

CLAIMS

1. A method of establishing a chicken methylation clock, the method comprising:
 - (a) determining the methylation ratio and the read coverage of the genomic CpG sites of an age-correlated training sample of a specific chicken tissue;
 - (b) defining a set of CpG sites having reliable methylation ratios in all training samples of step (a) using a cutoff value; and
 - (c) performing a penalized regression using the methylation ratios of step (b) as input and the age correlated to the training sample as dependent variable, by applying a penalized regression model;thereby obtaining a set of CpG sites with corresponding weighting factors and intercept of the linear model equation as parameters defining the chicken methylation clock.
2. The method according to claim 1, wherein the methylation ratio and the read coverage of the genomic CpG sites are determined in step (a) using bisulfite sequencing.
3. The method according to any one of the preceding claims, wherein the age-correlated training sample of a specific tissue used in step (a) is selected from the group consisting of gut tissue, muscle tissue, organ tissue or skin tissue.
4. The method according to any one of the preceding claims, wherein the coverage cutoff value defined in step (b) is 3.
5. The method according to any one of the preceding claims, the method further comprising
 - (d) optimizing the fit of the regression model such that the number of CpG sites is reduced to below 100.
6. The method according to claim 5, wherein for optimizing the fit of the regression model in step (d), the algorithm applies ridge regression in combination with lasso regression.
7. The method according to claim 6, wherein the methods of ridge regression and lasso regression are balanced using an alpha value of between 0 and 1.
8. An in vitro method for predicting the chronological age of chicken, the method comprising the steps of:
 - a) obtaining genomic chicken DNA from biological sample material deriving from the chicken subject or from the chicken population to be tested;
 - b) determining the methylation level of for the CpG sites indicated in Table 2 or, alternatively, for the CpG sites indicated in Table 3, in the genomic chicken DNA;

c) comparing the methylation levels of these CpG sites in the genomic chicken DNA from the sample to be tested with the methylation levels of the same CpG sites from an age-correlated reference sample,
and deducing therefrom the chronological age of the subject or the population to be tested.

5

9. A method for predicting the chronological age of a chicken tissue sample, the method comprising:

- (a) obtaining genomic DNA from the tissue sample material deriving from a chicken subject or from a chicken population to be tested,
- 10 (b) determining the methylation ratios for the CpG sites indicated in Table 2 or, alternatively, for the CpG sites indicated in Table 3, and multiplying same with their respective weighing factors to obtain the weighted methylation ratios of those CpG sites,
- (c) computing the sum over the weighted methylation ratios obtained in step (b) and adding the respective intercept of linear model equation,
- 15 thus predicting the chronological age for the chicken tissue sample.

10. The method according to claim 9, wherein the tissue sample material deriving from a chicken subject or from a chicken population to be tested used in step (a) is selected from the group consisting of gut tissue, muscle tissue, organ tissue or skin tissue.

20

11. The method according to any one of claims 9 and 10, wherein the tissue sample material deriving from a chicken subject or from a chicken population to be tested used in step (a) is gut tissue, preferably isolated from fecal sample material.

25 12. The method according to any one of claims 9 to 11, wherein the methylation ratios for the CpG sites in step (b) were determined using bisulfite sequencing.

13. Method for detecting accelerated aging in chicken tissue samples, the method comprising:

- 30 (a) obtaining genomic DNA from the tissue sample material deriving from a chicken subject or from a chicken population to be tested,
- (b) determining the methylation ratios for the CpG sites indicated in Table 2 or, alternatively, for the CpG sites indicated in Table 3, and multiplying same with their respective weighing factors to obtain the weighted methylation ratios of those CpG sites,
- (c) computing the sum over the weighted methylation ratios obtained in step (b) and adding the respective intercept of linear model equation, thus predicting the age for the chicken tissue sample (epigenetic age) and
- 35 (d) comparing the predicted age of step (c) with the actual chronological age of the tissue sample,

wherein a predicted age higher than the chronological age is indicative of accelerated aging in the
40 chicken tissue.