Abstract

Genetics and disease research has often been limited by the technologies and tools available to researchers. Tools such as DNA sequencing have been the primary means researchers employ to attempt to gain a better understanding not only of the form and content of the human genome, but also of the genetic differences that can be found in cases of unusual phenotypes and disease.

The field of DNA sequencing has evolved, growing to include technologies that can provide enormous amounts of sequence data. Certain technologies have struggled to provide data over large or complex regions in particular, and to do so with high accuracy. The past several years have shown tremendous progress in both read length and accuracy. Platforms such as the PacBio Sequel II System and the Oxford Nanopore Technologies © Nanopore-based systems often yield single-base accuracy as well as read lengths in excess of 200,000 bases.

The success of these platforms depends greatly on the ability to isolate and preserve large DNA fragments, commonly referred to as High Molecular Weight (HMW) DNA. Common methods are extremely resource-intensive, costly, or provide DNA that does not perform well in sequencing. In this study we demonstrate a new method that offers improvements in the ability to extract DNA that yields high performance in Long Read Sequencing (LRS) without unnecessary barriers in workflow or reagent cost.

Simple, Gentle Workflow to Preserve HMW DNA

The Promega Wizard® HMW DNA extraction workflow employs a precipitation-based approach with enhancements and proprietary reagents that contribute to maximizing the size of DNA that is obtained. Sample compatibility includes whole bacteria, and plant leaf tissue. Optimization of early processing and lysis steps, maximizing the size of DNA that is obtained.

HMW DNA Size Analysis by PFGE

Following extraction, DNA fragment size was evaluated using Pulsed Field Gel Electrophoresis (PFGE). PFGE gels are useful for approximating the fragment size range of HMW DNA samples, which do not properly migrate or separate using traditional gel electrophoresis methods.

Using the Wizard® HMW DNA extraction method, large fragments were isolated from a variety of samples. DNA fragment size varies by sample type, with fragments in excess of 500kb obtained in some cases. From fresh whole blood, overnight E. coli cultures, and freshly cultured mammalian cells, DNA in excess of 250kb was routinely obtained. The results with spinach are representative of extractions from plant samples, which require freezing with liquid nitrogen and subsequent processing with mortar and pestle. This yields somewhat smaller but still very large DNAs.

Large fragment size analysis by Capillary Electrophoresis (CE)

Differences in extraction method performance can be seen not only on PFGE gels, but also on more precise instruments such as the Agilent® FemoPulse System. The Wizard® HMW kit method is compared to a competing method in the respect below.

High Yields and Purities

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Extraction Technology Comparison

6. Extraction Technology Comparison

Existing commercial HMW DNA extraction kits are frequently used by researchers as a tool to sequence new and open source genome projects. However, the gold-standard gel plug method may come with excessive trade-offs. In the example of the QIAGEN® MagAttract HMW RNA Kit with the Promega Wizard® HMW DNA method, there are differences in not only yield and in the recovered fragment size.

7. Long-Read Sequencing

Physical analysis methods such as PFGE CE, and spectrophotometry can serve as useful in-process checks to assess the quality of an extraction or a sample’s potential suitability for molecular analysis. However, these methods may also fail to detect DNA damage, impurities, or inhibitors that could reduce performance in sequencing.

DNA extracted from samples of E. coli JM109 cells, fresh human blood, and spinach leaf using the Wizard® HMW kit was prepped for sequencing using the standard library prep protocol, and subsequently sequenced on Oxford Nanopore MinION® instrument.

8. Summary

Advances in long-read sequencing, including longer read lengths, higher throughput, and lower costs, are driving the field of genomics to embrace this technology. However, the reagents necessary to solve these problems are still emerging. The Wizard® HMW DNA extraction kit, however, provides researchers with a unique opportunity to take advantage of this technology.

The new Promega Wizard® HMW DNA extraction kit chemistry and method enables researchers to obtain high-mass DNA from a variety of samples that will give strong performance in long-read sequencing. As a cost-effective method, it will broaden the reach of long-read sequencing to a wider audience, including those with limited resources or less experience in extracting HMW DNA.

In subsequent studies, potential sequencing optimizations and sample types will be investigated. Of particular interest is the use of the rapid library prep method, which may better preserve large fragments that were successfully extracted than the traditional ligation-based method which employs bead-based clean-ups that are likely shearing DNA.