APPLICATION NOTE

Simplify Low-Input Small RNA-seq Library Prep & Improve Discovery

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Key Takeaways

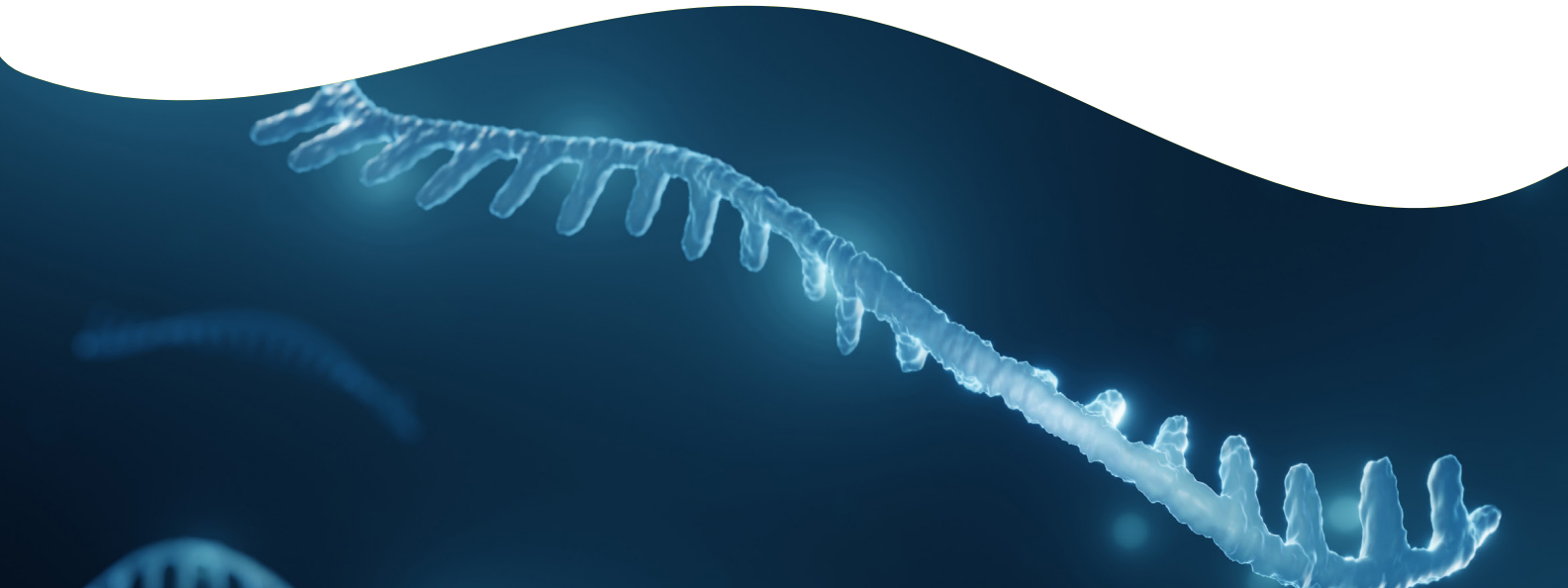
- Gel-free workflow from 1 ng of total RNA input
- Exceptional miRNA discovery

Introduction

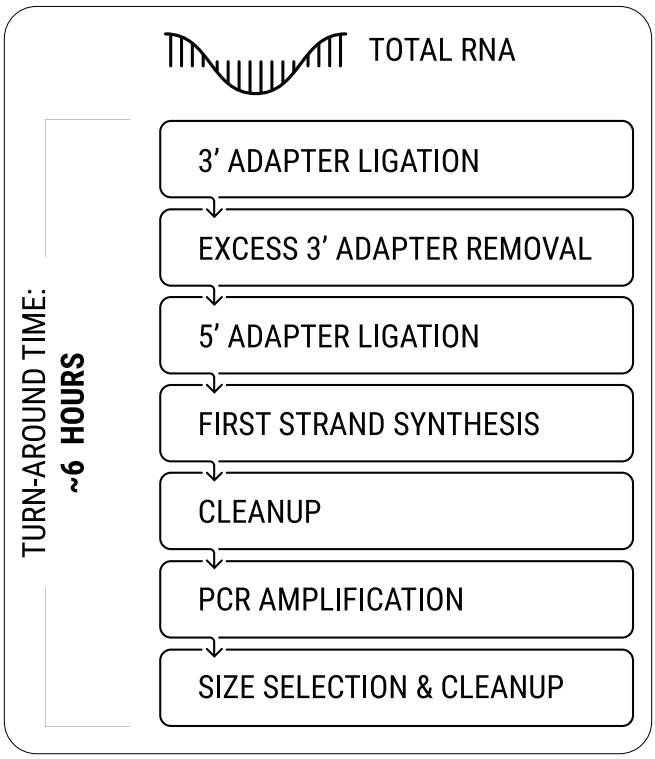
MicroRNAs (miRNA) are 18-22 nucleotide-long non-coding RNAs which play an important role in the regulation of protein levels. Many recent studies have proposed using miRNAs as biomarkers because they are tissue specific, stable in extracellular space and expression profiles can be linked to health status. They can be reliably detected in tissues and diverse body fluids, of which plasma, serum, and urine are frequently used as they are pretty much non-invasive sample types and easy to obtain. Over the last years we have observed a strong trend in the field where researchers interested in miRNA are moving from studying tissues to body fluids.

