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Targeted Bisulfite Pyrosequencing & Amplicon Bisulfite Sequencing Epigenetic Analysis

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Evaluation of Bisulfite Pyro Assays to Replicate DNAm Associated With Childhood Trauma

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Aims

ur objective is to optimize a targeted assay to study DNA methylation markers t selected loci recently identified as associated with childhood trauma.

Introduction

pigenetic modifications refer to changes in the DNA that do not involve the ucleotide sequence. These modifications provide phenotypic plasticity in sponse to the environment and include the closely related processes of DNA ethylation (DNAm), histone modification and chromatin remodeling[1]. In the se of DNAm, a methyl group is added to cytosine bases. Methylation is ssential for normal cell functioning and aberrant methylation has been ssociated with a wide variety of environmental factors and human diseases[2-7]. a recent methylome-wide association study several loci were identified as fferently methylated between children that had experienced childhood trauma s. children without any history of such trauma. In the current work, we present he approach for an investigation to replicate these methylation marks in an dependent sample.

Methodology

Primer Design] To target a region of interest, forward and reverse primers are rst designed using Pyromark Assay Design software **(Figure 1)**. Primer set andidates are chosen based out of a score of 100; scores are determined by otential for mispriming, likelihood for primer dimers, amplicon length etc. igher scores typically correlate to better PCR performance **(Figure 2)**.

Primer Evaluation] BiSearch, an online primer-design algorithm and search tool used to check primer sets in order to ameliorate PCR efficiency by avoiding on-specific PCR products due to genomic repetition. Another online primer-esign algorithm IDT OligoAnalyzer tool identifies oligo properties such as elting temperature, hairpins, dimers, and genomic mismatches **(Figure 3-I.)**. uccessfully designed and *in silico* evaluated primer sets are further evaluated in he lab. After amplification of bisulfite converted DNA the PCR product for each rimer pair is examined with 2% agarose gel electrophoresis to determine the pproximate amplicon length. Next, for amplicons of relevant length, Agilent oanalyzer chip-based capillary electrophoresis is performed in order to etermine if amplicons of the correct size were obtained **(Figure 4)**.

DNA] For each sample to be investigated, genomic DNA was extracted. Next, the enomic DNA was bisulfite converted using Zymo EZ DNA Methylation-Lightning ts (i.e., all unmethylated cytosines (C) were converted to uracil while ethylated cytosine bases (5-methylcytosine) persisted due to methyl steric ndrance **(Figure 3-II.)**.

Target Methylation Assay] To assess the DNA methylation status of the target cus the carefully evaluated primer set is used for amplification of the bisulfite onverted DNA for each sample. Next, the percentage of methylation (i.e. ercentage of original C that remained as C after bisulfite conversion) is assessed **igure 3, II.)** by pyrosequencing using a Pyromark Q96 sequencer (Qiagen).

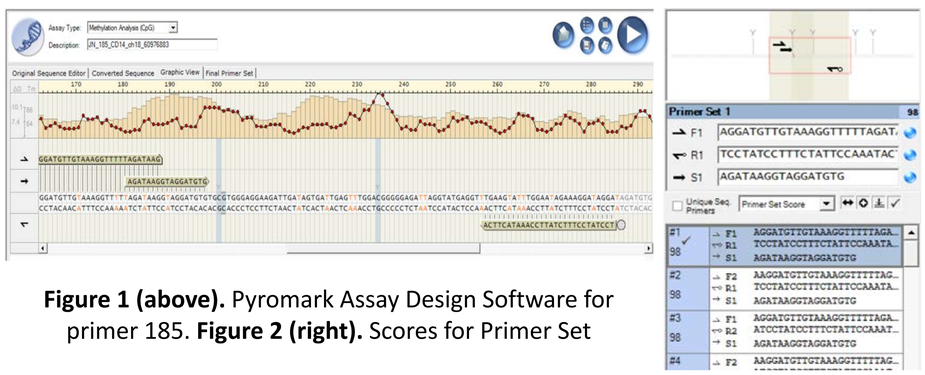

Figure 1 (above). Pyromark Assay Design Software for primer 185. **Figure 2 (right).** Scores for Primer Set

