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APPLICATION NOTE

# Innovative small RNA profiling with unique D-Plex technology on MGI DNBSEQ™ sequencers

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*The Diagenode D-Plex small RNA DNBSEQ™ kit is a tool designed for the study of the small non-coding transcriptome. It offers key advantages such as ultra-low input tolerance, liquid-biopsy compatibility and an easy-to-use protocol with minimal hands-on time. By applying a ligation-free preparation method, the final D-Plex libraries efficiently capture a vast spectrum of small non-coding RNAs while minimizing inherent ligation biases. Supported by its capability for high quality sequencing on MGI's DNBSEQ™ platforms, the D-Plex small RNA DNBSEQ™ kit is exceedingly suitable for discovery of circulating biomarkers in clinically relevant samples*

## Introduction

Small non-coding RNAs (sncRNAs) offer great hope as clinical biomarkers in the prediction and early detection of disease or in the investigation of response to treatment. This has been highlighted in the context of several medical conditions such as cancer, liver disease, cardiovascular disease, and central nervous system disorders, among many others. There are several types of sncRNAs, of which microRNAs (miRNAs) are the best known and the most frequently assessed for their potential role as biomarkers [1]. Other sncRNA species, such as PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs) and tRNA-derived small RNAs represent new potential classes of biomarkers to be explored as they have proved to be key components for cellular regulation in some diseases [2], [3].

Next-generation sequencing (NGS) exhibits an unprecedented opportunity to discover and quantify diverse kinds of sncRNAs. However, currently available library preparation methods do not provide optimal approaches to comprehensively analyze the whole small RNA spectrum of a sample of interest. In all small RNA library production protocols, the RNA fragment needs to be converted into cDNA, attached to adaptors on both extremities and enriched via DNA amplification to form a suitable library ready for a successful sequencing run. A main concern of the generally used ligation-based methods represents the heavy bias caused by an uneven affinity of RNA ligases towards the different template sequences and 5'-modifications for the addition of sequencing-adapters. These libraries show a clear enrichment for canonical miRNAs harboring 5'-mono-P and 3'-OH, which are precisely the biochemical prerequisites for the commonly used ligases, leading to an underrepresentation of the other small RNA species in the sample of interest [4]. Additionally, ligation-based methods need large amounts of starting materials (often >10 ng total RNA per sample) and require several purification steps which are challenging for low input and clinical-relevant circulating RNA samples.

Here, we aimed to optimize a sequencing library strategy, which (1) is suitable for ultra-low and circulating RNA inputs, (2) allows capture of the wide diversity of small RNAs and (3) is compatible for DNBSEQ™ sequencing on MGI sequencers. The D-Plex technology utilizes the innovative capture and amplification by tailing and switching, a ligation-free method for RNA library preparation [5]. The protocol is described in **Figure 1**. As a first step, the RNA fragment is polyadenylated and captured by a poly-dT primer which contains part of the 3'-adapter sequence and serves as the starting point for the reverse transcription. By applying a template-switch reaction, the synthesized cDNA strand is then elongated by the terminal 5'-adapter sequence and can be directly used for PCR amplification to enrich the library as a whole. The final library is then ready to be used for the DNA single-strand circularization step required for DNBSEQ™ sequencing on MGI sequencers [6]. Together, our D-Plex technology to generate complex small RNA libraries from clinical-relevant RNA samples in combination with MGI's highly efficient DNBSEQ™ techniques provides a promising tool for valuable small RNA profiling.

