Australia's National Science Agency

Update on epigenetic ageing of tuna

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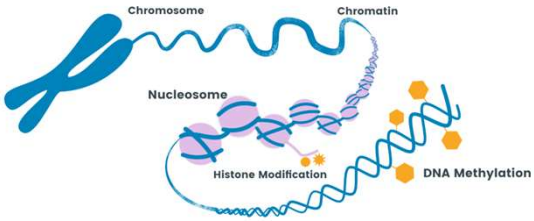
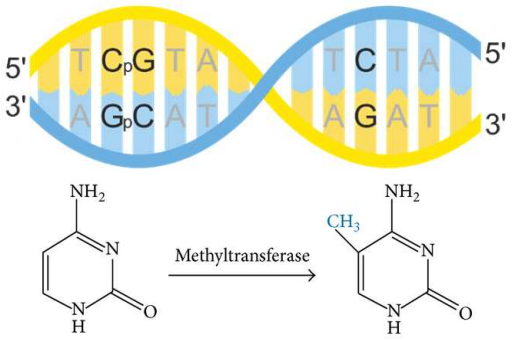
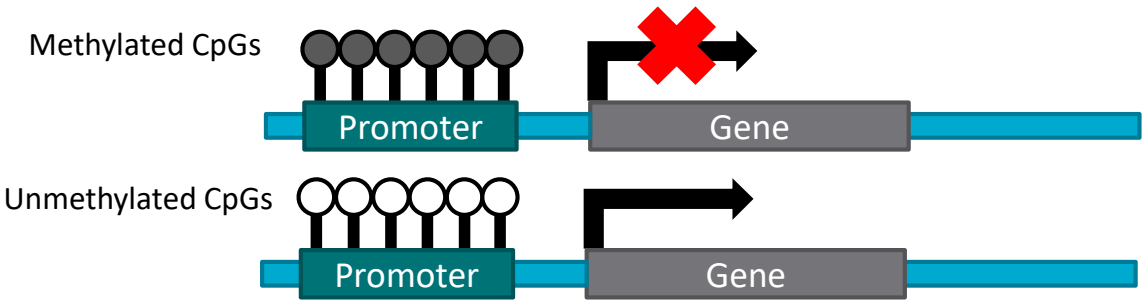
CSIRO Environomics Future Science Platform

What is DNA Methylation?



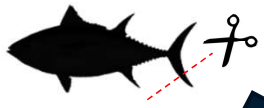


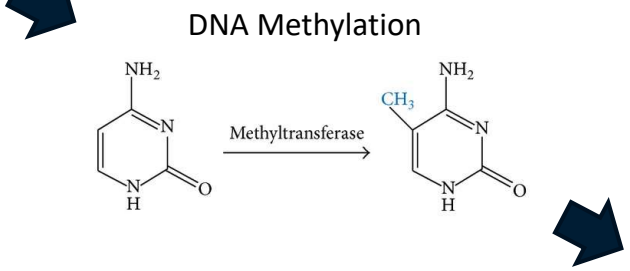


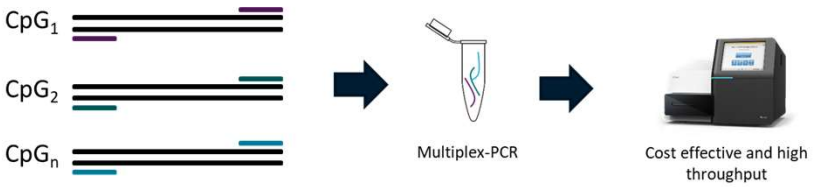
Images credit: www.labclinics.com

- DNA methylation is an epigenetic modification to DNA (does not modify the underlying sequence).
- DNA methylation is mostly known for its role in regulating gene expression through cytosine-phosphate-guanine (CpG) sites near the start of genes.
- It has also been shown that DNA methylation at specific CpG sites is age associated. The methylation either increases or decreases with age.
- By identifying the most age-associated CpG sites, machine learning can be done to generate models known as epigenetic clocks to predict age.

