

Mouse DNAge[®] Service Demo Report: Epigenetic Aging Clock

Workflow Checklist

Sample Received	✓
Sample Quality Check	✓
Sample Prepared for Sequencing	✓
Next-Gen Sequencing	✓
Sequence Quality Check	✓
Bioinformatics Analysis	✓
Data/Results	✓

Background

Recent publications reported reliable epigenetic age predictors including the Horvath clock – the most accurate molecular measurement of aging available – obtained by using linear regression of chronological age and DNA methylation at multiple CpG sites [1-5]. Those studies demonstrated that epigenetic age tightly correlates with chronological (or birth) age, especially in humans. More recently, scientists demonstrated that an epigenetic age clock can be built for the mouse model organism as well [6]. The current investigation utilized blood DNA samples to determine their DNA methylation age (DNAge®).

Materials & Methods

Sample preparation: 27 blood samples were collected from mice and stabilized for downstream processing using the DNA/RNA Shield™ reagent from Zymo Research Corp. (Zymo) (www.zymoresearch.com – Cat. No. R1150). Sample DNA was purified from blood using the Quick-DNA™ Miniprep Plus kit (Cat. No. D4068). Quantity and quality control checks were performed and summarized in a separate Excel file accompanying the data delivery email. Bisulfite conversion was performed using the EZ DNA Methylation-Lightning™ Kit (Cat. No. D5030) according to the standard protocol. Samples were then enriched for sequencing of >500 age-associated gene loci on an illumina® HiSeq instrument.

Sequence Alignments & Data Analysis: Sequence reads were identified using Illumina basecalling software and aligned to the reference genome using Bismark, an aligner optimized for bisulfite sequence data and methylation calling. The methylation level of each sampled cytosine was estimated as the number of reads reporting a C, divided by the total number of reads reporting a C or T. Calculated DNA methylation values obtained from the sequence data were used to assess DNAge® according to Zymo's proprietary DNAge® predictor.

Sample Summary

Table 1. Blood Sample Summary

Sample ID	Original Sample ID	Sample Type	Strain	Gender
ZR9999_1	A1	Blood	C57BL/6	Male
ZR9999_2	A2	Blood	C57BL/6	Male
ZR9999_3	A3	Blood	C57BL/6	Male
ZR9999_4	A4	Blood	C57BL/6	Male
ZR9999_5	A5	Blood	C57BL/6	Male
ZR9999_6	A6	Blood	C57BL/6	Male
ZR9999_7	A7	Blood	C57BL/6	Male
ZR9999_8	A8	Blood	C57BL/6	Male
ZR9999_9	A9	Blood	C57BL/6	Male
ZR9999_10	A10	Blood	C57BL/6	Male
ZR9999_11	A11	Blood	C57BL/6	Male
ZR9999_12	A12	Blood	C57BL/6	Male
ZR9999_13	A13	Blood	C57BL/6	Male
ZR9999_14	B1	Blood	SJL	Male
ZR9999_15	B2	Blood	SJL	Male
ZR9999_16	C1	Blood	AKR/J	Male
ZR9999_17	C2	Blood	AKR/J	Male
ZR9999_18	C3	Blood	AKR/J	Male
ZR9999_19	D1	Blood	129S6	Male
ZR9999_20	D2	Blood	129S6	Male
ZR9999_21	D3	Blood	129S6	Male
ZR9999_22	D4	Blood	129S6	Male
ZR9999_23	D5	Blood	129S6	Male
ZR9999_24	D6	Blood	129S6	Male
ZR9999_25	E1	Blood	ICR (CD-1)	Male
ZR9999_26	E2	Blood	ICR (CD-1)	Male
ZR9999_27	E3	Blood	ICR (CD-1)	Male

