Resource Summary Report

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Multi-step high purity high molecular weight DNA extraction protocol from challenging fungal tissues

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Protocol Information

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Group: High molecular weight DNA extraction from all kingdoms

Summary: This protocol is based on the fact that we struggled for a long time to obtain high purity DNA from the fungal material, especially from several rust species. We finally thought we made it when we obtained DNA with perfect QC measures using a CTAB based DNA precipitation. Yet we still got low yields caused by high amount of 'active feedback' during the sequencing run. We and many others had similar observations that awesome looking DNA doesn't sequence well (https://www.protocols.io/groups/awesome-DNA-from-all-kingdoms-of-life/discussions/awesome-dna-purity-measures-but-quickly-dying-pores). In this protocol, we combined several ideas we accumulated over the last 1.5 years since we started working on HMW DNA extractions for Nanopore sequencing. -> Different precipitants have different affinities for different contaminants.-> Some contaminants may have a higher affinity and lower solubility in NaCl/PEG/SPRI 'clean' up steps.-> Adding enzyme cocktails during the extraction may help to get rid of some contaminants. We combined all these three steps in the current protocol as combinatorial testing is currently cost prohibitive. It may well be that one of these steps is already enough.These ideas are laid out in more detail below and in publication soon to come. Our general recommendation is to test different buffer conditions and precipitants and if necessary combine them in a sequential manner. We hypothesize that different precipitants, e.g. NaCl/PEG, isopropanol, ethanol, or CTAB, display varying affinities for precipitating different contaminants. By applying them in a sequential manner it may be possible to obtain clean DNA via preferential precipitation of DNA over contaminants. In addition, in this newly developed protocol, we add enzyme mixes to the extraction buffer containing pectinases and cellulases reducing the amount of co-purifying contaminants

from the fungal tissue. In case of other tissue types, different enzymes may have to be tested. It is important to add these enzymes during the extraction and not apply them to the final DNA suspension as most are not completely pure enzyme preparations and contain traces of DNAase activity that degrades the DNA when applied in simple solutions like TE buffer. We (see above) and many others have reported that NaCl/PEG-SPRI bead solutions are not always ideally suited to clean up DNA as contaminants simply co-precipitate. Following a similar logic of preferential precipitation, We hypothesize that is possible to first precipitate contaminants onto SPRI beads at low NaCl/PEG concentrations when HMW DNA stays in solution. In a subsequent step, DNA can be precipitated out of the remaining supernatant by increasing NaCl/PEG concentration adding more of the initial NaCl/PEG-SPRI beads solution. Contaminants with higher affinity to SPRI beads and lower solubility than DNA can thereby be removed from the solution.It is important to mention that we have had DNA preparations that fulfilled all our recommended quality control criteria but did not sequence well on the MinION. This was likely caused by ‘invisible’ contaminants. However, applying a combination of the above-suggested approaches enabled us to overcome this problem with our latest protocol.

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