How?







$$Age = CpG_1x_1 + .. CpG_Nx_N + c$$

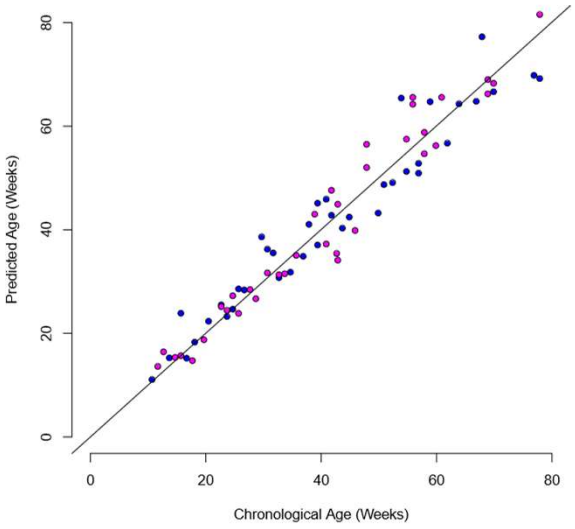
- In species such as humans, array based technology is commonly used for epigenetic ageing, however this is an expensive approach.
- We have generated a cost-effective approach, by targeting the minimum number of CpG sites required for age prediction by using multiplex PCR.
- To predict age from a fish, DNA is extracted and age-associated regions of DNA are amplified through multiplex PCR.
- By comparing the methylation to known ages, models can be constructed to predict age.

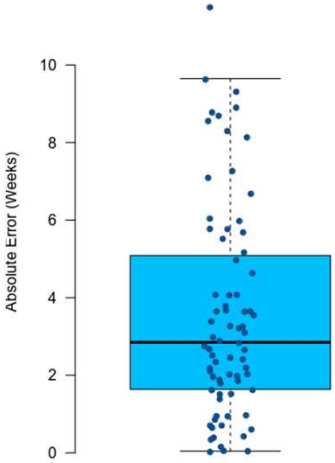
Age Prediction in Zebrafish

524,038 CpG sites

1,311 age-associated CpG sites

29 CpG sites





Mayne *et al.*, 2020, Aging

- We first generated an epigenetic clock on zebrafish.
- This model uses DNA from caudal fins and age-associated CpG sites were identified through genome wide sequencing.
- A total of 524,038 CpG sites were captured through DNA sequencing, of which, 1,311 CpG sites were associated with age (Pearson correlation, p-value < 0.01)
- Using a machine learning based approach (elastic net regression) 29 were identified to be the minimum number required to predict age.

Rapid epigenetic age estimation for SBT

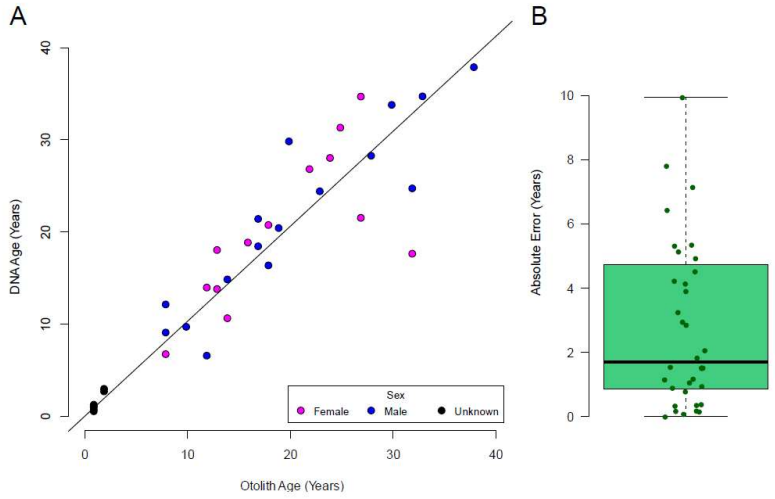
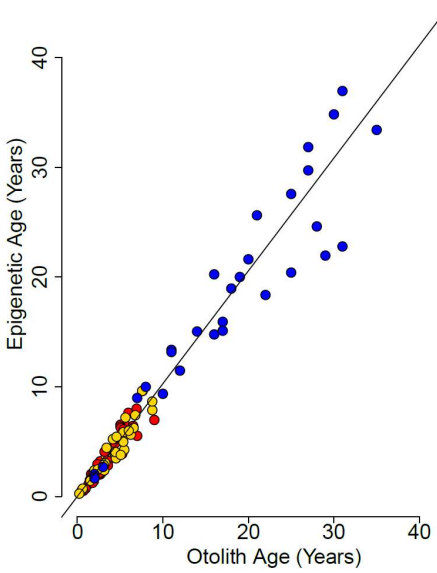


