RiboMarker®

RNA FRAGMENTOMICS LIBRARY PREPARATION KIT

RNA fragmentomics library preparation kit compatible with Illumina sequencing platforms

Comprehensive RNA profiling from any sample

Cell-free nucleic acids (cfDNA and cfRNA) are promising biomarkers for cancer and other diverse pathologies. Current methods using cfDNA analytes are not sensitive enough to reliably detect pathologies including cancer at early stages (when it is more treatable) or as a minimal residual disease after treatment when tumor-associated cfDNA are in several orders of magnitude lower abundance than background cfDNA that originated from non-cancerous cells. Recent studies indicate that cfRNA-based diagnostic assays can provide a higher sensitivity for these applications rather than cfDNA due to the larger abundance and diversity of RNA molecules involved in various biological functions. Importantly, the majority of cfRNA are small RNAs and RNA fragments of < 100 nt in length that together represent this novel diagnostic class.

Sequencing analysis of these molecules will improve our understanding of the role of these RNAs in various disease processes and aid in the discovery of novel RNA biomarkers with increased diagnostic sensitivity. However, most of the standard commercially available methods of NGS library preparation for short RNAs (< 100 nt) only detect RNAs having 5'-P and 3'-OH ends (e.g., miRNAs), **which account for ~10% of the entire small RNA pool, while the other 90% of RNAs are largely fragments with different 5' and 3' ends and are hidden from detection [1].**

The RiboMarker® platform enables a comprehensive analysis of the small RNA pool in any sample, encompassing: (i) miRNAs, tRNAs (tRFs), and piRNAs; (ii) a broad range of RNA fragments derived from "longer" RNAs; (iii) targeting of specific classes of RNA fragments to maximize the sensitivity of detection. RiboMarker® amplifies rare disease-associated RNA fragments by eliminating background noise from other, less informative RNA sequences. **To this end, we explored the potential of RiboMarker® for cancer detection, using cfRNA plasma samples from breast cancer patients and healthy donors.**

- **Captures all small RNAs and RNA fragments <100 nt in a sample**
- **Bias-free library construction**
- **Compatible with low input RNA from various sources including tissues, biofluids, soil, and more**

RiboMarker® Workflow

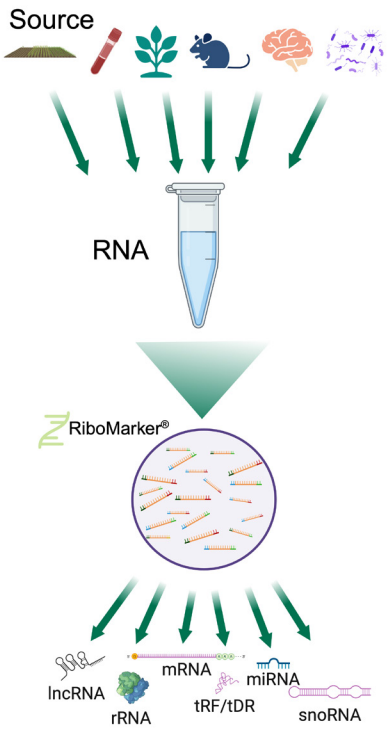

Figure 1 | RiboMarker® libraries are made with low RNA inputs and include small RNAs and RNA fragments with varying 5' and 3' end combinations up to 100 nt in length.

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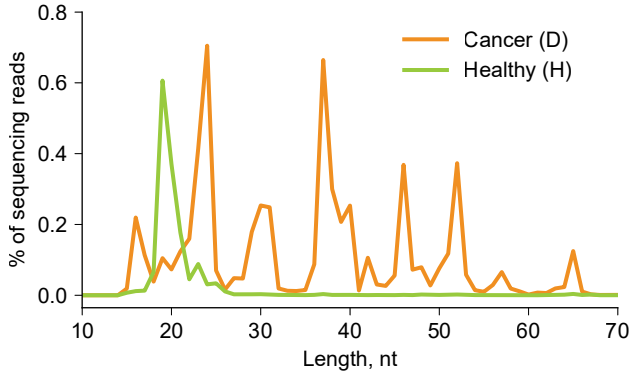
Case Study | RiboMarker sequencing of cancer plasma RNA

