Osiris Green

Report for Sample C22-5138

Background

To gather information for making our estimate of chronological age, we analyze the promoter regions of three different genes. The promoter region of a gene lies a short distance before the gene itself along the DNA double helix, and affects the way that gene behaves. Gene promoters generally contain several small segments of DNA called CpG islands, which are places where the DNA nucleotide cytosine (abbreviated as C) falls next to the nucleotide guanine (abbreviated as G). The "p" part of the term "CpG" stands for phosphate, which is one of the chemical elements of DNA that links nucleotides together. The cytosine part of a CpG island can be chemically modified by the cell to contain what is referred to as a methyl group (a carbon atom with three hydrogen atoms attached), and the addition of methyl groups (referred to as *methylation*) to CpG islands in promoter regions generally has an effect on the behavior of the associated gene. Often, more methylation corresponds to less activity of the gene.

In humans, several gene promoters have been shown to experience consistent methylation changes with age. We use three of these promoters regions to generate your age estimate – the promoters for the EDARADD gene, the NPTX2 gene, and the TOM1L1 gene. The EDARADD and TOM1L1 promoter regions exhibit a *reduction* in methylation with age, whereas NPTX2 shows an *increase* in age-related methylation. The rates of these changes also appears to be partially gender specific, which is why we ask you to report as male or female when making our age estimate. Additionally, it is not known if EDARADD, NPTX2, and TOM1L1 play any specific role in the aging process. The EDARADD gene functions in embryonic development, and NPTX2 and TOM1L1 contribute to neuronal synapses and cell signaling, respectively.

Shown below are the patterns of CpG methylation that we determined for these genes for your sample, with CpG islands highlighted in orange. Boxes have been placed

around CpG islands containing methyl groups, with the affected cytosine highlighted in red.

C22-5138 – EDARADD
AAGAGGAAGTTTATCCTCCCACCTACAAATTCCCCAGAGAGCTTTCATCT
AGAAGGTTTGACTCTGGCCAGACAACCAGCGAGCATCTTCTCGCAATCTG
TTGCTTCTTCCATGGCAAACTCCAGAGAATTAAGAAGCCAAACTCAACAT
CGCCATGGGCCTCAGGACGACTAAACAGATGGGGAGAGGCACT

3 out of 4 CpG islands methylated (75%)

C22-5138 – NPTX2
CTTAAGAAAGGGCGCGCGGACCCGGCAGGCCAGAGTGCCGAGCAGCGCGG
TGGGTGCGGCTGTGAGACGGCAGGAGACTTCTGCCCCGCGGTGCACGCGA
CCCTCGAGACGACAGCGCGGCTACTGCCAGCAGCGAAGGCGCCTCCCGCG
GAGCGCCCCGACGGCGCCCGCTCGCCCATGCCGAGCTGAGCGCGGCAGCG
GCGCGGCGGATGCTGGCGCTGCTGGCCGCCAGCGTGGCGCTCGCCGTGGCC
GCTGGGGCCCAGGACAGCC

3 out of 40 CpG islands methylated (7.5%)

C22-5138 – TOM1L1
AACTCACTGTAGAACCTCAGTCCTCAAAAATGTACCTTCCTTTCGATGCC
GCCTGGGGAGTGGAACCAAACAGGTGAACCGCGGGAGTCAGGCATGGAGT
GTTTGGGCCTCCACGAGGAGACACCAGAAACTTCTCGGTAGGGGAAGTTA
TTCCTAAAGGCACATTCTCCAGGGCACGGTATTGTTATGCCCGTTTTACA
GATCAGAAGAGGAG

2 out of 8 CpG islands methylated (25%)

Analysis

Going by the sequences identified above, 3 out of 4 of your EDARADD CpG sites contained methylation (75%), 3 out of 40 of your NPTX2 CpG sites contained methylation (7.5%), and 2 out of 8 of your TOM1L1 CpG sites contained methylation (25%). Using just these numbers, your EDARADD promoter region would predict an *exceptionally young* age of approximately 5 years of age. Your NPTX2 promoter region would predict an age of approximately 25 – 35 years of age, and your TOM1L1 promoter region would predict an age of approximately 45 years of age. These numbers are broad in estimate, and not particularly accurate. Part of the reason for this large degree of error is due to the inherent limitations of the technical process of analysis.

To determine locations of methylation, DNA is extracted from the buccal (cheek) cell sample that was mailed in. The DNA is then treated with a chemical called bisulfite that converts non-methylated cytosines into thymines, and leave methylated cytosines unaffected. This chemical conversion then allows the presence of methylation to be identified through the presence or absence of changes to the sequence of your DNA. Bisulfite treatment, however, is not guaranteed to be complete, meaning that some locations within your DNA that appear to have methylation have a likelihood of being the result of the failed chemical conversion of cytosine to thymine (false positives). The degree of error varies from batch-to-batch of processed samples, and we attempt to measure and correct for it statistically. The proper chemical conversion of cytosine to thymine for your sample averaged 64.8%, meaning that about one-third of the non-methylated cytosines in your DNA likely failed to properly convert. This amount somewhat high, but within the realm of what can be compensated for mathematically.

