

What is claimed is:

1. A method for calculating biological age of a subject, the method comprising the steps of:
 - (a) extracting DNA from a substrate from the subject;
 - (b) measuring DNA methylation in the extracted DNA from the substrate to obtain a DNA methylation profile;
 - (c) analyzing the DNA methylation profile of 13 human CG sites positioned in a putative antisense region to ElovL2 gene, ElovL2 AS1 region, as set forth in SEQ ID NO:1 to obtain a polygenic score using multiple linear regression equations or neural network analysis; and
 - (d) determining the biological age of the subject from the polygenic score, wherein the extracting DNA comprises extracting genomic DNA from saliva ~~or blood~~ obtained from the subject.
2. The method of claim 1, wherein the measuring DNA methylation is performed using methods comprising, DNA pyrosequencing, mass spectrometry based (Epityper™), PCR based methylation assays, targeted-amplicon next generation bisulfite sequencing on a platform selected from a group of HiSeq, MiniSeq, MiSeq, and NextSeq sequencers, Ion Torrent sequencing, methylated DNA Immunoprecipitation (MeDIP) sequencing, or hybridization with oligonucleotide arrays.
3. The method of claim 1, wherein the measuring DNA methylation is performed for polygenic DNA methylation biomarkers which comprises measuring the methylation status of CG sites within ~~any one of the 13 human CG sites and combinations thereof, which are~~ positioned in ~~a the~~ putative antisense region to ElovL2 gene, ElovL2 AS 1 region as set forth in SEQ ID NO: 1.
4. The method of claim 1, wherein the measuring DNA methylation is performed using DNA pyrosequencing comprising primers as set forth in SEQ ID N0:2 for the forward, biotinylated primer, SEQ ID N0:3 for the reverse primer, and SEQ ID N0:4 for the pyrosequencing primer.
5. The method of claim 1, wherein the measuring DNA methylation is performed using targeted-amplicon next generation bisulfite sequencing on a platform selected from a group of HiSeq, MiniSeq, MiSeq, and NextSeq sequencers, comprising primers as set forth in SEQ ID N0:5 for the forward primer and SEQ

ID NO:6 for the reverse primer.

6. The method of claim 1, wherein the measuring DNA methylation is performed using PCR based methylation assays selected from a group of methylation specific PCR and digital PCR.

~~7. The method of claim 1, wherein the analyzing the DNA methylation profile to obtain a polygenic score comprises using multiple linear regression equations or neural network analysis.~~

~~9.7.~~ A method for calculating biological age across multiple subjects, the method comprising the steps of:

- (a) extracting DNA from multiple substrates from multiple subjects;
- (b) measuring DNA methylation in the extracted DNA from multiple substrates to obtain a DNA methylation profile;
- (c) analyzing the DNA methylation profile of 13 human CG sites of a putative antisense region to ElovL2 gene, ElovL2 AS1 region, as set forth in SEQ ID NO:1 to obtain a polygenic score using multiple linear regression equations or neural network analysis; and
- (d) determining the biological age across multiple subjects from the polygenic score,

wherein the extracting DNA comprises extracting genomic DNA from saliva ~~or blood~~ obtained from a subject.

~~10.8.~~ The method of claim ~~87~~, wherein the measuring DNA methylation in the extracted DNA from multiple substrates comprises the steps of:

- (a) amplifying genomic DNA extracted from the multiple substrates with target specific primers to obtain PCR product 1;
- (b) amplifying the PCR product 1 of step (a) by barcoding primers to obtain PCR product 2;
- (c) performing multiplexed sequencing in a single next generation Miseq sequencing reaction using the PCR product 2 of step (b);
- (d) extracting data from the multiplexed sequencing of step (c); and
- (e) quantifying DNA methylation from the extracted data of step (d) to obtain a DNA methylation profile for each substrate,

wherein the extracting DNA comprises extracting genomic DNA from saliva ~~or blood~~ obtained from a subject.

~~109.~~ The method of claim ~~98~~, wherein the target specific primers to obtain PCR product 1 comprises primers as set forth in SEQ ID NO:5 for the forward primer

and SEQ ID N0:6 for the reverse primer, and wherein the barcoding primers to obtain PCR product 2 comprises primers as set forth in SEQ ID N0:7 for the forward primer and SEQ ID N0:8 for the reverse primer, which is the barcode index primer.

~~11. A combination of DNA methylation biomarkers for calculating biological age, wherein the combination of the of DNA methylation biomarkers comprises the human CG sites combinations thereof, which are positioned in a putative antisense region to ElovL2 gene, the ElovL2 AS 1 region as set forth in SEQ ID NO:1.~~

~~12. 10.~~ A kit for determining the biological age of a subject, comprising the means and reagents for collection and stabilizing of substrate from the subject; a scanner for reading a barcode on the kit; and instructions for collection and stabilizing of the substrate, wherein the substrate is saliva ~~or blood~~ of a subject, and wherein the stabilizing of substrate is for mailing in the collected substrate for extracting DNA for the measurement of DNA methylation in the extracted DNA from the substrate to obtain a DNA methylation profile of the subject to determine the biological age of the subject.