RiboMarker® captures the full spectrum of RNA 5' and 3' end combinations providing an enhanced capture of small RNAs and RNA fragments derived from a more diverse group of transcripts (Fig. 1). Leveraging this unique capability, we applied RiboMarker® to plasma samples collected from 3 healthy donors (H) and 3 breast cancer patients (D). Previous studies have implicated small nuclear RNAs (snRNAs) as a key discriminator between cancer patients and healthy donors. By examining RiboMarker® profiles for snRNAs from cancer and healthy patients we found distinct differences that enhanced their discrimination. Notably, RNA fragments longer than 30 nt were identified exclusively in cancer patients and was accompanied by large shifts in fragments of 19–24 nt which further distinguished cancer from healthy (Fig. 2). Looking at the specific snRNAs to assess their potential capability as biomarkers in this context, the one transcript which yielded the most robust discrimination betweeen cancer patients and healthy donors was the U2 snRNA (Fig. 3). Interestingly an assessment of read pileups from the U2 snRNA revealed two distinct RNA fragments: one derived internally and one originating from the 3' end and highly enriched in the cancer patients compared to the healthy donors (Fig. 3).

Together, these observations highlight the potential of U2 snRNA and other snRNA fragments as sensitive biomarkers for cancer detection, as revealed by RiboMarker®'s ability to capture distinct RNA fragment profiles. The enrichment of specific RNA fragments in cancer patients illustrates the power of comprehensive RNA fragmentomics for distinguishing disease states and advancing novel diagnostic tools.

RiboMarker® differentiates cancer patients & healthy donors

Figure 2 | Comparison of RiboMarker® RNA profiles of cfRNA plasma samples originated from breast cancer and healthy donors. Plasma samples were collected from 3 healthy donors (Healthy, H) and 3 patients diagnosed with breast cancer (Cancer, D). Length profiles are of the cfRNA sequences found for the selected snRNA class. The average distribution for cfRNA fragment lengths from all D (orange) and H (green) plasma samples is shown.



RiboMarker® enhances biomarker discovery

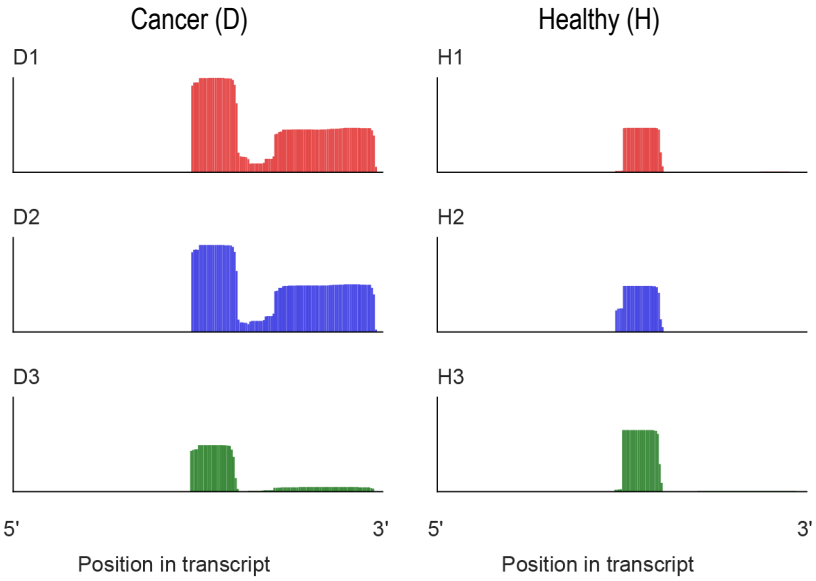


Figure 3 | Pileup of reads mapping to U2 snRNA found in cancer (D1, D2, D3) and healthy (H1, H2, H3) cfRNA plasma samples, corresponding to Figure 2. cfRNA profiles from the cancer patients (D) revealed an RNA fragment localized to the 3' half of the U2 snRNA transcript. This enhanced the discrimination of cfRNA from cancer plasma compared to healthy donor plasma which does not contain this 3'-derived RNA fragment.

REFERENCE

1. Shigematsu, M., Kirino, Y. 2022. Making invisible RNA visible: Discriminative sequencing methods for RNA molecules with specific terminal formations. Biomolecules 12: 611.

patent pending

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