A Novel Next-Gen Sequencing Library Preparation Method To Sequence Small, Long, And Low-Quality (Fragmented/ Degraded) RNA With low Quantity Of Templates (pico-grams) For improved Diagnostics, Biomarker Discovery, And Research.

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Objectives: There are several well-established Nest-gen-sequencing (NGS) methods in published literature and commercially available for sequencing total RNA, DNA, exome, small RNAs, and selected panels of gene/ loci to address specific issues.

However, it has been a great challenge to sequence highly fragmented and degraded DNA/RNA due to the limitations of library preparation methods. This affects the sequencing of clinical samples and biological research materials. Currently, it is also impossible to sequence small RNAs with pico-gram quantities of (low-input) total RNA templates.

Methods and results: We have addressed these limitations by developing a novel method to prepare NGS libraries. Our methods could efficiently capture RNA of all sizes (20bp and above) simultaneously and detect 2-10 times more unique transcripts at a similar sequencing depth than the current state-of-the-art methods.

Conclusions: Our method is applicable to highly fragmented and degraded samples like clinical samples, FFPE, cell-free RNA/DNA, liquid biopsies, archeological samples, and also normal samples. The variations and errors observed in NGS data due to different levels of degradations between samples are negligible in our method. We could generate 2-10 times more and better data with as little as 500pg of total RNA. Due to its high efficiency and sensitivity, our method will enable better diagnostics, biomarker discovery, and research.