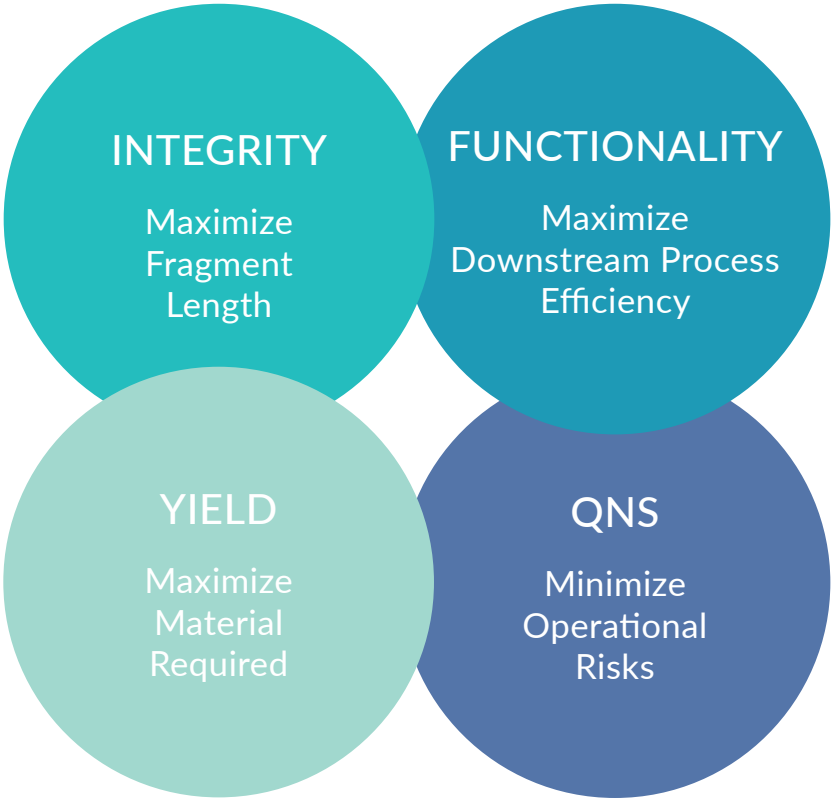
FFPE RNA Quality

Quality Metrics Explained for NGS

Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples have become highly valuable as a diagnostics offering. FFPE tissue samples offer labs the opportunity to correlate Pathomorphological analysis with the precision of modern genomic tools used for personalized treatments. Unfortunately, the methods incorporated for sample preservation lack the standardization and performance of most current extraction protocols, often limiting the use of the FFPE samples.



Need for a Multi-Parameter Quality Control Assessment

The nucleic acids content in FFPE tissue samples is damaged by the preservation method itself (chemical modifications, strand breaks). The traditional methods used to assess extraction performance and quality cannot directly be applied as they rely on proprieties of undamaged, intact nucleic acids and will be irrelevant in this context. For example, the Agilent RNA Integrity Number (RIN), which relies on a comparison of ribosomal RNAs abundance is not a reliable predictor of successful library preparation.

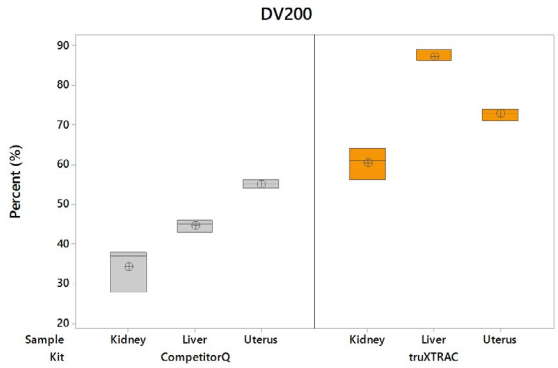
Here, we will discuss the specific metrics recommended to assess the quality of RNAs extracted from FFPE samples aimed to be sequenced.

Yield - Specific Fluorometric Quantitation

Accurate molarity is a key factor to develop a reliable NGS workflow. Covaris recommends the use of a Fluorometric method using a dye that specifically binds to RNAs. Within the context of quantitative biology, personalized medicine, and high-throughput automation needs, sample preparation is crucial, and the workflow's efficiency is less judged on extraction yield than on the percentage of samples that fail the quality threshold tests - Quantity Not Sufficient (QNS).

