Rachel Graziano
May 2020
Forensic Science and Biology Pre-Medical

DNA Methylation Patterns to Determine Donor Age

Mentor: Dr. Claire L. Glynn
Forensic Science Department

Within the forensic science community, there is a challenge that scientists have long sought to address that could prove useful in various cases. The ability to predict the chronological age of the donor of a biological sample could prove useful for investigative leads. Having this detail could, for example, help to determine the age of a minor working in a forced labor situation, or narrow down a suspect list in a homicide. Various methods have recently come forth as possible options for predicting the age. These include signal-joint T-cell receptor excision circle levels within cells, telomere length, and methylation pattern analysis (Ou et.al, Hong et.al). The aim of this research was to focus on methylation patterns for predicting donor age using quantitative polymerase chain reaction (qPCR) based methods. Based on its methodology, ease of use and cost-effectiveness this approach looked to be the most promising for forensic application. In the beginning of the project, three gene targets were going to be examined, and compared to a control gene, however, the focus shifted to one gene, FHL2, that would be compared to a control gene, EF1a.

After obtaining Institutional Review Board (IRB) approval, four buccal swabs each were collected from fourteen different donors, aged 18-85 using sterile cotton swabs, with written informed consent. The samples were stored at -20°C until extraction. The QIAmp DNA Investigator Extraction Kit (Qiagen®) was utilized to extract DNA from three of the swabs from each donor. Next, the DNA was quantified for each sample using the Nanodrop One^{C} UV-vis Spectrophotometer and the Qubit 3.0 Fluorometer. Following this, the EpiTect Bisulfite Kit (Qiagen®) was utilized to bisulfite convert the DNA samples. This a vital step when using qPCR as the methylation analysis tool. The bisulfite treatment converts unmethylated cytosines into uracils, allowing for methylation percentage detection. The bisulfite converted samples were amplified using the EpiTect Methylight PCR + Rox Vial Kit. Using this kit, and the specific primers and probes necessary for the gene targets, a cycle threshold (Ct) value was collected for both unmethylated and methylated regions of both target genes, which could then be used to determine the level of methylation present.

From each of the buccal swab samples, there was quantifiable DNA present. However, the results from the methylation analysis on the qPCR instrument were inconclusive. The samples were run in technical duplicates for both genes, for both the unmethylated and methylated primer/probes. Only five samples produced results in the duplicates, however the Ct values obtained within each duplicate varied considerably, and therefore showed inconsistency in the measurements. Due to the vast amount of undetected results, and the inter sample variation, it was determined that this methodology is not suitable for methylation analysis. It is suggested that the bisulfite conversion damages the samples to such an extent that reproducible results are not obtainable. Future research should continue to look into other methods, including pyrosequencing, and Sanger sequencing. If a suitable method can be developed then it would be important to look into genes that serve as good targets for determining age with methylation patterns.

Citations:

1.) Ou, X., Zhao, H., Sun, H., Wu, X., Xie, B., Shi, Y., & Wu, X. (n.d.)._Detection_and_quantification_of_the_age-related_sjTREC_decline_in_human_peripheral_blood&p=DevEx.LB.1,5069.1November 2010.International Journal of Legal Medicine 125(4):603-8
2.) Hong, Sae Rom, et al. "DNA Methylation-Based Age Prediction from Saliva: High Age Predictability by Combination of 7 CpG Markers." *Forensic Science International: Genetics*, vol. 29, 2017, pp. 118–125., doi:10.1016/j.fsigen.2017.04.006.
3.) Vidaki, Athina, and Manfred Kayser. "Recent Progress, Methods and Perspectives in Forensic Epigenetics." *Forensic Science International: Genetics*, vol. 37, 2018, pp. 180–195., doi:10.1016/j.fsigen.2018.08.008.