(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 December 2024 (19.12.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/258948 A1

(51) International Patent Classification:
C12Q 1/6876 (2018.01) *C12Q 1/686* (2018.01)

(21) International Application Number:
PCT/US2024/033579

(22) International Filing Date:
12 June 2024 (12.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/472,428 12 June 2023 (12.06.2023) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2024/258948 A1

(54) **Title:** METHOD FOR PREDICTING HEALTH OF A HUMAN SUBJECT

(57) **Abstract:** A method for predicting the health of a human subject includes steps of: (a) obtaining genomic DNA from a blood or saliva specimen of a human subject, (b) observing unmethylated CG loci, wherein said observing includes performing a bisulfite conversion process on the genomic DNA of the human subject, (c) comparing the unmethylated CG loci observed in (b) to unmethylated CG loci observed in genomic DNA collected from a reference group of human individuals, and (d) correlating the unmethylated CG loci observed in (b) with the unmethylated CG loci observed in the reference group of human individuals to predict health of the human subject.

IN THE UNITED STATES RECEIVING OFFICE

A UTILITY APPLICATION

For

METHOD FOR PREDICTING HEALTH OF A HUMAN SUBJECT

RELATED APPLICATION

[0001] This patent application claims priority to U. S. Provisional Patent Application Serial No. 63/472,428, filed on June 12, 2023, the full disclosure of which is incorporated herein by reference.

Technical Field

[0002] This document generally relates to the construction and validation of a DNA reference matrix focusing on certain unmethylated cytosine-phosphate-guanine sites /CG loci in order to (a) predict levels of a plurality of proteins and metabolites in a human subject to be tested and (b) correlate the predicted levels of the plurality of proteins and metabolites with disease risk for the human subject. In this way it is possible to better correlate patterns to disease and to create more complex phenotype (disease) prediction with more information through methylation.

BACKGROUND

[0003] When your body is aging faster than the calendar's progression of time, you are experiencing accelerated biological aging; the #1 predictor of chronic disease. Fortunately, there are lifestyle and medical interventions proven to help slow, stop, or even reverse the biological aging processes in your body. This document relates to a method for measuring baseline and

changes to an individual's pace of aging, giving that individual the power to steer his or her anti-aging or health and wellness journey in the right direction.

SUMMARY

[0004] A new and improved method is provided for predicting the health of a human subject. That method comprises, consists of or consists essentially of the steps of:

(a) obtaining genomic DNA from a blood or saliva specimen of the human subject in a manner known in the art;

(b) observing unmethylated CG loci, in the genomic DNA of the human subject, wherein said observing includes performing a bisulfite conversion process on the genomic DNA of the human subject so that cytosine residues in the genomic DNA of the human subject are transformed to uracil, while 5-methylcytosine residues in the genomic DNA of the human subject are not transformed to uracil;

(c) comparing the unmethylated CG loci observed in (b) to unmethylated CG loci observed in genomic DNA collected from a reference group of human individuals; and

(d) correlating the unmethylated CG loci observed in (b) with the unmethylated CG loci observed in the reference group of human individuals to predict health of the human subject wherein the CG loci are a plurality of CG loci selected from a group consisting of: cg00011943, cg00014020, cg00056433, cg00067133, cg00071360, cg00074145, cg00080105, cg00120123, cg00122614, cg00153759, cg00177243, cg00178119, cg00182994, cg00209129, cg00215550, cg00217225, cg00236988, cg00237714, cg00243480, cg00251125, cg00262132, cg00275686, cg00278547, cg00283022, cg00303773, cg00322993, cg00342468, cg00358895, cg00458337, cg00465312, cg00481382, cg00530438, cg00538458, cg00548856, cg00593298, cg00594167, cg00602326, cg00615271, cg00631329, cg00639633, cg00646555, cg00687674, cg00687962, cg00698355, cg00712841, cg00737155, cg00748449, cg00756943, cg00767058, cg00800095, cg00815399, cg00910115, cg00935026, cg00935421, cg00964035, cg00973876, cg00993310, cg01020263, cg01025883, cg01067216, cg01089474, cg01094440, cg01098936, cg01113710, cg01134012, cg01166985, cg01169463, cg01178063, cg01224520, cg01243823, cg01253289, cg01266159, cg01271129, cg01274656, cg01282852, cg01313513, cg01320510, cg01346718, cg01377932, cg01380346, cg01402735, cg01410359, cg01446515, cg01457077, cg01541424, cg01545493, cg01549940, cg01579765, cg01586635, cg01606057, cg01610915, cg01645869, cg01652244, cg01660630, cg01715525, cg01770232, cg01809675, cg01824466,