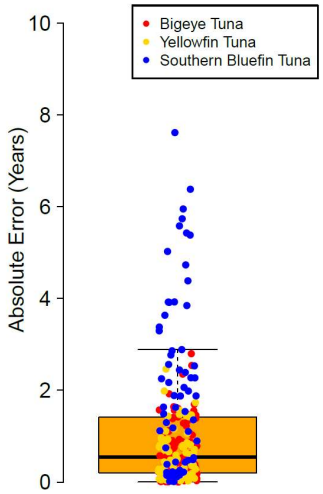
Figure 1. Performance of age prediction by DNA methylation in southern bluefin tuna. **A.** The correlation between the age derived from otoliths and predicted by DNA (Pearson correlation = 0.93, p-value = 1.23×10^{-16}). **B.** Absolute error rate between otolith age and DNA age (Median absolute error = 1.7 years).

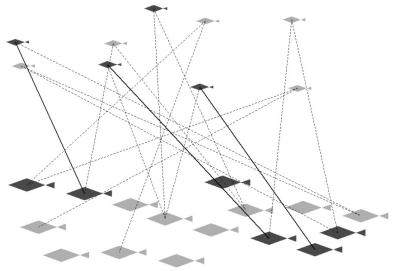
Mayne *et al.* (2021); CCSBT-ESC/2108/10

- To prevent the need for expensive genome wide sequencing, the conserved age-associated CpG sites between zebrafish and southern bluefin tuna (SBT) were identified by comparing the two genomes.
- A multiplex PCR assay was designed and allowed for age prediction from DNA extracted from tissue and DNA methylation at 22 CpG sites.
- This is to our knowledge is the first epigenetic clock for a commercial fish species.
- Preliminary results for SBT were presented to the CCSBT ESC in 2021 (CCSBT-ESC/2108/10).

