CLAIMS

1. A method for whole genome amplification and analysis of multiple target molecules in a biological sample including genomic DNA and target molecules comprising the steps of:

a) providing the biological sample;

b) contacting the biological sample with at least one binding agent, which is directed to at least one of the target molecules, conjugated with a tagged oligonucleotide, the tagged oligonucleotide comprising:

i) a payload sequence of nucleic acid (PL) comprising a binding-agent barcode sequence (BAB) and a unique molecular identifier sequence (UMI), and

ii) at least one first tagged oligonucleotide amplification sequence of nucleic acid (5-TOS);

so that – when at least one target molecule is present in the biological sample – the at least one binding agent binds to the at least one target molecule;

wherein the at least one binding agent is selected from the group consisting of:

a) an antibody or a functionally active fragment thereof,

b) an aptamer,

c) a small molecule,

d) a peptide, and

e) a protein;

wherein the target molecule is selected from the group

consisting of:

a) a protein,

b) a peptide,

c) a glycoprotein,

d) a carbohydrate,

e) a lipid, and

f) a combination thereof;

c) carrying out a separating step to selectively remove unbound binding agent thus obtaining a labeled biological sample;

d) carrying out on the labeled biological sample:

- a whole genome amplification of said genomic DNA by:

i) deterministic restriction-site whole genome amplification (DRS-WGA), or

ii) Multiple Annealing and Looping Based Amplification Cycles whole genome amplification (MALBAC), and

- an amplification of the tagged oligonucleotide conjugated with the at least one binding agent,

wherein whole genome amplification and amplification of the tagged oligonucleotide are carried out simultaneously;

e) preparing a massively parallel sequencing library from the amplified tagged oligonucleotide;

f) sequencing the massively parallel sequencing library;

g) retrieving the sequences of the binding-agent barcode sequence (BAB) and unique molecular identifier sequence (UMI) from each sequencing read;

h) counting the number of distinct unique molecular identifier sequences (UMI) for each binding agent.

2. The method according to claim 1, wherein the tagged oligonucleotide further comprises at least one second tagged oligonucleotide amplification sequence (3-TOS).

3. The method according to claim 1 or 2, wherein said unique molecular identifier sequence (UMI) is a degenerate or semi-degenerate sequence in the range from 10 to 30 nucleotides.

4. The method according to any of the preceding claims, wherein the method further comprises a step of isolating a single cell from the biological sample.

5. The method according to claim 4, wherein said step of isolating is performed by sorting cells.

6. The method according to claim 4, wherein said step of isolating is performed by partitioning cells into droplets.

7. The method according to any of claims 4 to 6, wherein said step of isolating is performed after step c) and before step d).

8. The method according to any of the preceding claims, further comprising a step of purifying the massively parallel sequencing library before step f).

9. The method according to any of claims 2 to 8, wherein the tagged oligonucleotide comprises from 5’ to 3’ at least:

a) the first tagged oligonucleotide amplification sequence of nucleic acid (5-TOS), comprising in turn a 5’ whole genome amplification handle sequence (5-WGAH) and a first amplification handle sequence (1AH);

b) the payload sequence (PL);

c) the second tagged oligonucleotide amplification sequence of nucleic acid (3-TOS), comprising in turn a second amplification handle sequence of nucleic acid (2AH) and a 3’ whole genome amplification handle sequence (3-WGAH).

10. The method according to any of the preceding claims, wherein the whole genome amplification and the amplification of the tagged oligo are carried out using a single primer.

11. The method according to any of claims 1 to 10, wherein the tagged oligonucleotide comprises from 5′ to 3′ at least:

a) the first tagged oligonucleotide amplification sequence of nucleic acid (5-TOS), comprising in turn a 5′ whole genome amplification handle sequence (5-WGAH) and a first amplification handle sequence (1AH);

b) the payload sequence (PL);

c) an annealing sequence (AS); and

wherein at least one primer is used for whole genome amplification and amplification of the tagged oligonucleotide and at least one oligonucleotide (E-p) is used

for the extension of the tagged oligonucleotide, said at least one oligonucleotide (E-p) comprising from 5′ to 3′ at least:

d) the 5′ whole genome amplification handle sequence (5-WGAH);

e) a spacer sequence (SS);

f) a second amplification handle sequence (2AH); and

g) a sequence reverse complementary to the annealing sequence (AS-RC) or a sequence reverse complementary to the binding-agent barcode sequence (BAB-RC).