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cg24976744, cg24982343, cg24997750, cg25047618, cg25063538, cg25114611, cg25146575, cg25148589, cg25156198, cg25161899, cg25181997, cg25189904, cg25202593, cg25259149, cg25259707, cg25330725, cg25331593, cg25361850, cg25375711, cg25397922, cg25418001, cg25418670, cg25420482, cg25420747, cg25436217, cg25499552, cg25504214, cg25560772, cg25563198, cg25569590, cg25623035, cg25638549, cg25639566, cg25670171, cg25671708, cg25703588, cg25717260, cg25738786, cg25745729, cg25784308, cg25786980, cg25843115, cg25843873, cg25903319, cg25916759, cg25927838, cg25928819, cg25943804, cg25945293, cg25952581, cg25952596, cg26029902, cg26044490, cg26071551, cg26093148, cg26116542, cg26116556, cg26156626, cg26229024, cg26239485, cg26267310, cg26276120, cg26301353, cg26314853, cg26315261, cg26441486, cg26442630, cg26443646, cg26479667, cg26499822, cg26500093, cg26501477, cg26528000, cg26544857, cg26577320, cg26599630, cg26606789, cg26676765, cg26691434, cg26702770, cg26760942, cg26764761, cg26767387, cg26805839, cg26818257, cg26839512, cg26883048, cg26962439, cg26992381, cg27021634, cg27066201, cg27079776, cg27127090, cg27128734, cg27130665, cg27131142, cg27152890, cg27154679, cg27154725, cg27208536, cg27229100, cg27245649, cg27256066, cg27296119, cg27315314, cg27336261, cg27348423, cg27354537, cg27357902, cg27368039, cg27374247, cg27395675, cg27449200, cg27454650, cg27461310, cg27488658, cg27498980, cg27500647, cg27553457, cg27589058, cg27632248.

[0005] The number of CG loci observed from the indicated group of CG loci may vary from embodiment to embodiment of the method. Thus, the plurality of CG loci may be any number between 2 CG loci and 1,352 (all) of the CG loci of the indicated group.

[0006] The method may include amplifying the genomic DNA of the blood or saliva specimen by a polymerase chain reaction.

[0007] The observing of the unmethylated CG loci, in the genomic DNA of the human subject may include hybridizing the genomic DNA to a beadchip to create a genomic DNA hybridized beadchip and then imaging the genomic DNA hybridized beadchip.

[0008] The method may include using a regression algorithm to correlate the unmethylated CG loci observed in (b) with the unmethylated GC loci observed in the reference group of human individuals.

[0009] The method may include performing, by computing device, a penalized regression model to create the regression algorithm of CpGs with relative weights wherein a methylation risk score (MRS) is defined by linear combination of m CpG site beta values c and weights w where: $MRS=\sum i=1mwici$.

DETAILED DESCRIPTION

[0010] A method for predicting the health of a human subject includes the steps of:

(a) obtaining genomic DNA from a blood or saliva specimen of the human subject;

(b) observing unmethylated CG loci, in the genomic DNA of the human subject, wherein said observing includes performing a bisulfite conversion process on the genomic DNA of the human subject so that cytosine residues in the genomic DNA of the human subject are transformed to uracil, while 5-methylcytosine residues in the genomic DNA of the human subject are not transformed to uracil;

(c) comparing the unmethylated CG loci observed in (b) to unmethylated CG loci observed in genomic DNA collected from a reference group of human individuals; and

