Technical Note

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

A validation study report

Introduction

Small RNAs are non-coding RNA (ncRNA) molecules that are less than 200nt in length[1] and 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 amounts for subsequent sequencing, leading to issues with unequal amplification[4].

Unique molecular identifiers (UMIs) can be used to eliminate undesirable PCR duplicates derived from a single molecule. After PCR, molecules sharing a UMI are assumed to be derived from the same input molecule. UMI small RNA sequencing quantifies small RNAs based on UMI species numbers instead of reads amount, leading to more accurate estimates of quantitative small RNA expression[3]. See Figure 1.

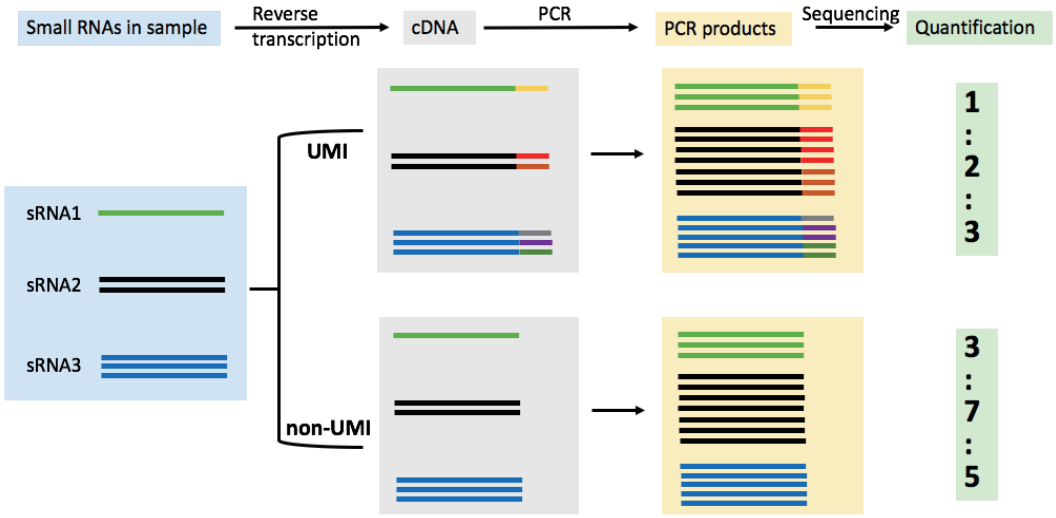

Figure 1. Principle of UMI technology in small RNA sequencing. sRNA: small RNA

DNBSEQ™ UMI Small RNA sequencing combines BGI's proprietary sequencing technology with the above principle, introducing UMIs 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 allows absolute quantification, resulting in high accuracy in small RNA identification, prediction, differential analysis, target gene prediction and functional analysis. The DNBSEQ™ UMI small RNA sequencing is especially beneficial to customers conducting 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 than quantification by counting reads
·As low as 1ng input
·Cost efficient

Case Study

Accuracy

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 data on the DNBSEQ™ technology platform. The data demonstrates that quantification by reads introduces excessive quantitative deviations, which increase with the number of reads, as shown in Figure 2.

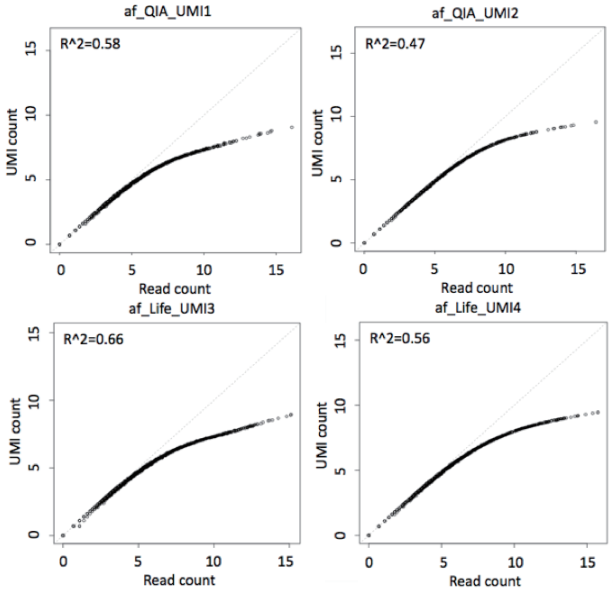

Figure 2. Quantification by reads introduces bias

High reproducibility and repeatability

The exosomes derived from the same human amniotic fluid sample were 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 provide greater accuracy.

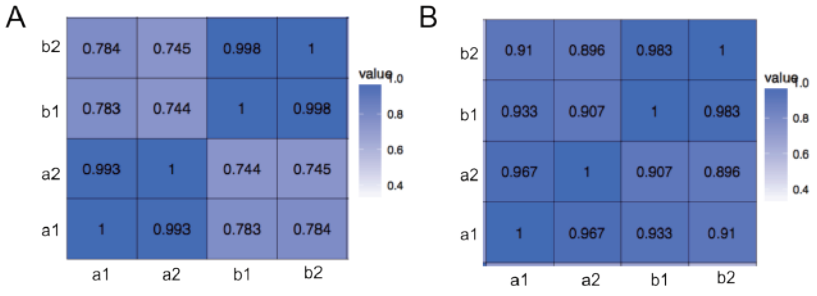

Figure 3. Technology repeatability comparison in reads (A) and UMI (B) quantification methods. A and B represent Qiagen and Life technologies exosome isolation kits respectively.

Lower input and high library success rate

DNBSEQ™ UMI Small RNA sequencing can effectively quantify low expression small RNA, with minimum input small RNA amounts as low as 1 ng for exosomes samples. BGI's experience is that 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 BGI's proprietary technology with UMI methodology, DNBSEQ™ Small RNA sequencing achieves precise quantification for low expression small RNAs as low as 1ng. Moreover, UMI methodology quantification eliminates the difference between technologies, leading to high quantification accuracy.

References

1 Storz G: An expanding universe of noncoding RNAs. Science 2002;296:1260-1263.

2 Neeb ZT, Zahler AM: An expanding world of small RNAs. Dev Cell 2014;28:111-112.

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.

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