Likewise, the process of determining your DNA sequence is also error-prone. Osiris Green only determined each DNA sequence once per sample in order to keep the cost of the service at a minimum. When a DNA sequence is determined, multiple random nucleotides are unidentifiable. As a result, there is the possibility that methylation exists at locations within each gene promoter that we failed to detect (false negatives). As with failed bisulfite conversion, we attempt to statistically correct for this degree of uncertainty. Shown below are the DNA sequences for your EDARADD, NPTX2, and TOM1L1 promoter regions with the unidentified nucleotides displayed in gray. Boxes have been placed around CpG islands that did not have enough associated information to show evidence for or against methylation.

C22-5138 - EDARADD

AAGAGGAAGTTTATCCTCCCACCTACAAATTCCCCAGAGAGCTTTCATCT
AGAAGGTTTGACTCTGGCCAGACAACCAGCGAGCATCTTCTCGCAATCTG
TTGCTTCTTCCATGGCAAACTCCAGAGAATTAAGAAGCCAAACTCAACAT
CGCCATGGGCCTCAGGACGACTAAACAGATGGGGAGAGGCACT

29 out of 193 nucleotides unidentified (15%)
4 out of 4 CpG islands accounted for (100%)

C22-5138 - NPTX2

CTTAAGAAAGGGCGCGCGGACCCGGCAGGCCAGAGTGCCGAGCAGCGCGG
TGGGTGCGGCTGTGAGACGGCAGGAGACTTCTGCCCCGCGGTGCACGCGA
CCCTCGAGACGACAGCGCGGCTACTGCCAGCAGCGAAGGCGCCTCCCGCG
GAGCGCCCCGACGGCGCCCGCTCGCCCATGCCGAGCTGAGCGCGGCAGCG
GCGGCGGGATGCTGGCGCTGCTGGCCGCCAGCGTGGCGCTCGCCGTGGCC
GCTGGGGCCCAGGACAGCC

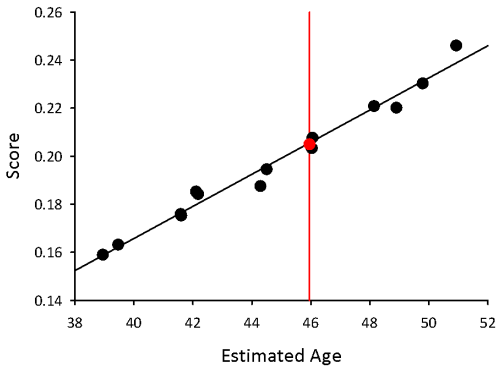
45 out of 269 nucleotides unidentified (16.7%)
34 out of 40 CpG islands accounted for (85%)

C22-5138 - TOM1L1

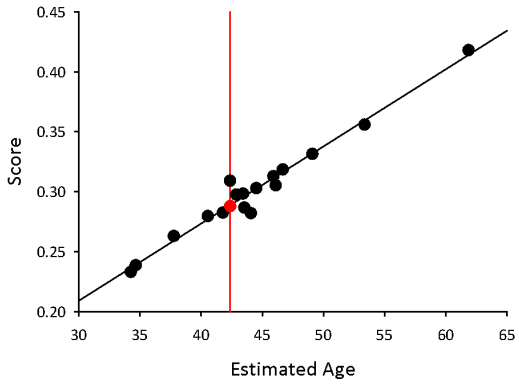
AACTCACTGTAGAACCTCAGTCCTCAAAAATGTACCTTCCTTTCGATGCC
GCCTGGGGAGTGGAACCAAACAGGTGAACCGCGGGAGTCAGGCATGGAGT
GTTTGGGCCTCCACGAGGAGACACCAGAAACTTCTCGGTAGGGGAAGTTA
TTCCTAAAGGCACATTCTCCAGGGCACGGTATTGTTATGCCCGTTTTACA
GATCAGAAGAGGAG

13 out of 214 nucleotides unidentified (6.1%)
7 out of 8 CpG islands accounted for (87.5%)

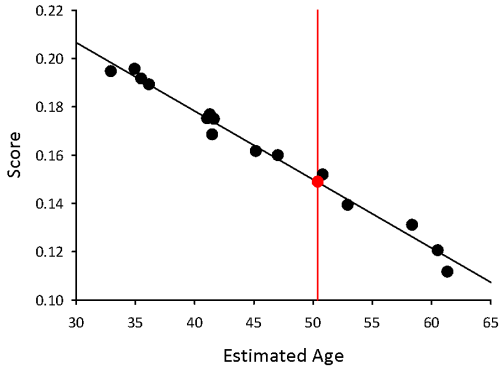
Given these sources of error, we do not make a direct comparison of the number of methylation events to age. Instead we calculate several statistical scores for each gene promoter based on our attempts to compensate for error. We then compile your age estimate as an average of those scores, weighted by how predictive they appear to be according to past data. Shown below are your most predictive scores, along with graphs demonstrating the connection between score and estimated age according to our current pool of male samples. In the graphs, your sample is displayed in red.



EDARADD
Score: 0.205
Estimated Age: 45.95



NPTX2
Score: 0.288
Estimated Age: 42.37



TOM1L1
Score: 0.149
Estimated Age: 50.38

Your overall estimated age is determined by taking a weighted average of your age estimates for the three genes (49.95 years, 42.37 years, and 50.38 years). As reflected on your Osiris Green customer account, is 47.4 years old, plus or minus 5.1 years.