



Successful Next-Generation Sequencing (NGS) for Exome Analysis from Formalin-Fixed and Paraffin-Embedded Tumor Tissue (FFPE) Samples

M^a Luisa Villahermosa

GENOMICA SAU (Pharmamar Group), Spain

Abstract:

Formalin-fixed, paraffin-embedded tumor tissue (FFPE) is likely to be the preferred choice for tissue preservation in clinical practice for the Next Generation Sequencing (NGS) analysis. The efficiency of Exome sequencing from FFPE tissue depends of the amount and quality of DNA extracted. To generate accurate NGS data, we have implemented a specific protocol in order to obtain accurate sequencing of the exomes. We analyzed 21 tissue samples obtained at diagnosis from patients with solid tumors using Ion ampliseq™ Exome RDY (ThermoFisher) with the objective of detecting germinal variants. Genomic DNA (gDNA) concentration was quantified using Qubit® fluorometry, and its quality was determined using the RNase P Taqman. Correct NGS libraries were obtained considering the degradation status: we increase the quantity of gDNA for performing

the library according with the data obtained with the RNase P analysis in each sample.

We obtained successful NGS results in 90.5% of cases: -15 samples showed Optimal results (Uniformity in sequence coverage >91.26%; Target base coverage at 20x >94%), being the quantity of gDNA 2-10ng/μl in 6 samples, and 10-50ng/μl in 9 samples; these samples showed optimal quality of gDNA. -5 samples showed Medium results (Uniformity in sequence coverage 50-80%; Target base coverage at 20x 60-85%), being the quantity of gDNA in all samples 2-5ng/μl, and the quality of the gDNA was mostly medium-low. -1 sample showed Low result (Uniformity in sequence coverage: 40%; Target base coverage at 20x 50%) being the quantity of gDNA 5 ng/μl, and the quality was very close to the medium values.

We have implemented an additional step before the Library generation, which allow us to increase the quantity of gDNA according with the obtained quantity/quality values. This assay may be mostly efficient for the clinical samples with high degradation and poor DNA quality.

Biography:

M^a Luisa Villahermosa is the Director of the R&D Department at GENOMICA, with 17 years' experience in developing innovative molecular diagnostic products in oncology and microbiology areas, from concept to Industry. She is creative and has an excellent understanding and product development know-how. The NGS technology was one of the innovation techniques that were introduced in the Department to offer services and to use it internally during the development and validation processes.