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Working

Experiment protocol: a syringe-filter based DNA extraction

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

1 Prepare the following solutions
a. TE buffer (10 mM Tris-HCl and 1 mM EDTA)
b. TE/lysozyme (TL) buffer (10 mM Tris-HCl, 1 mM EDTA, and 7.5 mg/mL lysozyme)
c. TE/proteinase K/SDS (TPS) buffer (10 mM Tris-HCl, 1 mM EDTA, 300 µg/mL proteinase K, and 1% SDS[w/v])

2 Filtration
The 10 mL sample or 100ml was passed through a 0.2 µm pore-sized syringe filter in the direction of the filtration.

3 TE buffer addition
A 1 mL of TE buffer was injected into the same filter, still in the direction of the filtration; the flow through was pooled into a sterile 15 mL tube.

4 Backwasing step
Backwashing was done in the opposite direction of filtration with the flow through pooled into the same 15 mL tube; the tubes were mixed by inversions for 5 mins in between each back-washing step:
a. A 1 mL of TL buffer was flushed through the filter then 300 µL of TL was added into the pooled flow-through.
b. Next, 1 mL of TPS buffer was flushed through the filter.
c. Lastly, 1 mL of TPS buffer and 3 times of 1 mL air was flushed and mixed to the flow through.
d. The filter is then discarded

5 Magnetic beads addition
A 50 µL of silica-coated magnetic beads solution (Dynabeads MyOne SILANE, Invitrogen) was added to the flow-through and was mixed by inversions for 5mins.

6 Washing
The beads were magnetized to the tube walls then the supernatant was discarded.

7 DNA elution and collection
a. The DNA was then eluted from the beads with 50 µL of TE buffer.
b. The magnetic beads were collected, and the DNA samples were recovered.
c. The recovered DNA was then diluted 3x with TE buffer to lessen possible inhibitions on downstream applications. The diluted DNA can also be stored in -20^{o}C.