Resource Summary Report

Generated by dkNET on Sep 16, 2026

DNA clean-up and size selection for long-read sequencing

DOI:10.17504/protocols.io.6kahcse
Type: Protocol

Proper Citation

Ashley Jones, Neeraj Purushotham, Jamila Nasim, Benjamin Schwessinger 2019. DNA clean-up and size selection for long-read sequencing. protocols.io dx.doi.org/10.17504/protocols.io.6kahcse

Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.6kahcse

Authors: Ashley Jones, Neeraj Purushotham, Jamila Nasim, Benjamin Schwessinger

Group: High molecular weight DNA extraction from all kingdoms

Summary: DNA extractions often contain impurities which limit the output of long-read sequencing technologies. Here a protocol is provided which removes impurities and size selects for longer fragments. To remove residual RNA and protein, an additional RNAse A and Proteinase K treatment is performed. A clean-up with chloroform: isoamyl alcohol (24:1) removes these proteins and other hydrophobic organics such as lipids. A low volume ethanol precipitation and wash is used to concentrate the DNA, hopefully also reducing polysaccharides. Finally, a Short Read Eliminator (SRE) kit by Circulomics is utilised for size selection, which also appears to clean the DNA. This has been trialled for the sorghum rot fungus Macrophomina phaseolina, providing highly promising results with an Oxford Nanopore MinION. One strain yielded 13.71 Gbases with an N50 of 21.75 kb, another strain yielded 9.72 Gbases with an N50 of 43.50 kb. Similar results are expected across many organisms, but have not been tested.

Affiliations: Australian National University, University of Southern Queensland, Australian National University, Australian National University

Version: 1

Publication Date: 2019

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Record Creation Time: 20210329T220523+0000

Record Last Update: 20210329T220859+0000

Data and Source Information

Source: