Agilent NGS FFPE QC Kit

The measure of quality, the measure of success!

Key Benefits

- Accurately qualify and quantify amplifiable DNA in challenging samples

- Optimized low input library prep workflow for improved complexity and target coverage

- Complete cancer research solutions from sample to data

Overview

Formalin-fixed paraffin-embedded tissue represents a valuable sample source for molecular cancer research. These samples, which numbers in the hundreds of millions, provide a contextual snapshot of the tissue at a specific timepoint and stage of normal biology or disease. With today's high resolution technologies such as next generation sequencing, greater information content may be extracted from these samples including signals from low frequency alleles that could easily be missed or dismissed as artifact. There are challenges in processing FFPE samples for this type of analysis. DNA derived from FFPE is oftentimes highly fragmented, cross-linked with protein and has a high proportion of single-stranded DNA. These features of FFPE DNA make it challenging for adaptor ligation and amplification, steps that are critical for successful preparation of sequencing libraries, impacting the overall library complexity and in turn, decreasing the sensitivity of variant calling and increasing the rate of false negatives.

Accurate qualification and quantitation of amplifiable DNA

The Agilent NGS FFPE QC kit is a qPCR-based assay that enables functional DNA quality assessment of input DNA prior to preparation of next generation sequencing libraries. This kit enables assessment of the integrity of DNA as well as accurate quantitation of amplifiable template going into library preparation. Sample integrity is assessed using two primer pairs that generate differently sized amplicons, a 42bp and a 123bp. This difference in the amplicon sizes allow for discrimination between samples that have sufficient intact amplifiable DNA and those that have a higher degree of fragmentation, effectively eliminating the need for agarose gel electrophoresis. In addition, since the assay is qPCR-based, functionality of the FFPE DNA as template for PCR is also assessed allowing for increased probability of successful preparation of next generation sequencing libraries (**Figure 1**).

Figure 1. Sample quality is determined based on the ΔΔCq between the sample and the reference. Briefly, amplification of two differentially sized amplicons are assessed. The ΔCq of the sample is the difference between the Cq of amplicon B (123 bp) and the Cq of amplicon A (42 bp). The quality score or ΔΔCq is then calculated as the difference between the ΔCq of the sample and the ΔCq of the reference. Quantitation, on the other hand, is based on the Cq of amplicon A alone.

Reference DNA (intact gDNA)

Amplicon A, 42 bp
Amplicon B, 123 bp

$$\Delta Cq_{ref} = Cq_B - Cq_A \cong 0$$

FFPE DNA (fragmented DNA)

Amplicon A, 42 bp
Amplicon B, 123 bp

$$\Delta Cq_{sample} = Cq_B - Cq_A > 0$$

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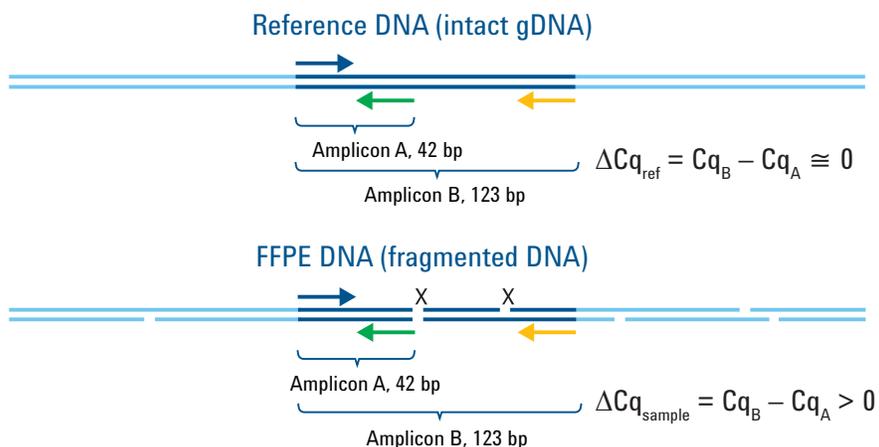
Optimized low input library prep workflow for improved complexity and target coverage

Sample pre-qualification is not sufficient to increase the probability of successful preparation of sequencing libraries. To maximize the information output from FFPE samples, the SureSelect[XT] protocols have been optimized, providing specific recommendations on amplification of pre-capture libraries as well as amount of sequencing to allocate per library based on the sample quality. These modifications ensure that there is sufficient representation of the molecules present in the starting sample going into the hybridization step which is critical to efficient enrichment of the targets. In addition, once these targets are enriched, the recommendations on sequencing depth should enable enough reads to ensure deep target coverage whether the starting sample is of higher or lower quality, for sensitive and accurate variant detection (**Figure 2**).

