Small RNA Sequencing and Biomarker Discovery

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1 Introduction

Small RNAs are promising non-invasive diagnostic biomarkers detectable in the cell-free fraction of body fluids, such as plasma, cerebrospinal fluid and urine for example The NEXTFLEX® Small RNA-seq kit v4 kit will be presented. The NEXTFLEX® Small RNA-seq kit v4 kit delivers reduction in ligation bias, low-input amounts, multiplexing capabilities) but with added design features such as completely gel-free protocol and greater discovery/detection rates to facilitate the study of microRNAs in body fluids.

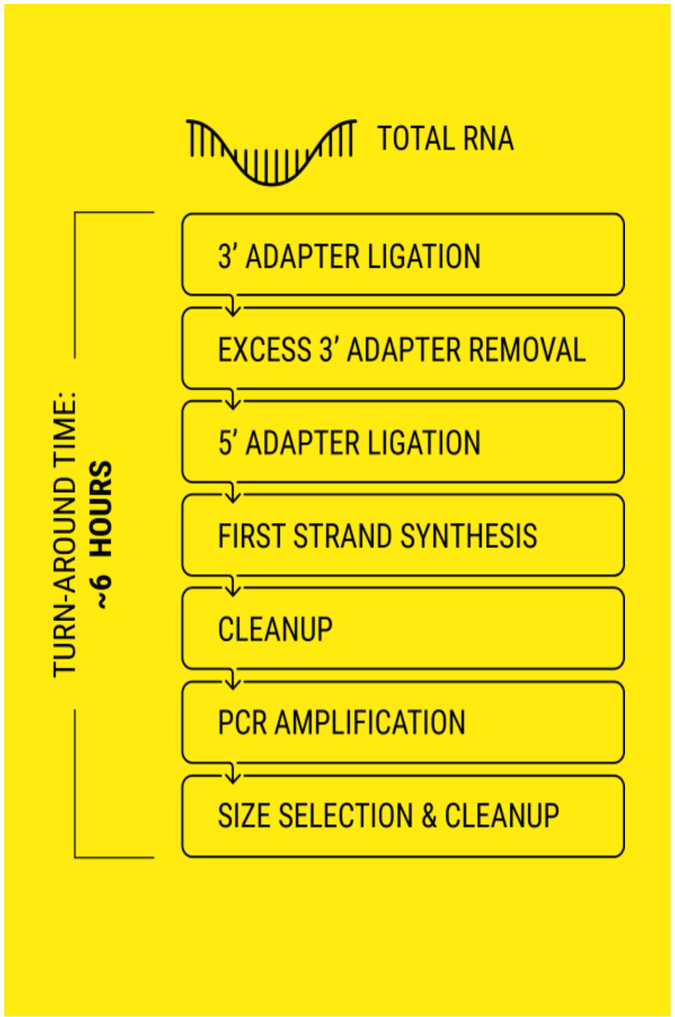
2 Methods

We performed a side-by-side comparison of NEXTFLEX Small RNA-Seq Kit v4 with Competitor A, which also offers a gel-free workflow even from 1 ng. To do so, we selected serum as input type. Serum is a challenging sample for small RNA-seq with a low proportion of reads aligning to miRNA. Additionally, serum samples have naturally a variable content on miRNA and differences are to be expected from different donors.

Five human serum samples (Zenbio) were extracted using the NextPrep™ Magnazol™ cfRNA isolation Kit. The RNA obtained was quantified with the Thermo Fisher Scientific Quibit® fluorometer. 1 ng of total RNA was used as input for either NEXTFLEX® Small RNA-Seq Kit v4 or Competitor A. Two different scientists performed the experiment to assess reproducibility.

Small RNA libraries were prepared manually according to the manufacturer's instructions and after Thermo Fisher® Scientific Quibit® fluorometer measuring and pooling they were run on an Illumina® MiSeq® platform at 1x75. Results were downsampled to the same number of reads for comparisons. Small RNA analysis was performed using a Revvity custom script. Alignment reference was mature miRNA from mirBase v22.1.

