Better Small RNA sequencing performance with Unique Molecular Identifiers (UMIs)

Technical Note

Introduction

Small RNAs as non-coding RNA (ncRNA) molecules that are less than 200nt in length[1], are often involved in gene silencing and post-transcriptional regulation of gene expression[2]. As such, small RNA sequencing is a powerful and quantitative tool to study gene regulation and function[3]. However, polymerase chain reaction (PCR) has to be applied to expand small RNA amount for the following sequencing, leading to unequally amplifications[4]. Unique molecular identifiers (UMIs) can be used to distinguish undesirable PCR duplicates derived from a single molecule and identical but biologically meaningful reads from different molecules[3]. UMI small RNA sequencing quantifies small RNAs based on UMI species numbers instead of reads amount. The following figure shows the principle of UMI technology.

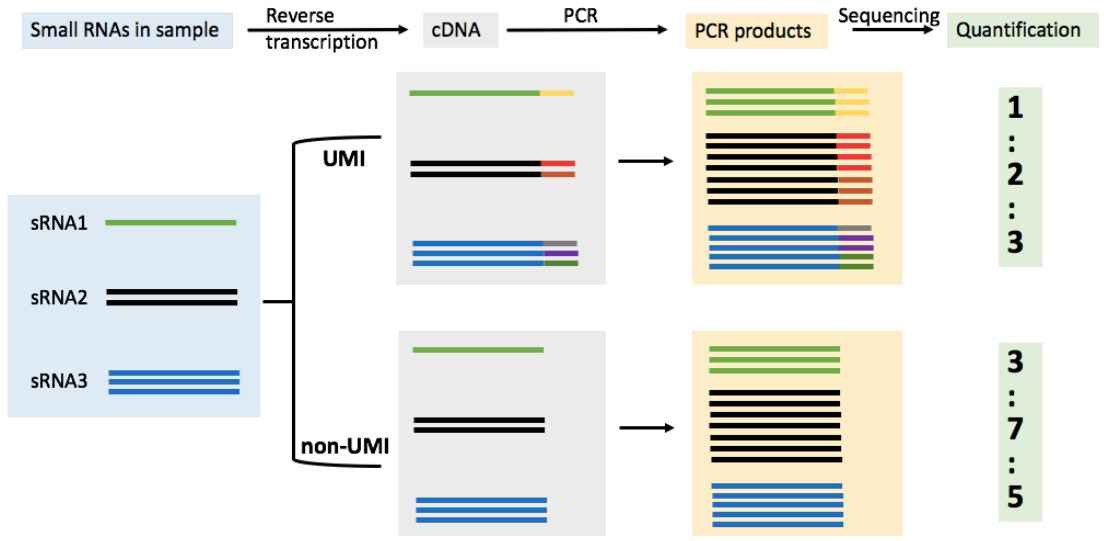

Figure 1. Principle of UMI technology in small RNA sequencing. sRNA: small RNA.

DNBseq UMI Small RNA sequencing introduces UMI during library construction to study the sequence information of small RNA fragments of a certain species in a specific space-time state. This sequencing service enables to achieve absolute quantification, resulting in high accuracy in small RNA identification, prediction,

differential analysis, target gene prediction and functional analysis. Owning to the high accuracy with low input small RNAs achieved by UMI technology, DNBseq UMI small RNA sequencing are especially beneficial to customers doing research on rare and precious samples or samples containing less RNAs such as exosomes.

Highlights

- More precise quantification than standard Small RNA sequencing
- Higher reproducibility and repeatability with quantification by reads
- As Low as 1ng input

Precise quantification

Exosomes derived from human amniotic fluid were isolated using both Life technologies and Qiagen commercial kits with two repeats. Small RNAs were then extracted from the exosomes samples for further library construction using BGI UMI library preparation kits and protocols. The sequencing libraries were sequenced using single-end 50bp sequencing to 20M reads of clean data on the DNBseq platform. Quantification by reads introduces excessive quantitative deviations which increase with the number of reads as shown in Figure 2A. While the accuracy is definitely higher by using UMI technology for RNA quantification. UMI corrects the quantitative bias caused by PCR amplification of more than70% small RNAs (Figure 2B).

