OPTIMIZING ULTRASONIC GDNA FRAGMENTATION FOR NGS SEQUENCING

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The fragmentation of genomic DNA (gDNA) is a crucial step in many Illumina library preparation protocols, including the TSO 500 assay, aimed at achieving fragment sizes ranging from 90 to 250 base pairs (bp) for next-generation sequencing (NGS). However, challenges arise in practice due to variations in fragmentation times, which are influenced by different extraction methods and sample sources.

To optimize ultrasonic gDNA fragmentation across various extraction methods for NGS sequencing applications.

Shearing of gDNA was performed using the Covaris S220 system, employing microtubes containing a total volume of 130 microliters of diluted Tris EDTA (pH 8.0) and 100 ng of gDNA, following the manufacturer's protocol. gDNA samples were extracted using three different kits: AllPrep DNA/RNA FFPE (Qiagen) from FFPE blocks, ReliaPrep FFPE gDNA Miniprep system (Promega) from glass slides, and Gentra Puregene (Qiagen) from cell lines.

The shearing times varied depending on the extraction method and sample source. gDNA extracted using the AllPrep DNA/RNA FFPE kit from FFPE blocks required shearing times ranging from 300 to 400 seconds. In contrast, gDNA extracted using the ReliaPrep FFPE gDNA Miniprep system from glass slides exhibited shearing times of approximately 480 to 500 seconds. Lastly, gDNA extracted using the Gentra Puregene kit from cell lines showed shearing times ranging from 150 to 180 seconds.

Our study demonstrates the influence of gDNA extraction methods and sample sources on ultrasonic fragmentation efficiency for NGS sequencing. The observed differences in shearing times highlight the need for method optimization to achieve consistent and optimal fragment sizes across diverse sample types. These findings contribute to the development of standardized protocols for gDNA fragmentation, facilitating reliable and reproducible NGS data generation in various research and clinical settings. Further investigations into the underlying factors affecting fragmentation efficiency are warranted to advance our understanding and improve NGS library preparation methodologies.