Tissue Specific Epigenetic Clocks Closely Correlate with Aging Progression and Predict Life Span

Xi Yu Jia*, Anand Gopalan, Paolo Piatti, Wei Guo, Jessica Valadez, Joe Ollar, Yap Chew, Xiaojing Yang
Zymo Research Corporation, Irvine, CA, USA
* Corresponding author's email: ljia@zymoresearch.com

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple®

Abstract

The associations between many aging related diseases, such as cancer, cardiovascular disease, and type 2 diabetes have been identified by numerous population studies in the past few years. Epigenetic age (DNAge®) has been recognized as a robust and reliable aging index for the determination of the biological age in human samples.

A robust DNAge® clock is essential for the accurate quantification of the epigenetic age in the model animal. We have developed a mouse DNAge® panel capable to monitor DNA methylation changes of over 2,000 genomic loci. Taking advantage of the robust targeted bisulfite sequencing approach, called SWARM® (Simplified Whole-panel Amplification Reaction Method), our mouse DNAge® panel generated highly reproducible DNA methylation data in a high-throughput manner. We also build tissue-specific epigenetic clocks for mouse whole blood, liver, muscle and brain in order to be able to study in detail the aging process in different tissues. To this end we used the elastic net regression of DNA methylation levels of the targeted loci in different tissues collected from 9 to 130 weeks old mice.

Interestingly, we found a strong correlation between DNAge® acceleration rate and life span among different mouse strains using our mouse DNAge® blood clock. We also discovered that DNAge® test was able to detect effects of different anti-aging intervention therapies performed in mice, and strongly correlates with lifespan. This demonstrates the sensitivity of tissue-specific epigenetic clocks in capturing aging progression. These reliable tissue-specific clocks will be important in understanding aging process and aid the development of effective anti-aging intervention.

Key Words: DNA methylation, targeted bisulfite sequencing, epigenetic age clock, DNAge®

The DNAge® Tests

The mouse DNAge® tests are built based on a principle similar to the one originally described in the Horvath Clock method, that utilizes DNA methylation levels of epigenetic markers for biological age determination. The current mouse tissue specific DNAge tests utilize SWARM® (Simplified Whole-panel Amplification Reaction Method) to analyze DNA methylation patterns of about 2,000 loci which are aging related markers based on literature search and internal discovery. We then further calibrated DNAge® clocks against different mice tissue, providing epigenetic age predictions in a tissue specific manner.
A penalized regression model's coefficients b_0, b_1, ..., b_n relate to transformed age as follow:

$$F(\text{chronological age}) = b_0 + b_1CpG_1 + \cdots + b_nCpG_n + \text{error}$$

DNAge™ is estimated as follow:

