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Molecular age estimation based on promotor CpG methylation using Methylation Sensitive PCR

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Introduction

Chronological age is a key factor in animal ecology, as many biological traits emerge and change over time. Such traits include: development, age of reproductive maturity, reproductive success, future reproductive potential, and mortality. Several molecular methods have emerged as potential vehicle for biological age determination. The aim of this experiment is to ascertain if a Methylation Sensitive PCR (MSP) could be developed to screen for methylation at a previously identified site in the GRIA2 gene.

Methods

- MSP was conducted with the EpiScope® MSP kit on the OneStep Plus real-time instrument following manufacturers' instructions, increasing the 1st denaturation to 1 min.
- Primers were designed by eye for both methylated and unmethylated target CpG's in the GRIA2 promoter identified in previous studies using PyroMark sequencing.
- EpiTect® PCR Control DNA was used to assay the specificity and selectivity of the methylated and unmethylated primer sets.

Results

Figure 1: Standard curve of unmethylated control DNA.

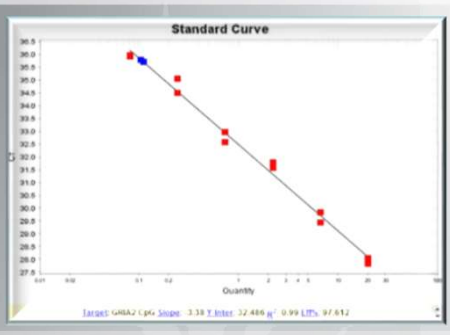


Figure 2: Amplification plot of control DNA (0.3–20 ng).

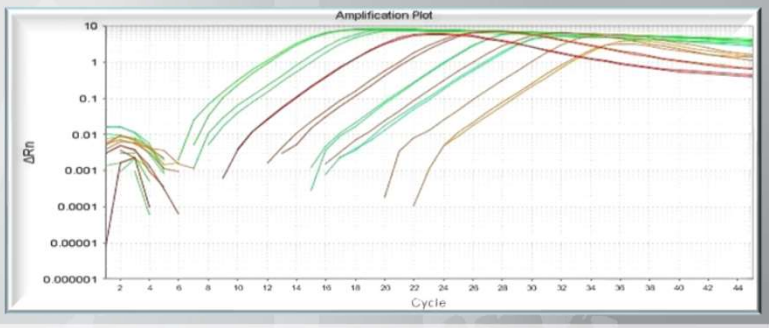
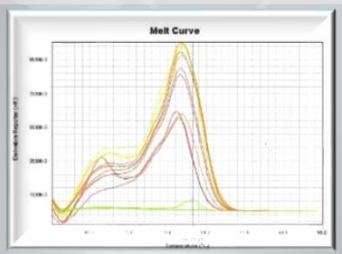


Figure 3: Melting curve of unmethylated DNA.



- After initial optimization, the assay was able to successfully amplify the unmethylated control DNA with an efficiency of 97.6% and R^2=99% (Fig. 1).
- Strong amplification plots (Fig. 2) were observed for a range of 0.3–20 ng input DNA.
- The melting curve (Fig 3.) showed a characteristic mixture of two products, with a single peak for the product.
- Identical results were obtained for methylated DNA.

Discussion & Conclusion

The primers designed for the GRIA2 CpG was able to amplify the selected CpG with great efficiency making MSP a promising method of methylation screening. However, primer design to assay a specific site faces many problems and selectivity for methylated vs. unmethylated may not be achievable.

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