Comparison of genomic DNA extraction methods in black tiger shrimp (*Penaeus monodon*) for long-read genome sequencing

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Abstract

Aquatic animals are important to human nutritional and economic needs. The availability of genome sequences will undoubtedly improve the aquatic animal production. However, only few reports of high-quality genome sequence of aquatic animals, especially crustaceans, are available. One of the key challenges for the success of genome sequencing in crustaceans is the difficulty in isolate high quantities of pure, intact, and high molecular weight (HMW) genomic DNA. In this study, five DNA extraction protocols (CTAB, Genomic-tip, Mollusc DNA, TIANamp Marine Animals DNA, and Sbeadex livestock kits) were evaluated for their effectiveness in extracting genomic DNA from black tiger shrimp (*Penaeus monodon*) for long-read sequencing platform. The quality and quantity of the differentially extracted DNA were assessed by NanoDrop spectrophotometer, Qubit fluorometer and pulsed-field gel electrophoresis. Among the five DNA extraction protocols, Genomic-tip kit gave high yielded genomic DNA with the highest quality. To evaluate whether the obtained genomic DNA could be used for the long-read sequencing platform, the DNA samples from top three extraction methods (CTAB method, Genomic-tip and Mollusc DNA kits) were used for Pacific Biosciences (PacBio) sequencing. While the genomic DNA form Genomic-tip and Mollusc DNA kits allowed successful library construction, the genomic DNA obtained from CTAB method did not. The sequencing of genomic DNA obtained from Genomic-tip kit yielded a higher number of long reads (N50 of 14.57 Kb) than those obtained from Mollusc DNA kit (N50 of 9.74 Kb). Therefore, an effective DNA extraction protocol could be further applied for extracting high quality genomic DNA for long-read sequencing of other aquatic animals.

Methods

Collect shrimp muscle

Immediately freeze in liquid nitrogen

Pulverize muscle sample in mortar containing liquid nitrogen

Extract DNA by the following methods:

A) Cetyltrimethyl ammonium bromide (CTAB) method
B) Genomic-tip 100/G kit (Qiagen, Germany)
C) E.Z.N.A. R Mollusc DNA kit (Omega bio-tek, USA)
D) TIANamp Marine Animals DNA kit (Tinagen, China)
E) Sbeadex livestock kit (LGC, Germany)

Purify DNA using AMPure PB bead (Pacific Biosciences, USA)

Check DNA quality and integrity by

- NanoDrop 8000 spectrophotometer
- Qubit dsDNA BR Assay kit
- Pulsed-field gel electrophoresis

DNA sequencing by PacBio sequencing platform

Results

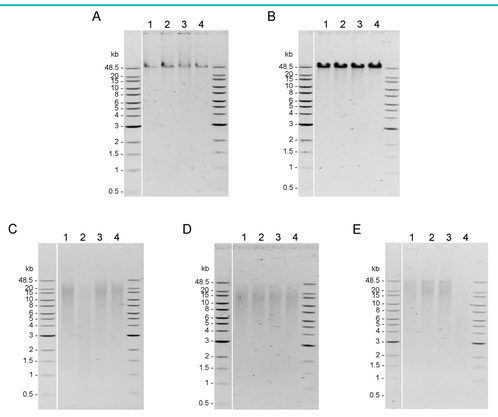

Figure 1. Quality assessment of genomic DNA of shrimp muscle extracted with different DNA extraction methods (n=4), including (A) CTAB method, (B) QIAGEN Genomic-tip 100/G kit, (C) E.Z.N.A. Mollusc DNA kit, (D) TIANamp Marine Animals DNA kit and (E) Sbeadex livestock kit. DNA samples were visualized on 0.75% pulsed-field gel electrophoresis. DNA size marker is Quick-Load 1 kb Extend DNA Ladder (New England BioLabs).

Table 1. Quality of gDNA extracted from shrimp muscle using different DNA extraction methods after purified with AMPure PB bead represented by an average a standard deviation value (SD).

NanoDrop spectrophotometer	Extraction method				
	CTAB	Genomic-tip 100/G kit	Mollusc DNA kit	TIANamp Marine Animals DNA kit	Sbeadex livestock kit
A260/A280	1.40 ± 0.09	1.84 ± 0.01	1.98 ± 0.04	1.63 ± 0.16	1.81 ± 0.03
A260/A230	0.40 ± 0.05	2.45 ± 0.03	2.01 ± 0.16	1.74 ± 0.22	1.85 ± 0.36

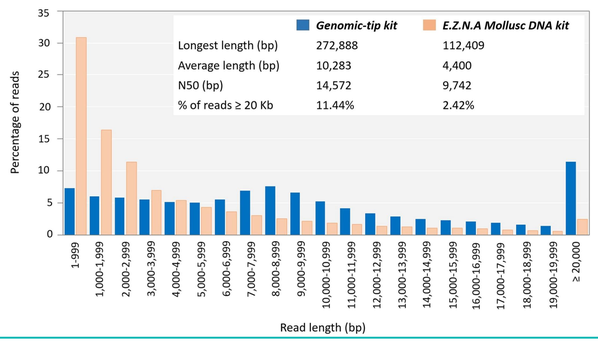

Figure 2. Read length distribution using DNA extracted by Mollusc DNA kit and Genomic-tip 100/G kit.

Conclusion

- The gravity-flow based method (Genomic-tip kit) was found to yield the highest quality genomic DNA and suitable for downstream whole genome sequencing application using the long-read sequencing platform.
- This method was successfully employed to extract high-quality genomic DNA for *P. monodon* resulting in a chromosome-level assembly of its genome.
- DNA extraction protocol could be further applied for extracting high quality genomic DNA for long-read sequencing of other aquatic animals.

References

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Acknowledgement

This project was funded by National Center for Genetic Engineering and Biotechnology (BIOTEC Platform No. P1651718), National Science and Technology Development Agency (TDG Food and Feed program No. P1950419) and supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skaodowska-Curie grant agreement No. 734486 (SAFE-Aqua). PA was supported by Postdoctoral Fellowship from BIOTEC.