

Resource Summary Report

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DNA size selection (>3-4kb) and purification of DNA using an improved homemade SPRI beads solution.

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Protocol Information

URL: <https://dx.doi.org/10.17504/protocols.io.n7hdhj6>

Authors: Ramawatar, Benjamin Schwessinger

Group: High molecular weight DNA extraction from all kingdoms

Summary: Most noncommercial tradition DNA extraction protocols result in a crude DNA preparation. If the DNA is intended to be used for a high-end application like Nanopore sequencing, it requires a thorough clean-up and size selection before it could be used for sequencing. Solid Phase Reversible Immobilisation (SPRI) magnetic beads is a quick and convenient way of purifying and size selecting intact double-stranded DNA from crude DNA. Most commercially available SPRI beads based DNA purification mix is quite expensive so our lab endeavored to develop an inexpensive beads mix which is as good as the commercially available ones. In this effort, our lab has optimized a beads mix for purifying and size selecting crude DNA extracted from eucalyptus and posted on protocol.io, <https://www.protocols.io/edit/high-purity-high-molecular-weight-dna-extraction-f-n5ydg7w?step=16>. However, this solution was not very effective in purifying crude DNA extracted from fungal material. DNA extracted from fungal material is highly viscous which is indicative of high levels of impurities in the DNA preparation. I tried to improve the beads mix for purifying rust DNA by adding 0.25 % (v/v) Tween-20 into the beads mix. I tested beads mix with Tween-20 to see if adding tween into the solution makes any difference in the recovery, purity and size selection. Turns out that bead solutions with Tween-20 make big difference in the size selection and recovery of the DNA compared to the bead solutions without. I calibrated/tested the bead solution with and without Tween-20 on the 1 kb DNA ladder to establish which DNA solution to beads volume ratio gives optimal recovery and size selection. We found that beads mix with 0.25 % Tween-20 works much better in size selection and recovery than beads mix without Tween-20. The best DNA to beads volume ratios were 1.0: 0.9 and 1.0: 1.0.

Associated Publications: Schalamun M, Nagar R, Kainer D, Beavan E, Eccles D, Rathjen JP, Lanfear R, Schwessinger B, Harnessing the MinION: An example of how to establish long-read sequencing in a laboratory using challenging plant tissue from . Molecular Ecology Resources 19(1). doi: [10.1111/1755-0998.12938](https://doi.org/10.1111/1755-0998.12938)

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