12. The method according to any of claims 1 to 8, wherein the tagged oligonucleotide comprises from 5’ to 3’ at least:

a) the first tagged oligonucleotide amplification sequence of nucleic acid (5-TOS) corresponding to a first amplification handle sequence (1AH);

b) the payload sequence (PL);

c) the second tagged oligonucleotide amplification sequence of nucleic acid (3-TOS) corresponding to a second amplification handle sequence (2AH); and

wherein at least one first primer is used for whole genome amplification and at least one second and one third primer are used for the amplification of the tagged oligonucleotides;

the at least one second primer having a sequence identical to the first amplification handle sequence (1AH) and the at least one third primer having a sequence reverse complementary to the second amplification handle sequence (2AH-RC).

13. The method according to claim 12, wherein the at least one second primer and at least one third primer are added in step d).

14. A method according to any of claims 9 to 13,

wherein said step e) of preparing a massively parallel sequencing library from the amplified tagged oligonucleotide is performed by a PCR reaction using at least one first library primer, comprising a 3′ sequence corresponding to the first amplification handle sequence (1AH), and at least one second library primer comprising a 3′ sequence corresponding to the sequence reverse complementary to the second amplification handle sequence (2AH-RC).

15. A kit for carrying out the method of any of claims 1 to 15 comprising:

a) at least one binding agent directed to at least one target molecule in a biological sample conjugated with a tagged oligonucleotide, the tagged oligonucleotide comprising:

i) a payload sequence of nucleic acid (PL) comprising a binding-agent barcode sequence (BAB) and a unique molecular identifier sequence (UMI),

ii) at least one first tagged oligonucleotide amplification sequence (5-TOS);

b) at least one primer for carrying out a whole genome amplification and at least one primer for carrying out an amplification of the tagged oligonucleotide, the at least one primer for carrying out a whole genome amplification and at least one primer for carrying out an amplification of the

tagged oligonucleotide having the same sequence;

wherein the at least one binding agent is selected from the group consisting of:

a) an antibody or a functionally active fragment thereof,

b) an aptamer,

c) a small molecule,

d) a peptide, and

e) a protein; and

wherein the target molecule is selected from the group consisting of:

a) a protein,

b) a peptide,

c) a glycoprotein,

d) a carbohydrate,

e) a lipid, and

f) a combination thereof.

16. A kit according to claim 15, further comprising an oligonucleotide for extending the tagged oligonucleotide.

17. A kit according to claim 15 or 16, wherein the at least one tagged oligonucleotide has a sequence corresponding to SEQ ID NO:1, the primers for carrying out the amplification of the tagged oligonucleotide are two and have respectively sequence SEQ ID NO:2 and SEQ ID NO:3.

18. A kit according to any of claims 15 to 17, further comprising one or more first library primer/s and one or more second library primer/s.

19. A kit according to claim 18, wherein said first library primer/s has/have a sequence selected from the group consisting of SEQ ID NO:8 to SEQ ID NO:15 and said second library primer/s has/have a sequence selected from the group consisting of SEQ ID NO:16 to SEQ ID NO:27.

20. A kit according to claim 15 or 16, wherein the at least one tagged oligonucleotide has a sequence corresponding to SEQ ID NO:28, and the primer for carrying out the amplification of the tagged oligonucleotide is one and has a sequence corresponding to SEQ ID NO:29.

21. The kit according to claim 20, further comprising one or more first library primer/s and one or more second library primer/s.

22. A kit according to claim 21, wherein said first library primer/s has/have a sequence selected from the group consisting of SEQ ID NO:30 to SEQ ID NO:37 and said second library primer/s has/have a sequence selected from the group consisting of SEQ ID NO:38 to SEQ ID NO:49.