miR-662, miR-663, miR-7, miR-9, miR-9*, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99 and miR-99b.

**[0010]** The aforesaid method for determining all detectable microRNAs stably existing in serum/plasma of a subject is one or more selected from the group consisting of RT-PCR method, Real-time PCR method, Northern blotting method, RNase protection assay, Solexa sequencing technology and biochip method.

**[0011]** The aforesaid RT-PCR method includes the following steps:

**[0012]** (1) extracting the total RNA from the serum/plasma of a subject and obtaining cDNA samples by RNA reverse transcription reaction; or collecting serum/plasma samples from the subject and conducting reverse transcription reaction with serum/plasma being a buffer so as to prepare cDNA samples;

**[0013]** (2) designing a primer by use of microRNAs and conducting PCR reaction;

**[0014]** (3) conducting agarose gel electrophoresis of PCR products;

**[0015]** (4) observing agarose gel under ultraviolet lamp after EB staining.

**[0016]** The aforesaid real-time PCR method includes the following steps:

**[0017]** (1) extracting the total RNA from the serum/plasma of a subject and obtaining cDNA samples by RNA reverse transcription reaction; or collecting serum/plasma samples from the subject and conducting reverse transcription reaction with serum/plasma being a buffer so as to prepare cDNA samples;

**[0018]** (2) designing a primer by use of microRNAs;

**[0019]** (3) adding a fluorescent probe to conduct PCR reaction;

**[0020]** (4) detecting and comparing the variation in levels of microRNAs in the serum/plasma relative to those of microRNAs in normal serum/plasma.

**[0021]** The present invention further provides a kit for evaluating physiological and/or pathological condition of a subject, wherein the kit comprises the tools for determining all detectable microRNAs stably existing in the serum/plasma of the subject. The kit may comprises the primers of all mature microRNAs in human serum/plasma, specifically comprises the primers of let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-100, miR-101, miR-103, miR-105, miR-106a, miR-106b, miR-107, miR-10a, miR-10b, miR-122a, miR-124a, miR-125a, miR-125b, miR-126, miR-126*, miR-127, miR-128a, miR-128b, miR-129, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-134, miR-135a, miR-135b, miR-136, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-144, miR-145, miR-146a, miR-146b, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-153, miR-154, miR-154*, miR-155, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-181a, miR-181b, miR-181c, miR-181d, miR-182, miR-182*, miR-183, miR-184, miR-185, miR-186, miR-187, miR-188, miR-189, miR-18a, miR-18a*, miR-18b, miR-190, miR-191, miR-191*, miR-192, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a, miR-199a*, miR-199b, miR-19a, miR-19b, miR-200a, miR-200a*, miR-200b, miR-200c, miR-202, miR-202*, miR-203, miR-204, miR-205, miR-206, miR-208, miR-20a, miR-20b, miR-21, miR-210, miR-211, miR-212, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-219, miR-22, miR-220, miR-221, miR-222, miR-223, miR-224, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-27b, miR-28, miR-296, miR-299-3p, miR-299-5p, miR-29a, miR-29b, miR-29c, miR-301, miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302c*, miR-302d, miR-30a-3p, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-3p, miR-30e-5p, miR-32, miR-320, miR-323, miR-324-3p, miR-324-5p, miR-325, miR-326, miR-328, miR-329, miR-33, miR-330, miR-331, miR-335, miR-337, miR-338, miR-339, miR-33b, miR-340, miR-342, miR-345, miR-346, miR-34a, miR-34b, miR-34c, miR-361, miR-362, miR-363, miR-363*, miR-365, miR-367, miR-368, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-372, miR-373, miR-373*, miR-374, miR-375, miR-376a, miR-376a*, miR-376b, miR-377, miR-378, miR-379, miR-380-3p, miR-380-5p, miR-381, miR-382, miR-383, miR-384, miR-409-3p, miR-409-5p, miR-410, miR-411, miR-412, miR-421, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-425-5p, miR-429, miR-431, miR-432, miR-432*, miR-433, miR-448, miR-449, miR-450, miR-451, miR-452, miR-452*, miR-453, miR-455, miR-483, miR-484, miR-485-3p, miR-485-5p, miR-486, miR-487a, miR-487b, miR-488, miR-489, miR-490, miR-491, miR-492, miR-493, miR-493-3p, miR-494, miR-495, miR-496, miR-497, miR-498, miR-499, miR-500, miR-501, miR-502, miR-503, miR-504, miR-505, miR-506, miR-507, miR-508, miR-509, miR-510, miR-511, miR-512-3p, miR-512-5p, miR-513, miR-514, miR-515-3p, miR-515-5p, miR-516-3p, miR-516-5p, miR-517*, miR-517a, miR-517b, miR-517c, miR-518a, miR-518a-2*, miR-518b, miR-518c, miR-518c*, miR-518d, miR-518e, miR-518f, miR-518f*, miR-519a, miR-519b, miR-519c, miR-519d, miR-519e, miR-519e*, miR-520a, miR-520a*, miR-520b, miR-520c, miR-520d, miR-520d*, miR-520e, miR-520f, miR-520g, miR-520h, miR-521, miR-522, miR-523, miR-524, miR-524*, miR-525, miR-525*, miR-526a, miR-526b, miR-526b* miR-526c, miR-527, miR-532, miR-542-3p, miR-542-5p, miR-544, miR-545, miR-548a, miR-548b, miR-548c, miR-548d, miR-549, miR-550, miR-551a, miR-552, miR-553, miR-554, miR-555, miR-556, miR-557, miR-558, miR-559, miR-560, miR-561, miR-562, miR-563, miR-564, miR-565, miR-566, miR-567, miR-568, miR-569, miR-570, miR-571, miR-572, miR-573, miR-574, miR-575, miR-576, miR-577, miR-578, miR-579, miR-580, miR-581, miR-582, miR-583, miR-584, miR-585, miR-586, miR-587, miR-588, miR-589, miR-590, miR-591, miR-592, miR-593, miR-594, miR-595, miR-596, miR-597, miR-598, miR-599, miR-600, miR-601, miR-602, miR-603, miR-604, miR-605, miR-606, miR-607, miR-608, miR-609, miR-610, miR-611, miR-612, miR-613, miR-614, miR-615, miR-616, miR-617, miR-618, miR-619, miR-620, miR-621, miR-622, miR-623, miR-624, miR-625, miR-626, miR-627, miR-628, miR-629, miR-630, miR-631, miR-632, miR-633, miR-634, miR-635, miR-636, miR-637, miR-638, miR-639, miR-640, miR-641, miR-642, miR-643, miR-644, miR-645, miR-646, miR-647, miR-648, miR-649, miR-650, miR-651, miR-652, miR-653, miR-654, miR-655, miR-656, miR-657, miR-658, miR-659, miR-660, miR-661, miR-662, miR-663, miR-7, miR-9, miR-9*, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99a and miR-99b.

**[0022]** The present invention also provides a biochip for evaluating physiological and/or pathological condition of a subject, wherein the biochip contains the components for determining all detectable microRNAs stably existing in the serum/plasma of the subject. The biochip may also contain

the probes for all mature microRNAs in human serum/plasma. The probes specifically include the probes as shown in Table 1.

