Extraction of high-molecular-weight genomic DNA for long-read sequencing of single molecules

PROTOCOL FOR:

Extraction of high-molecular-weight genomic DNA for long-read sequencing of single molecules

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See full protocol online: <https://benchtalk.biotechniques.com/users/8521-biotechniques-editors/posts/12778-protocol-for-extraction-of-high-molecular-weight-genomic-dna-for-long-read-sequencing-of-single-molecules>

Protocol Summary: Third-generation sequencing, with read lengths >10 kb, will improve the assembly of complex genomes, but these techniques require high-molecular-weight genomic DNA (gDNA), and gDNA extraction protocols used for obtaining smaller fragments for short-read sequencing are not suitable for this purpose. Methods of preparing gDNA for bacterial artificial chromosome (BAC) libraries could be adapted, but these approaches are time-consuming, and commercial kits for these methods are expensive. Here, we present a protocol for rapid, inexpensive extraction of high-molecular-weight gDNA from bacteria, plants, and animals. Our technique was validated using sunflower leaf samples, producing a mean read length of 12.6 kb and a maximum read length of 80 kb.

Protocol Schematic: An illustration of the genomic DNA (gDNA) extraction workflow. Key steps and times indicated.