With these types of samples, the available input is low and often close to 1 ng. NEXTFLEX® Small RNA-Seq Kit v3 has been successfully used to study miRNA in different kinds of body fluids. However, due to the low input amounts the workflow required PAGE purification to separate the adapter dimers from the final library product. This is time consuming, makes difficult the automation of the workflow and might lead to loss of miRNA diversity and yield. To address this problem, we developed the NEXTFLEX® Small RNA-Seq Kit v4.

Patent-pending strategies to reduce generation of adapter dimers were incorporated into this kit streamlining the workflow and reducing bias The ligation reaction was optimized, adapter depletion oligonucleotides were added, and the randomized adapter ends were removed.



Streamlined NEXTFLEX® small RNA-seq kit v4 workflow



Comparison of NEXTFLEX® small RNA-seq kit v4 with competitor A

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 from Revvity. The RNA obtained was quantified with the Thermo Fisher® Scientific Qubit® 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 Qubit® 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

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).

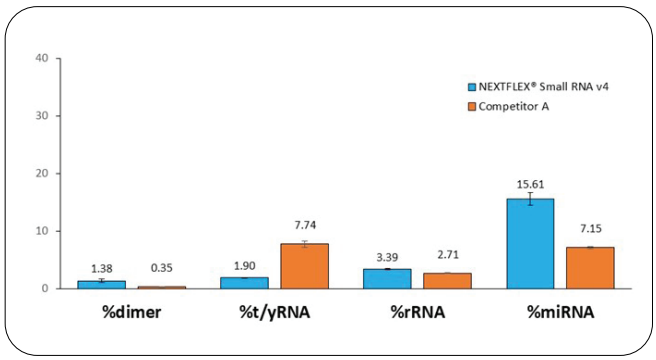


Figure 1. Proportion of reads aligning to adapter dimer and different RNA species.

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, sue ggesting 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%).

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).

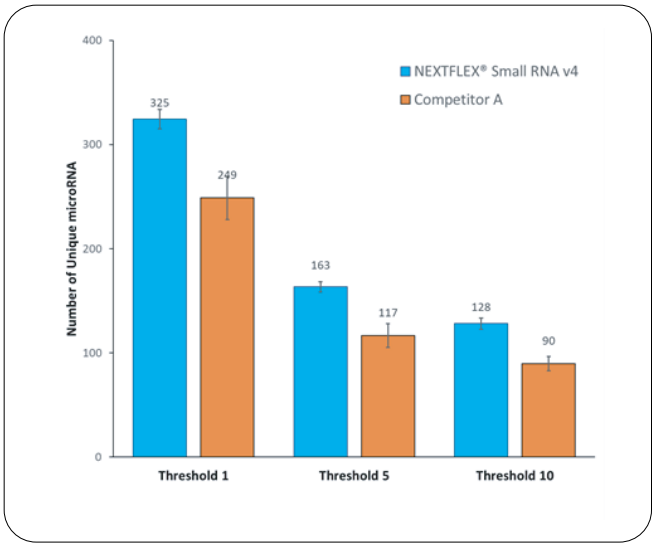


Figure 2. Average number of unique miRNA discovered per sample using 1 ng of serum as input.

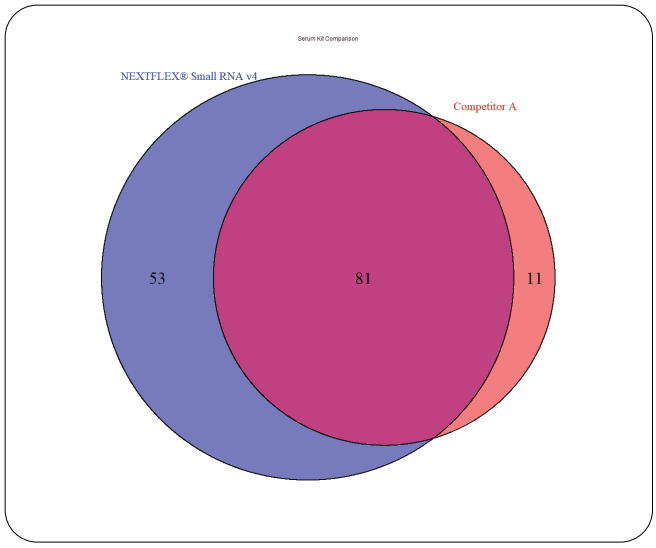


Figure 3. Venn diagram showing that 45% more miRNA were identified using the NEXTFLEX® Small RNA-Seq Kit v4 than Competitor A kit.

We find that 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 foundwith Competitor A and 53 miRNA only found with NEXTFLEX® Small RNA-Seq Kit v4.

Conclusion

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.

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