TABLE 1

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-let-7a | let-7a | AACTATACAACCTACTACCTCA |
| probe-let-7b | let-7b | AACCACACAACCTACTACCTCA |
| probe-let-7c | let-7c | AACCATACAACCTACTACCTCA |
| probe-let-7d | let-7d | ACTATGCAACCTACTACCTCT |
| probe-let-7e | let-7e | ACTATACAACCTCCTACCTCA |
| probe-let-7f | let-7f | AACTATACAATCTACTACCTCA |
| probe-let-7g | let-7g | ACTGTACAAACTACTACCTCA |
| probe-let-7i | let-7i | ACAGCACAAACTACTACCTCA |
| probe-miR-1 | miR-1 | TACATACTTCTTTACATTCCA |
| probe-miR-100 | miR-100 | CACAAGTTCGGATCTACGGGTT |
| probe-miR-101 | miR-101 | CTTCAGTTATCACAGTACTGTA |
| probe-miR-103 | miR-103 | TCATAGCCCTGTACAATGCTGCT |
| probe-miR-105 | miR-105 | ACAGGAGTCTGAGCATTTGA |
| probe-miR-106a | miR-106a | GCTACCTGCACTGTAAGCACTTTT |
| probe-miR-106b | miR-106b | ATCTGCACTGTCAGCACTTTA |
| probe-miR-107 | miR-107 | TGATAGCCCTGTACAATGCTGCT |
| probe-miR-10a | miR-10a | CACAAATTCGGATCTACAGGGTA |
| probe-miR-10b | miR-10b | ACAAATTCGGTTCTACAGGGTA |
| probe-miR-122a | miR-122a | ACAAACACCATTGTCACACTCCA |
| probe-miR-124a | miR-124a | TGGCATTCACCGCGTGCCTTAA |
| probe-miR-125a | miR-125a | CACAGGTTAAAGGGTCTCAGGGA |
| probe-miR-125b | miR-125b | TCACAAGTTAGGGTCTCAGGGA |
| probe-miR-126 | miR-126 | GCATTATTACTCACGGTACGA |
| probe-miR-126* | miR-126* | CGCGTACCAAAAGTAATAATG |
| probe-miR-127 | miR-127 | AGCCAAGCTCAGACGGATCCGA |
| probe-miR-128a | miR-128a | AAAAGAGACCGGTTCACTGTGA |
| probe-miR-128b | miR-128b | GAAAGAGACCGGTTCACTGTGA |
| probe-miR-129 | miR-129 | GCAAGCCCAGACCGCAAAAAG |
| probe-miR-130a | miR-130a | ATGCCCTTTTAACATTGCACTG |
| probe-miR-130b | miR-130b | ATGCCCTTTCATCATTGCACTG |
| probe-miR-132 | miR-132 | CGACCATGGCTGTAGACTGTTA |
| probe-miR-133a | miR-133a | ACAGCTGGTTGAAGGGGACCAA |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-133b | miR-133b | TAGCTGGTTGAAGGGGACCAA |
| probe-miR-134 | miR-134 | CCCTCTGGTCAACCAGTCACA |
| probe-miR-135a | miR-135a | TCACATAGGAATAAAAAGCCATA |
| probe-miR-135b | miR-135b | CACATAGGAATGAAAAGCCATA |
| probe-miR-136 | miR-136 | TCCATCATCAAAACAAATGGAGT |
| probe-miR-137 | miR-137 | CTACGCGTATTCTTAAGCAATA |
| probe-miR-138 | miR-138 | GATTCACAACACCAGCT |
| probe-miR-139 | miR-139 | AGACACGTGCACTGTAGA |
| probe-miR-140 | miR-140 | CTACCATAGGGTAAAACCACT |
| probe-miR-141 | miR-141 | CCATCTTTACCAGACAGTGTTA |
| probe-miR-142-3p | miR-142-3p | TCCATAAAGTAGGAAACACTACA |
| probe-miR-142-5p | miR-142-5p | GTAGTGCTTTCTACTTTATG |
| probe-miR-143 | miR-143 | TGAGCTACAGTGCTTCATCTCA |
| probe-miR-144 | miR-144 | CTAGTACATCATCTATACTGTA |
| probe-miR-145 | miR-145 | AAGGGATTCCTGGGAAAACTGGAC |
| probe-miR-146a | miR-146a | AACCCATGGAATTCAGTTCTCA |
| probe-miR-146b | miR-146b | AGCCTATGGAATTCAGTTCTCA |
| probe-miR-147 | miR-147 | GCAGAAGCATTTCCACACAC |
| probe-miR-148a | miR-148a | ACAAAGTTCTGTAGTGCACTGA |
| probe-miR-148b | miR-148b | ACAAAGTTCTGTGATGCACTGA |
| probe-miR-149 | miR-149 | GGAGTGAAGACACGGAGCCAGA |
| probe-miR-150 | miR-150 | CACTGGTACAAGGGTTGGGAGA |
| probe-miR-151 | miR-151 | CCTCAAGGAGCTTCAGTCTAGT |
| probe-miR-152 | miR-152 | CCCAAGTTCTGTCATGCACTGA |
| probe-miR-153 | miR-153 | TCACTTTTGTGACTATGCAA |
| probe-miR-154 | miR-154 | CGAAGGCAACACGGATAACCTA |
| probe-miR-154* | miR-154* | AATAGGTCAACCGTGTATGATT |
| probe-miR-155 | miR-155 | CCCCTATCACGATTAGCATTAA |
| probe-miR-15a | miR-15a | CACAAACCATTATGTGCTGCTA |
| probe-miR-15b | miR-15b | TGTAAACCATGATGTGCTGCTA |
| probe-miR-16 | miR-16 | CGCCAATATTTACGTGCTGCTA |
| probe-miR-17-3p | miR-17-3p | ACAAGTGCCTTCACTGCAGT |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-17-5p | miR-17-5p | ACTACCTGCACTGTAAGCACTTTG |
| probe-miR-181a | miR-181a | ACTCACCGACAGCGTTGAATGTT |
| probe-miR-181b | miR-181b | CCCACCGACAGCAATGAATGTT |
| probe-miR-181c | miR-181c | ACTCACCGACAGGTTGAATGTT |
| probe-miR-181d | miR-181d | AACCCACCGACAACAATGAATGTT |
| probe-miR-182 | miR-182 | TGTGAGTTCTACCATTGCCAAA |
| probe-miR-182* | miR-182* | TAGTTGGCAAGTCTAGAACCA |
| probe-miR-183 | miR-183 | CAGTGAATTCTACCAGTGCCATA |
| probe-miR-184 | miR-184 | ACCCTTATCAGTTCTCCGTCCA |
| probe-miR-185 | miR-185 | GAACTGCCTTTCTCTCCA |
| probe-miR-186 | miR-186 | AAGCCCAAAAGGAGAATTCTTTG |
| probe-miR-187 | miR-187 | CGGCTGCAACACAAGACACGA |
| probe-miR-188 | miR-188 | ACCCTCCACCATGCAAGGGATG |
| probe-miR-189 | miR-189 | ACTGATATCAGCTCAGTAGGCAC |
| probe-miR-18a | miR-18a | TATCTGCACTAGATGCACCTTA |
| probe-miR-18a* | miR-18a* | AGAAGGAGCACTTAGGGCAGT |
| probe-miR-18b | miR-18b | TAACTGCACTAGATGCACCTTA |
| probe-miR-190 | miR-190 | ACCTAATATATCAAACATATCA |
| probe-miR-191 | miR-191 | AGCTGCTTTTGGGATTCCGTTG |
| probe-miR-191* | miR-191* | GGGGACGAAATCCAAGCGCAGC |
| probe-miR-192 | miR-192 | GGCTGTCAATTCATAGGTCAG |
| probe-miR-193a | miR-193a | CTGGGACTTTGTAGGCCAGTT |
| probe-miR-193b | miR-193b | AAAGCGGGACTTTGAGGGCCAGTT |
| probe-miR-194 | miR-194 | TCCACATGGAGTTGCTGTTACA |
| probe-miR-195 | miR-195 | GCCAATATTTCTGTGCTGCTA |
| probe-miR-196a | miR-196a | CCAACAACATGAAACTACCTA |
| probe-miR-196b | miR-196b | CCAACAACAGGAAACTACCTA |
| probe-miR-197 | miR-197 | GCTGGGTGGAGAAGGTGGTGAA |
| probe-miR-198 | miR-198 | CCTATCTCCCCTCTGGACC |
| probe-miR-199a | miR-199a | GAACAGGTAGTCTGAACACTGGG |
| probe-miR-199a* | miR-199a* | AACCAATGTGCAGACTACTGTA |
| probe-miR-199b | miR-199b | GAACAGATAGTCTAAACACTGGG |
| probe-miR-19a | miR-19a | TCAGTTTTGCATAGATTTGCACA |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-19b | miR-19b | TCAGTTTTGCATGGATTTGCACA |
| probe-miR-200a | miR-200a | ACATCGTTACCAGACAGTGTTA |
| probe-miR-200a* | miR-200a* | TCCAGCACTGTCCGGTAAGATG |
| probe-miR-200b | miR-200b | GTCATCATTACCAGGCAGTATTA |
| probe-miR-200c | miR-200c | CCATCATTACCCGGCAGTATTA |
| probe-miR-202 | miR-202 | TTTTCCCATGCCCTATACCTCT |
| probe-miR-202* | miR-202* | AAAGAAGTATATGCATAGGAAA |
| probe-miR-203 | miR-203 | CTAGTGGTCCTAAACATTTCAC |
| probe-miR-204 | miR-204 | AGGCATAGGATGACAAAGGGAA |
| probe-miR-205 | miR-205 | CAGACTCCGGTGGAATGAAGGA |
| probe-miR-206 | miR-206 | CCACACACTTCCTTACATTCCA |
| probe-miR-208 | miR-208 | ACAAGCTTTTTGCTCGTCTTAT |
| probe-miR-20a | miR-20a | CTACCTGCACTATAAGCACTTTA |
| probe-miR-20b | miR-20b | CTACCTGCACTATGAGCACTTTG |
| probe-miR-21 | miR-21 | TCAACATCAGTCTGATAAGCTA |
| probe-miR-210 | miR-210 | TCAGCCGCTGTCACACGCACAG |
| probe-miR-211 | miR-211 | AGGCGAAGGATGACAAAGGGAA |
| probe-miR-212 | miR-212 | GGCCGTGACTGGAGACTGTTA |
| probe-miR-213 | miR-213 | GGTACAATCAACGGTCGATGGT |
| probe-miR-214 | miR-214 | CTGCCTGTCTGTGCCTGCTGT |
| probe-miR-215 | miR-215 | GTCTGTCAATTCATAGGTCAT |
| probe-miR-216 | miR-216 | CACAGTTGCCAGCTGAGATTA |
| probe-miR-217 | miR-217 | ATCCAATCAGTTCCTGATGCAGTA |
| probe-miR-218 | miR-218 | ACATGGTTAGATCAAGCACAA |
| probe-miR-219 | miR-219 | AGAATTGCGTTTGGACAATCA |
| probe-miR-22 | miR-22 | ACAGTTCTTCAACTGGCAGCTT |
| probe-miR-220 | miR-220 | AAAGTGTCAGATACGGTGTGG |
| probe-miR-221 | miR-221 | GAAACCCAGCAGACAATGTAGCT |
| probe-miR-222 | miR-222 | GAGACCCAGTAGCCAGATGTAGCT |
| probe-miR-223 | miR-223 | GGGGTATTTGACAAACTGACA |
| probe-miR-224 | miR-224 | TAAACGGAACCACTAGTGACTTG |
| probe-miR-23a | miR-23a | GGAAATCCCTGGCAATGTGAT |
| probe-miR-23b | miR-23b | GGTAATCCCTGGCAATGTGAT |
| probe-miR-24 | miR-24 | CTGTTCCTGCTGAACTGAGCCA |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-25 | miR-25 | TCAGACCGAGACAAGTGCAATG |
| probe-miR-26a | miR-26a | GCCTATCCTGGATTACTTGAA |
| probe-miR-26b | miR-26b | AACCTATCCTGAATTACTTGAA |
| probe-miR-27a | miR-27a | GCGGAACTTAGCCACTGTGAA |
| probe-miR-27b | miR-27b | GCAGAACTTAGCCACTGTGAA |
| probe-miR-28 | miR-28 | CTCAATAGACTGTGAGCTCCTT |
| probe-miR-296 | miR-296 | ACAGGATTGAGGGGGGGCCCT |
| probe-miR-299-3p | miR-299-3p | AAGCGGTTTACCATCCCACATA |
| probe-miR-299-5p | miR-299-5p | ATGTATGTGGGACGGTAAACCA |
| probe-miR-29a | miR-29a | AACCGATTTCAGATGGTGCTA |
| probe-miR-29b | miR-29b | AACACTGATTTCAAATGGTGCTA |
| probe-miR-29c | miR-29c | ACCGATTTCAAATGGTGCTA |
| probe-miR-301 | miR-301 | GCTTTGACAATACTATTGCACTG |
| probe-miR-302a | miR-302a | TCACCAAAACATGGAAGCACTTA |
| probe-miR-302a* | miR-302a* | AAAGCAAGTACATCCACGTTTA |
| probe-miR-302b | miR-302b | CTACTAAAACATGGAAGCACTTA |
| probe-miR-302b* | miR-302b* | AGAAAGCACTTCCATGTTAAAGT |
| probe-miR-302c | miR-302c | CCACTGAAACATGGAAGCACTTA |
| probe-miR-302c* | miR-302c* | CAGCAGGTACCCCCATGTTAAA |
| probe-miR-302d | miR-302d | ACACTCAAACATGGAAGCACTTA |
| probe-miR-30a-3p | miR-30a-3p | GCTGCAAACATCCGACTGAAAG |
| probe-miR-30a-5p | miR-30a-5p | CTTCCAGTCGAGGATGTTTACA |
| probe-miR-30b | miR-30b | AGCTGAGTGTAGGATGTTTACA |
| probe-miR-30c | miR-30c | GCTGAGAGTGTAGGATGTTTACA |
| probe-miR-30d | miR-30d | CTTCCAGTCGGGGATGTTTACA |
| probe-miR-30e-3p | miR-30e-3p | GCTGTAAACATCCGACTGAAAG |
| probe-miR-30e-5p | miR-30e-5p | TCCAGTCAAGGATGTTTACA |
| probe-miR-31 | miR-31 | CAGCTATGCCAGCATCTTGCC |
| probe-miR-32 | miR-32 | GCAACTTAGTAATGTGCAATA |
| probe-miR-320 | miR-320 | TTCGCCCTCTCAACCCAGCTTTT |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-323 | miR-323 | AGAGGTCGACCGTGTAATGTGC |
| probe-miR-324-3p | miR-324-3p | CCAGCAGCACCTGGGGCAGTGG |
| probe-miR-324-5p | miR-324-5p | ACACCAATGCCCTAGGGGATGCG |
| probe-miR-325 | miR-325 | ACACTTACTGGACACCTACTAGG |
| probe-miR-326 | miR-326 | CTGGAGGAAGGGCCCAGAGG |
| probe-miR-328 | miR-328 | ACGGAAGGGCAGAGAGGGCCAG |
| probe-miR-329 | miR-329 | AAAGAGGTTAACCAGGTGTGTT |
| probe-miR-33 | miR-33 | CAATGCAACTACAATGCAC |
| probe-miR-330 | miR-330 | TCTCTGCAGGCCGTGTGCTTTGC |
| probe-miR-331 | miR-331 | TTCTAGGATAGGCCCAGGGGC |
| probe-miR-335 | miR-335 | ACATTTTTCGTTATTGCTCTTGA |
| probe-miR-337 | miR-337 | AAAGGCATCATATAGGAGCTGGA |
| probe-miR-338 | miR-338 | TCAACAAAATCACTGATGCTGGA |
| probe-miR-339 | miR-339 | TGAGCTCCTGGAGGACAGGGA |
| probe-miR-33b | miR-33b | TGCAATGCAACAGCAATGCAC |
| probe-miR-340 | miR-340 | GGCTATAAAGTAACTGAGACGGA |
| probe-miR-342 | miR-342 | GACGGGTGCGATTTCTGTGTGAGA |
| probe-miR-345 | miR-345 | GCCCTGGACTAGGAGTCAGCA |
| probe-miR-346 | miR-346 | AGAGGCAGGCATGCGGGCAGACA |
| probe-miR-34a | miR-34a | AACAACCAGCTAAGACACTGCCA |
| probe-miR-34b | miR-34b | CAATCAGCTAATGACACTGCCTA |
| probe-miR-34c | miR-34c | GCAATCAGCTAACTACACTGCCT |
| probe-miR-361 | miR-361 | GTACCCCTGGAGATTCTGATAA |
| probe-miR-362 | miR-362 | CTCACACCTAGGTTCCAAGGATT |
| probe-miR-363 | miR-363 | TTACAGATGGATACCGTGCAAT |
| probe-miR-363* | miR-363* | AAATTGCATCGTGATCCACCCG |
| probe-miR-365 | miR-365 | ATAAGGATTTTTAGGGGCATTA |
| probe-miR-367 | miR-367 | TCACCATTGCTAAAGTGCAATT |
| probe-miR-368 | miR-368 | AAACGTGGAATTTCCTCTATGT |
| probe-miR-369-3p | miR-369-3p | AAAGATCAACCATGTATTATT |
| probe-miR-369-5p | miR-369-5p | GCGAATATAACACGGTCGATCT |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-370 | miR-370 | CCAGGTTCCACCCCAGCAGGC |
| probe-miR-371 | miR-371 | ACACTCAAAAGATGGCGGCAC |
| probe-miR-372 | miR-372 | ACGCTCAAATGTCGCAGCACTTT |
| probe-miR-373 | miR-373 | ACACCCCAAAATCGAAGCACTTC |
| probe-miR-373* | miR-373* | GGAAAGCGCCCCCATTTTGAGT |
| probe-miR-374 | miR-374 | CACTTATCAGGTTGTATTATAA |
| probe-miR-375 | miR-375 | TCACGCGAGCCGAACGAACAAA |
| probe-miR-376a | miR-376a | ACGTGGATTTTCCTCTATGAT |
| probe-miR-376a* | miR-376a* | CTCATAGAAGGAGAATCTACC |
| probe-miR-376b | miR-376b | AACATGGATTTTCCTCTATGAT |
| probe-miR-377 | miR-377 | ACAAAAGTTGCCTTTGTGTGAT |
| probe-miR-378 | miR-378 | ACACAGGACCTGGAGTCAGGAG |
| probe-miR-379 | miR-379 | TACGTTCCATAGTCTACCA |
| probe-miR-380-3p | miR-380-3p | AAGATGTGGACCATATTACATA |
| probe-miR-380-5p | miR-380-5p | GCGCATGTTCTATGGTCAACCA |
| probe-miR-381 | miR-381 | ACAGAGAGCTTGCCCTTGTATA |
| probe-miR-382 | miR-382 | CGAATCCACCACGAACAACTTC |
| probe-miR-383 | miR-383 | AGCCACAATCACCTTCTGATCT |
| probe-miR-384 | miR-384 | TATGAACAATTTCTAGGAAT |
| probe-miR-409-3p | miR-409-3p | AGGGGTTCACCGAGCAACATTCG |
| probe-miR-409-5p | miR-409-5p | TGCAAAGTTGCTCGGGTAACCT |
| probe-miR-410 | miR-410 | AACAGGCCATCTGTGTTATATT |
| probe-miR-411 | miR-411 | CGTACGCTATACGGTCTACTA |
| probe-miR-412 | miR-412 | ACGGCTAGTGGACCAGGTGAAGT |
| probe-miR-421 | miR-421 | GCGCCCAATTAATGTCTGTTGAT |
| probe-miR-422a | miR-422a | GGCCTTCTGACCCTAAGTCCAG |
| probe-miR-422b | miR-422b | GGCCTTCTGACTCCAAGTCCAG |
| probe-miR-423 | miR-423 | CTGAGGGGCCTCAGACCGAGCT |
| probe-miR-424 | miR-424 | TTCAAAACATGAATTGCTGCTG |
| probe-miR-425 | miR-425 | GGCGGACACGACATTCCCGAT |
| probe-miR-425-5p | miR-425-5p | TCAACGGGAGTGATCGTGTCATT |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-429 | miR-429 | ACGGTTTTACCAGACAGTATTA |
| probe-miR-431 | miR-431 | TGCATGACGGCCTGCAAGACA |
| probe-miR-432 | miR-432 | CCACCCAATGACCTACTCCAAGA |
| probe-miR-432* | miR-432* | AGACATGGAGGAGCCATCCAG |
| probe-miR-433 | miR-433 | ACACCGAGGAGCCCATCATGAT |
| probe-miR-448 | miR-448 | ATGGGACATCCTACATATGCAA |
| probe-miR-449 | miR-449 | ACCAGCTAACAATACACTGCCA |
| probe-miR-450 | miR-450 | TATTAGGAACACATCGCAAAAA |
| probe-miR-451 | miR-451 | AAACTCAGTAATGGTAACGGTTT |
| probe-miR-452 | miR-452 | GTCTCAGTTTCCTCTGCAAACA |
| probe-miR-452* | miR-452* | CTTCTTTGCAGATGAGACTGA |
| probe-miR-453 | miR-453 | CGAACTCACCACGGACAACCTC |
| probe-miR-455 | miR-455 | CGATGTAGTCCAAAGGCACATA |
| probe-miR-483 | miR-483 | AGAAGACGGGAGGAGAGGAGTGA |
| probe-miR-484 | miR-484 | ATCGGGAGGGGACTGAGCCTGA |
| probe-miR-485-3p | miR-485-3p | AGAGGAGAGCCGTGTATGAC |
| probe-miR-485-5p | miR-485-5p | GAATTCATCACGGCCAGCCTCT |
| probe-miR-486 | miR-486 | CTCGGGGCAGCTCAGTACAGGA |
| probe-miR-487a | miR-487a | AACTGGATGTCCCTGTATGATT |
| probe-miR-487b | miR-487b | AAGTGGATGACCCTGTACGATT |
| probe-miR-488 | miR-488 | TTGAGAGTGCCATTATCTGGG |
| probe-miR-489 | miR-489 | GCTGCCGTATATGTGATGTCACT |
| probe-miR-490 | miR-490 | CAGCATGGAGTCCTCCAGGTTG |
| probe-miR-491 | miR-491 | TCCTCATGGAAGGGTTCCCCACT |
| probe-miR-492 | miR-492 | AAGAATCTTGTCCCGCAGGTCCT |
| probe-miR-493 | miR-493 | AATGAAAGCCTACCATGTACAA |
| probe-miR-493-3p | miR-493-3p | CTGGCACACAGTAGACCTTCA |
| probe-miR-494 | miR-494 | AAGAGGTTTCCCGTGTATGTTTCA |
| probe-miR-495 | miR-495 | AAAGAAGTGCACCATGTTTGTTT |
| probe-miR-496 | miR-496 | GAGATTGGCCATGTAAT |
| probe-miR-497 | miR-497 | ACAAACCACAGTGTGCTGCTG |
| probe-miR-498 | miR-498 | GAAAAACGCCCCCTGGCTTGAAA |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-499 | miR-499 | TTAAACATCACTGCAAGTCTTAA |
| probe-miR-500 | miR-500 | CAGAATCCTTGCCCAGGTGCAT |
| probe-miR-501 | miR-501 | TCTCACCCAGGGACAAAGGATT |
| probe-miR-502 | miR-502 | TAGCACCCAGATAGCAAGGAT |
| probe-miR-503 | miR-503 | CTGCAGAACTGTTCCCGCTGCTA |
| probe-miR-504 | miR-504 | ATAGAGTGCAGACCAGGGTCT |
| probe-miR-505 | miR-505 | GAGGAAACCAGCAAGTGTTGAC |
| probe-miR-506 | miR-506 | TCTACTCAGAAGGGTGCCTTA |
| probe-miR-507 | miR-507 | TTCACTCCAAAAGGTGCAAAA |
| probe-miR-508 | miR-508 | TCTACTCCAAAAGGCTACAATCA |
| probe-miR-509 | miR-509 | TCTACCCACAGACGTACCAATCA |
| probe-miR-510 | miR-510 | TGTGATTGCCACTCTCCTGAGTA |
| probe-miR-511 | miR-511 | TGACTGCAGAGCAAAAGACAC |
| probe-miR-512-3p | miR-512-3p | GACCTCAGCTATGACAGCACTT |
| probe-miR-512-5p | miR-512-5p | GAAAGTGCCCTCAAGGCTGAGTG |
| probe-miR-513 | miR-513 | ATAAATGACACCTCCCTGTGAA |
| probe-miR-514 | miR-514 | CTACTCACAGAAGTGTCAAT |
| probe-miR-515-3p | miR-515-3p | ACGCTCCAAAAGAAGGCACTC |
| probe-miR-515-5p | miR-515-5p | CAGAAAGTGCTTTCTTTTGGAGAA |
| probe-miR-516-3p | miR-516-3p | ACCCTCTGAAAGGAAGCA |
| probe-miR-516-5p | miR-516-5p | AAAGTGCTTCTTACCTCCAGAT |
| probe-miR-517* | miR-517* | AGACAGTGCTTCCATCTAGAGG |
| probe-miR-517a | miR-517a | AACACTCTAAAGGGATGCACGAT |
| probe-miR-517b | miR-517b | AACACTCTAAAGGGATGCACGA |
| probe-miR-517c | miR-517c | ACACTCTAAAAGGATGCACGAT |
| probe-miR-518a | miR-518a | TCCAGCAAAGGGAAGCGCTTT |
| probe-miR-518a-2* | miR-518a-2* | AAAGGGCTTCCCTTTGCAGA |
| probe-miR-518b | miR-518b | ACCTCTAAAGGGGAGCGCTTTG |
| probe-miR-518c | miR-518c | CACTCTAAAGAGAAGCGCTTTG |
| probe-miR-518c* | miR-518c* | CAGAAAGTGCTTCCCTCCAGAGA |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-518d | miR-518d | GCTCCAAAGGGAAGCGCTTTG |
| probe-miR-518e | miR-518e | ACACTCTGAAGGGAAGCGCTTT |
| probe-miR-518f | miR-518f | TCCTCTAAAGAGAAGCGCTTT |
| probe-miR-518f* | miR-518f* | AGAGAAAGTGCTTCCCTCTAGAG |
| probe-miR-519a | miR-519a | GTAACACTCTAAAAGGATGCACTTT |
| probe-miR-519b | miR-519b | AAACCTCTAAAAGGATGCACTTT |
| probe-miR-519c | miR-519c | ATCCTCTAAAAAGATGCACTTT |
| probe-miR-519d | miR-519d | ACACTCTAAAGGGAGGCACTTTG |
| probe-miR-519e | miR-519e | ACACTCTAAAAGGAGGCACTTT |
| probe-miR-519e* | miR-519e* | GAAAGTGCTCCCTTTTGGAGAA |
| probe-miR-520a | miR-520a | ACAGTCCAAAGGGAAGCACTTT |
| probe-miR-520a* | miR-520a* | AGAAAGTACTTCCCTCTGGAG |
| probe-miR-520b | miR-520b | CCCTCTAAAAGGAAGCACTTT |
| probe-miR-520c | miR-520c | AACCCTCTAAAAGGAAGCACTTT |
| probe-miR-520d | miR-520d | AACCCACCAAAGAGAAGCACTTT |
| probe-miR-520d* | miR-520d* | CAGAAAGGGCTTCCCTTTGTAGA |
| probe-miR-520e | miR-520e | CCCTCAAAAAGGAAGCACTTT |
| probe-miR-520f | miR-520f | AACCCTCTAAAAGGAAGCACTT |
| probe-miR-520g | miR-520g | ACACTCTAAAGGGAAGCACTTTGT |
| probe-miR-520h | miR-520h | ACTCTAAAGGGAAGCACTTTGT |
| probe-miR-521 | miR-521 | ACACTCTAAAGGGAAGTGCGTT |
| probe-miR-522 | miR-522 | AACACTCTAAAGGGAACCATTTT |
| probe-miR-523 | miR-523 | CCCTCTATAGGGAAGCGCGTT |
| probe-miR-524 | miR-524 | ACTCCAAAGGGAAGCGCCTTC |
| probe-miR-524* | miR-524* | GAGAAAGTGCTTCCCTTTGTAG |
| probe-miR-525 | miR-525 | AGAAAGTGCATCCCTCTGGAG |
| probe-miR-525* | miR-525* | GCTCTAAAGGGAAGCGCCTTC |
| probe-miR-526a | miR-526a | AGAAAGTGCTTCCCTCTAGAG |
| probe-miR-526b | miR-526b | AACAGAAAGTGCTTCCCTCAAGAG |
| probe-miR-526b* | miR-526b* | GCCTCTAAAAGGAAGCACTTT |
| probe-miR-526c | miR-526c | AACAGAAAGCGCTTCCCTCTAGAG |
| probe-miR-527 | miR-527 | AGAAAGGGCTTCCCTTTGCAG |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-532 | miR-532 | ACGGTCCTACACTCAAGGCATG |
| probe-miR-542-3p | miR-542-3p | TTTCAGTTATCAATCTGTCACA |
| probe-miR-542-5p | miR-542-5p | CTCGTGACATGATGATCCCCGA |
| probe-miR-544 | miR-544 | ACTTGCTAAAAATGCAGAAT |
| probe-miR-545 | miR-545 | CACACAATAAATGTTTGCTGAT |
| probe-miR-548a | miR-548a | GCAAAAGTAATTGCCAGTTTTG |
| probe-miR-548b | miR-548b | ACAAAAGCAACTGAGGTTCTTG |
| probe-miR-548c | miR-548c | GCAAAAGTAATTGAGATTTTTG |
| probe-miR-548d | miR-548d | GCAAAAGAAACTGTGGTTTTTG |
| probe-miR-549 | miR-549 | AGAGCTCATCCATAGTTGTCA |
| probe-miR-550 | miR-550 | ATGTGCCTGAGGGAGTAAGACA |
| probe-miR-551a | miR-551a | TGGAAACCAAGAGTGGGTCGC |
| probe-miR-552 | miR-552 | TTGTCTAACCAGTCACCTGTT |
| probe-miR-553 | miR-553 | AAAACAAAATCTCACCGTTTT |
| probe-miR-554 | miR-554 | ACTGGCTGAGTCAGGACTAGC |
| probe-miR-555 | miR-555 | ATCAGAGGTTCAGCTTACCCT |
| probe-miR-556 | miR-556 | CATATTACAATGAGCTCATC |
| probe-miR-557 | miR-557 | AGACAAGGCCCACCCGTGCAAAC |
| probe-miR-558 | miR-558 | ATTTTGGTACAGCAGCTCA |
| probe-miR-559 | miR-559 | TTTTGGTGCATATTTACTTTA |
| probe-miR-560 | miR-560 | GGCGGCCGGCCGGCGCACGC |
| probe-miR-561 | miR-561 | ACTTCAAGGATCTTAAACTTTG |
| probe-miR-562 | miR-562 | GCAAATGGTACAGCTACTTT |
| probe-miR-563 | miR-563 | GGGAAACGTATGTCAACCT |
| probe-miR-564 | miR-564 | GCCTGCTGACACCGTGCCT |
| probe-miR-565 | miR-565 | AAACAGACATCGCGAGCCAGCC |
| probe-miR-566 | miR-566 | GTTGGGATCACAGGCGCCC |
| probe-miR-567 | miR-567 | GTTCTGTCCTGGAAGAACATACT |
| probe-miR-568 | miR-568 | GTGTGTATACATTTATACAT |
| probe-miR-569 | miR-569 | ACTTTCCAGGATTCATTAACT |
| probe-miR-570 | miR-570 | TGCAAAGGTAATTGCTGTTTTC |
| probe-miR-571 | miR-571 | CTCACTCAGATGGCCAACTCA |
| probe-miR-572 | miR-572 | TGGGCCACCGCCGAGCGGAC |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-573 | miR-573 | CTGATCAGTTACACATCACTTCAG |
| probe-miR-574 | miR-574 | GTGGGTGTGTGCATGAGCGTG |
| probe-miR-575 | miR-575 | GCTCCTGTCCAACTGGCTC |
| probe-miR-576 | miR-576 | CAAAGACGTGGAGAAATTAGAAT |
| probe-miR-577 | miR-577 | CAGGTACCAATATTTTATCTA |
| probe-miR-578 | miR-578 | ACAATCCTAGAGCACAAGAAG |
| probe-miR-579 | miR-579 | ATCGCGGTTTATACCAAATGAAT |
| probe-miR-580 | miR-580 | CCTAATGATTCATCATTCTCAA |
| probe-miR-581 | miR-581 | ACTGATCTAGAGAACACAAGA |
| probe-miR-582 | miR-582 | AGTAACTGGTTGAACAACTGTAA |
| probe-miR-583 | miR-583 | GTAATGGGACCTTCCTCTTTG |
| probe-miR-584 | miR-584 | CTCAGTCCCAGGCAAACCATAA |
| probe-miR-585 | miR-585 | TAGCATACAGATACGCCCA |
| probe-miR-586 | miR-586 | GGACCTAAAAATACAATGCATA |
| probe-miR-587 | miR-587 | GTGACTCATCACCTATGGAAA |
| probe-miR-588 | miR-588 | GTTCTAACCCATTGTGGCCAA |
| probe-miR-589 | miR-589 | TCTGGGAACCGGCATTTGTTCTGA |
| probe-miR-590 | miR-590 | CTGCACTTTTATGAATAAGCTC |
| probe-miR-591 | miR-591 | ACAATGAGAACCCATGGTCT |
| probe-miR-592 | miR-592 | ACATCATCGCATATTGACACAA |
| probe-miR-593 | miR-593 | GCTGAGCAATGCCTGGCTGGTGCCT |
| probe-miR-594 | miR-594 | AAAGTCACAGGCCACCCCAGATGGG |
| probe-miR-595 | miR-595 | AGACACACCACGGCACACTTC |
| probe-miR-596 | miR-596 | CCCGAGGAGCCGGGCAGGCTT |
| probe-miR-597 | miR-597 | ACAGTGGTCATCGAGTGACACA |
| probe-miR-598 | miR-598 | TGACGATGACAACGATGACGTA |
| probe-miR-599 | miR-599 | GTTTGATAAACTGACACAAC |
| probe-miR-600 | miR-600 | GAGCAAGGCTCTTGTCTGTAAGT |
| probe-miR-601 | miR-601 | CTCCTCCAACAATCCTAGACCA |
| probe-miR-602 | miR-602 | GGGCCGCAGCTGTCGCCCGTGTC |
| probe-miR-603 | miR-603 | GCAAAAGTAATTGCAGTGTGTG |
| probe-miR-604 | miR-604 | GTCCTGAATTCCGCAGCCT |
| probe-miR-605 | miR-605 | AGGAGAAGGCACCATGGGATTTA |
| probe-miR-606 | miR-606 | ATCTTTGATTTTCAGTAGTTT |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-607 | miR-607 | GTTATAGATCTGGATTTGAAC |
| probe-miR-608 | miR-608 | ACGGAGCTGTCCCAACACCACCCCT |
| probe-miR-609 | miR-609 | AGAGATGAGAGAAACACCCT |
| probe-miR-610 | miR-610 | TCCCAGCACACATTTAGCTCA |
| probe-miR-611 | miR-611 | GTCAGACCCCGAGGGGTCCTCGC |
| probe-miR-612 | miR-612 | AAGGAGCTCAGAAGCCCTGCCCAGC |
| probe-miR-613 | miR-613 | GGCAAAGAAGGAACATTCCT |
| probe-miR-614 | miR-614 | CCACCTGGCAAGAACAGGCGTTC |
| probe-miR-615 | miR-615 | AGAGGGAGACCCAGGCTCGGA |
| probe-miR-616 | miR-616 | AAGTCACTGAAGGGTTTTGAGT |
| probe-miR-617 | miR-617 | GCCACCTTCAAATGGGAAGTCT |
| probe-miR-618 | miR-618 | ACTCAGAAGGACAAGTAGAGTTT |
| probe-miR-619 | miR-619 | ACTGGGCACAAACATGTCCAGGTC |
| probe-miR-620 | miR-620 | ATTTCTATATCTATCTCCAT |
| probe-miR-621 | miR-621 | AGGTAAGCGCTGTTGCTAGCC |
| probe-miR-622 | miR-622 | GCTCCAACCTCAGCAGACTGT |
| probe-miR-623 | miR-623 | ACCCAACAGCCCCTGCAAGGGAT |
| probe-miR-624 | miR-624 | TGAACACAAGGTACTGGTACTA |
| probe-miR-625 | miR-625 | AGGACTATAGAACTTTCCCCCT |
| probe-miR-626 | miR-626 | AAGACATTTTCAGACAGCT |
| probe-miR-627 | miR-627 | TCCTCTTTTCTTAGAGACTCAC |
| probe-miR-628 | miR-628 | CGACTGCCACTCTTACTAGA |
| probe-miR-629 | miR-629 | GCTGGGCTTACGTTGGGAGAAC |
| probe-miR-630 | miR-630 | ACCTTCCCTGGTACAGAATACT |
| probe-miR-631 | miR-631 | GCTGAGGTCTGGGCCAGGTCT |
| probe-miR-632 | miR-632 | TCCCACAGGAAGCAGACAC |
| probe-miR-633 | miR-633 | TTTATTGTGGTAGATACTATTAG |
| probe-miR-634 | miR-634 | GTCCAAAGTTGGGGTGCTGGTT |
| probe-miR-635 | miR-635 | GGACATTGTTTCAGTGCCCAAGT |
| probe-miR-636 | miR-636 | CTGCGGGCGGGACGAGCAAGCACA |
| probe-miR-637 | miR-637 | ACGCAGAGCCCGAAAGCCCCCAGT |
| probe-miR-638 | miR-638 | AGGCCGCCACCCGCCCGCGATCCCT |
| probe-miR-639 | miR-639 | ACAGCGCTCGCAACCGCAGCGAT |
| probe-miR-640 | miR-640 | AGAGGCAGGTTCCTGGATCAT |

