



Figure 1S: Linear relationship between ΔC_t values and methylation concentrations in synthetic standard samples. The ΔC_t values were calculated by subtracting the C_t values of mSEPT9-specific amplification from the consistent C_t values of total SEPT9. The observed linear trend, with a regression coefficient of $R^2=0.9415$, confirms the reliability of ΔC_t as a robust metric for relative methylation quantification across a range of methylation concentrations. Error bars represent the standard deviation from duplicate measurements.