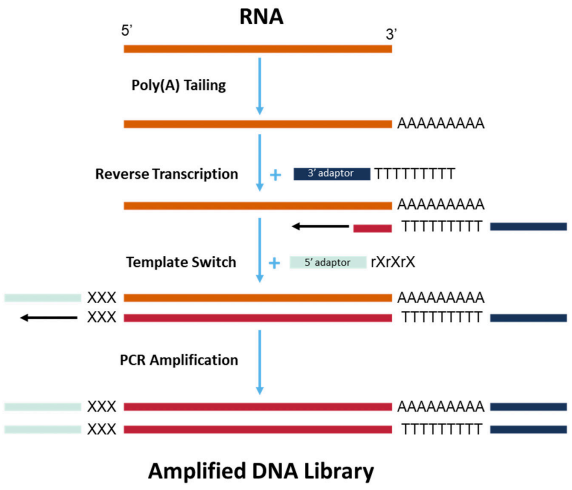


**Figure 1.** D-Plex Small RNA DNBSEQ library preparation workflow. Single stranded RNAs are polyadenylated at their 3'-end and primed with a poly-dT oligonucleotide containing the terminal 3'-adapter sequence. The addition of a template-switching oligonucleotide enables the elongation of the cDNA synthesis to fuse the terminal 5'-adapter sequence during the reverse transcription reaction. During PCR amplification, the required MGI's adapter sequences and barcodes are incorporated into the final library construct.

## Methods

Circulating RNA was extracted from human plasma (Seralab 23134, lot BRH1462944) using the Qiagen miRNeasy Mini Kit (Qiagen, 217004). The D-Plex small RNA DNBSEQ™ (Diagenode, C05030051 (Core module – 24 rxns), C05030060 (D-Plex 24 DNBSEQ Barcodes - Set A), C05030061 (D-Plex 24 DNBSEQ Barcodes - Set B)) protocol was followed to prepare RNA libraries and challenged by comparing very-low inputs (10 pg, 25 pg and 50 pg) with a higher reference input (2.5 ng). Circularization (MGIEasy Circularization Kit (MGI, 1000005259)), DNB production and sequencing were accomplished according to the corresponding MGI's protocols (High-throughput Sequencing Set (DNBSEQ-G400RS FCL SE50) (MGI, 1000017992)) on a DNBSEQ-G400 sequencer using the new CoolMPS sequencing technology, SE50 starting with 3 dark cycles. RNA-seq data were processed according to to the manufacturer's instructions (Diagenode, C05030060).

## Results

With more than 40 million reads per sample, the overall sequencing quality has exceeded expectations. Even before trimming of the A-tail, a very high per base sequence quality was achieved with an average score of 37 (**Figure 2**). Still 84%-92% of the reads remained after trimming and filtering with up to 70% mapping efficiency (**Figure 3**). Regarding the capture of the whole small non-coding RNA spectrum, detection of a large diversity of small RNAs could be confirmed (**Figure 4**). By efficiently incorporating miRNA, miscRNA, scaRNA, scRNA, snoRNA and snRNA in the libraries, the kit provides a high potential tool for clinical biomarker discovery. Importantly, the widespread diversity of information is maintained equally between the replicates and keeps further consistency even when decreasing the starting material from 2.5 ng down to ultra-low 10 pg circulating RNAs derived from human plasma samples. Outstanding ultra-low input performance could be additionally demonstrated by calculating the Pearson correlation coefficient between very low (10 pg, 25 pg and 50 pg) and high (2.5 ng) RNA inputs, convincing with an outcome of R=0.99 as shown in **Figure 5** for the 2.5 ng to 25 pg comparison.

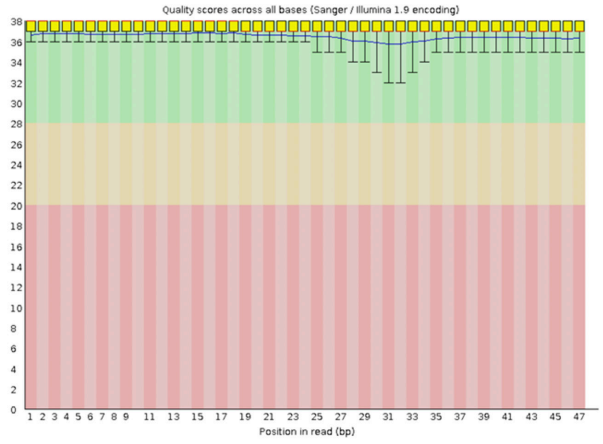


**Figure 2. Per base sequence quality before trimming.**

Data from 50 pg circulating RNAs derived from human plasma samples.

