# Gel-free library prep using miRNA isolated from exosomes.

## Key takeaways

- Gel-free workflow from RNA isolated from exosomes
- Exceptional miRNA discovery

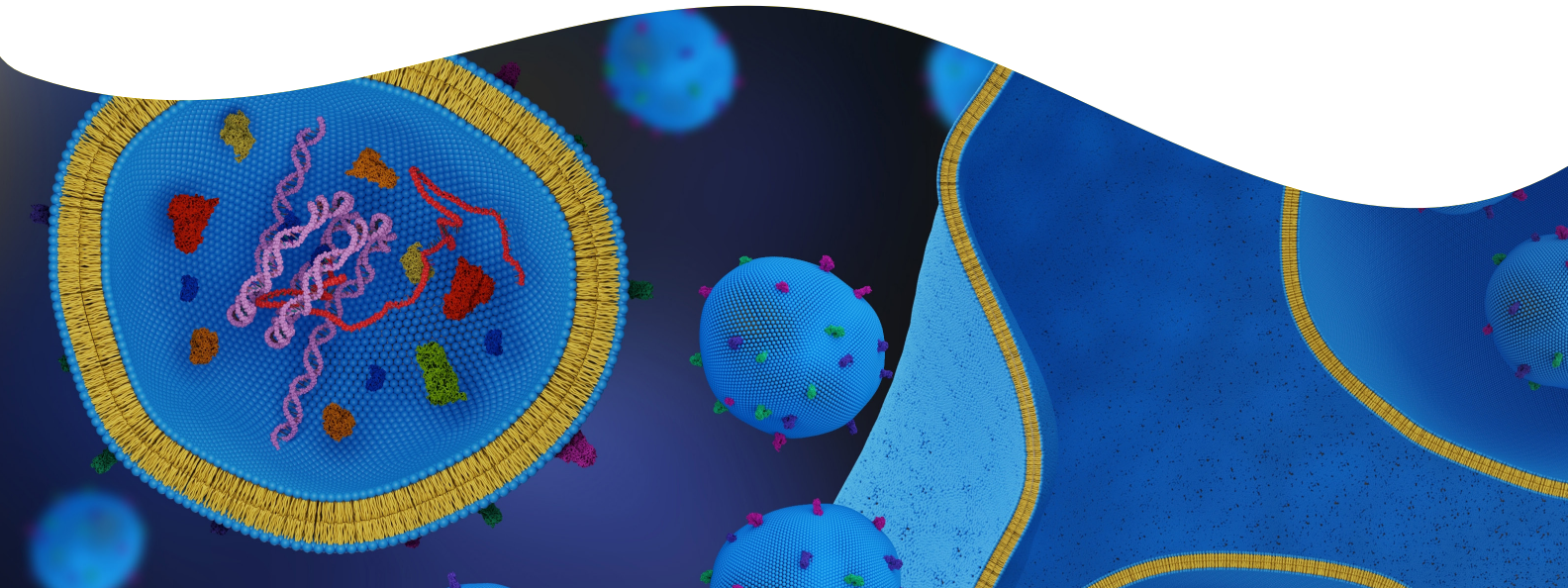
## Introduction

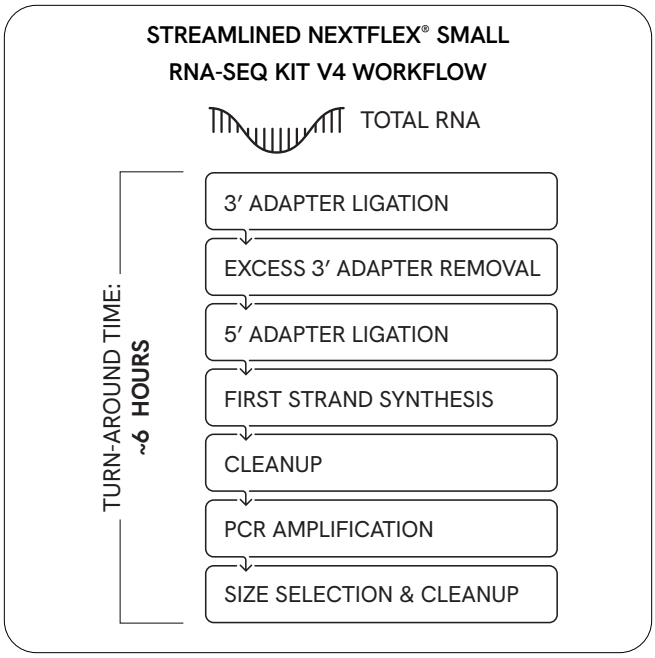
Extracellular vesicles are membrane-bound compartments which are being increasingly recognized as important players in cell-to-cell communication. They are secreted by most cell types and are present in many and perhaps all body fluids, including plasma. Extracellular vesicles are classified in different types according to their size. The most characterized are exosomes, which are 50-200 nm in size. Exosomes transport a cargo of different proteins and RNA (exoRNA), mainly mRNA and miRNA. miRNAs in particular are being studied as potential biomarkers for diseases. The fact that they are present in exosomes make them attractive candidates as biomarker molecule as this means that can be collected easily and in a minimally invasive way[1-3].

