

CLAIMS

1. A method of estimating a circulating tumor DNA (ctDNA) burden in a subject, the method comprising:
 - determining in a blood sample obtained from the subject, a level of cell free DNA (cfDNA) that maps to six or more nucleosome-depleted regions (NDR); and
 - estimating the ctDNA burden based on said level of cfDNA,wherein said NDRs
 - (i) comprises the NDRs of genes which transcripts are differentially expressed between healthy blood tissue and tumor tissue and/or
 - (ii) are degraded to different extents between healthy blood tissue and blood tissue of a tumor-bearing subject.
2. The method according to claim 1, wherein determining in a blood sample obtained from the subject, a level of cfDNA that maps to six or more NDRs comprises:
 - sequencing cfDNA fragments in the blood sample to obtain sequencing reads; and
 - determining the number of sequencing reads that align with the six or more NDRs to obtain said level of cfDNA that maps to six or more NDRs.
3. The method according to claim 2, further comprising
 - contacting the blood sample with six or more probes capable of binding to the six or more NDRs to capture cfDNA fragments comprising the six or more NDRs prior to the sequencing step.
4. The method according to any one of the preceding claims, wherein the NDRs are selected from the group consisting of: a promoter region, a first exon-intron junction and combinations thereof.
5. The method according to any one of the preceding claims, wherein the estimated ctDNA burden is positively associated with a tumor burden in the subject.
6. The method according to any one of the preceding claims, wherein said transcript that is differentially expressed between healthy blood tissue and tumor tissue comprises a

transcript which FPKM (Fragments Per Kilobase of transcript per Million) value differs by at least 10 times between healthy blood tissue and tumor tissue.

7. The method according to any one of the preceding claims, wherein said NDRs that are degraded to different extents between healthy blood tissue and blood tissue of a tumor-bearing subject comprises NDRs having different sequencing coverage in healthy blood tissue and in tumor tissue.
8. The method according to any one of the preceding claims, wherein said transcript that is differentially expressed in healthy blood tissue and tumor tissue is selected from the group consisting of: a transcript that is more highly expressed in healthy blood tissue than in tumor tissue, a transcript that is more highly expressed in tumor tissue than in healthy blood tissue and combinations thereof.
9. The method according to any one of the preceding claims, wherein said transcript which is differentially expressed between blood tissue and tumor tissue consists of transcript(s) that is more highly expressed in blood tissue than in tumor tissue.
10. The method according to any one of the preceding claims, wherein the six or more NDRs comprise six NDRs, further optionally ten NDRs.
11. The method according to any one of the preceding claims, wherein the total length of the six or more NDRs is no more than 30 kb.
12. The method according to any one of the preceding claims, wherein the NDRs comprise NDRs of genes comprise SLC11A1, NLRP12, PRTN3, HMBS, LILRB3, ACSL1, GP9, MX2, RASGRP4, ATG16L2 and combinations thereof.
13. The method according to any one of the preceding claims, wherein the method is a method of determining disease progression in a subject and the method further comprises:
 - determining in a subsequent blood sample obtained from the subject, a level of cfDNA that maps to six or more NDRs;
 - estimating the ctDNA burden based on said level of cfDNA;
 - comparing the ctDNA burden estimated from said subsequent blood sample with

the ctDNA burden estimated from said blood sample; and

identifying the subject as having disease progression if the ctDNA burden estimated from said subsequent blood sample is higher than the ctDNA burden estimated from said blood sample and identifying otherwise if the ctDNA burden estimated from said subsequent blood sample is not higher than the ctDNA burden estimated from said blood sample.

14. The method according to claim 13, the method further comprising changing the treatment regimen received by the subject if the subject is identified as having disease progression.
15. The method according to any one of the preceding claims, wherein the tumor comprises colorectal tumor.
16. The method according to claim 15, wherein the NDRs comprise SHKBP1, ACSL1, BCAR1, RAB25, PRTN3, LSR and combinations thereof.
17. A kit for estimating a ctDNA burden in a subject, the kit comprising probes that are capable of binding to NDRs, wherein said NDRs
 - (i) comprise the NDRs of genes which transcripts are differentially expressed between healthy blood tissue and tumor tissue and/or
 - (ii) are degraded to different extents between healthy blood tissue and blood tissue of a tumor-bearing subject,

wherein said NDRs comprise genes selected from the group comprising SLC11A1, NLRP12, PRTN3, HMBS, LILRB3, ACSL1, GP9, MX2, RASGRP4, ATG16L2 and combinations thereof.

18. A kit for estimating ctDNA burden in a subject, the kit comprising probes that are capable of binding to NDRs, wherein said NDRs
 - (i) comprise the NDRs of genes which transcripts are differentially expressed between healthy blood tissue and tumor tissue and/or
 - (ii) are degraded to different extents between healthy blood tissue and blood tissue of a tumor-bearing subject,

wherein said tumor comprises colorectal tumor and wherein said NDRs comprise SHKBP1, ACSL1, BCAR1, RAB25, PRTN3, LSR and combinations thereof.

19. The kit according to claim 17 or 18, wherein the probes comprise the sequence of SEQ ID NO: 1 to SEQ ID NO: 577, or a sequence sharing at least 75% sequence identity thereto.