

Figure 2: Comparison of nanopore sequencing and Qiagen<sup>®</sup> MGMT pyro kit of CpGs 76-79 in the *MGMT* CpG-island. Results show per-site methylation percentage of each CpG (a) or average values of 4 CpG sites analyzed by the MGMT pyro kit (b) in the "retrospective nCATs" samples. Black vertical line marks the 10 % cut-off value between methylated and unmethylated samples as measured by the MGMT pyro kit. (c) ROC curves for a logistic regression model based on average methylation of CpGs 76-79 by nanopore sequencing (Retrospective nCATs, 60 samples) with classification by the MGMT pyro kit as operator (solid black) and prediction of MGMT status of 98 samples (dashed grey). (d) Comparison of known *MGMT* status (methylated or unmethylated, x-axis) based on the optimal average methylation threshold of CpGs 76-79 derived from the training samples (retrospective nCATs) and model predictions (other samples). The Y-axis represents average methylation percentage of CpG sites 76-79 based on nanopore sequencing. Dashed horizontal line represents the optimal threshold of 22% methylated, grey box represents 1-95% confidence interval (<10% and >33%)