RNA Integrity - DV_{200}

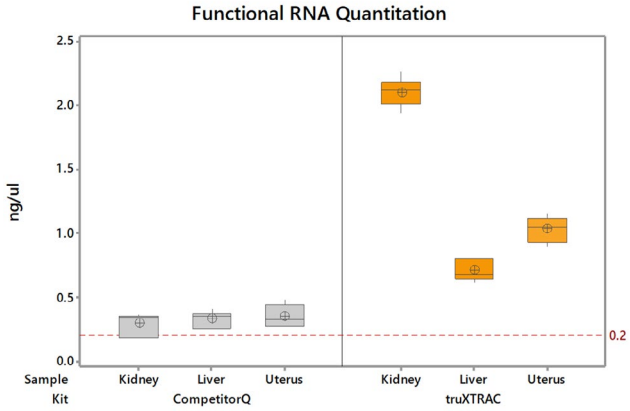
Sequencing technologies have improved in the past years, and NGS workflows are less dependent on RNA degradation. But there are still some limits on the minimum fragments size that can be sequenced and correctly mapped to a reference genome. Illumina recommends that at least 30% of the RNAs submitted to its NGS workflows should be longer than 200 bases (DV_{200}). This can be easily tested using a capillary electrophoresis method such as the Agilent Bioanalyzer.



DV200 scores for both Covaris truXTRAC® tNA Plus extraction kit and Competitor Q. RNA extracted with a Covaris solution shows better integrity.

RNA functionality - FRQ

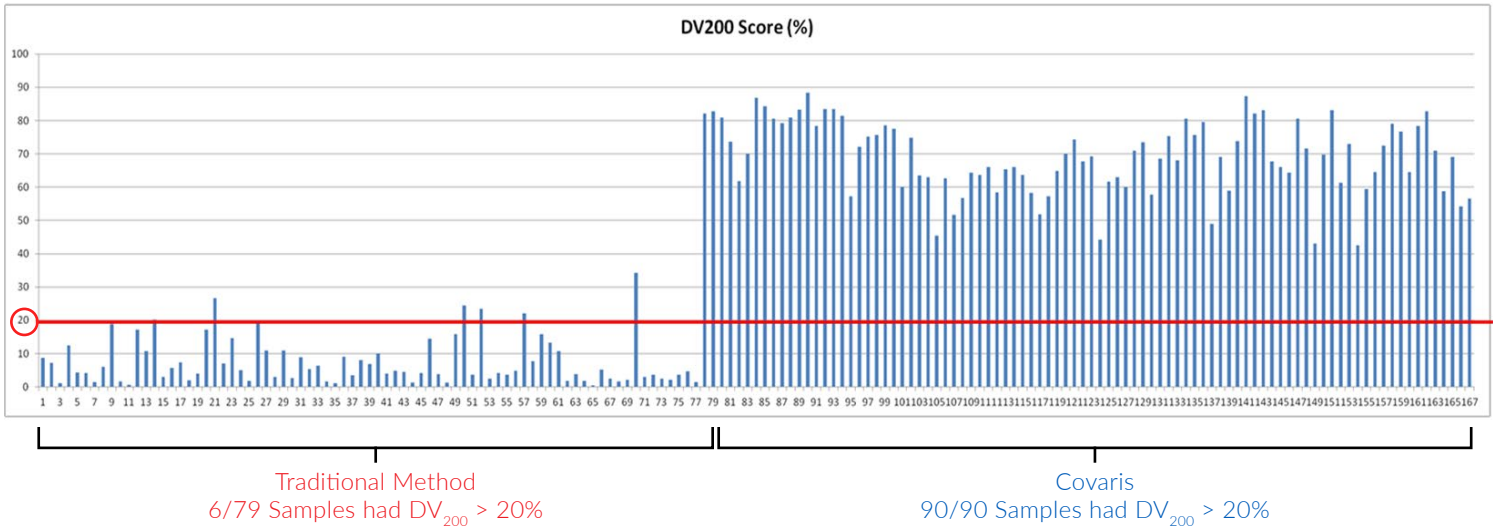
Downstream applications often require enzymatic reactions, which are very sensitive to the input quality. Thermo Fisher Scientific developed the Functional RNA Quantitation (FRQ) RT-qPCR based assay, which is highly relevant for challenging samples such as FFPE. Quantitation by qPCR is only possible for RNAs of adequate quality to be processed by the enzymes used in the assay. Thermo Fisher establishes that samples with FRQ <0.2 ng/µL are likely of poor quality and will not reliably produce good sequencing results on their platform.



Functional RNA Quantitation (FRQ) Score using Covaris truXTRAC tNA Plus extraction kit compared to Competitor Q. RNA extracted with a Covaris solution shows better functionality.

The Covaris Solution to Lowering the QNS Rate

The Covaris solution enables the use of clinical FFPE tissues samples in high-throughput labs. In collaboration with Hudson Alpha, automation of RNA FFPE diagnostic samples were achieved. “The majority of samples are now entering downstream analysis vs. the minority” - Dr. Shawn Levy



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