TABLE 1-continued

Probes of all mature microRNAs in human serum/plasma

| Probes | Corresponding micro-RNAs | Sequences of probes |
|---|---|---|
| probe-miR-641 | miR-641 | GAGGTGACTCTATCCTATGTCTTT |
| probe-miR-642 | miR-642 | CAAGACACATTTGGAGAGGGAC |
| probe-miR-643 | miR-643 | CTACCTGAGCTAGCATACAAGT |
| probe-miR-644 | miR-644 | GCTCTAAGAAAGCCACACT |
| probe-miR-645 | miR-645 | TCAGCAGTACCAGCCTAGA |
| probe-miR-646 | miR-646 | GCCTCAGAGGCAGCTGCTT |
| probe-miR-647 | miR-647 | GAAGGAAGTGAGTGCAGCCAC |
| probe-miR-648 | miR-648 | ACCAGTGCCCTGCACACTT |
| probe-miR-649 | miR-649 | GACTCTTGAACAACACAGGTTT |
| probe-miR-650 | miR-650 | GTCCTGAGAGCGCTGCCTCCT |
| probe-miR-651 | miR-651 | CAAAAGTCAAGCTTATCCTAAA |
| probe-miR-652 | miR-652 | TGCACAACCCTAGTGGCGCCATT |
| probe-miR-653 | miR-653 | GTTCAGTAGAGATTGTTTCAA |
| probe-miR-654 | miR-654 | GCACATGTTCTGCGGCCCACCA |
| probe-miR-655 | miR-655 | AAAGAGGTTAACCATGTATTAT |
| probe-miR-656 | miR-656 | AGAGGTTGACTGTATAATATT |
| probe-miR-657 | miR-657 | CCTAGAGAGGGTGAGAACCTGCC |
| probe-miR-658 | miR-658 | ACCAACGGACCTACTTCCCTCCGCC |
| probe-miR-659 | miR-659 | TGGGGACCCTCCCTGAACCAAG |
| probe-miR-660 | miR-660 | CAACTCCGATATGCAATGGGTA |
| probe-miR-661 | miR-661 | ACGCGCAGGCCAGAGACCCAGGCA |
| probe-miR-662 | miR-662 | CTGCTGGGCCACAACGTGGGA |
| probe-miR-663 | miR-663 | GCGGTCCCGCGGCGCCCCGCCT |
| probe-miR-7 | miR-7 | CAACAAAATCACTAGTCTTCCA |
| probe-miR-9 | miR-9 | TCATACAGCTAGATAACCAAAGA |
| probe-miR-9* | miR-9* | ACTTTCGGTTATCTAGCTTTA |
| probe-miR-92 | miR-92 | CAGGCCGGGACAAGTGCAATA |
| probe-miR-93 | miR-93 | CTACCTGCACGAACAGCACTTT |
| probe-miR-95 | miR-95 | TGCTCAATAAATACCCGTTGAA |
| probe-miR-96 | miR-96 | GCAAAAATGTGCTAGTGCCAAA |
| probe-miR-98 | miR-98 | AACAATACAACTTACTACCTCA |
| probe-miR-99a | miR-99a | CACAAGATCGGATCTACGGGTT |
| probe-miR-99b | miR-99b | CGCAAGGTCGGTTCTACGGGTG |

**[0023]** Specifically, among the above-mentioned combinations, methods, kits or biochips, the said evaluation of the physiological and/or pathological condition of a subject is to determine the physiological and/or pathological condition of the subject after being administrated a test sample, which is specifically useful for screening the test sample for the activities on the prevention and/or treatment of diseases; the said evaluation of the physiological and/or pathological condition of a subject is to diagnose and/or differentially diagnose the diseases of the subject; the said evaluation of the physiological and/or pathological condition of a subject is to evaluate the effectiveness of the treatment on the diseases of the subject; the said evaluation of the physiological and/or pathological condition of a subject is to predict the disease occurrence of the subject, which is specifically the occurrence of complications and/or the relapse of malignant diseases; the above-mentioned combinations, methods, kits or biochips can also be useful for detecting the subject for prohibited drugs-taking.

**[0024]** The above-mentioned diseases include a variety of tumors; various acute/chronic infectious diseases, e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute/chronic infectious diseases caused by various pathogenic microorganisms; other acute/chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular diseases, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary, diseases of reproductive system and diseases of locomotor system.

**[0025]** The above-mentioned serum/plasma derives from the living bodies, tissues, organs and/or corpuses of the subject.

**[0026]** The problems to be solved by the present invention include: (1) analyzing and identifying the microRNA molecules and their stability in serum/plasma of a variety of animals such as human, mice and rats; (2) studying the specificity changes of microRNAs in serum/plasma during the course of various clinical diseases including a variety of tumors; various acute/chronic infectious diseases, e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute/chronic infectious diseases caused by various pathogenic microorganisms; other acute/chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular diseases, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system; (3) detecting the respective changes of microRNAs in serum/plasma for different diseases through biochip and sequencing technology for microRNAs in serum/plasma; (4) screening a kind of microRNA molecules in serum/plasma which have relatively greater differential expression during the course of diseases and normal physiological conditions to develop detection technologies for serum/plasma microRNAs, and then preparing biochips and diagnostic kits useful for disease diagnosis etc.

**[0027]** Specifically, the present invention analyzes and identifies the existence of microRNA molecules in serum/plasma of various animals such as human, mice and rats through the methods of RT-PCR, Real-time PCR, Northern blotting, RNase protection assay, Solexa sequencing technology and biochip. The stability of microRNAs in serum/plasma is studied by comparing the changes of microRNAs by the effect of DNase and RNase. The existence of serum/plasma microRNAs molecules and the correctness of their sequences are further verified through sequencing and comparing the PCR products of serum/plasma microRNAs.

**[0028]** The detailed preparation and analysis for serum/plasma microRNAs are as follows:

**[0029]** RT-PCR method: collecting serum/plasma samples; conducting reverse transcription reaction on serum/plasma samples to prepare cDNA samples, or extracting total RNA of serum/plasma with Trizol reagent and then conducting reverse transcription reaction so as to prepare cDNA samples; designing a primer through mature microRNAs so as to conduct PCR reaction; carrying out agarose gel electrophoresis with the products of PCR; and observing and taking photographs for the results under ultraviolet lamp after EB staining.

**[0030]** Real-time PCR method: collecting serum/plasma samples; conducting reverse transcription reaction on serum/plasma samples to prepare cDNA samples, or extracting total RNA of serum/plasma with Trizol reagent and then conducting reverse transcription reaction so as to prepare cDNA samples; designing a primer of PCR through mature microRNAs and adding a fluorescent probe EVA GREEN so as to carry out PCR reaction; analyzing and processing the data and then comparing the results.

**[0031]** Northern blotting method: collecting serum/plasma samples; extracting total RNA of serum/plasma with Trizol reagent; conducting denaturing PAGE-electrophoresis and membrane transferring experiment; preparing isotope-labeled microRNA probes; conducting membrane hybridization reaction; detecting the isotope signal for results such as using phosphor-screen scanning technology.

**[0032]** RNase protection assay: firstly synthesizing an antisense RNA probe, labelling it with isotopes and purifying it; collecting serum/plasma samples and extracting RNA; dissolving the extracted DNA in a hybrid buffer and then adding an antisense RNA probe so as to conduct hybridization reaction; adding a RNase digestion solution to initate reaction; subjecting the resultant material to electrophoresis and radioautography; and analyzing the results.

**[0033]** Solexa sequencing technology: collecting serum/plasma samples; extracting total RNA of serum/plasma with Trizol reagent; conducting PAGE-electrophoresis to recover RNA molecules of 17~27 nt; enzyme-linking adaptor prime to the 3' and 5' end of small RNA molecules respectively; conducting RT-PCR reaction prior to sequencing; and analyzing and processing the data.

**[0034]** Biochip method: arraying a library of all over 500 mature microRNAs to prepare biochips; collecting serum/plasma samples; extracting total RNA of serum/plasma; separating microRNAs by column separation; fluorescently-labelling microRNAs by use of T4 RNA ligase; conducting hybridization reaction with a biochip; and detecting and analyzing the data.