(d) correlating the unmethylated CG loci observed in (b) with the unmethylated CG loci observed in the reference group of human individuals to predict health of the human subject wherein the CG loci are a plurality of CG loci selected from a group consisting of:

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[0011] The absence of methylation at any of the above identified CG loci correlates with and is predictive of one or more of the following diseases, disorders or conditions: alcohol liver disease, allergic rhinitis, aortic aneurysm dissection, arterial embolism thrombosis, asthma, bladder cancer, breast cancer, bronchiectasis, cancer, cardiomyopathy, chronic bronchitis, chronic kidney disease, chronic liver disease, chronic pulmonary heart disease, chronic viral hepatitis, cirrhosis, coin lesion lung disease, colon Rectum cancer, congestive heart failure, chronic obstructive pulmonary disease (COPD), coronary artery disease, cardiovascular disease (CVD) excluding stroke, depression, emphysema, hypertensive heart disease, interstitial lung disease, leukemia, liver cancer, lung cancer, melanoma, myocardial infraction, non-alcoholic fatty liver disease (NAFLD), non-Hodgkin lymphoma, other chronic hepatitis, ovarian cancer, pancreatic cancer, peripheral vascular disease, prostate cancer, stomach cancer, stroke, type 1 diabetes, type 2 diabetes, and uterine corpus cancer as verified by the genomic DNA collected from the reference group of individuals.

[0012] The number of CG loci observed from the indicated group of CG loci may vary from embodiment to embodiment of the method. Thus, the plurality of CG loci may be any number between 2 CG loci and 1,352 (all) of the CG loci of the indicated group. In one possible embodiment, a plurality of at least five CG loci are selected from the group. In another embodiment, at least ten CG loci are selected from the group. In another, at least 15 CG loci are selected from the group.

[0013] In yet another embodiment, a plurality of at least 25 CG loci are selected from the group. In another possible embodiment, a plurality of at least 50 CG loci are selected from the group. In another possible embodiment, a plurality of at least 75 CG loci are selected from the group. In another possible embodiment, a plurality of at least 100 CG loci are selected from the group. In another possible embodiment, a plurality of at least 150 CG loci are selected from the group.

[0014] In still another possible embodiment, a plurality of at least 200 CG loci are selected from the group. In another possible embodiment, a plurality of at least 250 CG loci are selected from the group. In another possible embodiment, a plurality of at least 500 CG loci are selected from the group. In another possible embodiment, a plurality of at least 1,000 CG loci are selected from the group. In yet another possible embodiment all 1,352 CG loci of the group are selected.

[0015] The bisulfite conversion process referenced in step (b) is known in the art. Kits useful for bisulfite conversion are commercially available from a number of manufacturers including Human Genetic Signatures' Methyleasy and Chemicon's CpGenome Modification Kit. See also, WO04096825A1, which describes bisulfite modification methods and Olek et al. Nuc. Acids Res. 24:5064-6 (1994), which discloses methods of performing bisulfite treatment and subsequent amplification.

[0016] The performing of the bisulfite conversion process may include the steps of amplifying the genomic DNA of the blood specimen by a polymerase chain reaction process, hybridizing the genomic DNA to a beadchip to create a genomic DNA hybridized beadchip, staining the genomic DNA hybridized beadchip and then imaging the genomic DNA hybridized beadchip.

[0017] The imaging of the genomic DNA hybridized beadchip may be performed using the Illumina EPIC850k array which provides for unambiguous CG loci identification for purposes of observing methylation at specific CG loci of the human DNA genome. Alternatively, other methods of epigenetic beta value collection may be used if desired.

[0018] The method also includes the step of using a regression algorithm to correlate the unmethylated CG loci observed in (b) with the unmethylated GC loci observed in the reference group of human individuals. This includes performing, by computing device, a penalized regression model to create the regression algorithm of CpGs with relative weights wherein a methylation risk score (MRS) is defined by linear combination of m CpG site beta values c and weights w where: MRS=$\sum i=1mwici$. It should be appreciated that different relative weights may be assigned to the GC loci.

