Introduction

Methylation of DNA is associated with cancer and aging. Promoter associated CpG islands are especially prone to hypermethylation and deactivation during the development of cancer.

Some non reproducible data, indicating technical

Methodology

Stage 1 – Identification of suitable Polymerase Chain Reaction (PCR) conditions for primers. This was done by varying the annealing temperature against a MgCl₂ concentration in the PCR mixture and then running the samples out on a DNA agarose electrophoresis gel to see which was most effective

Stage 2 – Testing the accuracy of the primers using DNA of known methylation levels to see if the assays produce methylation data corresponding to the known values. Carried out bisulphate modification to replenish stock of methylated DNA.

Stage 3 – Testing of 28 adult ALL samples set at diagnosis and relapse by PCR amplification and pyrosequencing.

Results

By taking the mean methylation values of the Cpg islands found in the promoter regions of my chosen genes at diagnosis and relapse a clear correlation should have been apparent. This pattern would show high methylation levels upon presentation, and an increased level of methylation upon relapse, however no clear conclusions can be drawn from my results. If my data had shown evidence of this the experiment would have been extended to 150 pairs of patient samples to confirm if high levels at presentation indicate likelihood of relapse in later life.

Mainly due to time constraints and technical errors I was unable to take repeat results of my data, and therefore my data is relatively unreliable. In addition to this some of the methylation values were highly unilkely, and therefore probably incorrect. For some of my data the pyrosequencer failed the results because of low peak values, this is most likely due to a problem with the bisulphate modification stage. Due to the small amount of sample available for each patient and limited time I was unable to modify more sample and repeat the process.

Setbacks:

- Was unable to find suitable PCR conditions for Them4, new forward and reverse primers were designed to fall in a different position along the promoter sequence.
- The Wnt7a sequencing primer for the pyrosequencer also required redesigning
- Bisulphate modification may not have been effective in some samples, causing low peak values in the pyrograms and resulting in a failed pyrosequencing, see figure 4
- Unknown human errors produced a lack of product either at the PCR amplification stage or during pyrosequencing, see figure 5
- DNA contamination of the blank control sample

Conclusions

- No overall trend
- Some non reproducible data, indicating technical flaws

References:


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