**[0035]** The change trend and change volume of serum/plasma microRNAs during various diseases and their relevancy with various diseases are analyzed through the above-mentioned technologies of RT-PCR, Real-time PCR, Northern blotting, RNase protection assay, Solexa sequencing technology, Biochip, etc. Among others, what to do firstly is to detect and analyze the changes of let-7a, let-7b, let-7c,

let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-100, miR-101, miR-103, miR-105, miR-106a, miR-106b, miR-107, miR-10a, miR-10b, miR-122a, miR-124a, miR-125a, miR-125b, miR-126, miR-126*, miR-127, miR-128a, miR-128b, miR-129, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-134, miR-135a, miR-135b, miR-136, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-144, miR-145, miR-146a, miR-146b, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-153, miR-154, miR-154*, miR-155, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-181a, miR-181b, miR-181c, miR-181d, miR-182, miR-182*, miR-183, miR-184, miR-185, miR-186, miR-187, miR-188, miR-189, miR-18a, miR-18a*, miR-18b, miR-190, miR-191, miR-191*, miR-192, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a, miR-199a*, miR-199b, miR-19a, miR-19b, miR-200a, miR-200a*, miR-200b, miR-200c, miR-202, miR-202*, miR-203, miR-204, miR-205, miR-206, miR-208, miR-20a, miR-20b, miR-21, miR-210, miR-211, miR-212, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-219, miR-22, miR-220, miR-221, miR-222, miR-223, miR-224, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-27b, miR-28, miR-296, miR-299-3p, miR-299-5p, miR-29a, miR-29b, miR-29c, miR-301, miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302c*, miR-302d, miR-30a-3p, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-3p, miR-30e-5p, miR-31, miR-32, miR-320, miR-323, miR-324-3p, miR-324-5p, miR-325, miR-326, miR-328, miR-329, miR-33, miR-330, miR-331, miR-335, miR-337, miR-338, miR-339, miR-33b, miR-340, miR-342, miR-345, miR-346, miR-34a, miR-34b, miR-34c, miR-361, miR-362, miR-363, miR-363*, miR-365, miR-367, miR-368, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-372, miR-373, miR-373*, miR-374, miR-375, miR-376a, miR-376a*, miR-376b, miR-377, miR-378, miR-379, miR-380-3p, miR-380-5p, miR-381, miR-382, miR-383, miR-384, miR-409-3p, miR-409-5p, miR-410, miR-411, miR-412, miR-421, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-425-5p, miR-429, miR-431, miR-432 miR-432*, miR-433, miR-448, miR-449, miR-450, miR-451, miR-452, miR-452*, miR-453, miR-455, miR-483, miR-484, miR-485-3p, miR-485-5p, miR-486, miR-487a, miR-487b, miR-488, miR-489, miR-490, miR-491, miR-492, miR-493, miR-493-3p, miR-494, miR-495, miR-496, miR-497, miR-498, miR-499, miR-500, miR-501, miR-502, miR-503, miR-504, miR-505, miR-506, miR-507, miR-508, miR-509, miR-510, miR-511, miR-512-3p, miR-512-5p, miR-513, miR-514, miR-515-3p, miR-515-5p, miR-516-3p, miR-516-5p, miR-517*, miR-517a, miR-517b, miR-517c, miR-518a, miR-518a-2*, miR-518b, miR-518c, miR-518c*, miR-518d, miR-518e, miR-518f, miR-518f*, miR-519a, miR-519b, miR-519c, miR-519d, miR-519e, miR-519e*, miR-520a, miR-520a*, miR-520b, miR-520c miR-520d, miR-520d*, miR-520e, miR-520f, miR-520g, miR-520h, miR-521, miR-522, miR-523, miR-524, miR-524*, miR-525, miR-525*, miR-526a, miR-526b, miR-526b*, miR-526c, miR-527, miR-532, miR-542-3p, miR-542-5p, miR-544, miR-545, miR-548a, miR-548b, miR-548c, miR-548d, miR-549, miR-550, miR-551a, miR-552, miR-553, miR-554, miR-555, miR-556, miR-557, miR-558, miR-559, miR-560, miR-561, miR-562, miR-563, miR-564, miR-565, miR-566, miR-567, miR-568, miR-569, miR-570, miR-571, miR-572, miR-573, miR-574, miR-575, miR-576, miR-577, miR-578, miR-579, miR-580, miR-581, miR-582, miR-583, miR-584, miR-585, miR-586, miR-587, miR-588, miR-589, miR-590, miR-591, miR-592, miR-593, miR-594, miR-595, miR-596, miR-597, miR-598, miR-599, miR-600, miR-601, miR-602, miR-603, miR-604, miR-605, miR-606, miR-607, miR-608, miR-609, miR-610, miR-611 miR-612, miR-613, miR-614, miR-615, miR-616, miR-617, miR-618, miR-619, miR-620, miR-621, miR-622, miR-623, miR-624, miR-625, miR-626, miR-627, miR-628, miR-629, miR-630, miR-631, miR-632, miR-633, miR-634, miR-635, miR-636, miR-637, miR-638, miR-639, miR-640, miR-641, miR-642, miR-643, miR-644, miR-645, miR-646, miR-647, miR-648, miR-649, miR-650, miR-651, miR-652, miR-653, miR-654, miR-655, miR-656, miR-657, miR-658, miR-659, miR-660, miR-661, miR-662, miR-663, miR-7, miR-9, miR-9*, miR-92, miR-93, miR-95, miR-98, miR-99a and miR-99b in various clinical diseases (including a variety of tumors; various acute/chronic infectious diseases, e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute/chronic infectious diseases caused by various pathogenic microorganisms; other acute/chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular diseases, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system); Biochips of serum/plasma microRNAs are prepared to determine the changes of serum/plasma microRNAs in different diseases, and meanwhile, Solexa sequencing and analysis on microRNAs in serum/plasma in different diseases are conducted.

**[0036]** The research and development of a technology for detecting disease-related serum/plasma microRNAs. Specifically, the microRNAs with disease-related specificity changes are screened out, their primers are collected into a PCR kit (RT-PCR or Real-time PCR) to prepare a disease-diagnostic kit, or their reverse complementary sequences are dripped on chips as probes so as to prepare the biochips for detecting serum/plasma microRNAs specific for a certain disease.

**[0037]** Presently, the technologies of traditional biochemistry and molecular biology for the clinical diagnosis of diseases are relatively complicated and insensitive. Novel techniques developed in recent years possibly useful for disease diagnosis are gene chip technique, protein (antibody) chip technique, etc. The changes at mRNA level measured through gene chips cannot completely reflect the actual changes at protein level, since the bioactivity of protein is closely related to post-transcriptional modification such as glycosylation and phosphorylation. In addition, for detection of many diseases, marker molecules in body fluids and blood cannot be detected through gene chip technology. Meanwhile, protein (antibody) chip technique and proteomic techniques also bear their limitations. In human body, especially in serum/plasma, there are tens of thousands of protein and polypeptide segments with extensively distributed concentrations, and the number of proteins definitely reported is very small, let alone those quantified. It is an extremely arduous task to find out those proteins having close relation with specific diseases from the large quantity of proteins and understand their roles in histopathologic changes. Moreover, lacking of complete antibody resources is the bottleneck restraining the development of antibody biochip technology. The detection technol-

ogy for serum/plasma microRNAs based on biochips of serum/plasma microRNAs and diagnostic kits skillfully combines the peculiar properties of serum/plasma microRNAs with conventional molecular biology detection technique together, which can rapidly analyze the respective constitution of serum/plasma microRNAs in respect of various diseases with high throughput and hence be of extremely clinical practicality. Since the changes of physiological conditions in organs and tissues will cause the constitutional changes of serum/plasma microRNAs, serum/plasma microRNAs can be used as "fingerprints for diseases" to realize early diagnosis of diseases.

**[0038]** The advantages of the technology of detecting serum/plasma microRNAs are as follows:

**[0039]** (1) As novel disease markers, serum/plasma microRNAs possess certain advantages such as extensive spectrum for detection, high sensitivity, low cost for detection, convenient sampling, easy preservation for samples (preserving serum/plasma at −20° C. will do), etc. This method can be widely used in general survey of diseases and other relevant tasks and has become an efficient means for early diagnosis of diseases.

**[0040]** (2) As novel disease markers, serum/plasma microRNAs will improve the low-specificity and low-sensitivity caused by individual differences which single markers are difficult to overcome, and notably increase the clinical detection rate of diseases so as to realize early diagnosis of diseases.

**[0041]** (3) The advantages of the technology of detecting serum/plasma microRNAs lie in that what to be detected is series of disease related markers, thus it can address the differences (i.e., age, sex, race, diet, circumstance, etc.) between individual patients, which are exactly a primary problem difficult to overcome by single disease markers.

**[0042]** In summary, utilizing the technology of detecting serum/plasma microRNAs can confirm diagnosis of histopathologic changes in early stage. These novel serum/plasma markers not only provide material foundation for people to comprehensively understand the mechanism of histopathologic changes in molecule level, but also accelerate the progress in diagnostics and therapeutics of clinical diseases. Of course, a majority of molecular diagnostic techniques used for disease detection in early period are at initial experimental stage and their validity needs to be further verified and improved. Moreover, since every disease has the characteristics of its own, this requires a peculiar method for the detection of said disease. In this manner, it is impossible for all diseases to be detected out only through one or only a few of detection methods. Nevertheless, based on the superiority of serum/plasma microRNAs, it is believed that, in the near future, the diagnostic technique of serum/plasma microRNAs for severe diseases such as cancer will become part of routine physical examination. In addition, microRNA related gene therapy will be widely utilized. Consequently, the overcoming of these diseases will come true, not just a dream.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0043]** The following are the detailed description of the embodiments of this invention with reference to the drawings, wherein:

**[0044]** FIG. **1** shows the RT-PCR result of partial microRNAs directly detected in the serum of a normal person.

**[0045]** FIG. **2** shows the RT-PCR results of the microRNAs in the RNA extracted from the serum of a normal person.

**[0046]** In FIG. **1** and FIG. **2**, U6 is a snRNA with a molecular weight of 100 bp, serving as an internal reference molecule in microRNAs experiments. The rest of 12 microRNAs are each miR-181a(181a), miR-181b(181b), miR-223(223), miR-142-3p(142-3p), miR-142-5p(142-5p), miR-150(150) with blood cell specificity; miR-1(1), miR-133a(133a), miR-206(206) from cardiac muscles and skeletal muscles; miR-9 (9), miR-124a(124a) from brain tissues; and miR-122a (122a) from liver.

**[0047]** FIG. **3** shows the RT-PCR results of partial microRNAs directly detected in the serum of mouse, rat, fetal bovine, calf and horse respectively.

**[0048]** FIG. **4** shows the variable quantity of the partial microRNAs in the serum of a patient suffering from the shown diseases compared with microRNAs in the serum of a normal person.

**[0049]** FIG. **5** shows the ratio between the quantities of macroRNAs and microRNAs in blood cells and serum.

**[0050]** FIG. **6** shows the enzyme digested results of macroRNAs and microRNAs.

## THE BEST MODE FOR CARRYING OUT THE INVENTION

### Example 1

**[0051]** The RT-PCR Experiments of MicroRNAs in Serum/Plasma

**[0052]** By using RT-PCR technique, it is found and proved that there stably exist various microRNAs in serum/plasma of both human beings and animals, and that their expression levels are considerably high. The specific RT-PCR steps are as follows:

**[0053]** (1) collecting serum/plasma of mice, rats, normal persons and some patients;

**[0054]** (2) preparing samples of cDNA. This operation has two options: one is to directly conduct reverse transcription reaction using 10 μl of serum/plasma; the other is to firstly extract the total RNA from serum/plasma (usually, about 10 μg of RNA can be enriched from 10 ml of serum/plasma) with Trizol reagent (Invitrogen Co.), subsequently obtain cDNA through RNA reverse transcription reaction. The reaction system of reverse transcription includes 4 μl 5×AMV buffer, 2 μl 10 mM each dNTP mixture (Takara Co.), 0.5 μl RNase Inhibitor (Takara Co.), 2 μl AMY (Takara Co.) and 1.5 μl gene specific reverse primers mixtures. The reaction steps successively include 15 minutes of incubation at 16° C., 1 hour of reaction at 42° C. and 5 minutes of incubation at 85° C.;

**[0055]** (3) PCR and Electrophoresis observation. The cDNA is diluted by 1/50. To 1 μl diluted cDNA are added 0.3 μl Taq polymerase (Takara Co.), 0.2 μl 10 μM forward primer, 0.20 μl 10 μM universal reverse primer, 1.2 μl 25 mM $MgCl_2$, 1.6 μl 2.5 mM each dNTP mixture (Takara Co.), 2 μl 10×PCR buffer, 13.5 μl $H_2O$, and PCR reaction is conducted in the 20 μl system. The PCR reaction is done under the following conditions: one cycle at 95° C. for 5 mins followed by 40 cycles at 95° C. for 15 seconds and 60° C. for 1 minute. 10 μl PCR product is subjected to 3% Agarose Gel Electrophoresis, which is observed under ultraviolet lamp after EB staining.

**[0056]** The detailed experimental results are shown in FIG. **1**. FIG. **1** shows the experimental results of RT-PCR directly conducted on the serum of normal persons. The all over 500 mature microRNAs in human being are selected for conducting RT-PCR reaction, of which 12 microRNAs are shown in FIG. **1** and each miR-181a, miR-181b, miR-223, miR-142-

3p, miR-142-5p, miR-150 with is blood cell specificity; miR-1, miR-133a, miR-206 from cardiac muscles and skeletal muscles; miR-9 and miR-124a from brain tissues; and miR-122a from liver. It can be seen from the results that all microRNAs from the above-mentioned four tissues are detectable in blood, and that not all over 500 mature microRNAs have high expression level in the serum/plasma, with some microRNAs being in fairly trace amount and even being normally nondetectable.

**[0057]** To further verify that there stably exist the microRNAs in serum/plasma, RNA is firstly extracted from the serum of normal persons, then all over 500 mature microRNAs of human are selected for PCR experiment. As shown in FIG. **2**, the results of FIG. **2** is quite consistent with that of FIG. **1**, the singleness of the PCR products indicating that both two assays can detect the expression and level of the microRNAs in people's serum/plasma, and proving that there stably exist microRNAs of various tissues sources in people's serum/plasma. In addition, the same method is used to detect the expression and level of over 500 microRNAs in the serum/plasma of mouse, rat, fetal bovine, calf and horse, it is also found that there is stable expression of microRNAs of various tissues sources in serum/plasma of mouse, rat, fetal bovine, calf and horse (see FIG. **3**).

## Example 2

### The Real-Time PCR Experiments of MicroRNAs in Serum/Plasma

**[0058]** Quantitative PCR experiments of microRNAs in serum/plasma are conducted to study the specific variation of microRNAs quantity in serum/plasma during the course of various diseases, including various tumors, various acute and chronic infectious diseases, e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute and chronic infectious diseases caused by various pathogenic microorganisms; other acute and chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular disease, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system. The experimental principles and experimental steps of quantitative PCR are basically the same as those of RT-PCR, with the only difference between them being the addition of a fluorescent dye EVA GREEN in the process of PCR. An ABI Prism 7300 fluorescent quantitative PCR instrument is used to conduct PCR reaction under the following conditions: one cycle at 95° C. for 5 mins followed by 40 cycles at 95° C. for 15 seconds and 60° C. for 1 minute. The data processing method used is ΔΔCT method, wherein CT is the number of cycles when the reaction reaches the threshold. The expression level of each microRNAs relative to that of internal standard reference can be expressed by the equation of 2-ACT, wherein $\Delta CT=CT_{sample}-CT_{internal\ reference}$. Reverse transcription reactions are directly conducted on serum/plasma samples of a patient and those of a normal person, and the quantities of microRNAs contained in each sample of serum/plasma are compared through quantitative PCR reactions.

**[0059]** Serum samples of patients who suffer from aplastic anemia, breast cancer, osteosarcoma, CNS (Central Nervous System) lymphoma, diabetes are selected, and at the same time, all over 500 mature microRNAs of human beings are used to conduct PCR reaction experiments. FIG. **4** shows the quantitative PCR experimental results of microRNAs within serum of patients and normal persons which include the above-mentioned miR-181a, miR-181b, miR-223, miR-142-3p, miR-142-5p, miR-150 with blood cell specificity; miR-1, miR-133a, miR-206 from cardiac muscles and skeletal muscles; miR-9, miR-124a from brain tissues; and miR-122a from liver. The ratio of the microRNAs quantity in serum between normal persons and patients suffering from aplastic anemia, breast cancer, osteosarcoma, CNS (Central Nervous System) lymphoma, diabetes are respectively up-regulated or down-regulated, and the variation extent of the microRNAs quantity from the same tissue source differs in patients with different diseases, indicating that there is specificity variation of microRNAs quantity in the serum/plasma of patients with different diseases. They can be taken as a type of novel markers for disease diagnosis.

## Example 3

### The Superiority of Serum/Plasma MicroRNAs as Disease Markers

**[0060]** Through detecting the quantities of microRNAs and macroRNAs in serum and blood cells, it is found that there is an abundant content of microRNAs in serum. See FIG. **5**. As represented by U6 molecules with a molecular weight of 100 bp and ribosomal RNA molecules with molecular weights being 18S and 28S respectively, the quantity of macroRNAs in blood cells is at least tens times that in serum; while the quantity of microRNAs in serum remains the same as that in blood cells except the microRNAs with blood cell specificity. Therefore, serum/plasma will specifically enrich small molecule RNAs, especially microRNAs.

