2018

Examination of Methylation Sites for Forensic Age Determination from Semen

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Introduction

- Age determination is critical to forensic sexual assault investigations; adequate age estimation would assist investigators with a correct identification
- Age-related changes in cytosine methylation (C → 5mC) at certain loci have been reported from blood and saliva in several studies
- Common methylation analysis involves BeadChip assays and pyrosequencing. Few forensic laboratories are equipped with these instruments and high costs prevents routine usage
- Methylation-sensitive high resolution melting (MS-HRM) measures methylation status easily and cost effectively, using bisulfite-treated and PCR amplified DNA
- Previous MS-HRM study has shown methylation at the ELOVL2 and FHL2 CpG islands in blood samples directly relate to age

ELOVL2

CGATTTGCAGGTCCAGC**CG**G**CG**C**CG**GTTT**CGCGCG**G**CG**GCTCAA**CG**GAGCCCCAG
GAATACCCACC**CG**CTGCCCAGATCGGCAGC

FHL2

TTTACCAAAACTCCTTTCTT**CG**TGCCCTC**CG**GGTCTTGGGAGCACAGTAGTTAT**CG**GG
AG**CG**CCTC**CG**G**CG**TGGGCTCT**CG**GG**CGCG**AGTTT**CG**GA**CG**AGGCCTGGG**CGCG**G
TGGCAGGGGTCTGCCCAC

- In bisulfite treatment, unmethylated cytosines convert to uracil, while methylated cytosines remain unaffected
- Bisulfite conversion and overall methylation status quantifiably modify the thermodynamic stability of the DNA sequence

Methods

Extraction

- Semen samples from 7 individuals were extracted using QIAamp DNA Investigator Kit

Bisulfite Conversion

- Bisulfite conversion of extracted DNA was conducted using EpiTect Bisulfite Kits

PCR amplification and High Resolution Melt Analysis

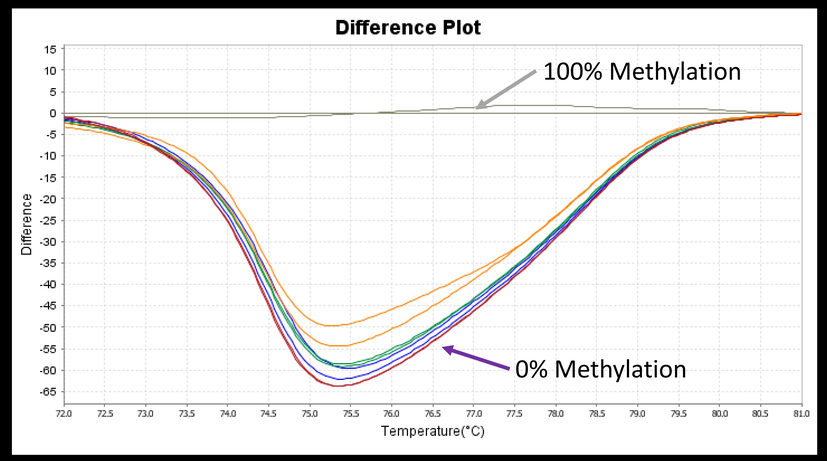
- Amplification was conducted using QuantStudio 6 Flex Real-Time PCR System
- DNA standards of known methylation values were co-analyzed: 0, 25%, 50%, 75%, 100%
- Melt Curve analysis was conducted using EpiTect HRM PCR Kit

Standard Curve Modeling and Methylation Prediction

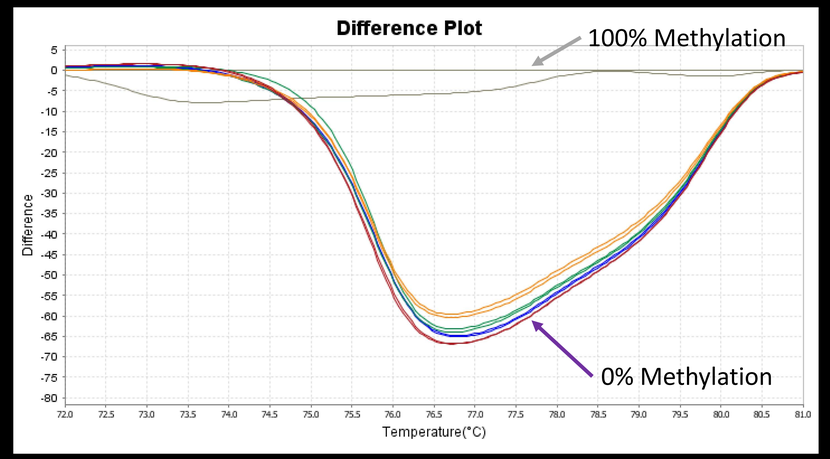
- Df value was assigned to absolute minimum value on difference plot with 100% methylation standard set as baseline
- Df values of DNA standards were used to generate reference standard curve

Results

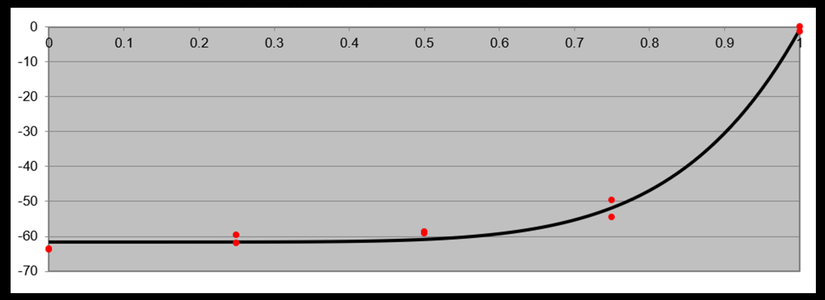
ELOVL2 Standards Melt Curve



FHL2 Standards Melt Curve



ELOVL2 Reference Standard Curve



Non-linear Regression with Df Values as a Function of Percent Methylation

$$y = d + \frac{a-d}{1+\left(\frac{x}{c}\right)^{b}}$$

Coefficient	ELOVL2	FHL2
a	2.95347	3.22090
b	6.56962	9.55867
c	6.51756	3.24281
d	13495287.1	4656593.3
x	% Methylation	% Methylation
y	Df Value	Df Value

Relationship Between Age and Methylation Status at Each Loci

Sample	AGE	METHYLATION ELOVL2	METHYLATION FHL2
5007	20	0.5329	UNKOWN
2271	25	UNKOWN	UNKOWN
5002	26	0.8208	0.8396
1025	32	UNKOWN	UNKOWN
3514	33	0.4904	UNKOWN
6034	33	0.5940	UNKOWN
7018	54	0.8970	0.8264

- Methylation values calculated as negative percentages using the standard curve regression were categorized as “Unknown”
- Minimal resolution for methylation values below 50% inhibited accurate quantification

Conclusions

- Results indicate that no correlation may exist between age and methylation status at these loci in semen
- Small sample size prohibits extrapolation to population

Potential Future direction

- Expand analysis of ELOVL2 and FHL2 loci to other forensically relevant body fluids; evaluate potential variability in methylation differences across male and female samples
- Evaluate ELOVL2 and FHL2 loci in blood to confirm published results
- Resources permitting, conduct an exploratory Whole-Genome Sequencing analysis for semen to identify semen-specific loci related to age

Acknowledgements

- Special thanks to the VCU Undergraduate Research Opportunities Program for providing funding and support for this project