

Robust whole exome sequencing from FFPE samples.

Introduction

Tissue samples have been stored in Formalin-Fixed Paraffin-Embedded (FFPE) for decades. Although well characterized FFPE samples are in abundance, they are seldom used in genomic studies because of the challenges of working with DNA isolated from this samples type. DNA extracted from FFPE samples can vary widely in quality due to age, fixation conditions, DNA-protein crosslinking, and inhibitors, which may impact downstream genomic analyses. Additionally, DNA extracted from these tissues is often of limited quantity.

NEXTFLEX[®] Human Whole Exome Sequencing (WES) Panel is a comprehensive solution that covers all target regions of major WES panels available in the market. With a target size of 37.1 Mb, the panel does not compromise performance in terms of coverage and uniformity, enabling efficient, cost-effective sequencing of the human whole exome. The probe design and reagent optimization technology incorporated in the NEXTFLEX[®] WES panels deliver robust target capture performance without masking or omitting hard-to-capture regions (such as GC- or AT-rich regions and homologous regions).

The NEXTFLEX[®] WES panel uses a streamlined protocol incorporating enzymatic fragmentation, bead-based concentration, and a bead-based normalization process, to greatly simplify WES. Additionally, the protocol is automated on the Sciclone[®] G3 NGSx workstation to increase throughput, minimize sample-to-sample variability and human errors, and reduce hands-on time.

In this application note we show that the NEXTFLEX[®] WES Panel is able to overcome the challenges inherent with FFPE samples to deliver robust WES performance.

For research use only. Not for use in diagnostic procedures.



Materials and methods

Third-party laboratories conducted a comparison study between the NEXTFLEX® WES Panel, Company A, and Company I panels. 200 ng of reference materials NA12878, NA12891, NA12892 for germline analysis, and HD832 for FFPE analysis were used. Illumina® platforms were used for the sequencing. The data from the three panels were downsampled to 5.4 Gb.

Results

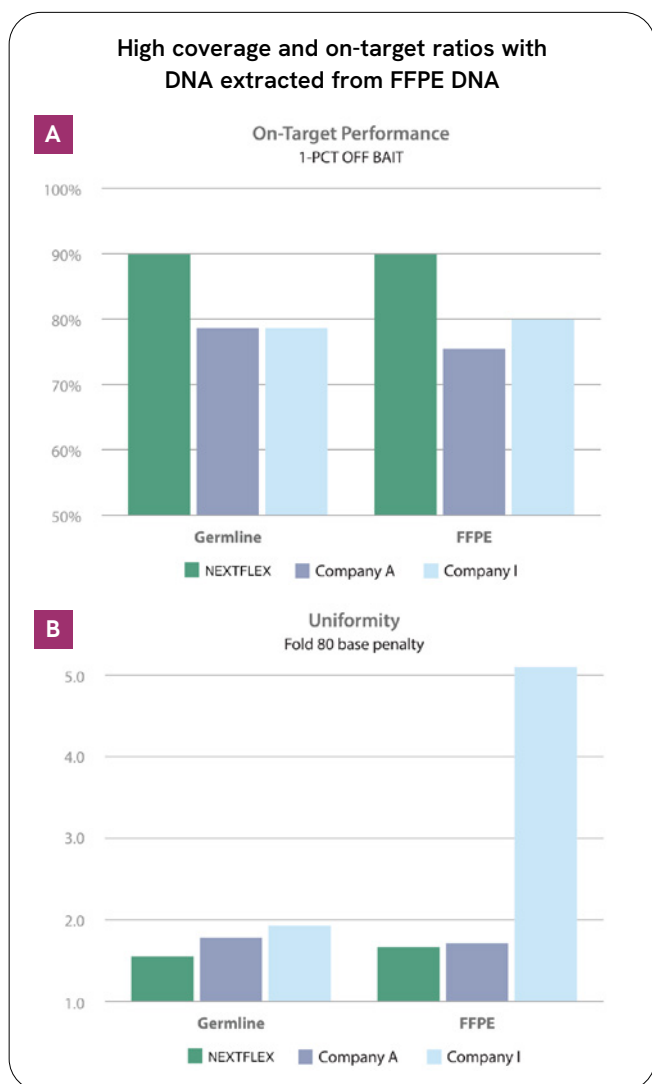


Figure 1. The NEXTFLEX® WES Panel shows exceptional performance compared to competitor products in both germline and FFPE analysis, measured by (A) 1-PCT OFF BAIT (higher is better), (B) Fold 80 base penalty (lower is better).

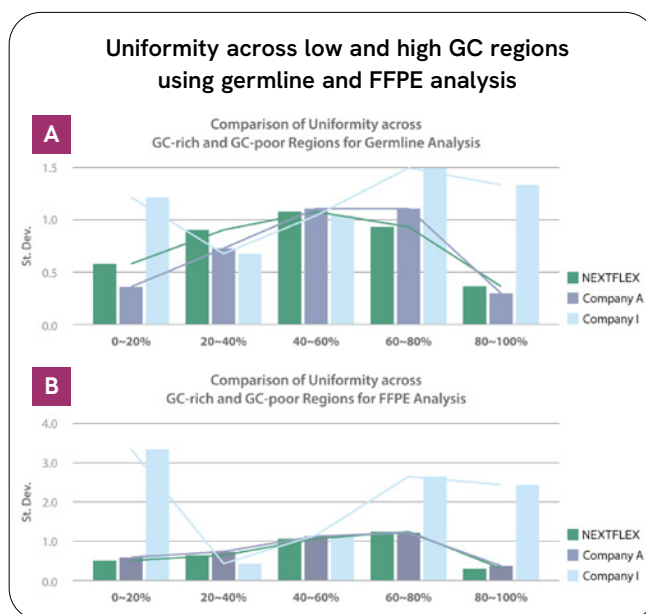


Figure 2. The NEXTFLEX® WES panel demonstrates consistent uniformity across a GC-rich and AT-rich regions for both germline (A) and FFPE samples (B), with minimal standard deviations in both cases.

Conclusions

The NEXTFLEX® WES panel allows for robust, cost-effective, time-saving sequencing of the whole exome from DNA isolated from FFPE samples.

It delivers better coverage, on-target performance, and uniformity with both germ line and FFPE samples when compared to competitors' solutions.

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