Sample Summary

Table 2. Genomic DNA Yield

Sample ID	Received Date	Processed Date	Processed Volume (µl)	Total DNA Recovery (ng)	260/280 Ratio	260/230 Ratio
ZR9999_1	1/1/2017	1/3/2017	400	3,922.2	1.86	2.70
ZR9999_2	1/1/2017	1/3/2017	400	3,481.3	1.86	2.73
ZR9999_3	1/1/2017	1/3/2017	400	4,728.0	1.87	2.64
ZR9999_4	1/1/2017	1/3/2017	400	4,896.0	1.86	2.62
ZR9999_5	1/1/2017	1/3/2017	400	3,034.4	1.84	2.61
ZR9999_6	1/1/2017	1/3/2017	400	1,359.0	1.87	2.16
ZR9999_7	1/1/2017	1/3/2017	400	1,844.6	1.89	2.54
ZR9999_8	1/1/2017	1/3/2017	400	705.9	2.01	2.28
ZR9999_9	1/1/2017	1/3/2017	400	754.5	2.01	2.31
ZR9999_10	1/1/2017	1/3/2017	400	489.9	2.00	2.01
ZR9999_11	1/1/2017	1/3/2017	400	535.1	2.05	2.23
ZR9999_12	1/1/2017	1/3/2017	400	1,511.6	1.96	2.11
ZR9999_13	1/1/2017	1/3/2017	400	784.1	1.89	2.47
ZR9999_14	1/1/2017	1/3/2017	400	1,531.7	1.97	2.27
ZR9999_15	1/1/2017	1/3/2017	400	1,187.3	1.92	2.30
ZR9999_16	1/1/2017	1/3/2017	400	1,850.0	1.83	2.37
ZR9999_17	1/1/2017	1/3/2017	400	663.9	1.82	2.14
ZR9999_18	1/1/2017	1/3/2017	400	165.0	1.77	1.69
ZR9999_19	1/1/2017	1/3/2017	200	248.4	1.82	1.74
ZR9999_20	1/1/2017	1/3/2017	200	516.5	1.97	1.72
ZR9999_21	1/1/2017	1/3/2017	200	576.3	2.03	1.81
ZR9999_22	1/1/2017	1/3/2017	200	598.8	1.93	1.92
ZR9999_23	1/1/2017	1/3/2017	200	1,055.4	1.86	2.25
ZR9999_24	1/1/2017	1/3/2017	200	578.3	1.81	2.17
ZR9999_25	1/1/2017	1/3/2017	400	200.0	1.70	1.69
ZR9999_26	1/1/2017	1/3/2017	400	1,137.2	1.81	2.70
ZR9999_27	1/1/2017	1/3/2017	400	961.4	1.87	2.55

Results

The DNAge® of 27 blood samples estimated by the DNAge® predictor following the procedure described in the Methods are as shown in Table 3.

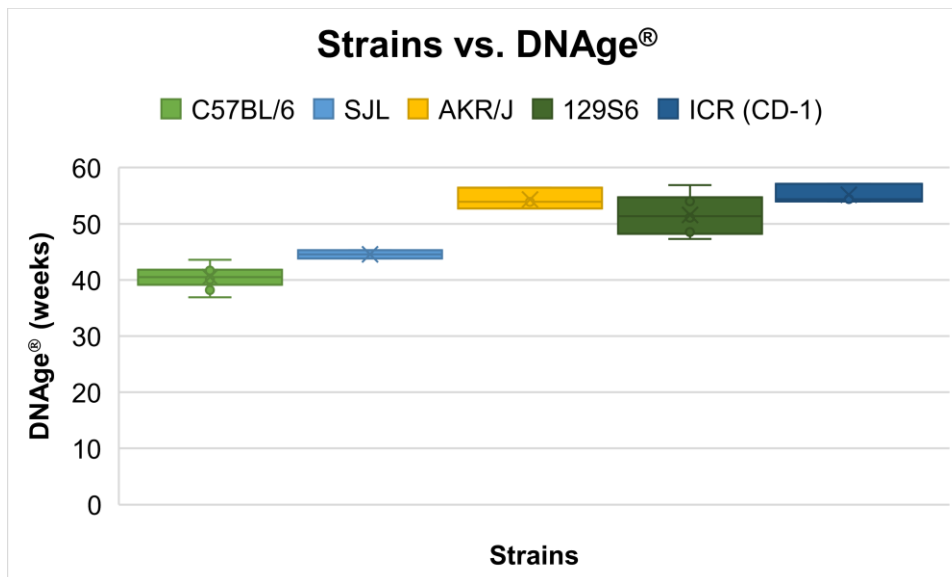
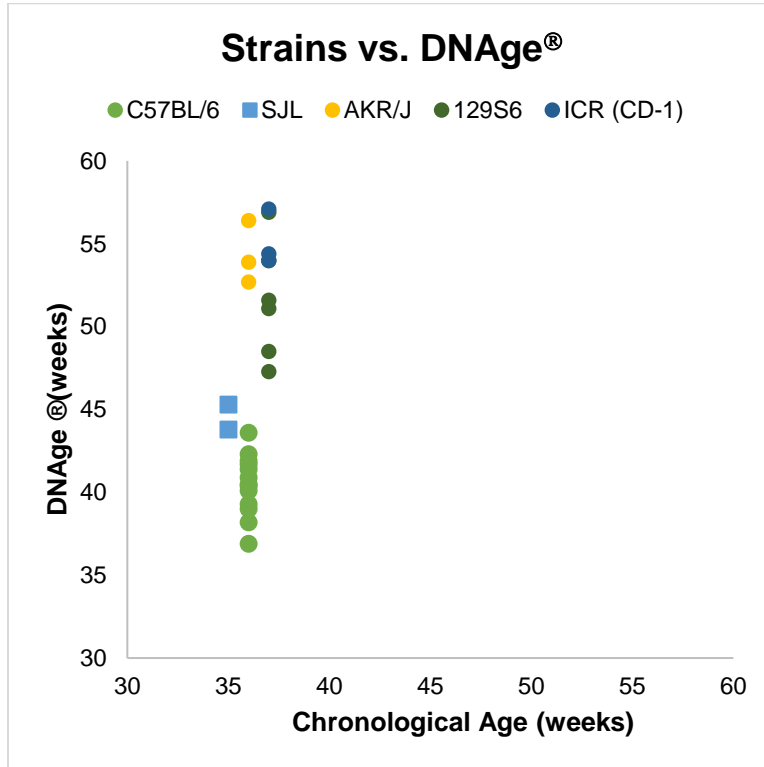
Table 3. The Estimated DNAge® *