**[0061]** It is also found that microRNAs are to some extent able to resist the action of endonuclease, which is possibly one of the reasons why microRNAs can stably exist in serum/plasma. Total RNAs extracted from cultured cell line are processed with endonuclease RNase A and the remaining quantity of macroRNAs and microRNAs are then detected. As shown in FIG. **6**, it is found that microRNAs can to some extent resist the degradation of endonuclease while the macroRNAs are substantially completely cut off. Therefore microRNAs can stably exist in serum/plasma.

**[0062]** Based on the two characteristics of abundance in content and stable existence of microRNAs in serum/plasma, microRNAs could be well applied in clinical test.

## Example 4

### Preparation of the Biochip of Serum/Plasma MicroRNAs Useful for Disease Diagnosis

**[0063]** A biochip of serum/plasma microRNAs is fabricated to verify the reliability of a kind of serum/plasma microRNAs probes relating to diseases which are selected through quantitative PCR method. The biochip contains all microRNAs probes that can be normally detected in people's serum/plasma, constituting a probe library. See Table 1.

**[0064]** When the probes are specifically applied in certain disease diagnosis or efficacy screening, some probes of the probe library are put together to construct a probe collection which makes it possible to quantitatively detect the variation of microRNAs in the specific conditions. For example, when

diagnosing colon cancer, the collection of probes that have interaction with microRNAs of numbers 17-5p, 21, 103, 106a, 107, 126*, 143, 145, 150, 155 and 210 is used. For another example, when diagnosing myocardial hypertrophy and chronic heart failure, the collection of probes that have interaction with microRNAs of numbers 21, 23a, 23b, 24, 27a, 27b, 125b, 195, 199a, 214, 217, 133a is used. In addition, the chip can also do high-throughput screening of the probes of microRNAs varying stably in serum/plasma, and diseases can be predicted and diagnosed based on the overall variation of microRNAs in serum/plasma.

**[0065]** Sequencing method or quantitative PCR method is firstly used to determine that there is more than one copy of microRNAs in serum/plasma, and then reverse complementary probes of these microRNAs are synthesized, after which these probes are spotted on a chemically-modified slide in a size of 75×25 mm using a biochip microarrayer SmartArray™. The samples spotted on the chip also include U6 and tRNA as internal standard, artificially-prepared external standard in length of 30 bases, Hex as positive control etc. The entire lattice is divided into 4 sub-lattices and each sub-lattice has 23 rows and 21 columns, wherein the spot distance is 185 mm and the spot diameter is about 130 μm and each probe was repeatly spotted for 3 times.

**[0066]** The operational procedure of the biochip is: (1) extracting the total RNA from serum/plasma and detecting its quality through formaldehyde denaturing gel electrophoresis; (2) separation of microRNAs: 50-100 μg total RNA is taken to separate microRNAs from total RNA with Ambion's miRNA Isolation Kit (Cat #. 1560); (3) fluorescently-labeling of microRNAs samples: microRNAs samples are fluorescently-labeling with T4 RNA ligase, then precipitated with absolute ethanol, and then blown to dryness for chip hybridization; (4) hybridization and cleaning: RNA is dissolved into 16 μL hybridizing solution (15% formamide, 0.2% SDS, 3×SSC and 50×Denhardt's solution), and hybridized at 420 overnight. After completion of the hybridization, it is washed in a solution containing 0.2% SDS and 2×SSC at about 42° C. for 4 minutes, and then washed in a solution containing 0.2× SSC at room temperature for 4 minutes. Thereafter, the slides can be used for scanning immediately after being dried; (5) chip scanning: the chip is scanned with two-channel laser scanner LuxScan 10K/A; (6) data extracting and analysis: the chip image is analyzed with an image analyzing software LuxScan 3.0, the image signal is transformed into digital signal, and finally differentially-expressed genes are analyzed and selected with SAM method.

**[0067]** A biochip is prepared as above by using a kind of serum/plasma microRNAs probes which express greatly differently under disease condition and normal physiological condition double-verified by quantitative PCR technique and biochip technique. As compared with the traditional chip, there is no significant improvement in the manufacturing process and operational procedure of this biochip, but this chip simplifies the probe library, thereby greatly reducing the manufacturing cost and production time of the chip, and hence is easy to prepare. Meanwhile it increases the pertinence and practicability of chip. The application of the chip in practice can detect diseases in an early phase with only need of the serum/plasma of a patient and no need of other tissues, which helps guide the diagnosis and treatment.

## Example 5

## Preparation of Kits of MicroRNAs Useful for Disease Diagnosis and Prediction

**[0068]** The manufacturing processed and operational procedures of microRNAs kits useful for diagnosis, prediction of complication occurrence and malignant disease relapse, evaluation of therapeutic effects, screening of pharmaceutical active ingredients, assessment of drug efficacy, forensic authentication and prohibited drug inspection, etc. of all diseases are based on quantitative PCR technique and semi-quantitative PCR technique and biochip technique. The above-mentioned diseases include various tumors; various acute/chronic infectious diseases, e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute/chronic infectious diseases caused by various pathogenic microorganisms; other acute/chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular diseases, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system.

**[0069]** Sequencing method or quantitative PCR method is firstly used to determine that there is more than one copy of microRNAs in serum/plasma. Then, a kind of serum/plasma microRNAs that have a big difference between the expression levels in disease condition and in normal physiological condition are screened out through the techniques of quantative PCR and biochip, which are taken as an indicator for predicting whether canceration or other disease occurs and diagnosing the pathological degree. Finally the number of screened corresponding serum/plasma microRNAs of each disease would be controlled to over ten to tens, which is the optimized condensement of the chip-probe library. The kit contains a batch of serum/plasma microRNAs primers, Taq polymerase, dNTP, etc. The value of the kit lies in making it possible to detect the changing trend of microRNAs through the most simplified probe library and with only need of serum/plasma and no need of any other tissue samples, and further predict the probability of occurrence of diseases or diagnose the pathological phase of diseases based on this changing trend detected. Thus, the application of this kit in practice can increase the possibility of discovering diseases in an early phase, which helps guide the diagnosis and treatment of diseases.

**1**. A combination of microRNAs for evaluating the physiological and/or pathological condition of a subject, wherein the combination comprises all detectable microRNAs stably existing in the serum/plasma of the subject.

**2**. The combination according to claim **1**, characterized in that said all detectable microRNAs stably existing in the serum/plasma of a subject are all mature microRNAs in human serum/plasma.

**3**. The combination according to claim **1**, characterized in that said all detectable microRNAs stably existing in the serum/plasma of a subject are let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-100, miR-101, miR-103, miR-105, miR-106a, miR-106b, miR-107, miR-10a, miR-10b, miR-122a, miR-124a, miR-125a, miR-125b, miR-126, miR-126*, miR-127, miR-128a, miR-128b, miR-129, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-134, miR-135a, miR-135b, miR-136, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-144, miR-145, miR-146a, miR-146b, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-153, miR-154, miR-154*, miR-155, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-181a,

miR-181b, miR-181c, miR-181d, miR-182, miR-182*, miR-183, miR-184, miR-185, miR-186, miR-187, miR-188, miR-189, miR-18a, miR-18a*, miR-18b, miR-190, miR-191, miR-191*, miR-192, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a, miR-199a*, miR-199b, miR-19a, miR-19b, miR-200a, miR-200a*, miR-200b, miR-200c, miR-202, miR-202*, miR-203, miR-204, miR-205, miR-206, miR-208, miR-20a, miR-20b, miR-21, miR-210, miR-211, miR-212, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-219, miR-22, miR-220, miR-221, miR-222, miR-223, miR-224, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-27b, miR-28, miR-296, miR-299-3p, miR-299-5p, miR-29a, miR-29b, miR-29c, miR-301, miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302c*, miR-302d, miR-30a-3p, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-3p, miR-30e-5p, miR-31, miR-32, miR-320, miR-323, miR-324-3p, miR-324-5p, miR-325, miR-326, miR-328, miR-329, miR-33, miR-330, miR-331, miR-335, miR-337, miR-338, miR-339, miR-33b, miR-340, miR-342, miR-345, miR-346, miR-34a, miR-34b, miR-34c, miR-361, miR-362, miR-363, miR-363*, miR-365, miR-367, miR-368, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-372, miR-373, miR-373*, miR-374, miR-375, miR-376a, miR-376a*, miR-376b, miR-377, miR-378, miR-379, miR-380-3p, miR-380-5p, miR-381, miR-382, miR-383, miR-384, miR-409-3p, miR-409-5p, miR-410, miR-411, miR-412, miR-421, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-425-5p, miR-429, miR-431, miR-432, miR-432*, miR-433, miR-448, miR-449, miR-450, miR-451, miR-452, miR-452*, miR-453, miR-455, miR-483, miR-484, miR-485-3p, miR-485-5p, miR-486, miR-487a, miR-487b, miR-488, miR-489, miR-490, miR-491, miR-492, miR-493, miR-493-3p, miR-494, miR-495, miR-496, miR-497, miR-498, miR-499, miR-500, miR-501, miR-502, miR-503, miR-504, miR-505, miR-506, miR-507, miR-508, miR-509, miR-510, miR-511, miR-512-3p, miR-512-5p, miR-513, miR-514, miR-515-3p, miR-515-5p, miR-516-3p, miR-516-5p, miR-517*, miR-517a, miR-517b, miR-517c, miR-518a, miR-518a-2*, miR-518b, miR-518c, miR-518c*, miR-518d, miR-518e, miR-518f, miR-518f*, miR-519a, miR-519b, miR-519c, miR-519d, miR-519e, miR-519e*, miR-520a, miR-520a*, miR-520b, miR-520c, miR-520d, miR-520d*, miR-520e, miR-520f, miR-520g, miR-520h, miR-521, miR-522, miR-523, miR-524, miR-524*, miR-525, miR-525*, miR-526a, miR-526b, miR-526b*, miR-526c, miR-527, miR-532, miR-542-3p, miR-542-5p, miR-544, miR-545, miR-548a, miR-548b, miR-548c, miR-548d, miR-549, miR-550, miR-551a, miR-552, miR-553, miR-554, miR-555, miR-556, miR-557, miR-558, miR-559, miR-560, miR-561, miR-562, miR-563, miR-564, miR-565, miR-566, miR-567, miR-568, miR-569, miR-570, miR-571, miR-572, miR-573, miR-574, miR-575, miR-576, miR-577, miR-578, miR-579, miR-580, miR-581, miR-582, miR-583, miR-584, miR-585, miR-586, miR-587, miR-588, miR-589, miR-590, miR-591, miR-592, miR-593, miR-594, miR-595, miR-596, miR-597, miR-598, miR-599, miR-600, miR-601, miR-602, miR-603, miR-604, miR-605, miR-606, miR-607, miR-608, miR-609, miR-610, miR-611, miR-612, miR-613, miR-615, miR-616, miR-617, miR-618, miR-619, miR-620, miR-621, miR-622, miR-623, miR-624, miR-625, miR-626, miR-627, miR-628, miR-629, miR-630, miR-631, miR-632, miR-633, miR-634, miR-635, miR-636, miR-637, miR-638, miR-639, miR-640, miR-641, miR-642, miR-643, miR-644, miR-645, miR-646, miR-647, miR-648, miR-649, miR-650, miR-651, miR-652, miR-653, miR-654, miR-655, miR-656, miR-657, miR-658, miR-659, miR-660, miR-661, miR-662, miR-663, miR-7, miR-9, miR-9*, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99a and miR-99b.

**4**. The combination according to claim **1**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to determine physiological and/or pathological condition of the subject after being administrated a test sample.

**5**. The combination according to claim **4**, characterized in that the combination is useful for screening the test sample for the activities on the prevention and/or treatment of diseases.

**6**. The combination according claim **1**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to diagnose and/or differentially diagnose the diseases of the subject.

**7**. The combination according to claim **1**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to evaluate the effectiveness of treating the diseases of the subject.

**8**. The combination of according to claim **1**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to predict the occurrence of diseases of the subject.

**9**. The combination according to claim **8**, characterized in that the occurrence of diseases is the occurrence of complications and/or the relapse of malignant diseases.

**10**. The combination according to claim **5**, characterized in that the diseases include various tumors; various acute and chronic infectious diseases, e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute and chronic infectious diseases caused by various pathogenic microorganisms; other acute and chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular diseases, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system.

**11**. The combination according to claim **1**, characterized in that the combination is useful for detecting the subject for prohibited drugs-taking.

**12**. The combination according to claim **1**, characterized in that the serum/plasma derives from living body, tissues, organs and/or corpuses of the subject.

**13**. A method for evaluating the physiological and/or pathological condition of a subject, wherein the method comprises determining all detectable microRNAs stably existing in serum/plasma of the subject.

**14**. The method according to claim **13**, characterized in that said all detectable microRNAs stably existing in serum/plasma of a subject are all mature microRNAs in human serum/plasma.

**15**. The method according to claim **13**, characterized in that said all detectable microRNAs stably existing in serum/plasma of a subjects are let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-100, miR-101, miR-103, miR-105, miR-106a, miR-106b, miR-107, miR-10a, miR-10b, miR-122a, miR-124a, miR-125a, miR-125b, miR-126, miR-126*, miR-127, miR-128a, miR-128b, miR-129, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-134,

miR-135a, miR-135b, miR-136, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-144, miR-145, miR-146a, miR-146b, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-153, miR-154, miR-154*, miR-155, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-181a, miR-181b, miR-181c, miR-181d, miR-182, miR-182*, miR-183, miR-184, miR-185, miR-186, miR-187, miR-188, miR-189, miR-18a, miR-18a*, miR-18b, miR-190, miR-191, miR-191*, miR-192, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a, miR-199a*, miR-199b, miR-19a, miR-19b, miR-200a, miR-200a*, miR-200b, miR-200c, miR-202, miR-202*, miR-203, miR-204, miR-205, miR-206, miR-208, miR-20a, miR-20b, miR-21, miR-210, miR-211, miR-212, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-219, miR-22, miR-220, miR-221, miR-222, miR-223, miR-224, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-27b, miR-28, miR-296, miR-299-3p, miR-299-5p, miR-29a, miR-29b, miR-29c, miR-301, miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302c*, miR-302d, miR-30a-3p, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-3p, miR-30e-5p, miR-31, miR-32, miR-320, miR-323, miR-324-3p, miR-324-5p, miR-325, miR-326, miR-328, miR-329, miR-33, miR-330, miR-331, miR-335, miR-337, miR-338, miR-339, miR-33b, miR-340, miR-342, miR-345, miR-346, miR-34a, miR-34b, miR-34c, miR-361, miR-362, miR-363, miR-363*, miR-365, miR-367, miR-368, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-372, miR-373, miR-373*, miR-374, miR-375, miR-376a, miR-376a*, miR-376b, miR-377, miR-378, miR-379, miR-380-3p, miR-380-5p, miR-381, miR-382, miR-383, miR-384, miR-409-3p, miR-409-5p, miR-410, miR-411, miR-412, miR-421, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-425-5p, miR-429, miR-431, miR-432, miR-432*, miR-433, miR-448, miR-449, miR-450, miR-451, miR-452, miR-452*, miR-453, miR-455, miR-483, miR-484, miR-485-3p, miR-485-5p, miR-486, miR-487a, miR-487b, miR-488, miR-489, miR-490, miR-491, miR-492, miR-493, miR-493-3p, miR-494, miR-495, miR-496, miR-497, miR-498, miR-499, miR-500, miR-501, miR-502, miR-503, miR-504, miR-505, miR-506, miR-507, miR-508, miR-509, miR-510, miR-511, miR-512-3p, miR-512-5p, miR-513, miR-514, miR-515-3p, miR-515-5p, miR-516-3p, miR-516-5p, miR-517*, miR-517a, miR-517b, miR-517c, miR-518a, miR-518a-2*, miR-518b, miR-518c, miR-518c*, miR-518d, miR-518e, miR-518f, miR-518f*, miR-519a, miR-519b, miR-519c, miR-519d, miR-519e, miR-519e*, miR-520a, miR-520a*, miR-520b, miR-520c, miR-520d, miR-520d*, miR-520e, miR-520f, miR-520g, miR-520h, miR-521, miR-522, miR-523, miR-524, miR-524*, miR-525, miR-525*, miR-526a, miR-526b, miR-526b*, miR-526c, miR-527, miR-532, miR-542-3p, miR-542-5p, miR-544, miR-545, miR-548a, miR-548b, miR-548c, miR-548d, miR-549, miR-550, miR-551a, miR-552, miR-553, miR-554, miR-555, miR-556, miR-557, miR-558, miR-559, miR-560, miR-561, miR-562, miR-563, miR-564, miR-565, miR-566, miR-567, miR-568, miR-569, miR-570, miR-571, miR-572, miR-573, miR-574, miR-575, miR-576, miR-577, miR-578, miR-579, miR-580, miR-581, miR-582, miR-583, miR-584, miR-585, miR-586, miR-587, miR-588, miR-589, miR-590, miR-591, miR-592, miR-593, miR-594, miR-595, miR-596, miR-597, miR-598, miR-599, miR-600, miR-601, miR-602, miR-603, miR-604, miR-605, miR-606, miR-607, miR-608, miR-609, miR-610, miR-611, miR-612, miR-613, miR-614, miR-615, miR-616, miR-617, miR-618, miR-619, ma-620, miR-621, miR-622, miR-623, miR-624, miR-625, miR-626, miR-627, miR-628, miR-629, miR-630, miR-631, miR-632, miR-633, miR-634, miR-635, miR-636, miR-637, miR-638, miR-639, miR-640, miR-641, miR-642, miR-643, miR-644, miR-645, miR-646, miR-647, miR-648, miR-649, miR-650, miR-651, miR-652, miR-653, miR-654, miR-655, miR-656, miR-657, miR-658, miR-659, miR-660, miR-661, miR-662, miR-663, miR-7, miR-9, miR-9*, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99a and miR-99b.