~~13. 11.~~ A computer-implemented method for providing recommendations for lifestyle changes, the method comprising the steps of:

- (a) assessing an entry in a computer-readable medium as obtained through sharing of user data from a subject;
- (b) matching the entry of step (a) to a kit of claim ~~12-10~~ as obtained from said subject for determining the biological age of said subject;
- (c) calculating the biological age of a subject using the method of claim 1 or 8 to obtain the calculated biological age;
- (d) integrating the calculated biological age of step (c) in ~~the a~~ machine learning model for said subject by performing statistical analysis using assessment of step (a) to obtain an integrated data report;
- (e) preparing a dynamic report for said subject by analyzing the integrated data report of step (d) with the progression of responses to the questionnaire as obtained through sharing of user data from said subject with time and comparing them to the recommendations of the national associations; and
- (f) sharing the dynamic report of step (e) on the computer-readable medium with said subject for providing recommendations for lifestyle changes.

~~14. 12.~~ The computer-implemented method of claim ~~13~~11, wherein the computer-readable medium comprises open source development tools to contain information on a

test for calculating biological age based on the method of claim 1 or 87, a virtual shopping cart for ordering said test, a scanning function for scanning a barcode of the kit of claim 1210, and a function for receiving test results from the lab, and wherein the open source development tools comprise questionnaires included in the computer-readable medium to probe lifestyle functions that impact on healthy aging, including basic physiological measures, weight, height blood pressure, heart rate, mood self-assessment, McGill pain questionnaire, diet and nutrition questionnaire, exercise questionnaire and lifestyle question including alcohol, drugs and smoking, and combination thereof.

~~15.~~ 13. The computer-implemented method of claim 1412, wherein the method comprises

the use of Android or Apple or WeChat miniprogram for personalized lifestyle recommendations, creating a health ecosystem focused on normalizing or slowing biological aging for a subject, or for storing data in an Object storage enterprise in a server or a cloud server including, Amazon, Ali cloud or Microsoft Azure using standard data pipeline and Management systems such as Cloud dataprep across multiple subjects.

~~16.~~ 14. The computer-implemented method of claim 1412, wherein the method comprises the use of set of artificial intelligence algorithms such as Random Forest (RF), Support Vector Machine (SVM), Linear Discriminant Analysis (LDA), Generalized Linear Model (GLM) and Deep Learning (DL) for calculating the weighted contribution of different lifestyle measures on the biological age of a subject or across multiple subjects which is dynamically updated to provide personalized lifestyle recommendations on lifestyle changes.

~~17. A method for developing a computer-readable medium, the method comprising the steps of:~~

- ~~—(a) storing the data derived from multiple subjects;~~
- ~~(s) analyzing the stored data of step (a); and~~

~~(x) building a model,~~

~~wherein the step of storing the data derived from multiple users comprises a cloud-based SQL data base,~~

~~wherein the step of analyzing the stored data comprises a group selected from — deep machine learning, reinforcement learning, and machine learning, or a combination thereof, and~~

~~wherein the step of building a model comprises correlating input questionnaire measurements and the difference between DNA methylation age and chronological age as an output as well as other physiological and psychological — outputs such as pain, blood pressure, BMI and mood.~~

~~30. The method for developing a computer-readable medium of claim 17, wherein the machine learning comprises a group selected from data mining algorithm comprising a random forest analysis or data mining algorithm comprising a K-Means Cluster Analysis or a platform comprising an Amazon Machine Learning — (AML) or a software comprising H2O.ai products on platforms including Apache Hadoop Distributed File system, Amazon EC2 Google compute Engine and Microsoft Azure, or a combination thereof.~~

~~32. 15.~~ The method of claim 1 or ~~8-7~~ for use in a method of assessing the effect of a biological intervention upon the biological age of a subject, the method

comprising the steps of:

- (a) calculating the biological age of a subject using the method of claim 1 or ~~8-7~~ to obtain the initial biological age before a biological intervention;
- (b) ~~performing a biological intervention~~administering a substance upon said subject;
- (c) repeating the step (a) on a subsequent substrate obtained from said subject after step (b) has been performed to obtain the biological age after the biological intervention;
- (d) integrating the biological age after the biological intervention in the machine learning model for said subject to assess the effect of the biological intervention upon the biological age of said subject,

wherein the ~~biological interventions~~substance of step (b) is selected from a group of nutritional supplements, vitamins, therapy, and ~~administration of a~~

test substance, ~~dietary manipulation, metabolic manipulation, surgical manipulation, social manipulation, behavioural manipulation, environmental manipulations, sensory manipulations, hormonal manipulation and epigenetic manipulation~~, or combinations thereof,

wherein the extracting DNA comprises extracting genomic DNA from saliva ~~or blood~~ obtained from a subject, and

wherein the integrating the biological age after the biological intervention in the machine learning model for said subject comprises the biological age assessed in step (c) and physiological parameters obtained through sharing of user data from said subject.

~~33.~~ 16. The method of claim 1 or ~~8-7~~ for use in a method of screening for an agent for being an anti-ageing agent, the method comprising the steps of:

~~(a)~~ (a) calculating the age of a substrate obtained from a subject using the method of claim 1 or ~~8-7~~ to obtain the initial biological age before administration of a test agent;

~~(b)~~ (b) administering a test agent to said subject;

~~(c)~~ (c) repeating the step (a) on a subsequent substrate obtained from said subject after step (b) has been performed to obtain the biological age after the administration of the test agent;

(d) integrating the biological age after the administration of the test agent in the machine learning model for said subject to assess whether a reduction in age has been calculated by integration in the machine learning model so to determine the test agent as an anti-ageing agent for said subject,

wherein the extracting DNA comprises extracting genomic DNA from saliva ~~or blood~~ obtained from a subject, and

wherein the integrating the biological age after the administration of the test agent in the machine learning model for said subject comprises the biological age assessed in step (c) and physiological parameters obtained through sharing of user data from said subject.