Sample ID	Original Sample ID	Strain	Chronol. Age (weeks)	DNAge® (weeks)	ΔAge (weeks)
ZR9999_1	A1	C57BL/6	36	40.1	4.1
ZR9999_2	A2	C57BL/6	36	42.3	6.3
ZR9999_3	A3	C57BL/6	36	40.9	4.9
ZR9999_4	A4	C57BL/6	36	39.3	3.3
ZR9999_5	A5	C57BL/6	36	39.0	3.0
ZR9999_6	A6	C57BL/6	36	36.9	0.9
ZR9999_7	A7	C57BL/6	36	38.2	2.2
ZR9999_8	A8	C57BL/6	36	43.6	7.6
ZR9999_9	A9	C57BL/6	36	41.7	5.7
ZR9999_10	A10	C57BL/6	36	40.4	4.4
ZR9999_11	A11	C57BL/6	36	40.5	4.5
ZR9999_12	A12	C57BL/6	36	41.4	5.4
ZR9999_13	A13	C57BL/6	36	41.9	5.9
ZR9999_14	B1	SJL	35	43.8	8.8
ZR9999_15	B2	SJL	35	45.3	10.3
ZR9999_16	C1	AKR/J	36	53.9	17.9
ZR9999_17	C2	AKR/J	36	52.7	16.7
ZR9999_18	C3	AKR/J	36	56.4	20.4
ZR9999_19	D1	129S6	37	51.1	14.1
ZR9999_20	D2	129S6	37	54.0	17.0
ZR9999_21	D3	129S6	37	56.9	19.9
ZR9999_22	D4	129S6	37	51.6	14.6
ZR9999_23	D5	129S6	37	48.5	11.5
ZR9999_24	D6	129S6	37	47.3	10.3
ZR9999_25	E1	ICR (CD-1)	37	57.1	20.1
ZR9999_26	E2	ICR (CD-1)	37	54.4	17.4
ZR9999_27	E3	ICR (CD-1)	37	54.0	17.0

* Please note that the data presented here is for research use only.