**16**. The method according to claim **13**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to determine the physiological and/or pathological condition of the subject after being administrated a test sample.

**17**. The method according to claim **16**, characterized in that the method is useful for screening the test sample for the activities on the prevention and/or treatment of diseases.

**18**. The method according to claim **13**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to diagnose and/or differentially diagnose the diseases of the subject.

**19**. The method according to claim **13**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to evaluate the effectiveness of treating the diseases of the subject.

**20**. The method according to claim **13**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to predict the occurrence of diseases of the subject.

**21**. The method according to claim **20**, characterized in that the occurrence of diseases is the occurrence of complications and/or the relapse of malignant diseases.

**22**. The method according to claim **17**, characterized in that the diseases include various tumors; various acute and chronic infectious diseases e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS; bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute and chronic infectious diseases caused by various pathogenic microorganisms; other acute and chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular disease, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system.

**23**. The method according to claim **13**, characterized in that the method is useful for detecting the subject for prohibited drugs-taking.

**24**. The method according to claim **13**, characterized in that the method for determining all detectable microRNAs stably existing in serum/plasma of a subject is one or more selected from the group consisting of RT-PCR method, Real-time PCR method, Northern blotting method, RNase protection assay, Solexa sequencing technology and biochip method.

**25**. The method according to claim **24**, characterized in that the method is RT-PCR method which includes the following steps:

(1) extracting the total RNA from the serum/plasma of the subject, and obtaining cDNA samples by RNA reverse transcription reaction; or collecting serum/plasma samples from the subject and conducting reverse tran-

scription reaction with serum/plasma being a buffer so as to prepare cDNA samples;

(2) designing a primer by use of microRNAs and conducting PCR reaction;

(3) conducting agarose gel electrophoresis of PCR products; and

(4) observing agarose gel under ultraviolet Lamp after EB staining.

**26**. The method according to claim **24**, characterized in that the method is Real-time PCR which includes the following steps:

(1) extracting the total RNA from the serum/plasma of the subject, and obtaining cDNA samples by RNA reverse transcription reaction; or collecting serum/plasma samples from the subject, preparing cDNA samples by reverse transcription reaction with serum/plasma being a buffer;

(2) designing a primer by use of microRNAs;

(3) adding a fluorescent probe to conduct PCR reaction;

(4) detecting and comparing the variation in levels of microRNAs in the serum/plasma samples relative to those of microRNAs in normal serum/plasma.

**27**. The method according to claim **13**, characterized in that the serum/plasma derives from living bodies, tissues, organs and/or corpuses of the subject.

**28**. A kit for evaluating the physiological and/or pathological condition of a subject, wherein the kit comprises the tools useful for determining all detectable microRNAs stably existing in the serum/plasma of the subject.

**29**. The kit according to claim **28**, characterized in that the kit comprises the primers of all mature microRNAs in human serum/plasma.

**30**. The kit according to claim **28**, characterized in that the kit comprises the primers of let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-1, miR-100, miR-101, miR-103, miR-105, miR-106a, miR-106b, miR-107, miR-10a, miR-10b, miR-122a, miR-124a, miR-125a, miR-125b, miR-126, miR-126*, miR-127, miR-128a, miR-128b, miR-129, miR-130a, miR-130b, miR-132, miR-133a, miR-133b, miR-134, miR-135a, miR-135b, miR-136, miR-137, miR-138, miR-139, miR-140, miR-141, miR-142-3p, miR-142-5p, miR-143, miR-144, miR-145, miR-146a, miR-146b, miR-147, miR-148a, miR-148b, miR-149, miR-150, miR-151, miR-152, miR-153, miR-154, miR-154*, miR-155, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-181a, miR-181b, miR-181c, miR-181d, miR-182, miR-182*, miR-183, miR-184, miR-185, miR-186, miR-187, miR-188, miR-189, miR-18a, miR-18a*, miR-18b, miR-190, miR-191, miR-191*, miR-192, miR-193a, miR-193b, miR-194, miR-195, miR-196a, miR-196b, miR-197, miR-198, miR-199a, miR-199a*, miR-199b, miR-19a, miR-19b, miR-200a, miR-200a*, miR-200b, miR-200c, miR-202, miR-202*, miR-203, miR-204, miR-205, miR-206, miR-208, miR-20a, miR-20b, miR-21, miR-210, miR-211, miR-212, miR-213, miR-214, miR-215, miR-216, miR-217, miR-218, miR-219, miR-22, miR-220, miR-221, miR-222, miR-223, miR-224, miR-23a, miR-23b, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-27b, miR-28, miR-296, miR-299-3p, miR-299-5p, miR-29a, miR-29b, miR-29c, miR-301, miR-302a, miR-302a*, miR-302b, miR-302b*, miR-302c, miR-302c*, miR-302d, miR-30a-3p, miR-30a-5p, miR-30b, miR-30c, miR-30d, miR-30e-3p, miR-30e-5p, miR-31, miR-32, miR-320, miR-323, miR-324-3p, miR-324-5p, miR-325, miR-326, miR-328, miR-329, miR-33, miR-330, miR-331, miR-335, miR-337, miR-338, miR-339, miR-33b, miR-340, miR-342, miR-345, miR-346, miR-34a, miR-34b, miR-34c, miR-361, miR-362, miR-363, miR-363*, miR-365, miR-367, miR-368, miR-369-3p, miR-369-5p, miR-370, miR-371, miR-372, miR-373, miR-373*, miR-374, miR-375, miR-376a, miR-376a*, miR-376b, miR-377, miR-378, miR-379, miR-380-3p, miR-380-5p, miR-381, miR-382, miR-383, miR-384, miR-409-3p, miR-409-5p, miR-410, miR-411, miR-412, miR-421, miR-422a, miR-422b, miR-423, miR-424, miR-425, miR-425-5p, miR-429, miR-431, miR-432, miR-432*, miR-433, miR-448, miR-449, miR-450, miR-451, miR-452, miR-452*, miR-453, miR-455, miR-483, miR-484, miR-485-3p, miR-485-5p, miR-486, miR-487a, miR-487b, miR-488, miR-489, miR-490, miR-491, miR-492, miR-493, miR-493-3p, miR-494, miR-495, miR-496, miR-497, miR-498, miR-499, miR-500, miR-501, miR-502, miR-503, miR-504, miR-505, miR-506, miR-507, miR-508, miR-509, miR-510, miR-511, miR-512-3p, miR-512-5p, miR-513, miR-514, miR-515-3p, miR-515-5p, miR-516-3p, miR-516-5p, miR-517*, miR-517a, miR-517b, miR-517c, miR-518a, miR-518a-2*, miR-518b, miR-518c, miR-518c*, miR-518d, miR-518e, miR-518f, miR-518f*, miR-519a, miR-519b, miR-519c, miR-519d, miR-519e, miR-519e*, miR-520a, miR-520a*, miR-520b, miR-520c, miR-520d, miR-520d*, miR-520e, miR-520f, miR-520g, miR-520h, miR-521, miR-522, miR-523, miR-524, miR-524*, miR-525, miR-525*, miR-526a, miR-526b, miR-526b*, miR-526c, miR-527, miR-532, miR-542-3p, miR-542-5p, miR-544, miR-545, miR-548a, miR-548b, miR-548c, miR-548d, miR-549, miR-550, miR-551a, miR-552, miR-553, miR-554, miR-555, miR-556, miR-557, miR-558, miR-559, miR-560, miR-561, miR-562, miR-563, miR-564, miR-565, miR-566, miR-567, miR-568, miR-569, miR-570, miR-571, miR-572, miR-573, miR-574, miR-575, miR-576, miR-577, miR-578, miR-579, miR-580, miR-581, miR-582, miR-583, miR-584, miR-585, miR-586, miR-587, miR-588, miR-589, miR-590, miR-591, miR-592, miR-593, miR-594, miR-595, miR-596, miR-597, miR-598, miR-599, miR-600, miR-601, miR-602, miR-603, miR-604, miR-605, miR-606, miR-607, miR-608, miR-609, miR-610, miR-611, miR-612, miR-613, miR-614, miR-615, miR-616, miR-617, miR-618, miR-619, miR-620, miR-621, miR-622, miR-623, miR-624, miR-625, miR-626, miR-627, miR-628, miR-629, miR-630, miR-631, miR-632, miR-633, miR-634, miR-635, miR-636, miR-637, miR-638, miR-639, miR-640, miR-641, miR-642, miR-643, miR-644, miR-645, miR-646, miR-647, miR-648, miR-649, miR-650, miR-651, miR-652, miR-653, miR-654, miR-655, miR-656, miR-657, miR-658, miR-659, miR-660, miR-661, miR-662, miR-663, miR-7, miR-9, miR-9*, miR-92, miR-93, miR-95, miR-96, miR-98, miR-99a and miR-99b.

**31**. The kit according to claim **28**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to determine the physiological and/or pathological condition of a subject after being administrated a test sample.

**32**. The kit according to claim **31**, characterized in that the kit is useful for screening the test sample for the activities on the prevention and/or treatment of diseases.

**33**. The kit according to claim **28**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to diagnose and/or differentially diagnose the diseases of the subject.

**34**. The kit according to claim **28**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to evaluate the effectiveness of treating the diseases of the subject.

**35**. The kit according to claim **28**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to predict the occurrence of diseases of the subject.

**36**. The kit according to claim **35**, characterized in that the occurrence of diseases is the occurrence of complications and/or the relapse of malignant diseases.

**37**. The kit according to claim **32**, characterized in that the diseases include various tumors, various acute and chronic infectious diseases e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute and chronic infectious diseases caused by various pathogenic microorganisms; other acute and chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular disease, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system.

**38**. The kit according to claim **28**, characterized in that the kit is useful for detecting the subject for prohibited drugs-taking.

**39**. The kit according to claim **28**, characterized in that the serum/plasma of the subject are from living bodies, tissues, organs and/or corpuses of the subject.

**40**. A biochip for evaluating the physiological and/or pathological condition of a subject, wherein the biochip contains components useful for determining all detectable microRNAs stably existing in the serum/plasma of the subject.

**41**. The biochip according to claim **40**, characterized in that the biochip contains the probes for all mature microRNAs in human serum/plasma.

