Quantitation of FFPE DNA

DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue is damaged in many aspects. There are, for instance, enzymatic digestion, shearing by pipetting, or the presence of abasic sites that induce strand breaks. Moreover, FFPE DNA is harmed by chemical alterations, such as cytosine deamination, and crosslinked to as well as contaminated with residual RNA, proteins, salts, organic solvents and PCR inhibitors. In summary, these changes and contaminants complicate correct DNA quantitation and successful PCR amplification.

Simbolo and colleagues[(1)] compared UV-VIS based NanoDrop, fluorochrome-based Qubit (both: Thermo Fisher Scientific) and quantitative real-time PCR (qPCR) for quantitation of FFPE DNA. They demonstrated that UV 260 nm measurement by spectrophotometry, such as NanoDrop, heavily overestimates DNA concentration because of DNA degradation and the presence of crosslinked RNA.

At ViennaLab we have performed similar experiments to compare UV-VIS, fluorometry (Qubit and Promega QuantiFluor systems) and qPCR for FFPE DNA quantitation. As shown in the Figure below, UV-VIS overestimates DNA concentrations by several orders of magnitude compared to qPCR and fluorometry.

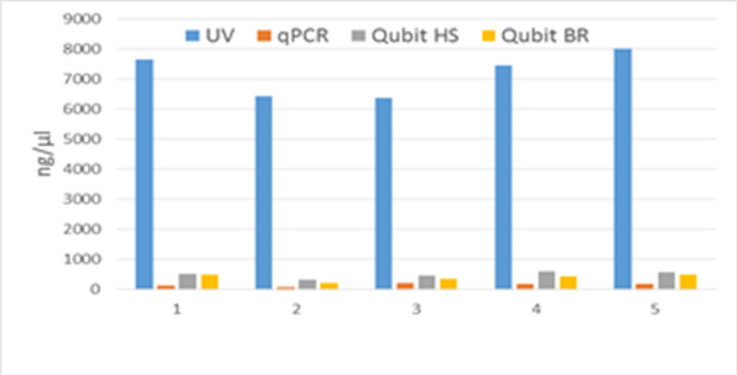

Figure: Comparison of 260/280nm Pharmacia GeneQuant RNA/DNA calculator, qPCR, Qubit Quant-iT HS ("high sensitivity") and BR ("broad range") assays. DNA was extracted from nineteen FFPE samples by using a commercially available FFPE DNA extraction kit. The result of five representative samples are shown.

When performing ViennaLab oncology StripAssays® (KRAS, NRAS, BRAF, EGFR) on FFPE tissue, a total of 5-50 ng DNA per reaction are required for reliable performance. Using FFPE DNA concentrations outside of that range will either outcompete the included sentinels or impair test performance in many aspects. It may, for example, result in reduced test sensitivity, the appearance of PCR Negative Control signals, weak or complete loss of PCR Positive Control amplification, or background staining at multiple mutant signals.

In conclusion, UV-VIS based systems are unsuitable for FFPE DNA quantitation. ViennaLab therefore recommends to use fluorometry, such as Qubit or QuantiFluor assays, or qPCR.

Reference: [(1)]Simbolo et al., 2013. DNA Qualification Workflow for Next Generation Sequencing of Histopathological Samples. PLoSOne 8(6):e62692.