Streamlined NEXTFLEX Small RNA-Seq Kit v4 Workflow



3 Results

miRNA Alignment

After filtering and mapping the data, we checked the proportion of reads that aligned with adapter dimer, tRNA, YRNA, rRNA and miRNA for both kits (Figure 1).

Both kits show a low proportion of adapter dimer or rRNA in the final data. A higher presence of reads corresponding tRNA and YRNA are seen on the libraries from Competitor A, suggesting that the mechanism to deplete those species is less efficient. Both kits presented a percentage of miRNA reads in agreement with published data for serum, although the NEXTFLEX Small RNA-Seq Kit v4 miRNA mapping rate was 2-fold higher than that obtained with Competitor A (15.61% vs 7.15%).

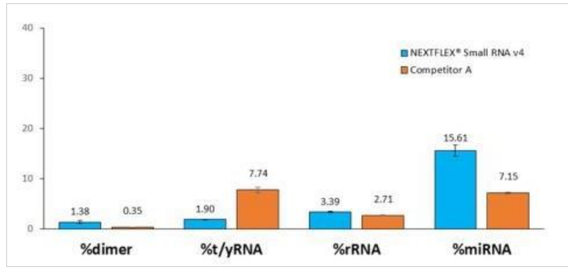


Figure 1. *Proportion of reads aligning to adapter dimer and different RNA species.*

miRNA Discovery

Performance of small RNA seq is known to be heavily dependent on the workflow used. To understand the relationship between the miRNA species identified by both kits we quantified how many were found and the similarities of the miRNA discovered in both workflows. To do so we looked first at the average number of unique miRNA discovered per sample (Figure 2).

The NEXTFLEX Small RNA-Seq Kit v4 has a higher miRNA discovery rate than Competitor A. Setting a threshold of 5 (at least 5 counts to call a specific miRNA), the average of unique miRNA discovered per sample is 163 vs 117, or 39% higher. If the threshold is more stringent and we set it at 10, then NEXTFLEX Small RNA-Seq Kit v4 can discover 128 vs 90, or 42% more unique miRNAs than Competitor A.

To investigate how comparable were the sets of miRNA found by both workflows we set threshold to 5 and determined the miRNA in common between replicates for each kit. This resulted in 134 miRNA for NEXTFLEX Small RNA-Seq Kit v4 and 92 for Competitor A. (Figure 3).

81 miRNA (88% of the miRNAs discovered by Competitor A) were also found by NEXTFLEX Small RNA-Seq Kit v4. 11 miRNA only found with Competitor A and 53 miRNA only found with NEXTFLEX Small RNA-Seq Kit v4.

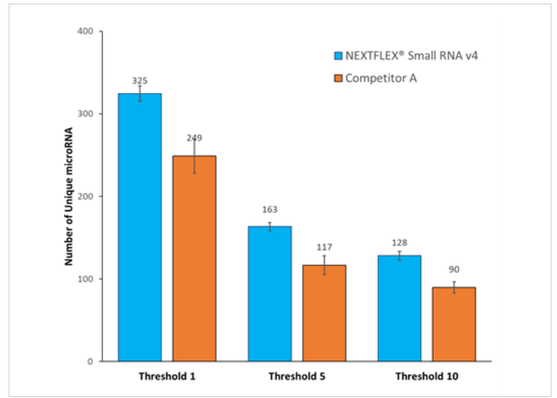


Figure 2. *Average number of unique miRNA discovered per sample using 1 ng of serum as input.*

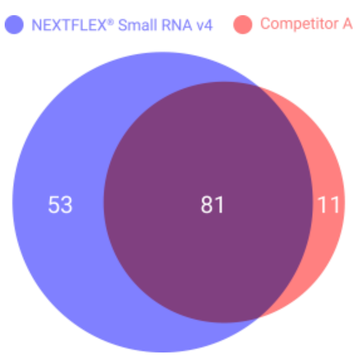


Figure 3. *Venn diagram showing that 45% more miRNA were identified using the NEXTFLEX Small RNA-Seq Kit v4 than Competitor A kit.*

4 Conclusions

NEXTFLEX® Small RNA-Seq Kit v4 has a streamlined, gel-free workflow delivering exceptional miRNA mapping and discovery rates even when working at low inputs with challenging samples such as serum.