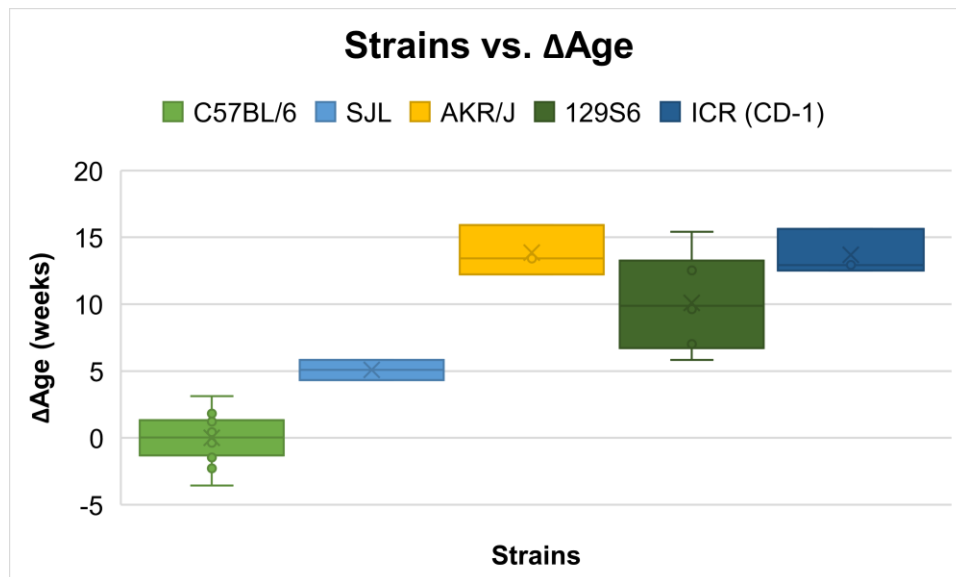
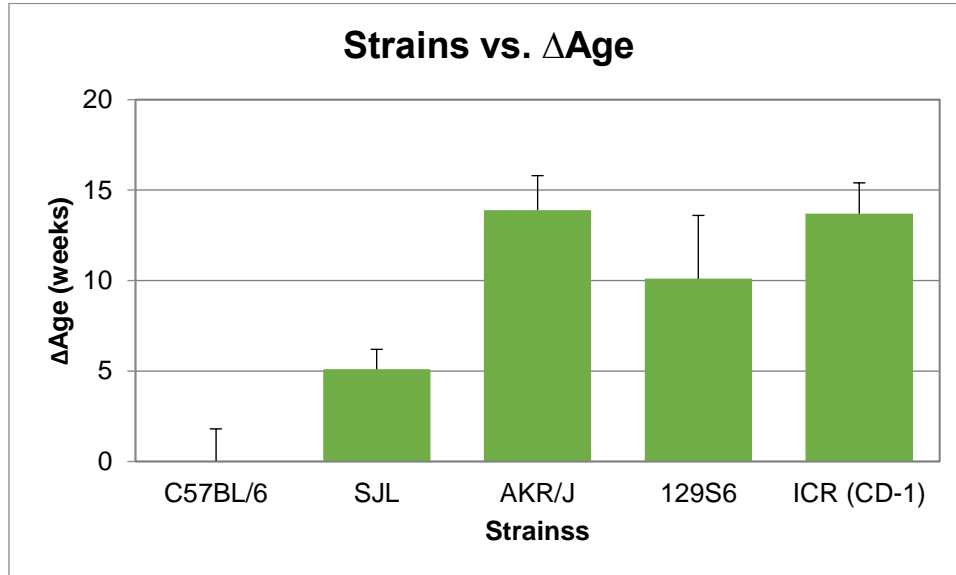
Results

Figure 1. The Estimated DNAge®* for blood samples collected from different strains of mice.



