CLAIMS

1. A method for estimating the age of a fish which is a member of the subclass Elasmobranchii, the method comprising:

analysing DNA obtained from the fish for the presence of a methylated cytosine at age-associated CpG sites; and

estimating the age of the fish based on methylated cytosine levels at the age-associated CpG sites.

2. The method of claim 1, wherein the age-associated CpG sites are selected from Table 8 or 9 or a homolog of one or more thereof.

3. The method of claim 1 or claim 2, wherein the fish is a shark.

4. The method of claim 3, wherein the shark is a school shark.

5. The method according to any one of claims 1 to 4, wherein the presence of methylated cytosine is analysed at five or more, 10 or more, 15 or more, 20 or more, 25 or more or 30 of the age-associated CpG sites.

6. The method according to any one of claims 1 to 5, wherein analysing DNA comprises multiplex PCR.

7. The method according to any one of claims 1 to 6, wherein analysing DNA comprises multiplex PCR and DNA sequencing.

8. The method according to claim 6 or claim 7, wherein the multiplex PCR uses two or more primer pairs configured to amplify a region of the DNA comprising the age-associated CpG sites.

9. The method according to any one of claims 1 to 8, wherein the age-associated CpG sites are identified by:

analysing DNA obtained from the species of fish of different chronological ages for the presence of methylated cytosine at CpG sites; and

using a statistical algorithm to identify age-associated CpG sites.

10. The method of claim 9, wherein analysing DNA comprises reduced representation bisulfite sequencing.

11. The method of claim 9 or claim 10, wherein the statistical algorithm is elastic net regression model.

12. A method for estimating the age of a fish comprising:

analysing DNA obtained from a fish for the presence of a methylated cytosine at age-associated CpG sites; and

estimating the age of the fish based on methylated cytosine levels at the age-associated CpG sites,

wherein the age-associated CpG sites are selected from

(i) Table 1, 2 or 3 or a homolog of one or more thereof;

(ii) Table 12 or a homolog of one or more thereof; or

(iii) Table 16 or a homolog of one or more thereof.

13. The method of claim 12, wherein the age-associated CpG sites are selected from Table 1, 2 or 3 or a homolog of one or more thereof.

14. The method of claim 12 or 13, wherein the age-associated CpG sites are comprised within one or more of the amplicons listed in Table 5.

15. The method according to any one of claims 12 to 14, wherein the presence of methylated cytosine is analysed at five or more, 10 or more, 15 or more, 20 or more, or 25 or more of the age-associated CpG sites.

16. The method according to any one of claims 12 to 15, wherein the fish is a member of the infraclass Teleostei.

17. The method according to any one of claims 12 to 16, wherein the fish is a Grouper, Tuna, Cobia, Sturgeon, Mahi-mahi, Bonito, Dhufish, Murray cod, Barramundi, Herring, Tra catfish, Mekong giant catfish, Cod, Pilchard, Pollock, Turbot, Hake, Anchovy, Haddock, Black carp, Grass carp, Eels, Koi Carp, Giant gourami, zebrafish, Mackerel, Australian lungfish, Mary river cod, Salmon or Trout.

18. The method of claim 17, wherein the fish is Zebrafish, Yellow fin tuna, Skipjack tuna, Atlantic cod, Atlantic herring, Alaska pollock, Australian lungfish, Mary River Cod or Atlantic Salmon.

19. The method according to any one of claims 12 to 18, wherein analysing DNA comprises multiplex PCR.

20. The method according to any one of claims 12 to 19, wherein analysing DNA comprises multiplex PCR and DNA sequencing.

21. The method according to claim 19 or claim 20, wherein the multiplex PCR uses two or more primer pairs configured to amplify a region of the DNA comprising the age-associated CpG sites.

22. The method of claim 21, wherein at least one of the primers (i) is selected from Table 4; and/or (ii) can be used to amplify the same CpG site as the primers of (i).

23. The method of claim 22, wherein at least one of the primers hybridizes to a region of the DNA within 100 or 50 or 20 base-pairs of a primer of (i).

24. The method according to any one of claims 19 to 23, wherein one or more or all of the primers pairs provided in Table 4 are used.

25. The method of claim 21, wherein at least one of the primers (i) is selected from Table 11; and/or (ii) can be used to amplify the same CpG site as the primers of (i).