**42**. The biochip according to claim **40**, characterized in that the biochip comprises the following probes:

| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-let-7a | let-7a | AACTATACAACCTACTACCTCA |
| probe-let-7b | let-7b | AACCACACAACCTACTACCTCA |
| probe-let-7c | let-7c | AACCATACAACCTACTACCTCA |
| probe-let-7d | let-7d | ACTATGCAACCTACTACCTCT |
| probe-let-7e | let-7e | ACTATACAACCTCCTACCTCA |
| probe-let-7f | let-7f | AACTATACAATCTACTACCTCA |
| probe-let-7g | let-7g | ACTGTACAAACTACTACCTCA |
| probe-let-7i | let-7i | ACAGCACAAACTACTACCTCA |
| probe-miR-1 | miR-1 | TACATACTTCTTTACATTCCA |
| probe-miR-100 | miR-100 | CACAAGTTCGGATCTACGGGTT |
| probe-miR-101 | miR-101 | CTTCAGTTATCACAGTACTGTA |
| probe-miR-103 | miR-103 | TCATAGCCCTGTACAATGCTGCT |
| probe-miR-105 | miR-105 | ACAGGAGTCTGAGCATTTGA |
| probe-miR-106a | miR-106a | GCTACCTGCACTGTAAGCACTTTT |
| probe-miR-106b | miR-106b | ATCTGCACTGTCAGCACTTTA |
| probe-miR-107 | miR-107 | TGATAGCCCTGTACAATGCTGCT |
| probe-miR-10a | miR-10a | CACAAATTCGGATCTACAGGGTA |
| probe-miR-10b | miR-10b | ACAAATTCGGTTCTACAGGGTA |
| probe-miR-122a | miR-122a | ACAAACACCATTGTCACACTCCA |
| probe-miR-124a | miR-124a | TGGCATTCACCGCGTGCCTTAA |
| probe-miR-125a | miR-125a | CACAGGTTAAAGGGTCTCAGGGA |
| probe-miR-125b | miR-125b | TCACAAGTTAGGGTCTCAGGGA |
| probe-miR-126 | miR-126 | GCATTATTACTCACGGTACGA |
| probe-miR-126* | miR-126* | CGCGTACCAAAAGTAATAATG |
| probe-miR-127 | miR-127 | AGCCAAGCTCAGACGGATCCGA |
| probe-miR-128a | miR-128a | AAAAGAGACCGGTTCACTGTGA |
| probe-miR-128b | miR-128b | GAAAGAGACCGGTTCACTGTGA |
| probe-miR-129 | miR-129 | GCAAGCCCAGACCGCAAAAAG |
| probe-miR-130a | miR-130a | ATGCCCTTTTAACATTGCACTG |
| probe-miR-130b | miR-130b | ATGCCCTTTCATCATTGCACTG |
| probe-miR-132 | miR-132 | CGACCATGGCTGTAGACTGTTA |
| probe-miR-133a | miR-133a | ACAGCTGGTTGAAGGGGACCAA |
| probe-miR-133b | miR-133b | TAGCTGGTTGAAGGGGACCAA |
| probe-miR-134 | miR-134 | CCCTCTGGTCAACCAGTCACA |
| probe-miR-135a | miR-135a | TCACATAGGAATAAAAAGCCATA |
| probe-miR-135b | miR-135b | CACATAGGAATGAAAAGCCATA |
| probe-miR-136 | miR-136 | TCCATCATCAAAACAAATGGAGT |
| probe-miR-137 | miR-137 | CTACGCGTATTCTTAAGCAATA |
| probe-miR-138 | miR-138 | GATTCACAACACCAGCT |
| probe-miR-139 | miR-139 | AGACACGTGCACTGTAGA |
| probe-miR-140 | miR-140 | CTACCATAGGGTAAAACCACT |
| probe-miR-141 | miR-141 | CCATCTTTACCAGACAGTGTTA |
| probe-miR-142-3p | miR-142-3p | TCCATAAAGTAGGAAACACTACA |
| probe-miR-142-5p | miR-142-5p | GTAGTGCTTTCTACTTTATG |
| probe-miR-143 | miR-143 | TGAGCTACAGTGCTTCATCTCA |
| probe-miR-144 | miR-144 | CTAGTACATCATCTATACTGTA |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-145 | miR-145 | AAGGGATTCCTGGGAAAACTGGAC |
| probe-miR-146a | miR-146a | AACCCATGGAATTCAGTTCTCA |
| probe-miR-146b | miR-146b | AGCCTATGGAATTCAGTTCTCA |
| probe-miR-147 | miR-147 | GCAGAAGCATTTCCACACAC |
| probe-miR-148a | miR-148a | ACAAAGTTCTGTAGTGCACTGA |
| probe-miR-148b | miR-148b | ACAAAGTTCTGTGATGCACTGA |
| probe-miR-149 | miR-149 | GGAGTGAAGACACGGAGCCAGA |
| probe-miR-150 | miR-150 | CACTGGTACAAGGGTTGGGAGA |
| probe-miR-151 | miR-151 | CCTCAAGGAGCTTCAGTCTAGT |
| probe-miR-152 | miR-152 | CCCAAGTTCTGTCATGCACTGA |
| probe-miR-153 | miR-153 | TCACTTTTGTGACTATGCAA |
| probe-miR-154 | miR-154 | CGAAGGCAACACGGATAACCTA |
| probe-miR-154* | miR-154* | AATAGGTCAACCGTGTATGATT |
| probe-miR-155 | miR-155 | CCCCTATCACGATTAGCATTAA |
| probe-miR-15a | miR-15a | CACAAACCATTATGTGCTGCTA |
| probe-miR-15b | miR-15b | TGTAAACCATGATGTGCTGCTA |
| probe-miR-16 | miR-16 | CGCCAATATTTACGTGCTGCTA |
| probe-miR-17-3p | miR-17-3p | ACAAGTGCCTTCACTGCAGT |
| probe-miR-17-5p | miR-17-5p | ACTACCTGCACTGTAAGCACTTTG |
| probe-miR-181a | miR-181a | ACTCACCGACAGCGTTGAATGTT |
| probe-miR-181b | miR-181b | CCCACCGACAGCAATGAATGTT |
| probe-miR-181c | miR-181c | ACTCACCGACAGGTTGAATGTT |
| probe-miR-181d | miR-181d | AACCCACCGACAACAATGAATGTT |
| probe-miR-182 | miR-182 | TGTGAGTTCTACCATTGCCAAA |
| probe-miR-182* | miR-182* | TAGTTGGCAAGTCTAGAACCA |
| probe-miR-183 | miR-183 | CAGTGAATTCTACCAGTGCCATA |
| probe-miR-184 | miR-184 | ACCCTTATCAGTTCTCCGTCCA |
| probe-miR-185 | miR-185 | GAACTGCCTTTCTCTCCA |
| probe-miR-186 | miR-186 | AAGCCCAAAAGGAGAATTCTTTG |
| probe-miR-187 | miR-187 | CGGCTGCAACACAAGACACGA |
| probe-miR-188 | miR-188 | ACCCTCCACCATGCAAGGGATG |
| probe-miR-189 | miR-189 | ACTGATATCAGCTCAGTAGGCAC |
| probe-miR-18a | miR-18a | TATCTGCACTAGATGCACCTTA |
| probe-miR-18a* | miR-18a* | AGAAGGAGCACTTAGGGCAGT |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-18b | miR-18b | TAACTGCACTAGATGCACCTTA |
| probe-miR-190 | miR-190 | ACCTAATATATCAAACATATCA |
| probe-miR-191 | miR-191 | AGCTGCTTTTGGGATTCCGTTG |
| probe-miR-191* | miR-191* | GGGGACGAAATCCAAGCGCAGC |
| probe-miR-192 | miR-192 | GGCTGTCAATTCATAGGTCAG |
| probe-miR-193a | miR-193a | CTGGGACTTTGTAGGCCAGTT |
| probe-miR-193b | miR-193b | AAAGCGGGACTTTGAGGGCCAGTT |
| probe-miR-194 | miR-194 | TCCACATGGAGTTGCTGTTACA |
| probe-miR-195 | miR-195 | GCCAATATTTCTGTGCTGCTA |
| probe-miR-196a | miR-196a | CCAACAACATGAAACTACCTA |
| probe-miR-196b | miR-196b | CCAACAACAGGAAACTACCTA |
| probe-miR-197 | miR-197 | GCTGGGTGGAGAAGGTGGTGAA |
| probe-miR-198 | miR-198 | CCTATCTCCCCTCTGGACC |
| probe-miR-199a | miR-199a | GAACAGGTAGTCTGAACACTGGG |
| probe-miR-199a* | miR-199a* | AACCAATGTGCAGACTACTGTA |
| probe-miR-199b | miR-199b | GAACAGATAGTCTAAACACTGGG |
| probe-miR-19a | miR-19a | TCAGTTTTGCATAGATTTGCACA |
| probe-miR-19b | miR-19b | TCAGTTTTGCATGGATTTGCACA |
| probe-miR-200a | miR-200a | ACATCGTTACCAGACAGTGTTA |
| probe-miR-200a* | miR-200a* | TCCAGCACTGTCCGGTAAGATG |
| probe-miR-200b | miR-200b | GTCATCATTACCAGGCAGTATTA |
| probe-miR-200c | miR-200c | CCATCATTACCCGGCAGTATTA |
| probe-miR-202 | miR-202 | TTTTCCCATGCCCTATACCTCT |
| probe-miR-202* | miR-202* | AAAGAAGTATATGCATAGGAAA |
| probe-miR-203 | miR-203 | CTAGTGGTCCTAAACATTTCAC |
| probe-miR-204 | miR-204 | AGGCATAGGATGACAAAGGGAA |
| probe-miR-205 | miR-205 | CAGACTCCGGTGGAATGAAGGA |
| probe-miR-206 | miR-206 | CCACACACTTCCTTACATTCCA |
| probe-miR-208 | miR-208 | ACAAGCTTTTTGCTCGTCTTAT |
| probe-miR-20a | miR-20a | CTACCTGCACTATAAGCACTTTA |
| probe-miR-20b | miR-20b | CTACCTGCACTATGAGCACTTTG |
| probe-miR-21 | miR-21 | TCAACATCAGTCTGATAAGCTA |
| probe-miR-210 | miR-210 | TCAGCCGCTGTCACACGCACAG |
| probe-miR-211 | miR-211 | AGGCGAAGGATGACAAAGGGAA |
| probe-miR-212 | miR-212 | GGCCGTGACTGGAGACTGTTA |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-213 | miR-213 | GGTACAATCAACGGTCGATGGT |
| probe-miR-214 | miR-214 | CTGCCTGTCTGTGCCTGCTGT |
| probe-miR-215 | miR-215 | GTCTGTCAATTCATAGGTCAT |
| probe-miR-216 | miR-216 | CACAGTTGCCAGCTGAGATTA |
| probe-miR-217 | miR-217 | ATCCAATCAGTTCCTGATGCAGTA |
| probe-miR-218 | miR-218 | ACATGGTTAGATCAAGCACAA |
| probe-miR-219 | miR-219 | AGAATTGCGTTTGGACAATCA |
| probe-miR-22 | miR-22 | ACAGTTCTTCAACTGGCAGCTT |
| probe-miR-220 | miR-220 | AAAGTGTCAGATACGGTGTGG |
| probe-miR-221 | miR-221 | GAAACCCAGCAGACAATGTAGCT |
| probe-miR-222 | miR-222 | GAGACCCAGTAGCCAGATGTAGCT |
| probe-miR-223 | miR-223 | GGGGTATTTGACAAACTGACA |
| probe-miR-224 | miR-224 | TAAACGGAACCACTAGTGACTTG |
| probe-miR-23a | miR-23a | GGAAATCCCTGGCAATGTGAT |
| probe-miR-23b | miR-23b | GGTAATCCCTGGCAATGTGAT |
| probe-miR-24 | miR-24 | CTGTTCCTGCTGAACTGAGCCA |
| probe-miR-25 | miR-25 | TCAGACCGAGACAAGTGCAATG |
| probe-miR-26a | miR-26a | GCCTATCCTGGATTACTTGAA |
| probe-miR-26b | miR-26b | AACCTATCCTGAATTACTTGAA |
| probe-miR-27a | miR-27a | GCGGAACTTAGCCACTGTGAA |
| probe-miR-27b | miR-27b | GCAGAACTTAGCCACTGTGAA |
| probe-miR-28 | miR-28 | CTCAATAGACTGTGAGCTCCTT |
| probe-miR-296 | miR-296 | ACAGGATTGAGGGGGGGCCCT |
| probe-miR-299-3p | miR-299-3p | AAGCGGTTTACCATCCCACATA |
| probe-miR-299-5p | miR-299-5p | ATGTATGTGGGACGGTAAACCA |
| probe-miR-29a | miR-29a | AACCGATTTCAGATGGTGCTA |
| probe-miR-29b | miR-29b | AACACTGATTTCAAATGGTGCTA |
| probe-miR-29c | miR-29c | ACCGATTTCAAATGGTGCTA |
| probe-miR-301 | miR-301 | GCTTTGACAATACTATTGCACTG |
| probe-miR-302a | miR-302a | TCACCAAAACATGGAAGCACTTA |
| probe-miR-302a | miR-302a* | AAAGCAAGTACATCCACGTTTA |
| probe-miR-302b | miR-302b | CTACTAAAACATGGAAGCACTTA |
| probe-miR-302b* | miR-302b* | AGAAAGCACTTCCATGTTAAAGT |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-302c | miR-302c | CCACTGAAACATGGAAGCACTTA |
| probe-miR-302c* | miR-302c* | CAGCAGGTACCCCCATGTTAAA |
| probe-miR-302d | miR-302d | ACACTCAAACATGGAAGCACTTA |
| probe-miR-30a-3p | miR-30a-3p | GCTGCAAACATCCGACTGAAAG |
| probe-miR-30a-5p | miR-30a-5p | CTTCCAGTCGAGGATGTTTACA |
| probe-miR-30b | miR-30b | AGCTGAGTGTAGGATGTTTACA |
| probe-miR-30c | miR-30c | GCTGAGAGTGTAGGATGTTTACA |
| probe-miR-30d | miR-30d | CTTCCAGTCGGGGATGTTTACA |
| probe-miR-30e-3p | miR-30e-3p | GCTGTAAACATCCGACTGAAAG |
| probe-miR-30e-5p | miR-30e-5p | TCCAGTCAAGGATGTTTACA |
| probe-miR-31 | miR-31 | CAGCTATGCCAGCATCTTGCC |
| probe-miR-32 | miR-32 | GCAACTTAGTAATGTGCAATA |
| probe-miR-320 | miR-320 | TTCGCCCTCTCAACCCAGCTTTT |
| probe-miR-323 | miR-323 | AGAGGTCGACCGTGTAATGTGC |
| probe-miR-324-3p | miR-324-3p | CCAGCAGCACCTGGGGCAGTGG |
| probe-miR-324-5p | miR-324-5p | ACACCAATGCCCTAGGGGATGCG |
| probe-miR-325 | miR-325 | ACACTTACTGGACACCTACTAGG |
| probe-miR-326 | miR-326 | CTGGAGGAAGGGCCCAGAGG |
| probe-miR-328 | miR-328 | ACGGAAGGGCAGAGAGGGCCAG |
| probe-miR-329 | miR-329 | AAAGAGGTTAACCAGGTGTGTT |
| probe-miR-33 | miR-33 | CAATGCAACTACAATGCAC |
| probe-miR-330 | miR-330 | TCTCTGCAGGCCGTGTGCTTTGC |
| probe-miR-331 | miR-331 | TTCTAGGATAGGCCCAGGGGC |
| probe-miR-335 | miR-335 | ACATTTTTCGTTATTGCTCTTGA |
| probe-miR-337 | miR-337 | AAAGGCATCATATAGGAGCTGGA |
| probe-miR-338 | miR-338 | TCAACAAAATCACTGATGCTGGA |
| probe-miR-339 | miR-339 | TGAGCTCCTGGAGGACAGGGA |
| probe-miR-33b | miR-33b | TGCAATGCAACAGCAATGCAC |
| probe-miR-340 | miR-340 | GGCTATAAAGTAACTGAGACGGA |
| probe-miR-342 | miR-342 | GACGGGTGCGATTTCTGTGTGAGA |
| probe-miR-345 | miR-345 | GCCCTGGACTAGGAGTCAGCA |
| probe-miR-346 | miR-346 | AGAGGCAGGCATGCGGGCAGACA |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-34a | miR-34a | AACAACCAGCTAAGACACTGCCA |
| probe-miR-34b | miR-34b | CAATCAGCTAATGACACTGCCTA |
| probe-miR-34c | miR-34c | GCAATCAGCTAACTACACTGCCT |
| probe-miR-361 | miR-361 | GTACCCCTGGAGATTCTGATAA |
| probe-miR-362 | miR-362 | CTCACACCTAGGTTCCAAGGATT |
| probe-miR-363 | miR-363 | TTACAGATGGATACCGTGCAAT |
| probe-miR-363* | miR-363* | AAATTGCATCGTGATCCACCCG |
| probe-miR-365 | miR-365 | ATAAGGATTTTTAGGGGCATTA |
| probe-miR-367 | miR-367 | TCACCATTGCTAAAGTGCAATT |
| probe-miR-368 | miR-368 | AAACGTGGAATTTCCTCTATGT |
| probe-miR-369-3p | miR-369-3p | AAAGATCAACCATGTATTATT |
| probe-miR-369-5p | miR-369-5p | GCGAATATAACACGGTCGATCT |
| probe-miR-370 | miR-370 | CCAGGTTCCACCCCAGCAGGC |
| probe-miR-371 | miR-371 | ACACTCAAAAGATGGCGGCAC |
| probe-miR-372 | miR-372 | ACGCTCAAATGTCGCAGCACTTT |
| probe-miR-373 | miR-373 | ACACCCCAAAATCGAAGCACTTC |
| probe-miR-373* | miR-373* | GGAAAGCGCCCCCATTTTGAGT |
| probe-miR-374 | miR-374 | CACTTATCAGGTTGTATTATAA |
| probe-miR-375 | miR-375 | TCACGCGAGCCGAACGAACAAA |
| probe-miR-376a | miR-376a | ACGTGGATTTTCCTCTATGAT |
| probe-miR-376a* | miR-376a* | CTCATAGAAGGAGAATCTACC |
| probe-miR-376b | miR-376b | AACATGGATTTTCCTCTATGAT |
| probe-miR-377 | miR-377 | ACAAAAGTTGCCTTTGTGTGAT |
| probe-miR-378 | miR-378 | ACACAGGACCTGGAGTCAGGAG |
| probe-miR-379 | miR-379 | TACGTTCCATAGTCTACCA |
| probe-miR-380-3p | miR-380-3p | AAGATGTGGACCATATTACATA |
| probe-miR-380-5p | miR-380-5p | GCGCATGTTCTATGGTCAACCA |
| probe-miR-381 | miR-381 | ACAGAGAGCTTGCCCTTGTATA |
| probe-miR-382 | miR-382 | CGAATCCACCACGAACAACTTC |
| probe-miR-383 | miR-383 | AGCCACAATCACCTTCTGATCT |
| probe-miR-384 | miR-384 | TATGAACAATTTCTAGGAAT |
| probe-miR-409-3p | miR-409-3p | AGGGGTTCACCGAGCAACATTCG |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-409-5p | miR-409-5p | TGCAAAGTTGCTCGGGTAACCT |
| probe-miR-410 | miR-410 | AACAGGCCATCTGTGTTATATT |
| probe-miR-411 | miR-411 | CGTACGCTATACGGTCTACTA |
| probe-miR-412 | miR-412 | ACGGCTAGTGGACCAGGTGAAGT |
| probe-miR-421 | miR-421 | GCGCCCAATTAATGTCTGTTGAT |
| probe-miR-422a | miR-422a | GGCCTTCTGACCCTAAGTCCAG |
| probe-miR-422b | miR-422b | GGCCTTCTGACTCCAAGTCCAG |
| probe-miR-423 | miR-423 | CTGAGGGGCCTCAGACCGAGCT |
| probe-miR-424 | miR-424 | TTCAAAACATGAATTGCTGCTG |
| probe-miR-425 | miR-425 | GGCGGACACGACATTCCCGAT |
| probe-miR-425-5p | miR-425-5p | TCAACGGGAGTGATCGTGTCATT |
| probe-miR-429 | miR-429 | ACGGTTTTACCAGACAGTATTA |
| probe-miR-431 | miR-431 | TGCATGACGGCCTGCAAGACA |
| probe-miR-432 | miR-432 | CCACCCAATGACCTACTCCAAGA |
| nrobe-miR-432* | miR-432* | AGACATGGAGGAGCCATCCAG |
| probe-miR-433 | miR-433 | ACACCGAGGAGCCCATCATGAT |
| probe-miR-448 | miR-448 | ATGGGACATCCTACATATGCAA |
| probe-miR-449 | miR-449 | ACCAGCTAACAATACACTGCCA |
| probe-miR-450 | miR-450 | TATTAGGAACACATCGCAAAAA |
| probe-miR-451 | miR-451 | AAACTCAGTAATGGTAACGGTTT |
| probe-miR-452 | miR-452 | GTCTCAGTTTCCTCTGCAAACA |
| probe-miR-452* | miR-452* | CTTCTTTGCAGATGAGACTGA |
| probe-miR-453 | miR-453 | CGAACTCACCACGGACAACCTC |
| probe-miR-455 | miR-455 | CGATGTAGTCCAAAGGCACATA |
| probe-miR-483 | miR-483 | AGAAGACGGGAGGAGAGGAGTGA |
| probe-miR-484 | miR-484 | ATCGGGAGGGGACTGAGCCTGA |
| probe-miR-485-3p | miR-485-3p | AGAGGAGAGCCGTGTATGAC |
| probe-miR-485-5p | miR-485-5p | GAATTCATCACGGCCAGCCTCT |
| probe-miR-486 | miR-486 | CTCGGGGCAGCTCAGTACAGGA |
| probe-miR-487a | miR-487a | AACTGGATGTCCCTGTATGATT |
| probe-miR-487b | miR-487b | AAGTGGATGACCCTGTACGATT |
| probe-miR-488 | miR-488 | TTGAGAGTGCCATTATCTGGG |
| probe-miR-489 | miR-489 | GCTGCCGTATATGTGATGTCACT |
| probe-miR-490 | miR-490 | CAGCATGGAGTCCTCCAGGTTG |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-491 | miR-491 | TCCTCATGGAAGGGTTCCCCACT |
| probe-miR-492 | miR-492 | AAGAATCTTGTCCCGCAGGTCCT |
| probe-miR-493 | miR-493 | AATGAAAGCCTACCATGTACAA |
| probe-miR-493-3p | miR-493-3p | CTGGCACACAGTAGACCTTCA |
| probe-miR-494 | miR-494 | AAGAGGTTTCCCGTGTATGTTTCA |
| probe-miR-495 | miR-495 | AAAGAAGTGCACCATGTTTGTTT |
| probe-miR-496 | miR-496 | GAGATTGGCCATGTAAT |
| probe-miR-497 | miR-497 | ACAAACCACAGTGTGCTGCTG |
| probe-miR-498 | miR-498 | GAAAAACGCCCCCTGGCTTGAAA |
| probe-miR-499 | miR-499 | TTAAACATCACTGCAAGTCTTAA |
| probe-miR-500 | miR-500 | CAGAATCCTTGCCCAGGTGCAT |
| probe-miR-501 | miR-501 | TCTCACCCAGGGACAAAGGATT |
| probe-miR-502 | miR-502 | TAGCACCCAGATAGCAAGGAT |
| probe-miR-503 | miR-503 | CTGCAGAACTGTTCCCGCTGCTA |
| probe-miR-504 | miR-504 | ATAGAGTGCAGACCAGGGTCT |
| probe-miR-505 | miR-505 | GAGGAAACCAGCAAGTGTTGAC |
| probe-miR-506 | miR-506 | TCTACTCAGAAGGGTGCCTTA |
| probe-miR-507 | miR-507 | TTCACTCCAAAAGGTGCAAAA |
| probe-miR-508 | miR-508 | TCTACTCCAAAAGGCTACAATCA |
| probe-miR-509 | miR-509 | TCTACCCACAGACGTACCAATCA |
| probe-miR-510 | miR-510 | TGTGATTGCCACTCTCCTGAGTA |
| probe-miR-511 | miR-511 | TGACTGCAGAGCAAAAGACAC |
| probe-miR-512-3p | miR-512-3p | GACCTCAGCTATGACAGCACTT |
| probe-miR-512-5p | miR-512-5p | GAAAGTGCCCTCAAGGCTGAGTG |
| probe-miR-513 | miR-513 | ATAAATGACACCTCCCTGTGAA |
| probe-miR-514 | miR-514 | CTACTCACAGAAGTGTCAAT |
| probe-miR-515-3p | miR-515-3p | ACGCTCCAAAAGAAGGCACTC |
| probe-miR-515-5p | miR-515-5p | CAGAAAGTGCTTTCTTTTGGAGAA |
| probe-miR-516-3p | miR-516-3p | ACCCTCTGAAAGGAAGCA |
| probe-miR-516-5p | miR-516-5p | AAAGTGCTTCTTACCTCCAGAT |
| probe-miR-517* | miR-517* | AGACAGTGCTTCCATCTAGAGG |
| probe-miR-517a | miR-517a | AACACTCTAAAGGGATGCACGAT |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-517b | miR-517b | AACACTCTAAAGGGATGCACGA |
| probe-miR-517c | miR-517c | ACACTCTAAAAGGATGCACGAT |
| probe-miR-518a | miR-518a | TCCAGCAAAGGGAAGCGCTTT |
| probe-miR-518a-2* | miR-518a-2* | AAAGGGCTTCCCTTTGCAGA |
| probe-miR-518b | miR-518b | ACCTCTAAAGGGGAGCGCTTTG |
| probe-miR-518c | miR-518c | CACTCTAAAGAGAAGCGCTTTG |
| probe-miR-518c* | miR-518c* | CAGAAAGTGCTTCCCTCCAGAGA |
| probe-miR-518d | miR-518d | GCTCCAAAGGGAAGCGCTTTG |
| probe-miR-518e | miR-518e | ACACTCTGAAGGGAAGCGCTTT |
| probe-miR-518f | miR-518f | TCCTCTAAAGAGAAGCGCTTT |
| probe-miR-518f* | miR-518f* | AGAGAAAGTGCTTCCCTCTAGAG |
| probe-miR-519a | miR-519a | GTAACACTCTAAAAGGATGCACTTT |
| probe-miR-519b | miR-519b | AAACCTCTAAAAGGATGCACTTT |
| probe-miR-519c | miR-519c | ATCCTCTAAAAAGATGCACTTT |
| probe-miR-519d | miR-519d | ACACTCTAAAGGGAGGCACTTTG |
| probe-miR-519e | miR-519e | ACACTCTAAAAGGAGGCACTTT |
| probe-miR-519e* | miR-519e* | GAAAGTGCTCCCTTTTGGAGAA |
| probe-miR-520a | miR-520a | ACAGTCCAAAGGGAAGCACTTT |
| probe-miR-520a* | miR-520a* | AGAAAGTACTTCCCTCTGGAG |
| probe-miR-520b | miR-520b | CCCTCTAAAAGGAAGCACTTT |
| probe-miR-520c | miR-520c | AACCCTCTAAAAGGAAGCACTTT |
| probe-miR-520d | miR-520d | AACCCACCAAAGAGAAGCACTTT |
| probe-miR-520d* | miR-520d* | CAGAAAGGGCTTCCCTTTGTAGA |
| probe-miR-520e | miR-520e | CCCTCAAAAAGGAAGCACTTT |
| probe-miR-520f | miR-520f | AACCCTCTAAAAGGAAGCACTT |
| probe-miR-520g | miR-520g | ACACTCTAAAGGGAAGCACTTTGT |
| probe-miR-520h | miR-520h | ACTCTAAAGGGAAGCACTTTGT |
| probe-miR-521 | miR-521 | ACACTCTAAAGGGAAGTGCGTT |
| probe-miR-522 | miR-522 | AACACTCTAAAGGGAACCATTTT |
| probe-miR-523 | miR-523 | CCCTCTATAGGGAAGCGCGTT |
| probe-miR-524 | miR-524 | ACTCCAAAGGGAAGCGCCTTC |
| probe-miR-524* | miR-524* | GAGAAAGTGCTTCCCTTTGTAG |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-525 | miR-525 | AGAAAGTGCATCCCTCTGGAG |
| probe-miR-525* | miR-525* | GCTCTAAAGGGAAGCGCCTTC |
| probe-miR-526a | miR-526a | AGAAAGTGCTTCCCTCTAGAG |
| probe-miR-526b | miR-526b | AACAGAAAGTGCTTCCCTCAAGAG |
| probe-miR-526b* | miR-526b* | GCCTCTAAAAGGAAGCACTTT |
| probe-miR-526c | miR-526c | AACAGAAAGCGCTTCCCTCTAGAG |
| probe-miR-527 | miR-527 | AGAAAGGGCTTCCCTTTGCAG |
| probe-miR-532 | miR-532 | ACGGTCCTACACTCAAGGCATG |
| probe-miR-542-3p | miR-542-3p | TTTCAGTTATCAATCTGTCACA |
| probe-miR-542-5p | miR-542-5p | CTCGTGACATGATGATCCCCGA |
| probe-miR-544 | miR-544 | ACTTGCTAAAAATGCAGAAT |
| probe-miR-545 | miR-545 | CACACAATAAATGTTTGCTGAT |
| probe-miR-548a | miR-548a | GCAAAAGTAATTGCCAGTTTTG |
| probe-miR-548b | miR-548b | ACAAAAGCAACTGAGGTTCTTG |
| probe-miR-548c | miR-548c | GCAAAAGTAATTGAGATTTTTG |
| probe-miR-548d | miR-548d | GCAAAAGAAACTGTGGTTTTTG |
| probe-miR-549 | miR-549 | AGAGCTCATCCATAGTTGTCA |
| probe-miR-550 | miR-550 | ATGTGCCTGAGGGAGTAAGACA |
| probe-miR-551a | miR-551a | TGGAAACCAAGAGTGGGTCGC |
| probe-miR-552 | miR-552 | TTGTCTAACCAGTCACCTGTT |
| probe-miR-553 | miR-553 | AAAACAAAATCTCACCGTTTT |
| probe-miR-554 | miR-554 | ACTGGCTGAGTCAGGACTAGC |
| probe-miR-555 | miR-555 | ATCAGAGGTTCAGCTTACCCT |
| probe-miR-556 | miR-556 | CATATTACAATGAGCTCATC |
| probe-miR-557 | miR-557 | AGACAAGGCCCACCCGTGCAAAC |
| probe-miR-558 | miR-558 | ATTTTGGTACAGCAGCTCA |
| probe-miR-559 | miR-559 | TTTTGGTGCATATTTACTTTA |
| probe-miR-560 | miR-560 | GGCGGCCGGCCGGCGCACGC |
| probe-miR-561 | miR-561 | ACTTCAAGGATCTTAAACTTTG |
| probe-miR-562 | miR-562 | GCAAATGGTACAGCTACTTT |
| probe-miR-563 | miR-563 | GGGAAACGTATGTCAACCT |
| probe-miR-564 | miR-564 | GCCTGCTGACACCGTGCCT |
| probe-miR-565 | miR-565 | AAACAGACATCGCGAGCCAGCC |
| probe-miR-566 | miR-566 | GTTGGGATCACAGGCGCCC |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-567 | miR-567 | GTTCTGTCCTGGAAGAACATACT |
| probe-miR-568 | miR-568 | GTGTGTATACATTTATACAT |
| probe-miR-569 | miR-569 | ACTTTCCAGGATTCATTAACT |
| probe-miR-570 | miR-570 | TGCAAAGGTAATTGCTGTTTTC |
| probe-miR-571 | miR-571 | CTCACTCAGATGGCCAACTCA |
| probe-miR-572 | miR-572 | TGGGCCACCGCCGAGCGGAC |
| probe-miR-573 | miR-573 | CTGATCAGTTACACATCACTTCAG |
| probe-miR-574 | miR-574 | GTGGGTGTGTGCATGAGCGTG |
| probe-miR-575 | miR-575 | GCTCCTGTCCAACTGGCTC |
| probe-miR-576 | miR-576 | CAAAGACGTGGAGAAATTAGAAT |
| probe-miR-577 | miR-577 | CAGGTACCAATATTTTATCTA |
| probe-miR-578 | miR-578 | ACAATCCTAGAGCACAAGAAG |
| probe-miR-579 | miR-579 | ATCGCGGTTTATACCAAATGAAT |
| probe-miR-580 | miR-580 | CCTAATGATTCATCATTCTCAA |
| probe-miR-581 | miR-581 | ACTGATCTAGAGAACACAAGA |
| probe-miR-582 | miR-582 | AGTAACTGGTTGAACAACTGTAA |
| probe-miR-583 | miR-583 | GTAATGGGACCTTCCTCTTTG |
| probe-miR-584 | miR-584 | CTCAGTCCCAGGCAAACCATAA |
| probe-miR-585 | miR-585 | TAGCATACAGATACGCCCA |
| probe-miR-586 | miR-586 | GGACCTAAAAATACAATGCATA |
| probe-miR-587 | miR-587 | GTGACTCATCACCTATGGAAA |
| probe-miR-588 | miR-588 | GTTCTAACCCATTGTGGCCAA |
| probe-miR-589 | miR-589 | TCTGGGAACCGGCATTTGTTCTGA |
| probe-miR-590 | miR-590 | CTGCACTTTTATGAATAAGCTC |
| probe-miR-591 | miR-591 | ACAATGAGAACCCATGGTCT |
| probe-miR-592 | miR-592 | ACATCATCGCATATTGACACAA |
| probe-miR-593 | miR-593 | GCTGAGCAATGCCTGGCTGGTGCCT |
| probe-miR-594 | miR-594 | AAAGTCACAGGCCACCCCAGATGGG |
| probe-miR-595 | miR-595 | AGACACACCACGGCACACTTC |
| probe-miR-596 | miR-596 | CCCGAGGAGCCGGGCAGGCTT |
| probe-miR-597 | miR-597 | ACAGTGGTCATCGAGTGACACA |
| probe-miR-598 | miR-598 | TGACGATGACAACGATGACGTA |
| probe-miR-599 | miR-599 | GTTTGATAAACTGACACAAC |
| probe-miR-600 | miR-600 | GAGCAAGGCTCTTGTCTGTAAGT |
| probe-miR-601 | miR-601 | CTCCTCCAACAATCCTAGACCA |
| probe-miR-602 | miR-602 | GGGCCGCAGCTGTCGCCCGTGTC |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-603 | miR-603 | GCAAAAGTAATTGCAGTGTGTG |
| probe-miR-604 | miR-604 | GTCCTGAATTCCGCAGCCT |
| probe-miR-605 | miR-605 | AGGAGAAGGCACCATGGGATTTA |
| probe-miR-606 | miR-606 | ATCTTTGATTTTCAGTAGTTT |
| probe-miR-607 | miR-607 | GTTATAGATCTGGATTTGAAC |
| probe-miR-608 | miR-608 | ACGGAGCTGTCCCAACACCACCCCT |
| probe-miR-609 | miR-609 | AGAGATGAGAGAAACACCCT |
| probe-miR-610 | miR-610 | TCCCAGCACACATTTAGCTCA |
| probe-miR-611 | miR-611 | GTCAGACCCCGAGGGGTCCTCGC |
| probe-miR-612 | miR-612 | AAGGAGCTCAGAAGCCCTGCCCAGC |
| probe-miR-613 | miR-613 | GGCAAAGAAGGAACATTCCT |
| probe-miR-614 | miR-614 | CCACCTGGCAAGAACAGGCGTTC |
| probe-miR-615 | miR-615 | AGAGGGAGACCCAGGCTCGGA |
| probe-miR-616 | miR-616 | AAGTCACTGAAGGGTTTTGAGT |
| probe-miR-617 | miR-617 | GCCACCTTCAAATGGGAAGTCT |
| probe-miR-618 | miR-618 | ACTCAGAAGGACAAGTAGAGTTT |
| probe-miR-619 | miR-619 | ACTGGGCACAAACATGTCCAGGTC |
| probe-miR-620 | miR-620 | ATTTCTATATCTATCTCCAT |
| probe-miR-621 | miR-621 | AGGTAAGCGCTGTTGCTAGCC |
| probe-miR-622 | miR-622 | GCTCCAACCTCAGCAGACTGT |
| probe-miR-623 | miR-623 | ACCCAACAGCCCCTGCAAGGGAT |
| probe-miR-624 | miR-624 | TGAACACAAGGTACTGGTACTA |
| probe-miR-625 | miR-625 | AGGACTATAGAACTTTCCCCCT |
| probe-miR-626 | miR-626 | AAGACATTTTCAGACAGCT |
| probe-miR-627 | miR-627 | TCCTCTTTTCTTAGAGACTCAC |
| probe-miR-628 | miR-628 | CGACTGCCACTCTTACTAGA |
| probe-miR-629 | miR-629 | GCTGGGCTTACGTTGGGAGAAC |
| probe-miR-630 | miR-630 | ACCTTCCCTGGTACAGAATACT |
| probe-miR-631 | miR-631 | GCTGAGGTCTGGGCCAGGTCT |
| probe-miR-632 | miR-632 | TCCCACAGGAAGCAGACAC |
| probe-miR-633 | miR-633 | TTTATTGTGGTAGATACTATTAG |
| probe-miR-634 | miR-634 | GTCCAAAGTTGGGGTGCTGGTT |
| probe-miR-635 | miR-635 | GGACATTGTTTCAGTGCCCAAGT |
| probe-miR-636 | miR-636 | CTGCGGGCGGGACGAGCAAGCACA |
| probe-miR-637 | miR-637 | ACGCAGAGCCCGAAAGCCCCCAGT |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-638 | miR-638 | AGGCCGCCACCCGCCCGCGATCCCT |
| probe-miR-639 | miR-639 | ACAGCGCTCGCAACCGCAGCGAT |
| probe-miR-640 | miR-640 | AGAGGCAGGTTCCTGGATCAT |
| probe-miR-641 | miR-641 | GAGGTGACTCTATCCTATGTCTTT |
| probe-miR-642 | miR-642 | CAAGACACATTTGGAGAGGGAC |
| probe-miR-643 | miR-643 | CTACCTGAGCTAGCATACAAGT |
| probe-miR-644 | miR-644 | GCTCTAAGAAAGCCACACT |
| probe-miR-645 | miR-645 | TCAGCAGTACCAGCCTAGA |
| probe-miR-646 | miR-646 | GCCTCAGAGGCAGCTGCTT |
| probe-miR-647 | miR-647 | GAAGGAAGTGAGTGCAGCCAC |
| probe-miR-648 | miR-648 | ACCAGTGCCCTGCACACTT |
| probe-miR-649 | miR-649 | GACTCTTGAACAACACAGGTTT |
| probe-miR-650 | miR-650 | GTCCTGAGAGCGCTGCCTCCT |
| probe-miR-651 | miR-651 | CAAAAGTCAAGCTTATCCTAAA |
| probe-miR-652 | miR-652 | TGCACAACCCTAGTGGCGCCATT |
| probe-miR-653 | miR-653 | GTTCAGTAGAGATTGTTTCAA |
| probe-miR-654 | miR-654 | GCACATGTTCTGCGGCCCACCA |
| probe-miR-655 | miR-655 | AAAGAGGTTAACCATGTATTAT |
| probe-miR-656 | miR-656 | AGAGGTTGACTGTATAATATT |
| probe-miR-657 | miR-657 | CCTAGAGAGGGTGAGAACCTGCC |
| probe-miR-658 | miR-658 | ACCAACGGACCTACTTCCCTCCGCC |
| probe-miR-659 | miR-659 | TGGGGACCCTCCCTGAACCAAG |
| probe-miR-660 | miR-660 | CAACTCCGATATGCAATGGGTA |
| probe-miR-661 | miR-661 | ACGCGCAGGCCAGAGACCCAGGCA |
| probe-miR-662 | miR-662 | CTGCTGGGCCACAACGTGGGA |
| probe-miR-663 | miR-663 | GCGGTCCCGCGGCGCCCCGCCT |
| probe-miR-7 | miR-7 | CAACAAAATCACTAGTCTTCCA |
| probe-miR-9 | miR-9 | TCATACAGCTAGATAACCAAAGA |
| probe-miR-9* | miR-9* | ACTTTCGGTTATCTAGCTTTA |
| probe-miR-92 | miR-92 | CAGGCCGGGACAAGTGCAATA |
| probe-miR-93 | miR-93 | CTACCTGCACGAACAGCACTTT |
| probe-miR-95 | miR-95 | TGCTCAATAAATACCCGTTGAA |
| probe-miR-96 | miR-96 | GCAAAAATGTGCTAGTGCCAAA |
| probe-miR-98 | miR-98 | AACAATACAACTTACTACCTCA |