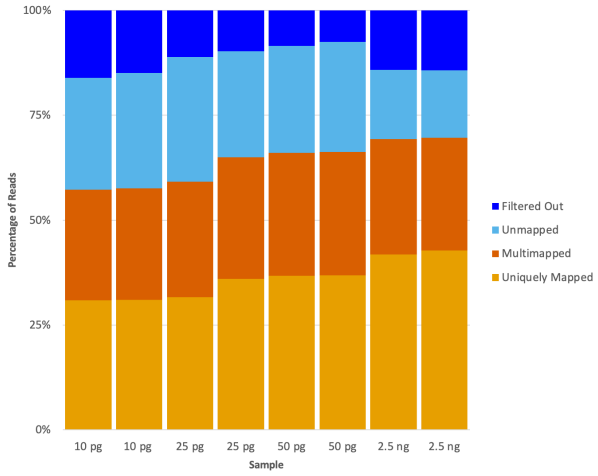


**Figure 3. Sequencing statistics.**

Filtering and mapping outputs for all RNA inputs (10 pg, 25 pg, 50 pg, and 2.5 ng) derived from human plasma samples.

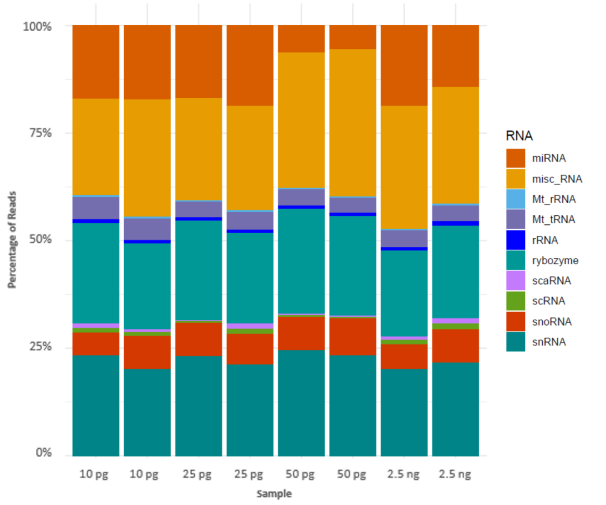


**Figure 4. Diversity of small non-coding RNAs.**

Biotype profiles for all RNA inputs (10 pg, 25 pg, 50 pg, and 2.5 ng) derived from human plasma samples.

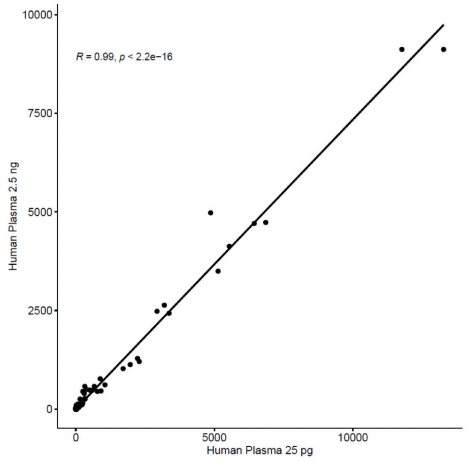


**Figure 5. Ultra-low input performance.**

Person correlation between ultra-low (25 pg) and high (2.5 ng) RNA inputs derived from human plasma samples considering the normalized counts of small non-coding transcripts.

## Conclusion

The D-Plex Small RNA DNBSEQ™ library preparation protocol represents a fast and easy-to-use, one-day one-tube method for unbiased small non-coding RNA library production with ultra-low input capability. Supported by high quality sequencing on MGI's DNBSEQ™ platforms, the D-Plex Small RNA DNBSEQ™ kit is suitable for biomarker discovery in clinically relevant samples.

## References

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