There is a need of a convenient method to construct libraries from exoRNA. The amount of RNA obtained from exosomes is low, with just a few picograms corresponding to miRNA. Based on quantitative analysis this can be as little as 1 miRNA molecule/100 exosomes[4], making this a very challenging sample type.

Here, we describe a simplified, commercially available protocol encompassing exoRNA extraction and preparation of exosomal miRNA-seq libraries from serum and bone marrow.

The NEXTFLEX® Small RNA-Seq Kit v4 is a gel-free solution that was shown previously to produce high rates of miRNA mapping and discovery when working with low input samples such as plasma and serum. Here we wanted to verify that NEXTFLEX® Small RNA-Seq Kit can also be used to investigate microRNAs in a population of purified exosomes. Additionally, the protocol is completely automated protocol from library prep to normalization pooling on the Sciclone® G3 NGS/NGSx workstations and Zephyr® G3 NGS workstations.






## Methods

Exosomes from pooled human serum healthy donors and human bone marrow were obtained from System Biosciences. From each sample, 10 µg of purified exosomes was extracted using the NextPrep™ Magnazol™ cfRNA Isolation Kit from **Revvity.** This corresponds to approximately 2x $10^9$ vesicles[5].

12 µL of purified RNA were split into triplicate for sample preparation, equivalent to roughly to 10 pg of input of microRNA per library if we assume 1 miRNA/100 exosomes.

Small RNA libraries were prepared manually using the NEXTFLEX® Small RNA-Seq Kit v4 according to the manufacturer's instructions except the adapters were used at ¼ dilution. Once libraries were prepared, they were quantified with Thermo Fisher® Scientific Qubit® fluorometer, pooled, and run on an Illumina® MiSeq® platform at 1x75 bp read lengths. Small RNA analysis was performed using a **Revvity** custom script. Alignment reference was mature miRNA from mirBase v22.1.

## Results

The NEXTFLEX® Small RNA-Seq Kit v4 was able to generate gel-free libraries for all samples. After sequencing, filtering, and mapping the data, the proportion of reads that aligned with adapter dimer, tRNA, YRNA, rRNA and miRNA for both sample types were determined. Reads mapping to adapter dimer were below 3% in all cases.

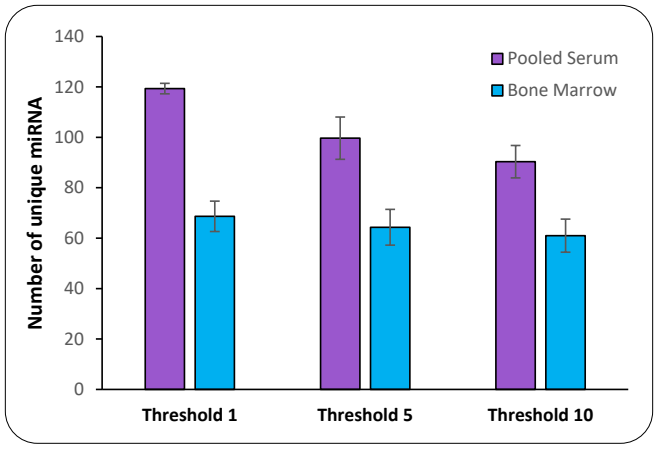



Figure 1: Average number of unique microRNA discovered from each sample

Unique miRNA species were identified and quantified in each sample at different thresholds (Figure 1). Even with the relatively low sequencing depth used in this experiment the diversity of the miRNA found in the human serum samples are in agreement with the values reported in the literature[6].

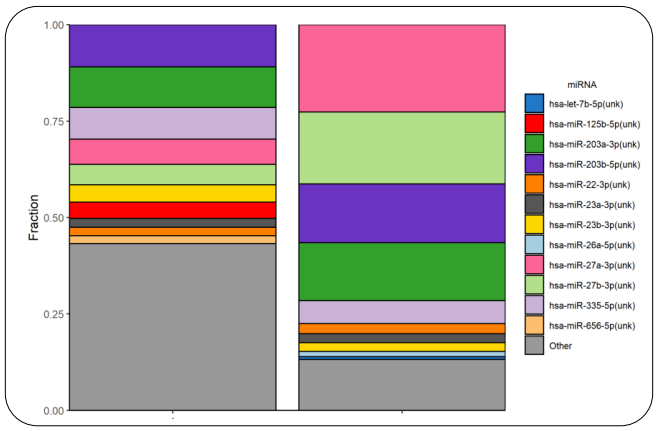



Figure 2: Average top 10 miRNA observed with exosomes from bone marrow and pooled serum samples.

Finally, we looked at the top 10 miRNA expressed on each of the replicates of the exosome samples, to illustrate the differences in the content of each type of exosome (Figure 2).

## Conclusion

In the present study, we presented a simplified, commercially available protocol encompassing exoRNA extraction and preparation of exosomal miRNA-Seq libraries from serum and bone marrow. This convenient gel-free workflow enables the construction of libraries from exoRNA. Using this workflow, researchers can achieve a high number of reads aligning to mature miRNA and low adapter dimers, even with the very low miRNA inputs characteristic of exosome samples.

## References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