$$DNAge^{TM} = inverse.F(b_0 + b_1CpG_1 + \cdots + b_nCpG_n)$$

Sample Collection to Build DNAge® Clocks

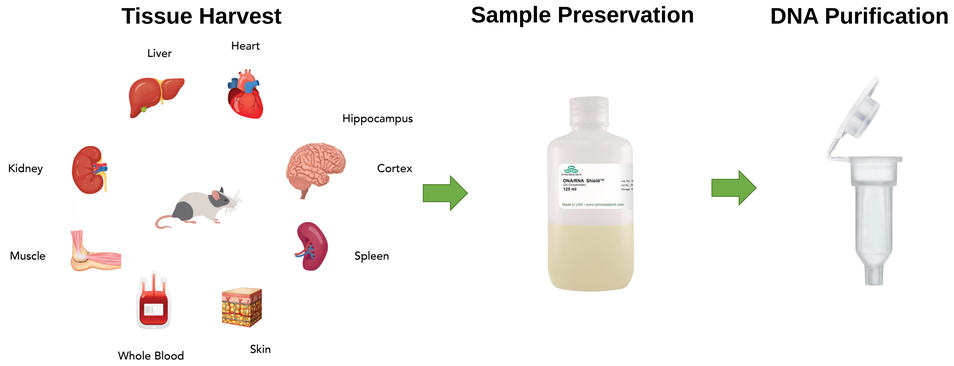
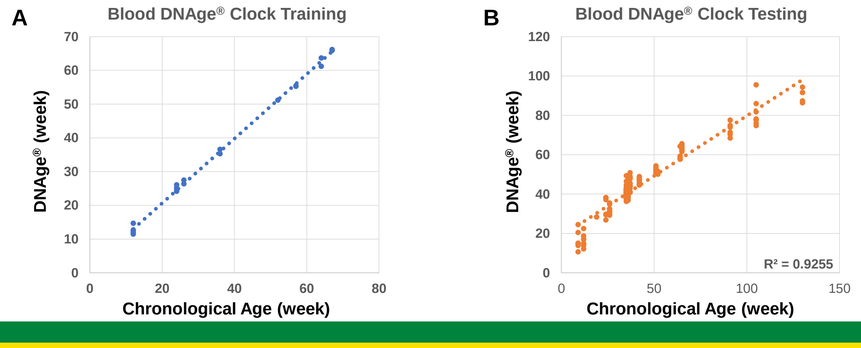


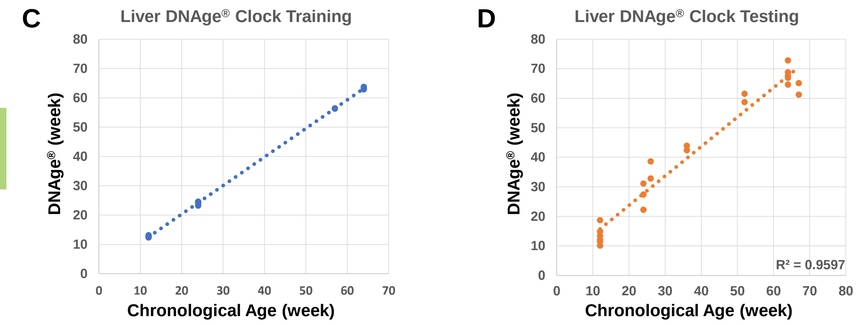
Figure 1. Mice tissue collection, preservation and purification. Indicated tissue samples and whole blood samples are collected from mice between 9 to 130 weeks old following IRB approved protocols. The samples are immediately preserved in DNA/RNA shield for long term storage at room temperature and genome DNA samples are purified following optimized protocols for different tissue types.

Multi-tissue DNAge® Clock

Blood DNAge® Clock



Liver DNAge® Clock



Muscle DNAge® Clock

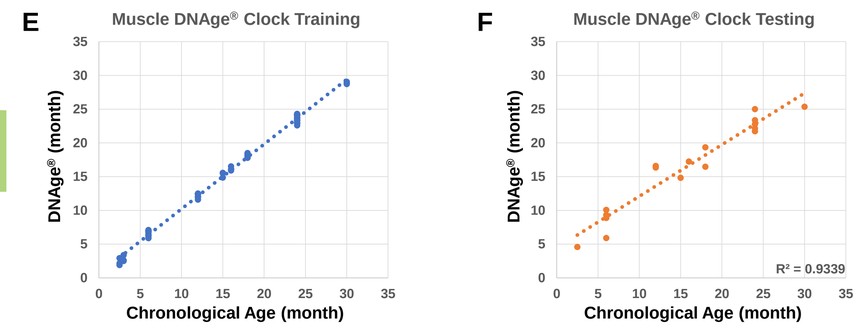
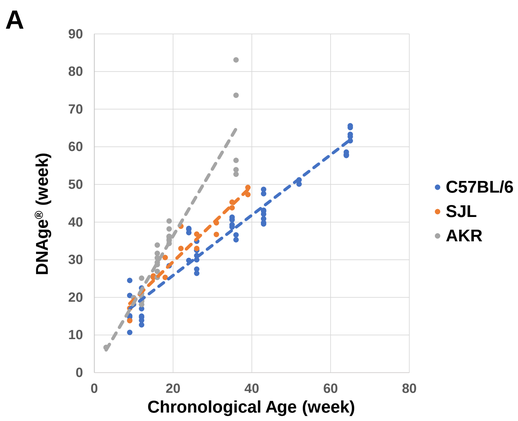