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| Probes | Corresponding micro-RNas | Sequences of probes |
|---|---|---|
| probe-miR-99a | miR-99a | CACAAGATCGGATCTACGGGTT |
| probe-miR-99b | miR-99b | CGCAAGGTCGGTTCTACGGGTG |

**43**. The biochip according to claim **40**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to determine the physiological and/or pathological condition of the subject after being administrated a test sample.

**44**. The biochip according to claims **43**, characterized in that the biochip are useful for screening the test sample for the activities on the prevention and/or treatment of diseases.

**45**. The biochip according to claim **40**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to diagnose and/or differentially diagnose the diseases of the subject.

**46**. The biochip according to claim **40**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to evaluate the effectiveness of treating the diseases of the subject.

**47**. The biochip according to claim **40**, characterized in that said evaluating the physiological and/or pathological condition of a subject is to predict the occurrence of diseases of the subject.

**48**. The biochip according to claim **47**, characterized in that the occurrence of diseases is the occurrence of complications and/or the relapse of malignant diseases.

**49**. The biochip according to claim **44**, characterized in that the diseases include various tumors, various acute and chronic infectious diseases e.g. viral diseases such as viral influenza, viral hepatitis, AIDS, SARS, bacterial diseases such as tuberculosis, bacterial pneumonia, and other acute and chronic infectious diseases caused by various pathogenic microorganisms; other acute and chronic diseases such as diseases of respiratory system, diseases of immune system, diseases of blood and hematopoietic system, diseases of circulatory system such as cardio-cerebrovascular disease, metabolic diseases of endocrine system, diseases of digestive system, diseases of nervous system, diseases of urinary system, diseases of reproductive system and diseases of locomotor system.

**50**. The biochip according to claim **40**, characterized, in that the biochip are useful for detecting the subject for prohibited drugs-taking.

**51**. The chip according to claim **40**, characterized in that the serum/plasma the subject is from living bodies, tissues, organs and/or corpuses of the subject.

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