DNA Methylation Patterns for Donor Age Estimation

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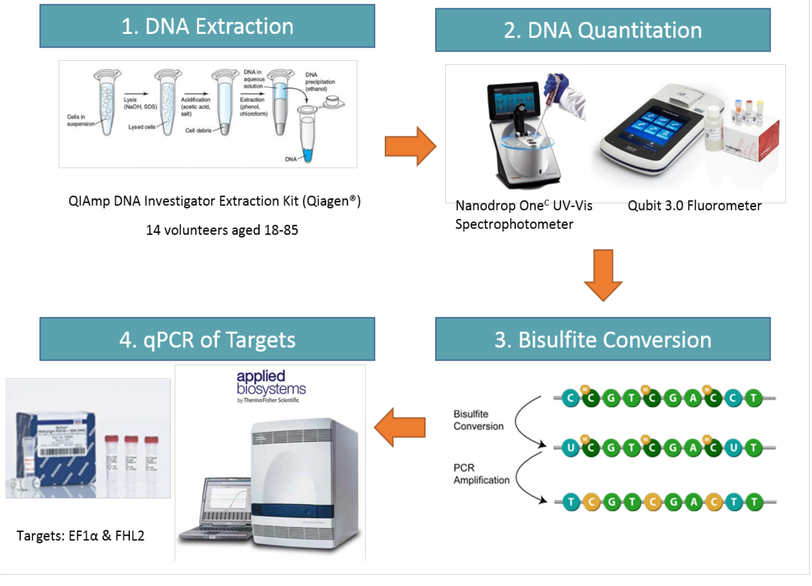
Background

Being able to determine the age of a person through a DNA sample is a feat that current DNA profiling is not able to accomplish. Recent studies have shown that DNA methylation patterns can be used to predict the chronological age of the donor of the sample. Several genes have shown promise in their ability to be related to age, including ELOVL2, KLF14, and FHL2. There have not been many studies that have used the method involing qPCR analysis with bisulfite conversion, as bisulfite conversion is known to be damaging to DNA samples. Therefore, it is important to test the validity of this method. Advancements in this venture will prove useful in human trafficking, sex trafficking and wide suspect list cases.

Research Aims

1) Determine the validity of using the EpiTect Methylight PCR + Rox Vial Kit (Qiagen®) for methylation analysis.
2) Validation, using RT-qPCR, of target CpG sites within the FHL2 gene as being useful in methylation analysis for age determination.

Methodology



Results

Ct (cycle threshold) values are the results given by the instrument and represent the expression (methylated or unmethylated) levels of that target.

	Average Ct Values			
Sample	FHL2Unmeth	FHL2Meth.	EF1AUnmeth.	EFIAMeth.
1B	20.08*	10.29	undetected	16.31*
2A	17.79*	24.32*	6.44	15.67
3A	26.07*	undetected	undetected	undetected
7A	15.49	17.71	21.51	undetected
8A	undetected	undetected	undetected	undetected
12B	undetected	undetected	undetected	undetected
13A	undetected	undetected	undetected	undetected
14A	undetected	undetected	undetected	undetected
BLANK	29.39	undetected	undetected	undetected

Table 1 : Methylation Results

- (*) Denotes that this is the average of a doublet set

Sample	Age
1B	18
2A	85
3A	76

Table 2 : Age List

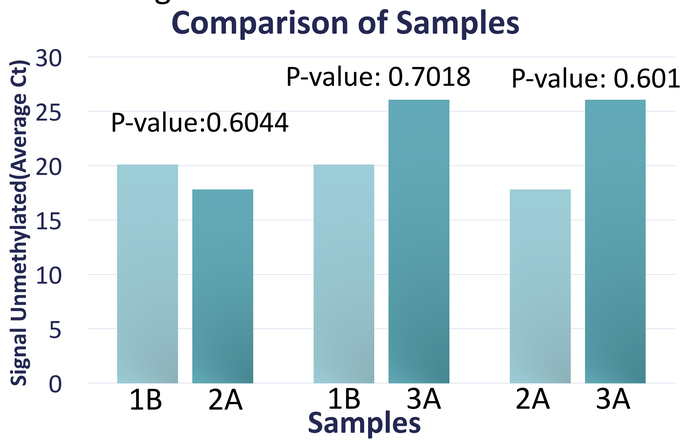


Table 3: Sample Comparison Significance

Average Ct values for data obtained from FHL2 unmethylated collections. ($p<0.05$ is significant)

Discussion

- All buccal swab collections yielded quantifiable DNA.
- Methylation status was unable to be detected in the target gene for all but 3 sample doublets, and only in the unmethylated state.
- In the 3 doublets with measurements, there is variability between them, which shows inconsistency in measurement.
- When compared to the other samples that had complete methylation analysis in both pairs for unmethylated signal, none showed significant differences compared to one another
- The EF1α gene targets did not produce results in all but 2 samples in both the methylated and unmethylated regions, which shows it may not be a usable target for an endogenous control.
- Results with the FHL2 gene target showed that this target is not a suitable candidate for age determination through methylation analysis.

Conclusion

Based on the vast amount of undetected results, this research highlights the fact that this method may not be suitable. PIRO sequencing may be a better option to eliminate the damaging bisulfite step which could have caused the varied results. Further studies should be done to explore further viable methods for methylation analysis, as well as age determination techniques. If viable methylation techniques are tested then further research should be done for suitable gene targets.

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