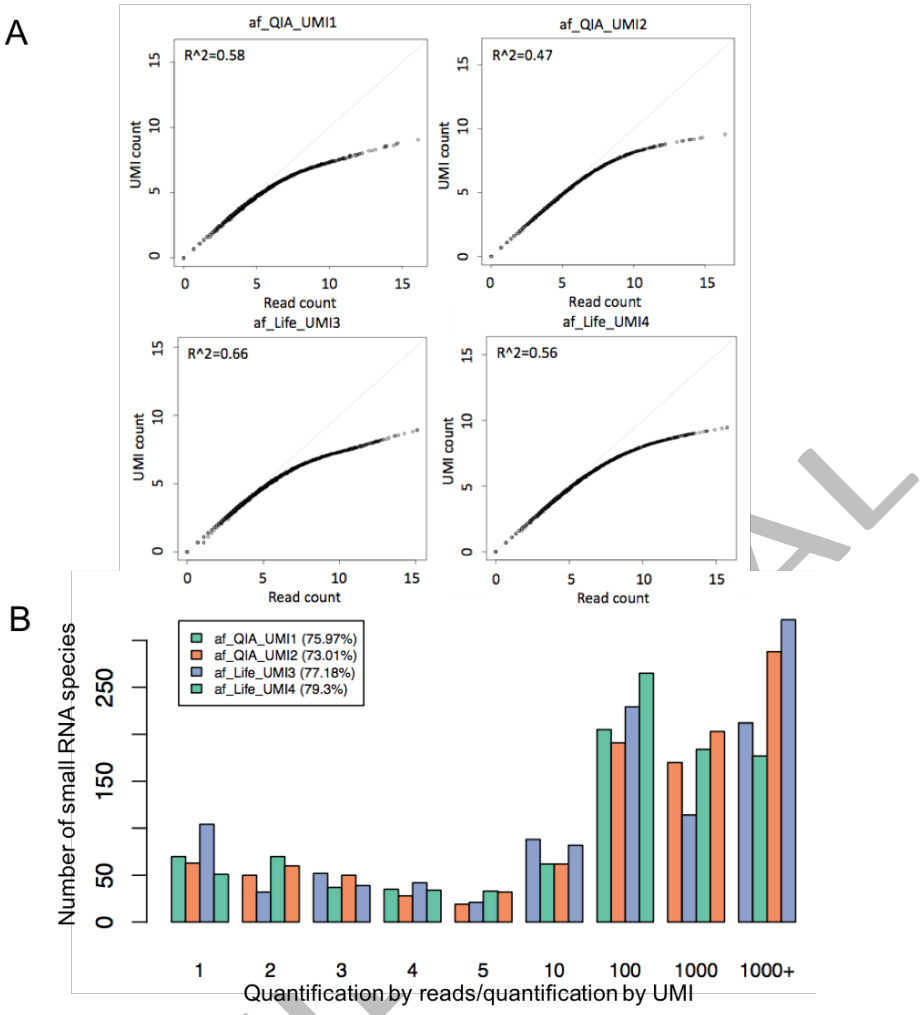

Figure 2. Comparison of reads and UMI quantification methods demonstrates superior quantification precision of UMI technology

High reproducibility and repeatability

The exosomes derived from the same human amniotic fluid sample was extracted with two different kits as mentioned before. The correlation between different extraction methods is about 0.75 in quantification by reads. While the UMI quantitative correlation between different technologies is as high as 0.9 or more, indicating that UMI quantification can eliminate the difference between technologies and be more accurate.

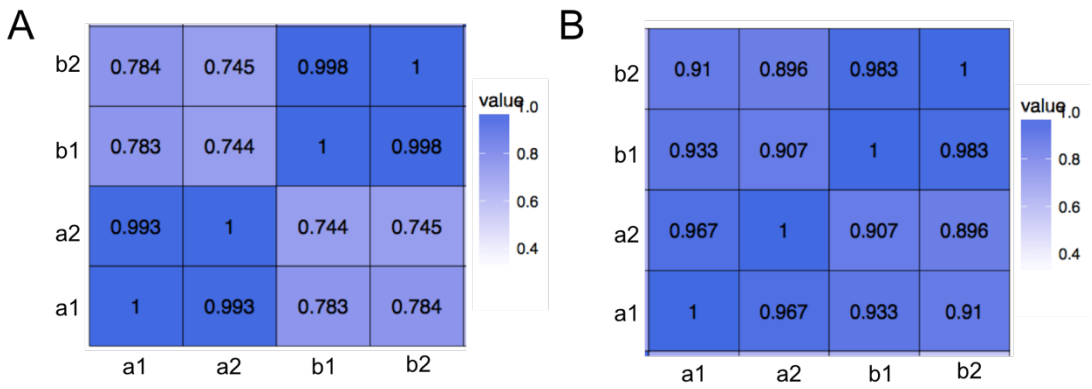

Figure 3. Technology repeatability comparison in reads (A) and UMI (B) quantification methods. a and b represent Qiagen and Life technologies exosome isolation kits respectively.

Lower input and high library success rate

DNBseq UMI Small RNA sequencing can effectively quantify low expression small RNA and the minimum input small RNA amount is 1 ng. The success rate of library construction is over 95%, which is beneficial for research on rare and precious samples.

Conclusion

DNBseq UMI Small RNA sequencing delivers accurate, affordable and high quality sequencing data to support academic and clinical research applications. Combining with UMI technology, DNBseq Small RNA sequencing achieves precise quantification for low expression small RNAs as low as 1ng. Moreover, UMI quantification eliminates the difference between technologies, leading to high quantification accuracy.

References

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3 Fu Y, Wu PH, Beane T, Zamore PD, Weng Z: Elimination of PCR duplicates in RNA-seq and small RNA-seq using unique molecular identifiers. BMC Genomics 2018;19:531.
4 Raabe CA, Tang TH, Brosius J, Rozhdestvensky TS: Biases in small RNA deep sequencing data. Nucleic Acids Res 2014;42:1414-1426.

For More Information

To learn more about our DNBseq UMI Small RNA seuqencing service, other sequencing services or about our DNBseq™ technology, please visit www.bgi.com, email info@bgi-international.com or contact your local BGI representative.

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