

Mar 03, 2019 Version 2

## Experiment protocol: a syringe-filter based DNA extraction V.2

DOI

[dx.doi.org/10.17504/protocols.io.ysqfwdw](https://dx.doi.org/10.17504/protocols.io.ysqfwdw)

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**Protocol Citation:** Sungwoo Bae 2019. Experiment protocol: a syringe-filter based DNA extraction. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.ysqfwdw>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** March 03, 2019

**Last Modified:** March 03, 2019

**Protocol Integer ID:** 21040

- 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 100µl 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 Backwashing 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

Optional: Washing buffer (1 mL) (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 70% ethanol) was added and mixed by pipetting the mixture several times.

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°C.