26. The method of claim 21, wherein at least one of the primers (i) is selected from Table 15; and/or (ii) can be used to amplify the same CpG site as the primers of (i).

27. The method according to any one of claims 1 to 26, wherein analysing DNA comprises determining the methylation beta value of the age associated CpG sites.

28. The method according to any one of claims 1 to 27, wherein estimating the age of the fish or reptile comprises comparing to an age correlated reference population.

29. The method according to any one of claims 1 to 28, wherein estimating the age of the fish or reptile comprises determining a methylation profile.

30. The method of claim 29, wherein the methylation profile is the sum of raw summed methylation beta values for the age-associated CpG sites.

31. The method of claim 30, wherein estimating the age of the fish comprises comparing the methylation profile for the DNA to a methylation profile from an age correlated reference population determined using the same age-associated CpG sites.

32. The method according to any one of claims 1 to 31, wherein the DNA analysed is from caudal fin.

33. The method according to any one of claims 1 to 32, wherein the DNA analysed is from a skin biopsy.

34. The method according to any one of claims 1 to 33 which further comprises obtaining a biological sample comprising the DNA from the fish.

35. The method according to any one of claims 1 to 34, wherein the correlation between chronological age and estimated age is at least 90%, or at least 95%.

36. Use of two or more primer pairs for amplifying one or more age-associated CpG sites listed in Table 1, 2, 3, 8, 9, 12, 16, 19 or 20.

37. A method of identifying an age-associated CpG site for a second species of fish comprising

(i) analysing DNA of the second fish species for a candidate age-associated CpG site corresponding to an age-associated CpG site identified for a first species of fish;

(ii) analysing the methylation patterns of a candidate age-associated CpG site identified in (i) in different ages of the second species of fish to determine if it is an age-associated CpG site in that second fish species.

38. The method of claim 37, wherein step (i) comprises a pairwise analysis of the DNA of the first fish species with the DNA of the second fish species.

39. The method of claim 37 or claim 38, wherein the first fish species is zebrafish and step (i) comprises analysing DNA of the second fish species for a candidate age-associated CpG site corresponding to an age-associated CpG site listed in Table 1, 2 or 3.

40. The method of claim 39, wherein the second fish species is a member of the infraclass Teleostei.

41. The method of claim 39, wherein the second fish species is a Murray cod, Mary River cod or an Australian lungfish.

42. The method of claim 37 or claim 38, wherein the first fish species is a shark species and step (i) comprises analysing DNA of the second fish species for a candidate age-associated CpG site corresponding to an age-associated CpG site listed in Table 8 or 9.

43. The method according to any one of claims 1 to 11, wherein the age associated CpG sites are identified by the method according to any one of claims 37, 38 and 42.

44. The method according to any one of claims 12 to 35, wherein the age associated CpG sites are identified by the method according to any one of claims 37 to 41.

45. A kit for estimating the age of a fish or reptile comprising one or more primer pairs or probes for detecting the presence of a methylated cytosine at age-associated CpG sites.

46. A method for estimating the age of a reptile comprising:

analysing DNA obtained from a reptile for the presence of a methylated cytosine at age-associated CpG sites; and

estimating the age of the reptile based on methylated cytosine levels at the age-associated CpG sites.

47. The method of claim 46, wherein the age-associated CpG sites are selected from Table 19 or 20 or a homolog of one or more thereof.

48. The method of claim 47, wherein the age-associated CpG sites are selected from Table 20 or a homolog of one or more thereof.

49. The method of claim 48, wherein the presence of methylated cytosine is analysed at five or more, 10 or more, 15 or more, 20 or more, 25 or more or all of the age-associated CpG sites listed in Table 20.

50. The method according to any one of claims 46 to 49, wherein the reptile is a marine turtle.

~~51. The method of claim 50, wherein the marine turtle is selected from the group consisting of Green sea turtle, Flatback turtle, Hawksbill turtle, Leatherback turtle, Loggerhead turtle and Olive Ridley turtle.~~

~~52. A method of identifying an age-associated CpG site for a second species of reptile comprising~~

~~(i) analysing DNA of the second reptile species for a candidate age associated CpG site corresponding to an age-associated CpG site identified for a first species of reptile;~~

~~(ii) analysing the methylation patterns of a candidate age-associated CpG site identified in (i) in different ages of the second species of reptile to determine if it is an age-associated CpG site in that second reptile species.~~

