CLAIMS:

1. A nucleic acid array for detecting spatial information of a nucleic acid in a sample, which comprises a solid support with multiple kinds of carrier sequences attached to its surface, in which each kind of carrier sequence occupies a different position in the array, said each kind of carrier sequence comprises a plurality of copies of the carrier sequence, and the carrier sequence in the direction from 5' to 3' comprises a positioning sequence and a first immobilization sequence, wherein,

the positioning sequence has a unique nucleotide sequence corresponding to the position of the kind of carrier sequence on the array;

the first immobilization sequence allows annealing to its complementary nucleotide sequence and initiating an extension reaction;

wherein the multiple copies of carrier sequence are a DNA nanoball (DNB) formed by rolling circle amplification using a complementary sequence of the carrier sequence as a template;

and wherein said each kind of carrier sequence comprising the same positioning sequence occupies an area having a diameter of less than 1 micrometer;

and wherein the carrier sequence further comprises a capture sequence template located upstream of the positioning sequence, the capture sequence template comprises a complementary sequence of the capture sequence, and the capture sequence is capable of hybridizing with the whole or part of the nucleic acid to be captured, which comprises: (a) an oligonucleotide sequence capable of capturing mRNA; (b) a random or degenerate oligonucleotide sequence; or, (c) a specific sequence for a specific target nucleic acid;

and, the first immobilization sequence of the carrier sequence also comprises a cleavage site, and the cleavage is selected from enzymatic cleavage with nicking enzyme, enzymatic cleavage with USER enzyme, photocleavage, chemical cleavage or CRISPR-based cleavage;

and, the nucleic acid array further comprises a first nucleic acid molecule, and the first nucleic acid molecule in the direction from 5' to 3' comprises: a binding region, a cleavage region, and a carrier sequence complementary region,

the binding region comprises a linker capable of ligating to the surface of the solid support;

the cleavage region comprises a cleavage site;

the carrier sequence complementary region comprises a sequence that is complementary to the carrier sequence, and in the direction from 5' to 3', comprises: a complement of the first

immobilization sequence, a complement of the positioning sequence, and a capture sequence; and, the capture sequence has a free 3' end to enable the first nucleic acid molecule to function as an extension primer;

and, the carrier sequence complementary region of the first nucleic acid molecule hybridizes to the carrier sequence to form a double strand;

and wherein each copy of each kind of carrier sequence comprises a first nucleic acid molecule hybridized therewith.

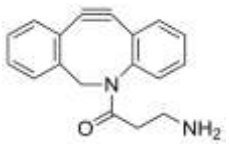
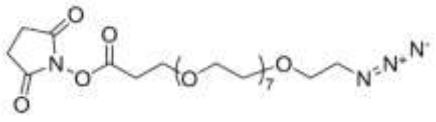
2. The nucleic acid array according to claim 1, wherein the carrier sequence further comprises a complement of UMI sequence located downstream of the capture sequence template and upstream of the first immobilization sequence, the complement of UMI sequence is complementary to the UMI sequence, the UMI sequence is a nucleotide sequence consisting of at least 1, at least 2, at least 3, at least 4, or at least 5nucleotides N, and each N is independently any one of A, C, G and T;

and, the carrier sequence complementary region of the first nucleic acid molecule further comprises the UMI sequence located upstream of the capture sequence and downstream of the complement of first immobilization sequence;

and wherein each copy of each kind of carrier sequence (i.e., carrier sequences comprising the same positioning sequence) comprises a complement of UMI sequence different from each other.

3. The nucleic acid array according to claim 1 or 2, wherein the linker of the first nucleic acid molecule is a linking group capable of coupling with an activating group, and the surface of the solid support is modified by the activating group.

4. The nucleic acid array according to claim 3, wherein the linker comprises -SH, -DBCO or -NHS.

5. The nucleic acid array according to claim 3, wherein the linker is  (DBCO), and  (Azido-dPEG®8-NHS ester) is attached to the surface of the solid support.

6. The nucleic acid array according to any one of claims 1 to 5, wherein the cleavage site contained in the cleavage region of the first nucleic acid molecule is a site where controlled cleavage is performed by a chemical, enzymatic, or photochemical method.

7. The nucleic acid array according to claim 6, wherein the cleavage region of the first nucleic acid molecule is different from the cleavage site contained in the first immobilization sequence of the carrier sequence.

8. The nucleic acid array according to any one of claims 1 to 7, wherein the solid support is a sequencing chip.

9. The nucleic acid array according to any one of claims 1 to 8, wherein the oligonucleotide sequence capable of capturing mRNA comprises a poly-T oligonucleotide sequence.