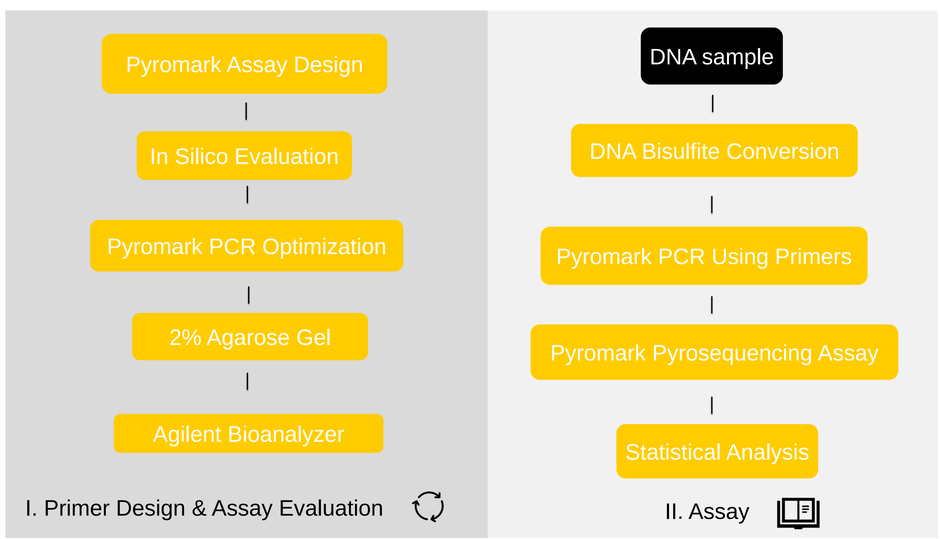

Figure 3. Steps in which loci of interests are bisulfite converted in order to determine which parts of DNA segment are methylated.

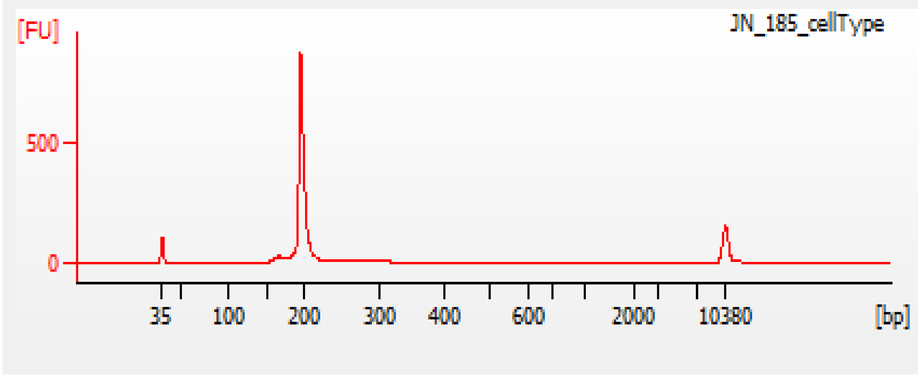

Figure 4. Primer analyses results of primer 185, by Agilent Bioanalyzer with major peak displaying DNA fragment size.

Results/Discussion

We attempted to design pyrosequencing assays for 3 loci. For each loci we created and evaluated multiple designs. Our evaluations resulted in high quality assays for two of the target loci, which were used for Pyromark Q96 sequencing analysis in 302 bisulfite converted DNA samples each.

For the third loci we were not able to design a suitable assay due to non-specific binding of the PCR primers across the genome.

Conclusion

Utilizing *in silico* and hands on lab technical primer evaluation we were able to design primer sets of high specificity to the target regions. In conclusion we have optimized and successfully evaluated 2 of 3 assays for the Pyromark Q96 platform that, in the next step, will be used to assess the replication of loci of interest in trauma associated methylation biomarkers.

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References

1. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. *Cell* 2007; **128**(4)**:** 669-681.
2. Champagne FA. Epigenetics and developmental plasticity across species. *Developmental Psychobiology* 2013; **55**(1)**:** 33-41.
3. Cross SH, Bird AP. CpG islands and genes. *Current Opinion in Genetics and Development* 1995; **5:** 309-314.
4. Aberg KA, McClay JL, Nerella S, Clark S, Kumar G, Chen W *et al.* Methylome-wide association study of schizophrenia: identifying blood biomarker signatures of environmental insults. *JAMA Psychiatry* 2014; **71**(3)**:** 255-264.
5. Li LC, Carroll PR, Dahiya R. Epigenetic changes in prostate cancer: implication for diagnosis and treatment. *Journal of the National Cancer Institute* 2005; **97**(2)**:** 103-115.
6. Mill J, Tang T, Kaminsky Z, Khare T, Yazdanpanah S, Bouchard L *et al.* Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. *Am J Hum Genet* 2008; **82**(3)**:** 696-711.
7. Singal R, Ginder GD. DNA methylation. *Blood* 1999; **93**(2)**:** 4059-4070.