Application Note

Validation of the PyroMark® Q48 Autoprep compared with the PyroMark Q24 system for methylation based age estimation

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Introduction

An age prediction method has previously been established using Pyrosequencing® and the PyroMark Q24 platform (1). The method is based on determination of the methylation level of a set of markers in genomic DNA extracted from the blood of an individual. Introduction of a new version of the analytical platform, the PyroMark Q48 Autoprep System, prompted us to validate the age prediction algorithm on this new automated higher-throughput platform.

For starting material in the validation we used the same set of over 100 samples of genomic DNA obtained from men and women aged from 2 to 75 years and stored at –70°C for over one year. This set of samples was used previously to validate the PyroMark Q24 age prediction algorithm. All genomic materials were bisulfite converted. Markers located at five loci (ELOVL2 on 6p24.2, C1orf132 on 1q32.2, TRIM59 on 3q25.33, KLF14 on 7q32.3 and FHL2 on 2q12.2) were amplified by singleplex and multiplex PCR, and their methylation level was established with the Pyrosequencing method on the PyroMark Q48 Autoprep platform.

Based on the study results, using the PyroMark Q24 and PyroMark Q48 Autoprep platforms, the mean absolute deviation (MAD) of the standard error of age prediction between the two platforms was 1.7 years. This value is almost two times lower than the MAD of the age estimation error on the PyroMark Q24 platform alone. Based on these data we conclude that the age prediction algorithm developed on the PyroMark Q24 platform can be used for methylation data obtained using the PyroMark Q48 Autoprep without any modification.

Materials

Blood samples were collected in EDTA-blood tubes from volunteers who had signed informed consent statements prior to sample donation. In total approximately 120 unrelated males and females, aged between 18 and 75 years, were analyzed. Additionally, samples from children ▷

aged 2–17 years were collected, and written consents were obtained from their parents. Genomic DNA was extracted from whole blood using the phenol–chloroform method following a standard protocol or with a commercially available kit according to the manufacturer's protocol and stored at –70°C for over one and half years.

Finally, data from 91 samples were used for comparison of both platforms.

Methods

Bisulfite conversion

Un-methylated cytosines in the extracted genomic material were converted to uracils using the EpiTect® Fast 96 Bisulfite Kit following the manufacturer's instructions. PCR amplification of selected markers was performed using an AgePlex Kit,* with reactions carried out in a total volume of 25 µl or 2 x 25 µl (5-plex). Reactions contained 0.2 mM of each of the primers, 20 ng of template DNA, and the PyroMark PCR Master Mix from the QIAGEN® PyroMark PCR Kit.

Pyrosequencing

Pyrosequencing was performed using PyroMark Q48 Advanced CpG Reagents (4 x 48) on a PyroMark Q48 Autoprep instrument following the manufacturer's instructions. 10 µl of PCR product dedicated for each marker plus 25 µl of AgePlex Kit sequencing primer were downloaded on the instrument in the 48-well plate. All additional steps were carried out automatically by the instrument. Pyrograms® generated by the instrument were automatically analyzed using PyroMark Q48 Autoprep software.

Data analysis

Estimation of age of the 91 samples, which had the methylation level of their markers determined using PyroMark Q48 Autoprep platform and chemistry, was done based on the linear regression model developed on the PyroMark Q24 platform. The age estimation error for the same set of 91 samples was assessed on the PyroMark Q48 Autoprep and PyroMark Q24 platforms using the mean absolute deviation (MAD) of the age estimation error. In addition, the correlation coefficient for age estimations data obtained on the PyroMark Q24 and PyroMark Q48 Autoprep platforms was established.

Results

The difference in the age estimation of the 91-sample set using the PyroMark Q24 and the PyroMark Q48 Autoprep platforms and chemistries is shown in Figure 1. Over 95% of age estimation differences were in the ±6 years range. The MAD of the age estimation difference was 1.7 years, which is over two times lower than the overall method accuracy of 3.9 years, as established on the PyroMark Q24 platform.

* AgePlex Kits, including PCR primers and sequencing primers, can be obtained from BioVectis. See **www.biovectis.com/forensic/biological-age-prediction** for more information.

