

CLAIMS

1. A method for analysing loss-of-heterozygosity (LoH) in at least one sample comprising genomic DNA, the method comprising the steps of:

5 a. providing the at least one sample comprising genomic DNA;

b. carrying out a deterministic restriction-site whole genome amplification (DRS-WGA) of said genomic DNA;

10 c. preparing a massively parallel sequencing library from the product of said DRS-WGA;

d. carrying out low-pass whole genome sequencing at a mean coverage depth of < 1 on said massively parallel sequencing library;

15 e. aligning the reads obtained in step d. on a reference genome for said at least one sample;

f. extracting the allelic content at a plurality of loci, wherein said plurality of loci comprises polymorphic loci and/or heterozygous loci;

20 g. assigning an LoH score to at least one genomic window of said reference genome for said at least one sample as a function of the number of loci with at least two different alleles in said plurality of loci.

25 2. The method according to claim 1, wherein a step of size selection is performed before, during or after said step c. of preparing a massively parallel sequencing library and said step of preparing a massively parallel sequencing library does not include a random fragmentation step.

30 3. The method according to claim 2, wherein said step of size-selection retains fragments in the range from 100 to 800 base pairs.

4. The method according to claim 3, wherein said step of size selection retains fragments in the range from 300 to

450 base pairs.

5. The method according to claim 3, wherein the peak of fragments retained in said step of size-selection is centered on a base pair range from 150bp to 600bp.

5 6. The method according to claim 5, wherein said step of size selection step retains fragments in the range 425-575 base pairs.

7. The method according to any of claims 1 to 6, wherein said at least one genomic window has a constant width in
10 base pairs.

8. The method according to any of claims 1 to 6, wherein said at least one genomic window has a constant number of said plurality of loci.

9. The method according to any of claims 1 to 6, wherein
15 said at least one genomic window is selected from the group consisting of a chromosome, a chromosome arm, and a segmented copy-number region.

10. The method according to any of the preceding claims, wherein said plurality of loci comprises polymorphic
20 loci for the reference genome for said at least one sample.

11. The method according to claims 7, 8 or 10, wherein said LoH score corresponds to the number of heterozygous loci in said at least one genomic window.

12. The method according to claim 10, wherein said LoH
25 score corresponds to the proportion of heterozygous loci with respect to the total number of said polymorphic loci in the at least one genomic window.

13. The method according to claim 10, wherein said LoH score corresponds to the p-value of a statistical test.

30 14. The method according to claim 13, wherein said statistical test assesses the significance of over-representation of biallelic loci with respect to sequencing

and WGA error rates.

15. The method according to claim 13, wherein said statistical test assesses the significance of under-representation of biallelic loci with respect to a control
5 sample.

16. The method according to claim 15, wherein said control sample comprises at least one genomic region at main ploidy from said at least one sample.

17. The method according to claim 15 wherein said
10 control sample is an at least one normal sample.

18. The method according to claim 17, wherein said at least one normal sample is obtained from the same individual under test from which said at least one sample was obtained.

19. The method according to claim 15, wherein said
15 control sample is a maternal or paternal sample, for said at least one sample.

20. The method according to any one of claim 11 to 13, wherein, if said LoH score passes a threshold for a genomic window, said genomic window is called as being in LoH.

20 21. The method according to claim 20, further comprising a step of assigning an LoH status to at least one genomic region if the LoH scores for each genomic window comprised in that region passes said threshold.

22. The method according to claims 20, further
25 comprising a step of assigning an LoH status to at least one genomic region as a function of the LoH status of genomic windows comprised in that region.

23. The method according to claims 21 or 22 wherein said at least one genomic region comprises a tumor suppressor
30 gene.

24. The method according to claim 23, wherein said tumor suppressor gene is selected from the group consisting

of:

- a. BRCA1
- b. BRCA2
- c. PALB2
- 5 d. TP53
- e. CDKN2A
- f. RB1
- g. APC
- h. PTEN
- 10 i. CDKN1B
- j. DMP1
- k. NF1
- l. AML1
- m. EGR1
- 15 n. TGFBR1
- o. TGFBR2
- p. SMAD4

25. The method according to any of the preceding claims, wherein said at least one sample has a purity of at least 50%.

- 20 26. The method according to claim 25, wherein said at least one sample is a single cell.

- 27. The method according to any of the preceding claims, wherein in step d low-pass whole genome sequencing is carried out at a mean coverage depth of < 0.05 on said massively parallel
25 sequencing library.

- 28. The method according to any of the preceding claims, wherein in step d low-pass whole genome sequencing is carried out at a mean coverage depth of < 0.01 on said massively parallel
30 sequencing library.