[0019] To ensure the methylation risk score added predictive value over commonly captured features (e.g., age and sex), we created a baseline predictive model that included patients' age, sex, reference-based methylation cell-type composition estimates, self-reported race-ethnicity, self-reported smoking status, and the first ten genetic principal components. We fit the baseline model using a linear or logistic regression model depending on whether the outcome was continuous or binary. We compared the baseline model to models that included the baseline features as well as either methylation or genotype data. For the MRS, we used regression with LASSO, elastic net, and ridge regularization over the genomic features while treating the baseline features as fixed covariates. We fit all models using 10-fold double cross-validation, wherein each training set an additional cross-validation was performed for hyperparameter selection, then this training-set cross-validated model was used to predict the held-out test set. We tested for significance using an association test (via linear regression) between the cross-validated predicted outcome (i.e., the concatenated predictor across all folds) and the true outcome.

[0020] Still further, the method includes predicting proteomic and metabolomic data as surrogate biomarkers. For instance, we are using DNA methylation to predict things like C-Reactive protein which is a classical clinical marker. Toward this end, a plurality of known proteins and metabolites of medical interest for diagnosing alcohol liver disease, allergic rhinitis, aortic aneurysm dissection, arterial embolism thrombosis, asthma, bladder cancer, breast cancer,

bronchiectasis, cancer, cardiomyopathy, chronic bronchitis, chronic kidney disease, chronic liver disease, chronic pulmonary heart disease, chronic viral hepatitis, cirrhosis, coin lesion lung disease, colon Rectum cancer, congestive heart failure, chronic obstructive pulmonary disease (COPD), coronary artery disease, cardiovascular disease (CVD) excluding stroke, depression, emphysema, hypertensive heart disease, interstitial lung disease, leukemia, liver cancer, lung cancer, melanoma, myocardial infraction, non-alcoholic fatty liver disease (NAFLD), non-Hodgkin lymphoma, other chronic hepatitis, ovarian cancer, pancreatic cancer, peripheral vascular disease, prostate cancer, stomach cancer, stroke, type 1 diabetes, type 2 diabetes, and uterine corpus cancer are initially used to construct a baseline CG loci reference matrix from a reference group of individuals with known health histories. The method is then used to predict the levels of those plurality of proteins and metabolites in the human subject by correlating the unmethylated CG loci observed in (b) with the unmethylated CG loci observed in the reference group of human individuals to predict disease risk or health of the human subject.

[0021] Each of the following terms written in singular grammatical form: "a", "an", and "the", as used herein, means "at least one", or "one or more". Use of the phrase "One or more" herein does not alter this intended meaning of "a", "an", or "the". Accordingly, the terms "a", "an", and "the", as used herein, may also refer to, and encompass, a plurality of the stated entity or object, unless otherwise specifically defined or stated herein, or, unless the context clearly dictates otherwise. For example, the phrase: "a protein", as used herein, may also refer to, and encompass, a plurality of proteins.

[0022] Each of the following terms: "includes", "including", "has", "having", "comprises", and "comprising", and, their linguistic/grammatical variants, derivatives, or/and conjugates, as used herein, means "including, but not limited to", and is to be taken as specifying the stated component(s), feature(s), characteristic(s), parameter(s), integer(s), or step(s), and does not preclude addition of one or more additional component(s), feature(s), characteristic(s), parameter(s), integer(s), step(s), or groups thereof.

[0023] The phrase "consisting of", as used herein, is closed-ended and excludes any element, step, or ingredient not specifically mentioned. The phrase "consisting essentially of", as used

herein, is a semi-closed term indicating that an item is limited to the components specified and those that do not materially affect the basic and novel characteristic(s) of what is specified.

[0024] Terms of approximation, such as the terms about, substantially, approximately, etc., as used herein, refers to ± 10 % of the stated numerical value.

[0025] Although the method of this disclosure have been illustratively described and presented by way of specific exemplary embodiments, and examples thereof, it is evident that many alternatives, modifications, or/and variations, thereof, will be apparent to those skilled in the art. Accordingly, it is intended that all such alternatives, modifications, or/and variations, fall within the spirit of, and are encompassed by, the broad scope of the appended claims.