Tuna species







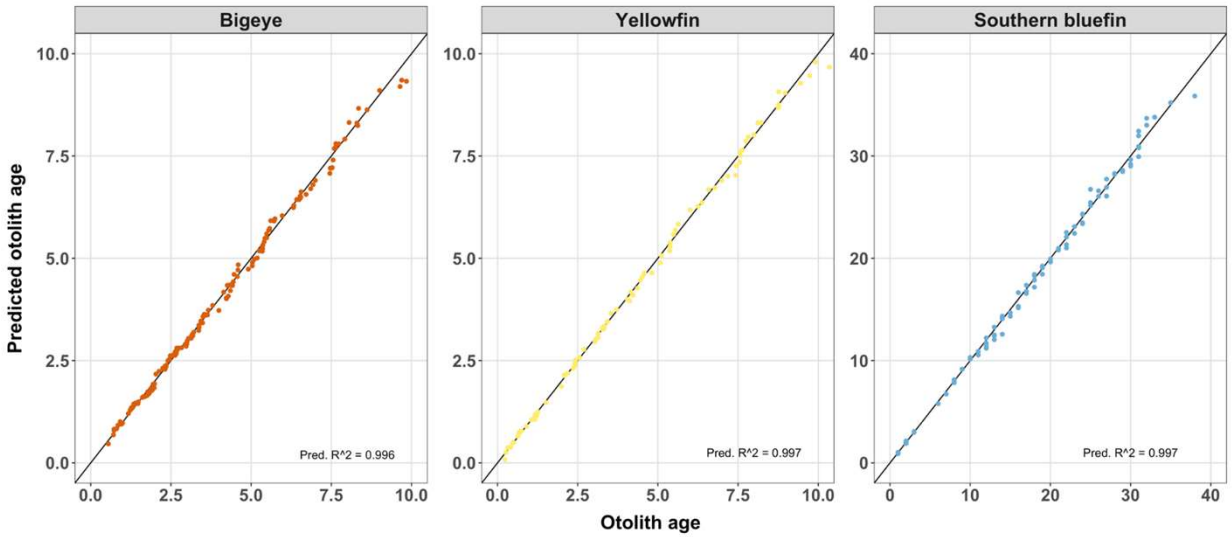
Bravington *et al., 2016*

10,000 + Samples

Mayne *et al.,* In preparation, Images Credit: rules.fish.wa.gov.au

- The tuna epigenetic clock was further expanded to incorporate bigeye and yellowfin tuna, using the same 22 CpG sites.
- This work still uses an elastic net regression, a common algorithm in epigenetic clock research.
- The aim is to incorporate the epigenetic age assay into the close kin mark recapture work. However, this requires further development to the assay to make it high throughput.

Improved statistical models

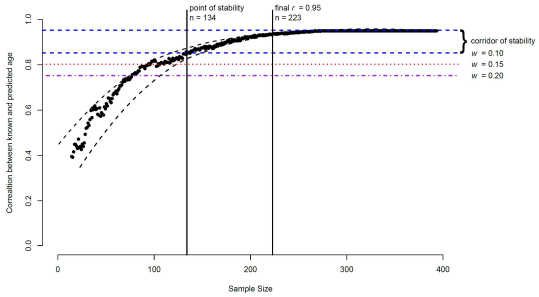


Predicted otolith ages calculated using the NL-INV species-specific epigenetic clock against observed otolith ages from five- fold cross-validation. *The predictions from the five cross-validation test sets are presented as an aggregated plot for each of the species. The solid black line is the y = x line. The prediction R^2 values were generated by regressing the aggregated cross-validation test set predictions on the observed otolith ages. Samples sizes for each species were N=158 for bigeye, N=94 for yellowfin and N=96 for southern bluefin.*

Mayne & Lloyd-Jones *et al.,* In preparation

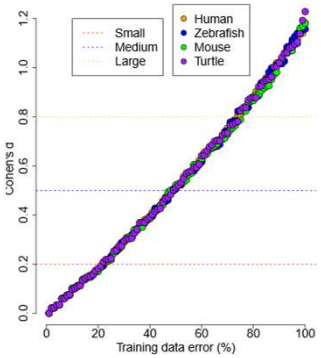
- Additional modelling has improved the epigenetic clock age prediction.
- This new modelling uses shape-constrained generalised additive models. Briefly these models take into account any nonlinear relationship between methylation and age, whereas previous models do not. This has improved the overall model for tuna epigenetic age prediction.

Epigenetic Clock Requirements



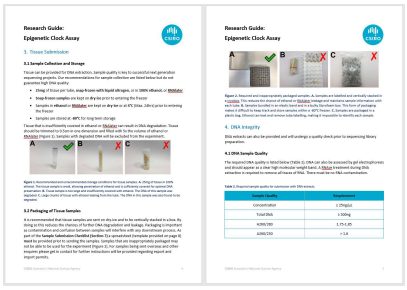
Require large sample sizes for calibration

Mayne *et al.*, 2021, Molecular Ecology Resources



< 23% Error in calibration dataset

Mayne *et al.*, 2023, Evolutionary Applications



Research Guides

- We continue to determine the optimal parameters for epigenetic clock design.
- We have also provided guides in sample collection for external collaborators .

Thank you

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