Figure 2. Building and validation of mouse DNAge clocks using SWARM® technology. All samples are collected from C57BL/6J mice. A,C,E,G show the DNAge® data of training samples in the indicated tissue types. B,D,F,G present DNAge results of testing samples in the indicated tissue types. Dotted line: the regression line of DNAge®.

Blood DNAge® Acceleration Predicts Life Span in Mouse



B

Strain name	Median lifespan(days)	Liner Regression
C57BL/6J	901	y = 0.8005x + 9.8136
SJL/J	514	y = 1.0068x + 9.3087
AKR/J	288	y = 1.7817x + 0.6645

Figure 3. DNAge®. acceleration rate in different mouse strains. (A) Whole blood samples from different strains of mouse are collected at indicated time points. The DNAge®. was assessed using Zymo Mouse Aging clock (Blood). (B) The aging acceleration rates are associated with reported median lifespan.

Changes in Blood DNAge® Correlate with Life Expectancy

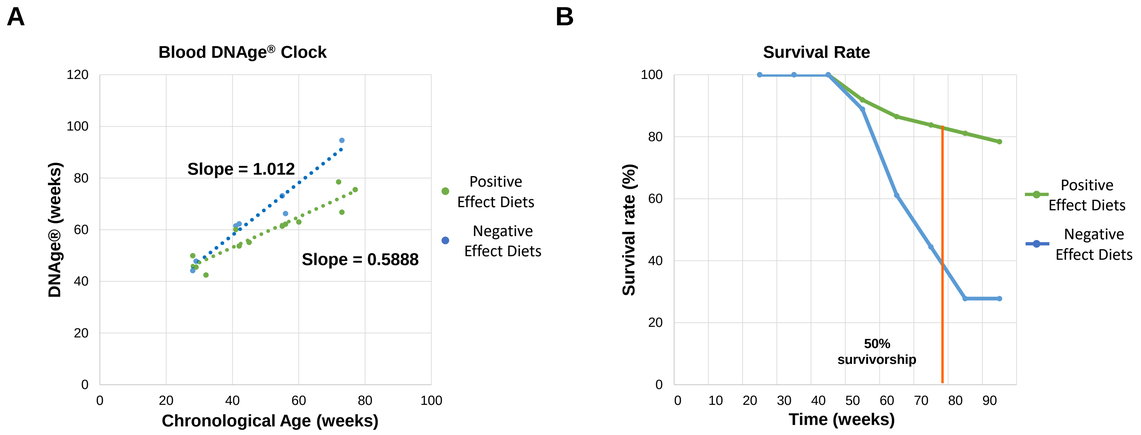


Figure 4. DNAge® analysis of Swiss Webster mice treated with different antiaging intervention therapies. (A) Differences in blood DNAge® progression between mice treated with beneficial (Positive Effect) and detrimental (Negative Effect) diets (n=124 and n=46 respectively) (B) Survival rate of the mice (n=37) fed with beneficial (Positive Effect) diets in comparison with mice (n=18) treated with detrimental (Negative Effect) diets. The red bar indicates the typical 50% survivorship of Swiss Webster (78 weeks).

Conclusion

- The mouse DNAge® test is based on our newly developed targeted bisulfite sequencing platform – SWARM®, which is a flexible, low cost technology that requires relatively low DNA starting material.
- The mouse DNAge® test is an invaluable tool for age quantification and monitoring, and can be apply to study the aging processes in different tissues.
- Remarkably, mouse DNAge® strongly correlates (and can predict) with life expectancy in different mouse strains, and in mice treated with antiaging diets.