Supplementary Materials and Methods

Development of a nested real-time qPCR assay for the relative quantification of circ-PRMT1 expression

The circ-PRMT1 amplicon spanning the back-splice junction of circ-PRMT1 was validated using Sanger sequencing. Then, a nested real-time qPCR assay, preceded by a pre-amplification step, was designed and optimized for the accurate quantification of circ-PRMT1 in samples of low total RNA mass. For this purpose, we performed standardization experiments with regard to cDNA input, primer concentration, and annealing temperature, $MgCl_2$ concentration, number of thermal cycles during the 1st PCR, and dilution of the pre-amplified template of the nested real-time qPCR. Next, triplicate reactions of serial dilutions of the DLD-1 cDNA were used to generate specific melt curves for circ-PRMT1 and *GAPDH* amplicons, to validate the qPCR efficiencies. The unique melt curve of each amplicon served as evidence of the specificity of the assay, and the mean C_t values were plotted against the $\log_{10}$ of DLD-1 cDNA quantity.