What is Claimed:

1. A method, comprising:

(a) obtaining genomic DNA from a blood or saliva specimen of a human subject;

(b) observing unmethylated CG loci, in the genomic DNA of the human subject, wherein said observing includes performing a bisulfite conversion process on the genomic DNA of the human subject so that cytosine residues in the genomic DNA of the human subject are transformed to uracil, while 5-methylcytosine residues in the genomic DNA of the human subject are not transformed to uracil;

(c) comparing the unmethylated CG loci observed in (b) to unmethylated CG loci observed in genomic DNA collected from a reference group of human individuals; and

(d) correlating the unmethylated CG loci observed in (b) with the unmethylated CG loci observed in the reference group of human individuals to predict health of the human subject wherein the CG loci are a plurality of at least five CG loci selected from a group consisting of cg00011943, cg00014020, cg00056433, cg00067133, cg00071360, cg00074145, cg00080105, cg00120123, cg00122614, cg00153759, cg00177243, cg00178119, cg00182994, cg00209129, cg00215550, cg00217225, cg00236988, cg00237714, cg00243480, cg00251125, cg00262132, cg00275686, cg00278547, cg00283022, cg00303773, cg00322993, cg00342468, cg00358895, cg00458337, cg00465312, cg00481382, cg00530438, cg00538458, cg00548856, cg00593298, cg00594167, cg00602326, cg00615271, cg00631329, cg00639633, cg00646555, cg00687674, cg00687962, cg00698355, cg00712841, cg00737155, cg00748449, cg00756943, cg00767058, cg00800095, cg00815399, cg00910115, cg00935026, cg00935421, cg00964035, cg00973876, cg00993310, cg01020263, cg01025883, cg01067216, cg01089474, cg01094440, cg01098936, cg01113710, cg01134012, cg01166985, cg01169463, cg01178063, cg01224520, cg01243823, cg01253289, cg01266159, cg01271129, cg01274656, cg01282852, cg01313513, cg01320510, cg01346718, cg01377932, cg01380346, cg01402735, cg01410359, cg01446515, cg01457077, cg01541424, cg01545493, cg01549940, cg01579765, cg01586635, cg01606057, cg01610915, cg01645869, cg01652244, cg01660630, cg01715525, cg01770232, cg01809675, cg01824466, cg01826979, cg01844642, cg01849284, cg01857379, cg01858517, cg01865937, cg01892689, cg01893681, cg01894498, cg01929138, cg01930417, cg01956293, cg02001279, cg02007867, cg02042600, cg02048674, cg02055264, cg02056921, cg02058108, cg02081263, cg02084118, cg02084211, cg02094681, cg02106682, cg02132714, cg02217269, cg02246605, cg02310386,

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2. The method of claim 1, wherein a plurality of at least ten CG loci are selected from the group.

3. The method of claim 1, wherein a plurality of at least 15 CG loci are selected from the group.

4. The method of claim 1, wherein a plurality of at least 25 CG loci are selected from the group.

5. The method of claim 1, wherein a plurality of at least 50 CG loci are selected from the group.

6. The method of claim 1, wherein a plurality of at least 75 CG loci are selected from the group.

7. The method of claim 1, wherein a plurality of at least 100 CG loci are selected from the group.

8. The method of claim 1, wherein a plurality of at least 150 CG loci are selected from the group.

9. The method of claim 1, wherein a plurality of at least 200 CG loci are selected from the group.

10 The method of claim 1, wherein a plurality of at least 250 CG loci are selected from the group.

11. The method of claim 1, wherein a plurality of at least 500 CG loci are selected from the group.

12. The method of claim 1, wherein a plurality of at least 1000 CG loci are selected from the group.

13. The method of claim 1, wherein all 1,352 CG loci are selected from the group.

14. The method of item 1, including amplifying the genomic DNA of the blood or saliva specimen by a polymerase chain reaction.

15. The method of item 14, wherein the observing of the unmethylated CG loci, in the genomic DNA of the human subject includes hybridizing the genomic DNA to a beadchip to create a genomic DNA hybridized beadchip and then imaging the genomic DNA hybridized beadchip.

