Feb 26, 2021

🌐 High-molecular weight DNA extraction, clean-up and size selection for long-read sequencing

Ashley Jones[1], Cynthia Torkel[1], David Stanley[1], Jamila Nasim[1], Justin Borevitz[1],
Benjamin Schwessinger[1]

[1]Research School of Biology, Australian National University, Canberra, ACT, Australia

1 | Works for me   dx.doi.org/10.17504/protocols.io.bss7nehn

High molecular weight DNA extraction from all kingdoms
Tech. support email: **See@each.protocol**

Ashley Jones
Australian National University

SUBMIT TO PLOS ONE

ABSTRACT

Rapid advancements in long-read sequencing technologies have transformed sequencing read lengths from bps to Mbps, which has enabled chromosome-scale genome assemblies. However, read lengths are now becoming limited by the extraction of pure high-molecular weight DNA suitable for long-read sequencing, which is particularly challenging in plants and fungi. To overcome this, we present a protocol collection; high-molecular weight DNA extraction, clean-up and size selection for long-read sequencing. We optimised a gentle magnetic bead based high-molecular weight DNA extraction, which is presented here in detail. The protocol circumvents spin columns and high-centrifugation, to limit DNA fragmentation. The protocol is scalable based on tissue input, which can be used on many species of plants, fungi, reptiles, insects and bacteria. It is also cost effective compared to kit-based protocols and hence applicable at scale at low resource settings. An optional sorbitol wash is listed and is highly recommended for plant and fungal tissues. To further remove any remaining contaminants such as phenols and polysaccharides, optional DNA clean-up and size selection strategies are given. This protocol collection is suitable for all common long-read sequencing platforms, such as technologies offered by PacBio and Nanopore. Using these protocols, sequencing on the Oxford Nanopore MinION can achieve read length N50 values of 30-50 kb, with reads exceeding 200 kb and outputs ranging from 15-30 Gbp. This has been routinely achieved with eucalypts, acacias, rice, themeda, wheat, wheat rusts, various other fungi, geckos, skinks, ticks, ladybird beetles, caterpillars and E. coli.

CREATED

Feb 26, 2021

LAST MODIFIED

Feb 26, 2021

COLLECTION INTEGER ID

47679

ABSTRACT

Rapid advancements in long-read sequencing technologies have transformed sequencing read lengths from bps to Mbps, which has enabled chromosome-scale genome assemblies. However, read lengths are now becoming limited by the extraction of pure high-molecular weight DNA suitable for long-read sequencing, which is particularly challenging in plants and fungi. To overcome this, we present a protocol collection; high-molecular weight DNA extraction, clean-up and size selection for long-read sequencing. We optimised a gentle magnetic bead based high-molecular weight DNA extraction, which is presented here in detail. The protocol circumvents spin columns and high-centrifugation, to limit DNA fragmentation. The protocol is scalable based on tissue input, which can be used on many species of plants, fungi, reptiles, insects and bacteria. It is also cost effective compared to kit-based protocols and hence applicable at scale at low resource settings. An optional sorbitol wash is listed and is highly recommended for plant and fungal tissues. To further remove any remaining contaminants such as phenols and polysaccharides, optional DNA clean-up and size selection strategies are given. This protocol collection is suitable for all common long-read sequencing platforms, such as technologies offered by PacBio and Nanopore. Using these protocols, sequencing on the Oxford Nanopore MinION can achieve read length N50 values of 30-50 kb, with reads exceeding 200 kb and outputs ranging from 15-30 Gbp. This has been routinely achieved with eucalypts, acacias, rice, themeda, wheat, wheat rusts, various other fungi, geckos, skinks, ticks, ladybird beetles, caterpillars and E. coli.

FILES

Scalable high-molecular weight DNA extraction for long-read sequencing
Version 1
by Ashley Jones, Australian National University

Sorbitol washing complex homogenate for improved DNA extractions
Version 1
by Ashley Jones, Australian National University

DNA clean-up and size selection for long-read sequencing
Version 3
by Ashley Jones, Australian National University