Claims

1. A kit for multiplex analyte encoding, comprising

(A) at least twenty (20) different sets of analyte-specific probes for encoding of at least 20 different analytes, each set of analyte-specific probes interacting with a different analyte, wherein if the analyte is a nucleic acid each set of analyte-specific probes comprises at least five (5) analyte-specific probes which specifically interact with different sub-structures of the same analyte, each analyte-specific probe comprising

(aa) a binding element (S) that specifically interacts with one of the different analytes to be encoded, and

(bb) an identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence),

wherein the analyte-specific probes of a particular set of analyte-specific probes differ from the analyte-specific probes of another set of analyte-specific probes in the nucleotide sequence of the identifier element (T),

wherein the analyte-specific probes in each set of analyte-specific probes binds to the same analyte and comprises the same nucleotide sequence of the identifier element (T) which is unique to said analyte; and

(B) at least one set of decoding oligonucleotides per analyte, wherein in each set of decoding oligonucleotides for an individual analyte each decoding oligonucleotide comprises:

(aa) an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

(bb) a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide;

wherein the decoding oligonucleotides of a set for an individual analyte differ from the decoding oligonucleotides of another set for a different analyte in the identifier connect element (t); and

(C) a set of signal oligonucleotides, each signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of a translator element (c) comprised in a decoding oligonucleotide, and
(bb) a signal element.

2. The kit according to claim 1, wherein the kit comprises at least two different sets of decoding oligonucleotides per analyte,

wherein the decoding oligonucleotides comprised in these different sets comprise the same identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

wherein the decoding oligonucleotides of the different sets per analyte differ in the translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

3. The kit according to any one of claims 1 to 2, wherein the kit comprises at least two different sets of decoding oligonucleotides per analyte,

wherein the decoding oligonucleotides comprised in these different sets comprise the same identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

wherein the decoding oligonucleotides of the different sets for at least one analyte differ in the translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide.

4. The kit according to of any one of claims 1 to 3, wherein the number of different sets of decoding oligonucleotides per analyte comprising different translator elements (c) corresponds to the number of different sets of signal oligonucleotides comprising different connector elements (C).

5. The kit according to any one of claims 1 to 4, wherein the kit comprises:

(E) a set of non-signal oligonucleotides, each non-signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and

(bb) a quencher (Q), a signal element and a quencher (Q), or does not comprise a signal element.

6. A multiplex method for detecting different analytes in a sample by sequential signal-encoding of said analytes, comprising:

(A) contacting the sample with at least twenty (20) different sets of analyte-specific probes for encoding of at least 20 different analytes, each set of analyte-specific probes interacting with a different analyte, wherein if the analyte is a nucleic acid each set of analyte-specific probes comprises at least five (5) analyte-specific probes which specifically interact with different sub-structures of the same analyte, each analyte-specific probe comprising

(aa) a binding element (S) that specifically interacts with one of the different analytes to be encoded, and

(bb) an identifier element (T) comprising a nucleotide sequence which is unique to the analyte to be encoded (unique identifier sequence),

wherein the analyte-specific probes of a particular set of analyte-specific probes differ from the analyte-specific probes of another set of analyte-specific probes in the nucleotide sequence of the identifier element (T),

wherein the analyte-specific probes in each set of analyte-specific probes binds to the same analyte and comprises the same nucleotide sequence of the identifier element (T) which is unique to said analyte; and

(B) contacting the sample with at least one set of decoding oligonucleotides per analyte, wherein in each set of decoding oligonucleotides for an individual analyte each decoding oligonucleotide comprises:

(aa) an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of the corresponding analyte-specific probe set, and

(bb) a translator element (c) comprising a nucleotide sequence allowing a specific hybridization of a signal oligonucleotide;

wherein the decoding oligonucleotides of a set for an individual analyte differ from the decoding oligonucleotides of another set for a different analyte in the first connect element (t); and

(C) contacting the sample with at least a set of signal oligonucleotides, each signal oligonucleotide comprising:

(aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of a translator element (c) comprised in a decoding oligonucleotide, and
(bb) a signal element.

(D) Detecting the signal caused by the signal element;

(E) selectively removing the decoding oligonucleotides and signal oligonucleotides from the sample, thereby essentially maintaining the specific binding of the analyte-specific probes to the analytes to be encoded;

(F) Performing at least three (3) further cycles comprising steps B) to E) to generate an encoding scheme with a code word per analyte, wherein in particular the last cycle may stop with step (D).

7. The method according to claim 6, wherein all steps are automated, in particular wherein steps B) to F) are automated, in particular by using a robotic system.

8. The method according to any one of claims 6 to 7, wherein each analyte is associated with a specific code word, wherein said code word comprise a number of positions, and wherein each position corresponds to one cycle resulting in a plurality of distinguishable encoding schemes with the plurality of code words.

9. The method according to any one of claims 6 to 8, wherein no signal is detected for at least one analyte within at least one cycle.

10. The method according to any one of claims 6 to 9, wherein for at least for one individual analyte a position of the code word is zero (0).

11. The method according to of any one of claims 6 to 10, wherein the code word zero (0) is generated by using no decoding oligonucleotides having an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of a corresponding analyte-specific probe for an individual analyte.

12. The method according to of any one of claims 6 to 11, wherein if at least for one individual analyte a position of the code word is zero (0) in this cycle no corresponding decoding oligonucleotides having an identifier connector element (t) comprising a nucleotide sequence which is essentially complementary to at least a section of the unique identifier sequence of the identifier element (T) of a corresponding analyte-specific probe for an individual analyte are used.

13. The method according to any one of claims 6 to 12, wherein the sample is contacted with:

    at least two sets of non-signal oligonucleotides, each non-signal oligonucleotide comprising:

        (aa) a translator connector element (C) comprising a nucleotide sequence which is essentially complementary to at least a section of the nucleotide sequence of the translator element (c), and

        (bb) a quencher (Q), a signal element and a quencher (Q), or does not comprise a signal element.

14. An *in vitro* method for diagnosis of a disease selected from the group comprising cancer, neuronal diseases, cardiovascular diseases, inflammatory diseases, autoimmune diseases, diseases due to a viral or bacterial infection, skin diseases, skeletal muscle diseases, dental diseases and prenatal diseases comprising the use of the multiplex method according to any one of claims 6 to 13.

15. An *in vitro* method for diagnosis of a disease in plants selected from the group comprising: diseases caused by biotic stress, preferably by infectious and/or parasitic origin, or diseases caused by abiotic stress, preferably caused by nutritional deficiencies and/or unfavorable environment, said method comprising the use of the multiplex method according to any one of claims 6 to 13.

16. An optical multiplexing system suitable for the method according to any one of claims 6 to 13, comprising at least:
    - one reaction vessel for containing the kits or part of the kits according to any one of claims 1 to 5;
    - a detection unit comprising a microscope, in particular a fluorescence microscope
    - a camera
    - a liquid handling device.

17. An *in vitro* method for screening, identifying and/or testing a substance and/or drug comprising:
    (a) contacting a test sample comprising a sample with a substance and/or drug
    (b) detecting different analytes in a sample by sequential signal-encoding of said analytes with a method according to any one of claims 6 to 13.