17. The method of item 16, including using a regression algorithm to correlate the unmethylated CG loci observed in (b) with the unmethylated GC loci observed in the reference group of human individuals.

19. The method of claim 18, including performing, by computing device, a penalized regression model to create the regression algorithm of CpGs with relative weights wherein a methylation risk score (MRS) is defined by linear combination of m CpG site beta values c and weights w where: $MRS=\sum i=1mwici$.

20. The method of item 1, wherein the observing of the unmethylated CG loci, in the genomic DNA of the human subject includes hybridizing the genomic DNA to a beadchip to create a genomic DNA hybridized bead chip and then imaging the genomic DNA hybridized beadchip.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033579

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C12Q 1/6876*** (2024.01); ***C12Q 1/686*** (2024.01)

CPC: ***C12Q 1/6876; C12Q 1/686;*** *C12Q 2500/00; C12Q 2600/154*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2012/162660 A2 (BROWN UNIVERSITY et al.) 29 November 2012 (29.11.2012) entire document	1, 14, 15, 17, 19, 20
A	US 2013/0129668 A1 (FIRESTEIN et al.) 23 May 2013 (23.05.2013) entire document	1, 14, 15, 17, 19, 20
A	US 2008/0254447 A1 (FOEKENS et al.) 16 October 2008 (16.10.2008) entire document	1, 14, 15, 17, 19, 20
A	US 2023/0098195 A1 (TRU DIAGNOSTICS INC.) 30 March 2023 (30.03.2023) entire document	1, 14, 15, 17, 19, 20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 August 2024 (08.08.2024)	**06 November 2024 (06.11.2024)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450 UNITED STATES OF AMERICA**	**TAINA MATOS**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033579

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-15, 17, 19, and 20 are drawn to methods.

The first invention of Group I+ is restricted to CG loci selected to be cg00011943, cg00014020, cg00056433, cg00067133, cg00071360 and methods comprising the same. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named invention was selected based on the first listed CG loci species presented in the claims (see claim 1). It is believed that claims 1 and 14, 15, 17, 19, and 20 read on this first named invention and thus these claims will be searched without fee to the extent that they read on cg00011943, cg00014020, cg00056433, cg00067133, and cg00071360.

Applicant is invited to elect additional CG loci to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be CG loci selected to be cg00074147, cg00080105, cg00120123, cg00122614, cg00153759 and methods comprising the same. Additional CG loci will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/ examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for unmethylated CG loci correlated to human health requiring the selection of alternative CG loci where "wherein the CG loci are a plurality of at least five CG loci selected from a group consisting of cg00011943, cg00014020, cg00056433, cg00067133, cg00071360, cg00074145, cg00080105, cg00120123, cg00122614, cg00153759, cg00177243, cg00178119, cg00182994, cg00209129, cg00215550, cg00217225, cg00236988, cg00237714, cg00243480, cg00251125, cg00262132, cg00275686, cg00278547, cg00283022, cg00303773, cg00322993, cg00342468, cg00358895, cg00458337, cg00465312, cg00481382, cg00530438, cg00538458, cg00548856, cg00593298, cg00594167, cg00602326, cg00615271, cg00631329, cg00639633,cg00646555,cg00687674, cg00687962, cg00698355, cg00712841, cg00737155, cg00748449, cg00756943, cg00767058, cg00800095, cg00815399, cg00910115, cg00935026, cg00935421, cg00964035, cg00973876, cg00993310, cg01020263, cg01025883, cg01067216, cg01089474, cg01094440, cg01098936, cg01113710, cg01134012, cg01166985, cg01169463, cg01178063, cg01224520, cg01243823, cg01253289, cg01266159, cg01271129, cg01274656, cg01282852, cg01313513, cg01320510, cg01346718, cg01377932, cg01380346, cg01402735, cg01410359, cg01446515, cg01457077, cg01541424, cg01545493, cg01549940, cg01579765, cg01586635, cg01606057, cg01610915, cg01645869, cg01652244, cg01660630, cg01715525, cg01770232, cg01809675, cg01824466, cg01826979, cg01844642, cg01849284, cg01857379, cg01858517, cg01865937, cg01892689, cg01893681, cg01894498, cg01929138, cg01930417, cg01956293, cg02001279, cg02007867, cg02042600, cg02048674, cg02055264, cg02056921, cg02058108, cg02081263, cg02084118, cg02084211, cg02094681, cg02106682, cg02132714, cg02217269, cg02246605, cg02310386,..., cg27632248."

