

Resource Summary Report

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High quality DNA from Fungi for long read sequencing e.g. PacBio, Nanopore MinION

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Proper Citation

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.k6qczdw>

Authors: Benjamin Schwessinger and Megan McDonald

Group: Solomon Lab - Australian National University, High molecular weight DNA extraction from all kingdoms, Zymoseptoria community protocols (STBnet)

Summary: Extraction of high quality DNA for long read sequencing e.g. PacBio and MinION. Optimized for DNA extraction from *Bipolaris sorokiniana*. Also tested on *Parastagonospora nodorum*, *Zymoseptoria tritici*, wheat stripe rust, barley stripe rust and *Pyrenophora tritici-repentis*. Buffers are best when fresh and not older than 3-6 months. Buffered Phenol:Chloroform:Isoamylalcohol (25:24:1) should not be older than 3 months. Critical steps to obtain high quality DNA: Do NOT heat samples during DNA extractions! Perform all steps at RT or 4°C as indicated. Do NOT incubate samples with KAc for prolonged time periods. Perform two steps of buffered Phenol:Chloroform:Isoamylalcohol purification to reduce co-purifying metabolites. DNA fragments were well above the 40kb mark based on Pippin Pulse Gels. The sequencing center performed a second AMPure purification step before library construction.

Affiliations: Australian National University

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