Complete cancer research solutions from sample to data

Optimized workflows are critical to providing comprehensive variant detection and reduced turn-around time from sample to data. The Agilent NGS FFPE QC kit completes the sample to sequencing workflow for FFPE samples. Combined with the efficient SureSelect[XT] workflows that generate high complexity libraries appropriate for highly heterogenous samples, capture libraries optimized for enrichment of targets relevant in cancer research such as the ClearSeq Comprehensive Cancer panel and the SureSelect Human All Exon V6 + COSMIC, deep target coverage is achieved. For data analysis, SureCall enables analysis of paired tumor and normal samples, typical in cancer research, allowing for sensitive variant profiling of FFPE samples. (**Figure 3**).
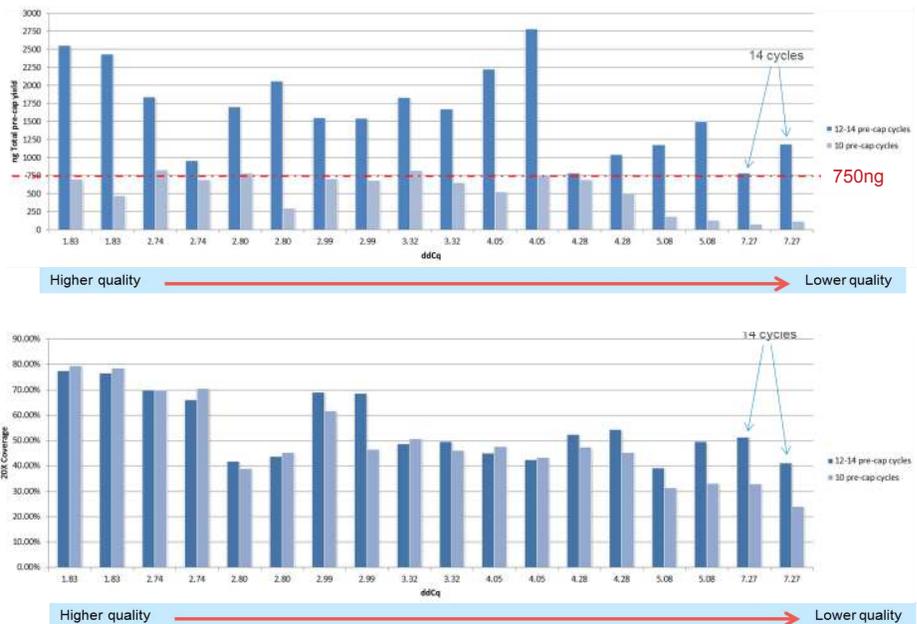

Figure 2. The Agilent NGS FFPE QC protocol provides recommendations to optimize preparation of enriched libraries for sequencing based on the quality score ($\Delta\Delta Cq$). Optimizations for lower quality FFPE samples include increasing amplification cycles for the pre-capture library to ensure sufficient template molecules are introduced into the hybridization, and increasing sequencing depth to enable better target coverage.
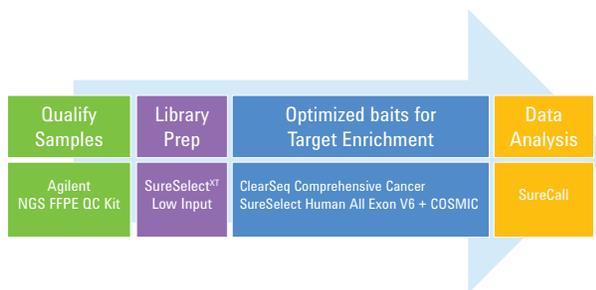

Figure 3. The Agilent NGS FFPE QC kit is part of the complete workflow solution that enables optimal data generation from even low quality FFPE samples. Complementary solutions for library prep, enrichment and data analysis are available providing support from sample pre-qualification all the way to variant calling.

Ordering Information:

	Part Number
Agilent NGS FFPE QC Kit, 16	G9700A
Agilent NGS FFPE QC Kit, 96	G9700B

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