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Human 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 have reported reliable epigenetic age predictors including the Horvath clock, obtained by using linear regression of chronological age and DNA methylation at 353 CpG sites. Differential DNA methylation has been observed between diseases such as HIV and controls and is modifiable by environmental factors such as diet, exercise, and drugs. The current project using urine DNA samples is aimed to determine the DNAge® (DNA methylation age) of these samples.

Materials & Methods

Sample Preparation: 29 urine samples were collected using the Urine Collection kit with Urine Conditioning Buffer from Zymo Research (www.zymoresearch.com – Cat. No. D3062). Sample DNA was purified from urine using the *Quick*-DNA™ Urine Kit (Cat. No. D3061). Quality control checks were performed using qPCR instruments. The results are summarized in Table 1 below. 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. Urine sample DNA methylation values were obtained from the sequence data and used to assess DNA age according to Zymo Research's proprietary myDNAge™ predictor.

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 sequencing 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 sequencing data were used to assess DNAge® according to Zymo's proprietary DNAge® predictor.

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Sample Summary

Table 1. Urine Sample Summary

Sample ID	Sample Type	Gender	Received Date	Processed Date	Processed Volume (mL)	qPCR Conc. (ng/µL)	Total (ng)
BCa1	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa2	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa3	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa4	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa5	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa6	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa7	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa8	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa9	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa10	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa11	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa12	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
BCa13	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl1	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl2	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl3	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl4	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl5	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl6	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl7	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl8	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl9	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl10	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl11	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl12	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl13	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl14	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl15	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x
Ctrl16	Urine	Male	xx/xx/20xx	xx/xx/20xx	xxx	xx.xx	xxx.x

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Results

The DNAge® estimates of 29 urine samples estimated by the DNAge® predictor following the procedure described in the Methods are as shown in Table 2.
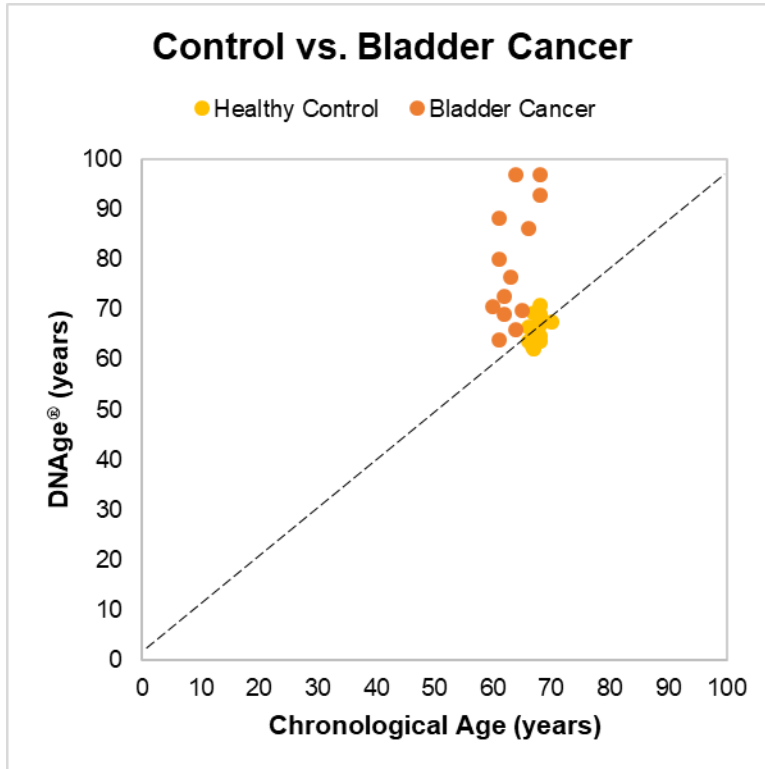
Table 2. The Estimated DNAge® *