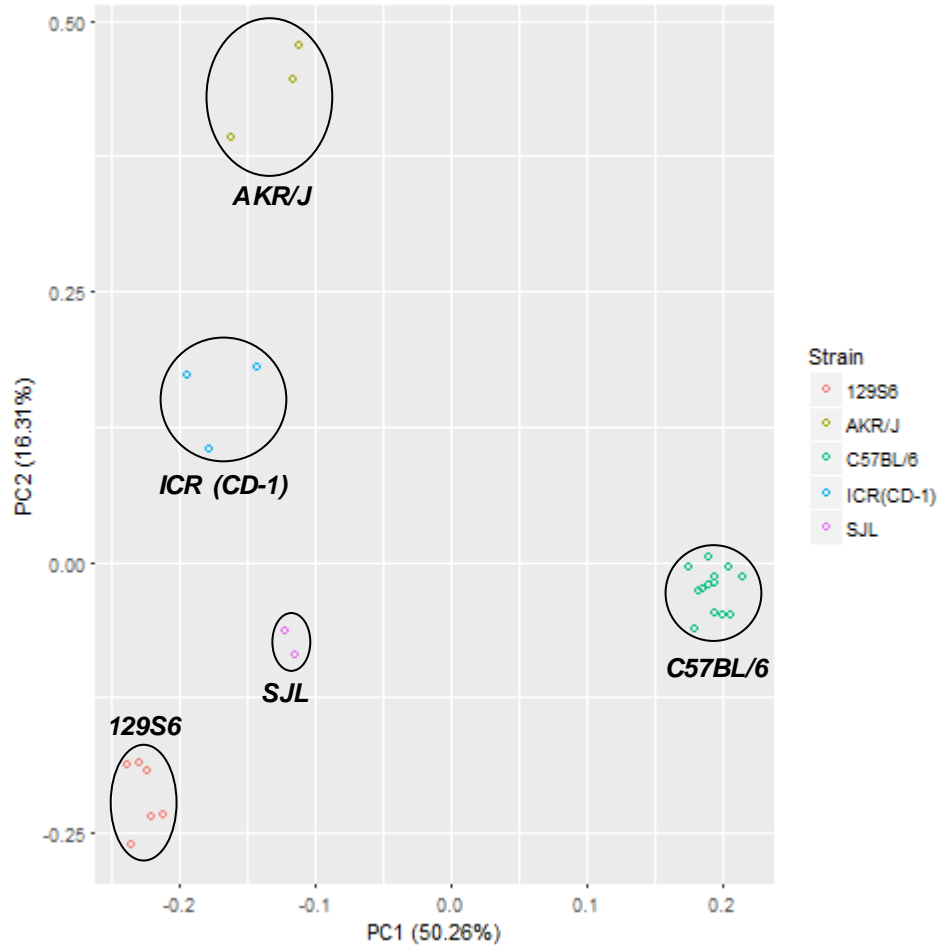
Results

Figure 2. The Δ Age (different between DNAge^{®*} and chronological age) in different mice strains in comparison with C567BL/6.



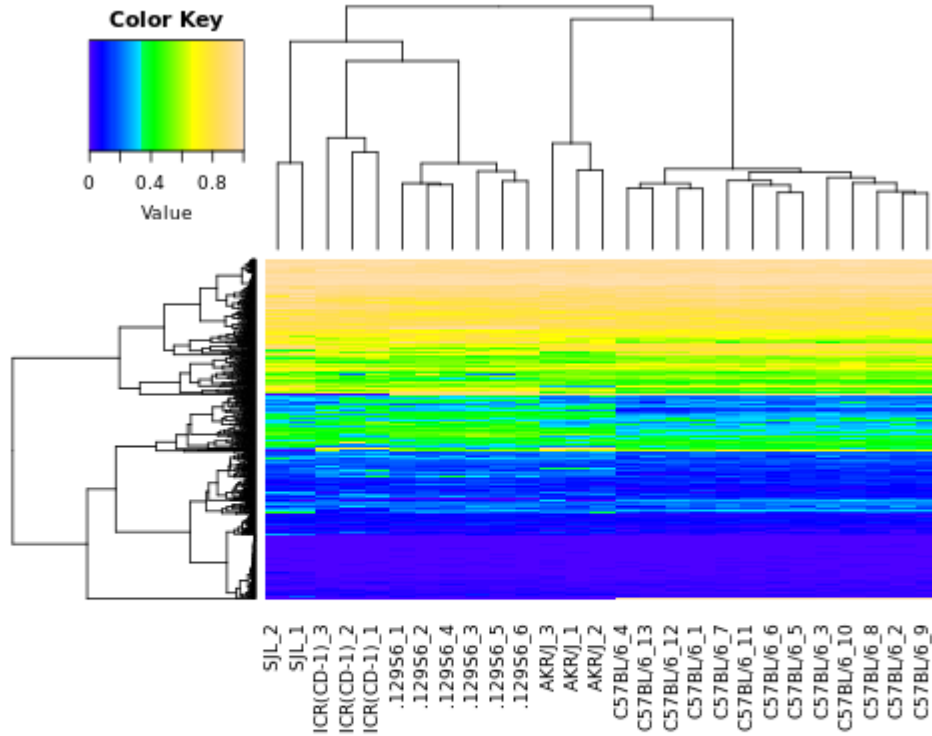
Results

Figure 3. Clustering analysis of DNA methylation value from xxx age-associated loci in 27 mouse blood samples using principal component analysis.



Results

Figure 4. Two-dimensional hierarchical clustering of the top xxx differentially methylated loci in 5 different mouse strains (p-value <0.05).



Reference

- [1] Horvath S. DNA methylation age of human tissues and cell types. *Genome Biology*. 2013;14(10): R115. doi:10.1186/gb-2013-14-10-r115.
- [2] Gibbs WW. Biomarkers and ageing: The clock-watcher. *Nature*. 2014 Apr 10;508(7495):168-70. doi: 10.1038/508168a.
- [3] Jones MJ, Goodman SJ, Kobor MS. DNA methylation and healthy human aging. *Aging Cell*. 2015;14(6):924-932. doi:10.1111/acer.12349.
- [4] Jylhävä J, Pedersen, NL, Hägg S. Biological Age Predictors. *EBioMedicine*. 2017 Jul;21:29-36. doi: 10.1016/j.ebiom.2017.03.046.
- [5] Marioni RE, *et al.* DNA methylation of blood predicts all-cause mortality in later life. *Genome Biology* 2015 Jan 30;16:25. doi: 10.1186/s13059-015-0584-6.
- [6] Petkovich DA, *et al.* Using DNA Methylation Profiling to Evaluate Biological Age and Longevity Interventions. *Cell Metabolism* 2017 Apr 4; 25, 954-960.