~~53. The method of claim 52, wherein step (i) comprises a pairwise analysis of the DNA of the first reptile species with the DNA of the second reptile species.~~

~~54. The method of claim 52 or claim 53, wherein the first reptile species is green sea turtle and step (i) comprises analysing DNA of the second reptile species for a candidate age-associated CpG site corresponding to an age-associated CpG site listed in Table 19 or 20.~~

~~55. The method of claim 54, wherein the second reptile species is a marine turtle.~~

~~56. The method of claim 55, wherein the marine turtle is selected from the group consisting of Flatback turtle, Hawksbill turtle, Leatherback turtle, Loggerhead turtle and Olive Ridley turtle.~~

~~57.~~1. A method for estimating the age of a fish ~~or reptile~~ comprising:

analysing DNA obtained from a fish ~~or reptile~~ for the presence of a methylated cytosine at age-associated CpG sites; and

estimating the age of the fish ~~or reptile~~ based on methylated cytosine levels at the age-associated CpG sites.

~~58. A computer-readable medium which comprises a training data set comprising one or more or all of the CpG sites listed in Tables 1, 2, 3, 8, 9, 12, 16, 19 or 20 or a homolog of one or more thereof.~~

2. The method of claim 1, wherein the age-associated CpG sites are selected from
(i) Table 8 or 9 or a homolog of one or more thereof;
(ii) Table 1, 2 or 3 or a homolog of one or more thereof;
(iii) Table 12 or a homolog of one or more thereof; or
(iv) Table 16 or a homolog of one or more thereof.

3. The method of claim 1, wherein the age-associated CpG sites are selected from Table 8 or 9 or a homolog of one or more thereof.

4. The method of claim 1, wherein the age-associated CpG sites are selected from Table 1, 2 or 3 or a homolog of one or more thereof.

5. The method of claim 1, wherein the age-associated CpG sites are comprised within one or more of the amplicons listed in Table 5.

6. The method of claim 1, wherein the presence of methylated cytosine is analysed at five or more, 10 or more, 15 or more, 20 or more, or 25 or more of the age-associated CpG sites.

7. The method of claim 1, wherein analysing DNA comprises multiplex PCR and DNA sequencing.

8. The method of claim 7, wherein the multiplex PCR uses two or more primer pairs configured to amplify a region of the DNA comprising the age-associated CpG sites.

9. The method of claim 8, wherein at least one of the primers (i) is selected from Table 4; and/or (ii) can be used to amplify the same CpG site as the primers of (i); and/or (iii) hybridizes to a region of the DNA within 100 or 50 or 20 base-pairs of a primer of (i).

10. The method of claim 1, wherein analysing DNA comprises determining the methylation beta value of the age associated CpG sites.

11. The method of claim 1, wherein the DNA analysed is from caudal fin and/or a skin biopsy.

12. The method of claim 1, wherein the fish is a member of the subclass Elasmobranchii.

13. The method of claim 12, wherein the fish is a shark.

14. The method of claim 1, wherein the fish is a member of the infraclass Teleostei.

15. The method of claim 14, wherein the fish is Zebrafish, Yellow fin tuna, Skipjack tuna, Atlantic cod, Atlantic herring, Alaska pollock, Australian lungfish, Mary River Cod or Atlantic Salmon.

16. The method of claim 1, wherein the age-associated CpG sites are identified by:

analysing DNA obtained from the species of fish of different chronological ages for the presence of methylated cytosine at CpG sites; and

using a statistical algorithm to identify age-associated CpG sites.

17. A method of identifying an age-associated CpG site for a second species of fish comprising

(i) analysing DNA of the second fish species for a candidate age-associated CpG site corresponding to an age-associated CpG site identified for a first species of fish;

(ii) analysing the methylation patterns of a candidate age-associated CpG site identified in (i) in different ages of the second species of fish to determine if it is an age-associated CpG site in that second fish species.

18. The method of claim 17, wherein the first fish species is zebrafish and step (i) comprises analysing DNA of the second fish species for a candidate age-associated CpG site corresponding to an age-associated CpG site listed in Table 1, 2 or 3.

19. The method of claim 17, wherein the first fish species is a shark species and step (i) comprises analysing DNA of the second fish species for a candidate age-associated CpG site corresponding to an age-associated CpG site listed in Table 8 or 9.

20. The method of claim 1, wherein the age-associated CpG sites are identified by the method according to claim 17.