

A Novel Approach for Rapid and Reliable Purification of High Molecular Weight Genomic DNA

Genomics

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We have developed a rapid and reliable purification methodology for isolation of ultra-high molecular weight (UHMW) genomic DNA, with fragment sizes into the Mb range for cells, blood, tissue, and bacteria. Key feature of the method is attachment of HMW gDNA to large glass beads. The procedure is fast and requires minimal handling steps, enabling preps to be done in 30 minutes (cells) and 90 minutes (tissues/bacteria). Only standard laboratory equipment is required.

The isolated DNA fragment length is tunable based on the agitation speed employed during lysis. Lower agitation speeds results in Mb-sized DNA, while maximum agitation speeds produce DNA of 50-250 kb, ideal for ligation-based nanopore sequencing. DNA elution from the glass beads is rapid and efficient, the resulting yields are often up to 50% higher than comparable HMW DNA isolation products or silica kits. The optimized chemistry enables the system to product highly pure DNA with A260/A280 ratios 1.80-1.90 and A260/A230 2.1-2.5; Nanopore sequencing with HMW DNA extracted with this method consistently produces N50 values between 35-55 kb depending on the sample type. The extracted DNA enables accumulation of large data amounts; often >10 Gb (Q-scores >13) can be obtained without re-loading the flow cells.

Here, we show examples of the benefits provided by this technology for long read sequencing workflows. This includes demonstration of successful extraction of HMW DNA from very small tissue amounts and low cell inputs, providing extraction solutions even when limited input material is available. Furthermore, we provide a use case showing how this extraction method reduces the workflow time for Cas9 enrichment sequencing-based assessment of transgene insertion sites in mice by up to 3 days. DNA purified using this technology is more easily dissolved and requires significantly less time to “rest” before use, which reduces workflow times regardless of downstream application.