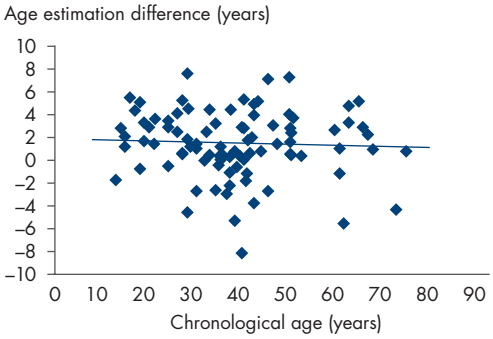

Figure 1. The MAD of age estimation difference using the PyroMark Q24 and the PyroMark Q48 Autoprep platforms is 1.7 years. The difference in age estimation for the set of 91 samples representing the 15 to 75 year old subjects was plotted as the function of the chronological age. Over 95% of age estimation differences were in the ±6 years range. The MAD of the age estimation difference was 1.7 years, which is over two times lower than the overall method accuracy of 3.9 years, as established on the PyroMark Q24 platform.

The age estimations of this set of 91 samples, analyzed over a 1.5 year time span using the two different Pyrosequencing platforms (PyroMark Q24 and PyroMark Q48 Autoprep), shows a very high correlation coefficient of 0.987 (Figure 2). The correlation coefficient of the error of the age estimation (0.991) by these two platforms was even higher than the estimated correlation coefficient (0.987).

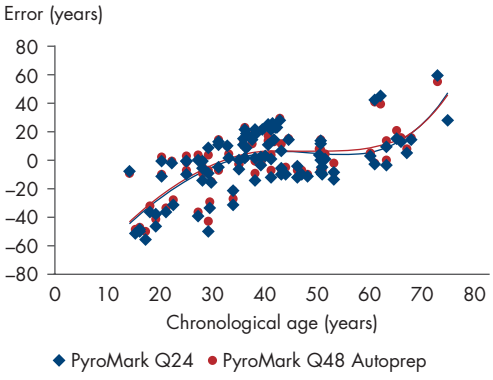

Figure 2. High correlation of chronological age to estimated age and correlation between the two PyroMark platforms used. The correlation coefficient of age estimated using the PyroMark Q24 and PyroMark Q48 Autoprep platforms is 0.987 whereas the correlation coefficient of the error of the age estimation by these two platforms is 0.991.

Conclusion

Use of the PyroMark Q48 Autoprep platform and chemistry for DNA methylation determination at five markers facilitated the human chronological age prediction. Using the algorithm developed on the PyroMark Q24 platform and chemistry, analysis on the PyroMark Q48 Autoprep system showed an accuracy of ±1.7 years. This error range of the age estimation is over two times lower them the overall method accuracy of ±3.9 years established on the PyroMark Q24 platform.

In addition to the different chemistry and instrument used for the Pyrosequencing based methylation level determination, two other factors could influence the overall age estimation error between the two platforms:

- storage of the samples (genomic DNA) at –70°C for over 1.5 years between analyses
- different human operators performing the analytical procedures

The high correlation in the age estimation obtained by those two platforms (0.987), and more importantly, the even higher correlation of errors of age estimation by those two platforms for the same set of samples (0.991) confirms the consistency of data from the two platforms.

We conclude that the PyroMark Q48 Autoprep instrument and chemistry can be used for methylation level based age estimation using the algorithm developed on the PyroMark Q24.

Reference

1. Zbiec´-Piekarska Renata et al. (2016) Use of Methylation Markers for Age Estimation of an Unknown Individual Based on Biological Traces, QIAGEN Application Note.

Ordering Information

Product	Contents	Cat. no.
PyroMark Q48 Autoprep System	PyroMark Q48 Instrument, multistep pipet, software, documentation and installation	9002470
PyroMark Q48 Advanced CpG Reagents (4 x 48)	Reagents for 4 x 48 PyroMark Q48 Autoprep CpG and long-read reactions: PyroMark Advanced Enzyme Mix, PyroMark Advanced Substrate Mix, Denaturation Solution, Annealing Buffer, Binding Buffer, Nucleotides	974022
EpiTect Fast 96 Bisulfite Kit	2 x EpiTect 96-well Plates, Bisulfite Solution, DNA Protect Buffer, Carrier RNA, Buffers	59720
PyroMark PCR Kit (200)	For 200 reactions: 2x PyroMark PCR Master Mix (includes HotStarTaq® DNA Polymerase and optimized PyroMark Reaction Buffer containing 3 mM $MgCl_2$ and dNTPs), 10x CoralLoad® Concentrate, 5x Q-Solution®, 25 mM $MgCl_2$, and RNase-Free Water	978703

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