Additionally, even if Groups I+ were considered to share the technical features of a method, comprising: (a) obtaining genomic DNA from a blood or saliva specimen of a human subject; (b) observing unmethylated CG loci, in the genomic DNA of the human subject, wherein said observing includes performing a bisulfite conversion process on the genomic DNA of the human subject so that cytosine residues in the genomic DNA of the human subject are transformed to uracil, while 5-methylcytosine residues in the genomic DNA of the human subject are not transformed to uracil; (c) comparing the unmethylated CG loci observed in (b) to unmethylated CG loci observed in genomic DNA collected from a reference group of human individuals; and (d) correlating the unmethylated CG loci observed in (b) with the unmethylated CG loci observed in the reference group of

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

human individuals to predict health of the human subject wherein the CG loci are a plurality of at least five CG loci. However, these shared technical features do not represent a contribution over the prior art.

US 2023/0098195 A1 to Tru Diagnostics, Inc. (hereinafter, "Tru") teaches a method (a method; Para. [0007]), comprising: (a) obtaining genomic DNA from a blood or saliva specimen of a human subject (determining a percentage of cell-types in a saliva specimen; Para. [0007]; the genomic DNA of the saliva specimen is derived form a human subject; Para. [0013]); (b) observing methylated CG loci, in the genomic DNA of the human subject (obtaining genomic DNA from the saliva specimen and observing cytosine methylation at a plurality of CG loci in the genomic DNA of the saliva specimen; Para. [0007]), wherein said observing includes performing a bisulfite conversion process on the genomic DNA of the human subject so that cytosine residues in the genomic DNA of the human subject are transformed to uracil (wherein said observing includes performing a bisulfate conversion process on the genomic DNA of the saliva specimen so that cytosine residues in the genomic DNA of the saliva specimen are transformed to uracil; Para. [0007]), while 5-methylcytosine residues in the genomic DNA of the human subject are not transformed to uracil (while 5-mehtylcytosine residues in the genomic DNA of the saliva specimen are not transformed to uracil; Para. [0007]); (c) comparing the methylated CG loci observed in (b) to methylated CG loci observed in genomic DNA collected from a reference group of human individuals (comparing the CG loci methylation observed to the CG loci methylation observed in genomic DNA collected from a reference group of saliva specimens; Para. [0008]; the genomic DNA of the saliva specimen is derived form a human subject; Para. [0013]); and (d) correlating the methylated CG loci observed in (b) with the methylated CG loci observed in the reference group of human individuals (correlating the CG loci methylation observed with the CG methylation observed in the reference group of saliva specimens to determine the percentage of cell-types in the saliva sample; Para. [0008]) wherein the CG loci are a plurality of at least five CG loci (the plurality of CG loci in the DNA of the human subject includes 157 CG loci; Para. [0009]).

WO 2012/162660 A2 to Brown University et al. (hereinafter, "Brown") teaches unmethylated CG loci (assessing a disease condition of the subject by analyzing differential methylation pattern…an unmethylated form of a sequence having a CpG dinucleotide; Pg. 7, Lns. 26-35); predict health of the human subject (assessing a disease condition of the subject by analyzing differential methylation pattern of CpG dinucleotides…DNA probes hybridize to a DNA sequence of each of a methylated form and unmethylated form of a sequence having a CpG dinucleotide…detecting the methylation status of each CpG dinucleotide…thereby estimating proportions of types of leukocytes in the sample from the subject for assessing the disease condition of the subject; Pg. 7, Lns. 26-35; Pg. 8, Lns. 1-3; comparing the signatures of a human patient and comparing to a signature from a healthy control human; Pg. 10, Lns. 16-27).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033579

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1, 14, 15, 17, 19, 20**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.