Original Sample ID	Sample ID	Chronol. Age (years)	DNAge® (years)
BCa1	ZR9999_1	60	xx.x
BCa2	ZR9999_2	61	xx.x
BCa3	ZR9999_3	61	xx.x
BCa4	ZR9999_4	61	xx.x
BCa5	ZR9999_5	62	xx.x
BCa6	ZR9999_6	62	xx.x
BCa7	ZR9999_7	63	xx.x
BCa8	ZR9999_8	64	xx.x
BCa9	ZR9999_9	64	xx.x
BCa10	ZR9999_10	65	xx.x
BCa11	ZR9999_11	66	xx.x
BCa12	ZR9999_12	68	xx.x
BCa13	ZR9999_13	68	xx.x
Ctrl1	ZR9999_14	67	xx.x
Ctrl2	ZR9999_15	68	xx.x
Ctrl3	ZR9999_16	67	xx.x
Ctrl4	ZR9999_17	68	xx.x
Ctrl5	ZR9999_18	67	xx.x
Ctrl6	ZR9999_19	70	xx.x
Ctrl7	ZR9999_20	66	xx.x
Ctrl8	ZR9999_21	67	xx.x
Ctrl9	ZR9999_22	67	xx.x
Ctrl10	ZR9999_23	67	xx.x
Ctrl11	ZR9999_24	68	xx.x
Ctrl12	ZR9999_25	68	xx.x
Ctrl13	ZR9999_26	66	xx.x
Ctrl14	ZR9999_27	68	xx.x
Ctrl15	ZR9999_28	67	xx.x
Ctrl16	ZR9999_29	67	xx.x

* Please note that the data presented here is for research use only, not for clinical applications. Any change in exercise, diet or medication regimes should not be undertaken without first consulting a physician.
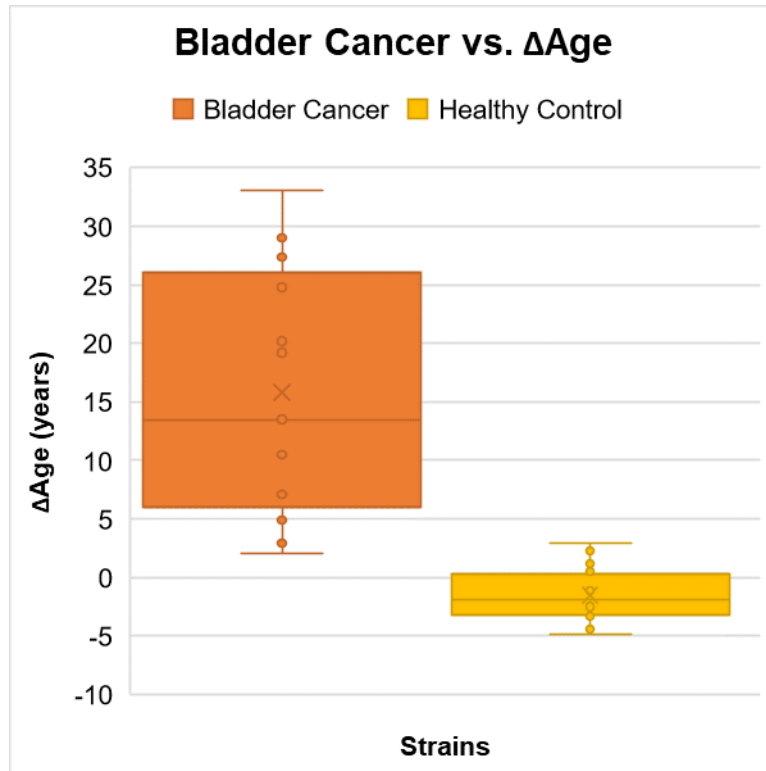
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Results

Figure 1. The Estimated DNAge®* for urine samples collected from healthy individuals and bladder cancer patients.

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Figure 2. The ΔAge (different between DNAge®* and chronological age) in bladder cancer patients in comparison with healthy individuals.

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