10. A method for generating a nucleic acid array for detecting spatial information of a nucleic acid in a biological sample, which comprises the following steps:

(1) providing multiple kinds of carrier sequences, each kind of carrier sequence comprises a plurality of copies of the carrier sequence, and the carrier sequence in the direction from 5' to 3' comprises a positioning sequence and a first immobilization sequence,

the positioning sequence has a unique nucleotide sequence corresponding to the position of the kind of carrier sequence on the array;

the first immobilization sequence allows annealing to its complementary nucleotide sequence and initiating an extension reaction;

(2) ligating the multiple kinds of carrier sequences to a surface of a solid support;

(3) providing a first primer, and perform a primer extension reaction by using the carrier sequence as a template, so that a region of the first immobilization sequence and the positioning sequence of the carrier sequence forms a double strand, wherein the strand that hybridizes to the carrier sequence is a first nucleic acid molecule, the first nucleic acid molecule in the direction from 5' to 3' comprises a complementary sequence of the first immobilization sequence and a complementary sequence of the positioning sequence; wherein, the first primer comprises a first immobilization sequence complementary region at its 3' end, the first immobilization sequence complementary region comprises a complementary sequence of the first immobilization sequence or a fragment thereof, and has a free 3' end;

and wherein in step (1), the multiple kinds of carrier sequences are provided by the following steps:

(i) providing multiple kinds of carrier sequence templates, the carrier sequence template comprising a complementary sequence of the carrier sequence;

(ii) perform a nucleic acid amplification reaction by using each kind of carrier sequence template as a template, to obtain an amplification product of each kind of carrier sequence template, the amplification product comprising a plurality of copies of the carrier sequence;

and wherein said each kind of carrier sequence comprising the same positioning sequence occupies an area having a diameter of less than 1 micrometer;

and wherein rolling circle amplification is performed to obtain a DNB formed by a concatemer of the carrier sequence;

and wherein:

in step (1), the carrier sequence further comprises a capture sequence template located upstream of the positioning sequence, the capture sequence template comprises a complementary sequence of the capture sequence, and the capture sequence can hybridize to the whole or part of the nucleic acid to be captured, which comprises: (a) an oligonucleotide sequence capable of capturing mRNA; (b) a random or degenerate oligonucleotide sequence; or, (c) a specific sequence for a specific target nucleic acid; and the first immobilization sequence of the carrier sequence also comprises a cleavage site, and the cleavage is selected from enzymatic cleavage with nicking enzyme, enzymatic cleavage with USER enzyme, photocleavage, chemical cleavage or CRISPR-based cleavage;

in step (3), a region of the first immobilization sequence, the positioning sequence and the capture sequence template of the carrier sequence forms a double strand, so that the first nucleic acid molecule in the direction from 5' to 3' comprises a complement of first immobilization sequence, a complement of positioning sequence and a capture sequence; wherein,

the first primer in the direction from 5' to 3' comprises a binding region, an cleavage region, and a first immobilization sequence complementary region, the binding region comprises a linker capable of ligating to the surface of the solid support, and the cleavage region comprises a cleavage site;

and, the method further comprises the following steps:

(4) ligating the first primer to the surface of the solid support; wherein, steps (3) and (4) are performed in any order.

11. The method according to claim 10, which further comprising:

(5) performing cleavage at the cleavage site contained in the first immobilization sequence of the carrier sequence to digest the carrier sequence, so that the extension product in step (3) is separated from the template where such extension product is formed (i.e., carrier sequence), and the first nucleic acid molecule is therefore ligated to the surface of the solid support.

12. The method according to any one of claims 10-11, wherein, in step (1), the cleavage site contained in the first immobilization sequence is a cleavage site of nicking enzyme.

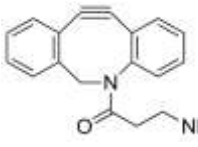
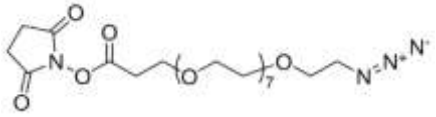
13. The method according to any one of claims 10-12, wherein, in step (1), the carrier sequence further comprises a complement of UMI sequence located downstream of the capture sequence template and upstream of the first immobilization sequence, the complement of UMI sequence is complementary to a UMI sequence, and the UMI sequence is a nucleotide sequence consisting of at least 1, at least 2, at least 3, at least 4, or at least 5 nucleotides N, each N is independently any one of A, C, G and T;

and, in step (3), a region of the first immobilization sequence, the positioning sequence, the capture sequence template, and the complement of UMI sequence of the carrier sequence forms a double strand, so that the first nucleic acid molecule in the direction from 5' to 3' comprises the complement of first immobilization sequence, the complement of positioning sequence and the

capture sequence, and the UMI sequence located upstream of the capture sequence and downstream of the complement of first immobilization sequence.

14. The method according to any one of claims 10-13, wherein the linker of the first primer is a linking group capable of coupling with an activating group, and the surface of the solid support is modified by the activating group.

15. The method according to claim 14, wherein the linker comprises -SH, -DBCO or -NHS.

16. The method according to claim 14, wherein the linker is  (DBCO), and  (Azido-dPEG®8-NHS ester) is attached to the surface of the solid support.

17. The method according to any one of claims 10-16, wherein the cleavage site contained in the cleavage region of the first primer is a site where controlled cleavage is performed by a chemical, enzymatic, or photochemical method;

and wherein the cleavage region of the first primer is different from the cleavage site contained in the first immobilization sequence of the carrier sequence.

18. A method for detecting spatial information of a nucleic acid in a sample, which comprises the following steps:

(1) providing the nucleic acid array according to any one of claims 1 to 9, or obtaining a nucleic acid array by the method according to any one of claims 10 to 17; wherein the nucleic acid array comprises a solid support with multiple kinds of carrier sequences attached to its surface, each kind of carrier sequence occupies a different position in the array, and said each kind of carrier sequence comprises a plurality of copies of the carrier sequence;

each carrier sequence comprises a first nucleic acid molecule hybridized therewith, and the first nucleic acid molecule comprises a complement of positioning sequence corresponding to the

position of the kind of carrier sequence on the array and a capture sequence capable of capturing the nucleic acid in the sample;

and wherein said each kind of carrier sequence comprising the same positioning sequence occupies an area having a diameter of less than 1 micrometer;(2) contacting the nucleic acid array with the sample to be tested under a condition that allows annealing, so that the nucleic acid in the sample to be tested anneals to the capture sequence of the first nucleic acid molecule, and thus the position of the nucleic acid is correlated with the position of the first nucleic acid molecule on the nucleic acid array;

(3) performing a primer extension reaction by using the first nucleic acid molecule as a primer and using the captured nucleic acid molecule as a template under a condition that allows the primer extension, to produce an extension product, in which the strand that hybridizes to the captured nucleic acid molecule has the complement of positioning sequence contained in the first nucleic acid molecule as a spatial information tag;

(4) releasing at least part of the nucleic acid molecules labeled with the spatial information tags from the surface of the array, wherein the part comprises the positioning sequence or its complementary strand and the captured nucleic acid molecule or its complementary strand; and

(5) directly or indirectly analyzing the sequence of the nucleic acid molecule released in step (4);

and wherein the spatial information of the nucleic acid comprises the location, distribution or expression of the nucleic acid.

19. The method according to claim 18, in which the method is used to detect a transcriptome in a sample, wherein:

in step (3), generating a cDNA molecule from the captured RNA molecule by using the first nucleic acid molecule as an RT primer, the cDNA molecule has the complement of positioning sequence contained in the first nucleic acid molecule as a spatial information tag;

in step (4), releasing at least part of the cDNA molecules or their amplicons from the surface of the array, wherein the released nucleic acid molecule may be the first or second strand of the cDNA molecule or an amplicon thereof, and wherein the part comprises the positioning sequence or its complementary strand;

and wherein in step (1), the capture sequence comprises an oligonucleotide sequence capable of capturing mRNA.

20. The method according to any one of claims 18-19, wherein the sample is a tissue sample.

21. The method according to any one of claims 18-20, wherein the sample is a formalin-fixed paraffin-embedded (FFPE) tissue or deep-frozen tissue.

22. The method according to any one of claims 18-21, in step (5), the sequence analysis comprises sequencing or a sequence-specific PCR reaction.

23. The method according to any one of claims 18-22, in which the method further comprises step (6): correlating the sequence analysis information obtained in step (5) to an image of the sample, wherein the sample is imaged before or after step (3);

and wherein the imaging of the sample uses light, bright field, dark field, phase contrast, fluorescence, reflection, interference, confocal microscopy or a combination thereof.

24. The method according to any one of claims 18-23, wherein before or after the nucleic acid molecule labeled with spatial information tag or the cDNA molecule labeled with spatial information tag is released from the surface of the array, the complementary strand or the second strand cDNA is generated;

and wherein the synthesis of the complementary strand or the second strand uses a random primer and a strand displacement polymerase.

25. The method according to any one of claims 18-24, wherein before the sequence analysis, the method further comprises a step of amplifying the nucleic acid molecule or cDNA molecule labeled with the spatial information tag;

and wherein the amplification step is performed after the nucleic acid molecule or cDNA molecule labeled with the spatial information tag is released from the array, or the amplification step is performed in situ on the array;

and wherein the amplification step comprises PCR.

26. The method according to any one of claims 18-25, wherein, before the sequence analysis, the method further comprises a step of purifying the released nucleic acid molecule.

27. The method according to any one of claims 18-26, wherein before step (4), the method further comprises a step of washing the array to remove a residue of the sample.

28. The method according to any one of claims 18-27, wherein in step (4), the nucleic acid molecule is released from the surface of the array by the following method: (i) nucleic acid cleavage; (ii) denaturation; or (iii) physical method.