

# DNA extraction protocol for low-biomass environmental samples: adapted from the Lucigen MasterPure Complete DNA and RNA Purification Kit manual

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## Method Article

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# Abstract

Microbial samples collected from built environment surfaces can be very low-biomass. This poses a critical challenge to extract valid signals out of the background noises for downstream analyses. Upon comparing and optimizing several extraction methods, our results demonstrated that bead-beating and heat lysis followed by liquid-liquid extraction was the optimal method, as opposed to widely used column- and magnetic bead-based methods. Adapted from the Lucigen MasterPure Complete DNA and RNA Purification Kit manual, this protocol demonstrates detailed points that merit special attention for low-biomass environmental samples.

## Introduction

## Reagents

### Material

- o Ice
- o 1.5 mL sterile microcentrifuge tube (No. = 2×sample No.)
- o O ring tube (Cat# 10831; BIOSPEC PRODUCTS INC)
- o 0.1 MM ZIRCONIA/SILICA BEADS (Cat# 11079101Z; BIOSPEC PRODUCTS INC)
  - 0.2 mL beads for 0.5 mL liquid
  - Fill with 0.2 mL autoclaved PCR tubes
  - Use sterile forceps to handle
  - Fill in biosafety cabinet

### Reagent

- o Isopropanol
- o Lucigen MasterPure Complete DNA and RNA Purification Kit (Cat# MC85200; Lucigen Biosearch Technologies)
- o Invitrogen™ Nuclease-Free Water (not DEPC-Treated) (Cat# AM9937)

## Volume of sample each tube

- o 250  $\mu$ L

## Equipment

1. Eppendorf ThermoMixer
2. Eppendorf centrifuge 5424
3. Mini-Beadbeater-96 (Cat# 1001, BioSpec Products)
4. Fisherbrand™ Analog Vortex Mixer (Cat# 02-215-414)
5. Eppendorf concentrator plus/vacufuge plus

## Procedure

### Do in advance

1. Thaw samples
2. Get ice
3. Set thermomixer to be 65 °C
4. Set centrifuge to be 4°C

### Lysis of samples

Thoroughly mix the various Lysis Solutions to ensure uniform composition before dispensing.

1. Dilute 1.666  $\mu$ L of Proteinase K into 250  $\mu$ L of 2X T and C Lysis Solution for each sample.
2. Transfer 250  $\mu$ L of the fluid sample to an O ring tube (prefilled with beads), add 250  $\mu$ L of 2X T and C Lysis Solution containing the Proteinase K, and mix thoroughly using vortex.
3. Bead-beating lysis for 5 min (no more than 12 tubes each time).

4. Incubate at 65°C for 25 minutes in ThermoMixer; vortex every 5 minutes; set the shaking speed at 300 rpm during the incubation.
5. Centrifuge the tubes at 12,000 x g for 3 min at room temperature.
6. Transfer 350+10 µL supernatant to a new microcentrifuge tube.
  - Use different pipettes (1000 µL + 20 µL) to recover the maximum possible volume.
  - This volume was determined upon testing. This could be adjusted according to sample characteristics.
  - Recommend documenting this volume for future trace-back.
7. Cool the samples to 37°C (just change the temperature of the ThermoMixer to 37°C and wait until 37°C is reached) and add 1.666 µL of 5 mg/mL RNase A to the sample. Mix thoroughly.
8. Incubate at 37°C for 50 minutes, still shaking at 300 rpm.
9. Place the samples on ice for 5-8 minutes and then proceed with total DNA precipitation.

### **Total DNA precipitation**

10. Add 330 µL of MPC Protein Precipitation Reagent to lysed sample and vortex vigorously for 10 seconds.
11. Pellet the debris by centrifugation at 4°C for 18 minutes at 16,000 x g in a microcentrifuge.
  - If the resultant pellet is clear, small, or loose, add an additional 25 µL of MPC Protein Precipitation Reagent, mix, and pellet the debris again.
  - This protocol has already added 80 µL more MPC reagent compared to the kit manual.
12. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet. Use different pipettes (1000 µL + 20 µL) to slowly and gently suction the maximum possible volume while maintaining the pellet intact.
  - Followed by 1000 µL pipette, 20 µL pipette is used because the pellet is easy to be dislodged by the disturbance from the strong suction force of 1000 µL pipette. By using pipettes with a smaller volume, we can recover more liquid while avoiding contaminants in the pellet.
  - In theory, expect ~670 µL of the supernatant, but in practice, I only recovered 600+20 µL supernatant. This volume could be adjusted according to sample characteristics. Recommend documenting this volume for future trace-back.

13. Add 834  $\mu\text{L}$  of isopropanol to the recovered supernatant. Invert the tube 50-60 times.
14. Pellet the DNA by centrifugation (16,000 x g) at 4°C for 18 minutes in a microcentrifuge.
15. Carefully remove the isopropanol without dislodging the DNA pellet, using a pipette.
16. Rinse twice with fresh 70% ethanol, being careful not to dislodge the pellet. Centrifuge briefly in between each washing step (2 min; 16,000 x g). Remove all of the residual ethanol with a pipette.
17. Vacuum centrifuge the sample at room temperature for 2 min under V-AQ mode. The indicator is that the DNA pellet turns from white to transparent.
  - This time could be adjusted. Try adding 1 min (or even 30 s) each time at the beginning of working with a new sample to avoid over-drying.
  - For reference, I vacuum centrifuged 2 min + 1 min for my samples.
18. Resuspend the DNA in 50  $\mu\text{L}$  of DNase-free water. Disrupt the DNA pellet gently by gently flicking the tube. If necessary, leave the DNA at 4 °C overnight to rehydrate.

## Troubleshooting

## Time Taken

## Anticipated Results

## References

1. Lucigen MasterPure™ Complete DNA and RNA Purification Kit manual: [MA110E-MasterPure-Complete](#)
2. Blaustein, R. A., Michelitsch, L. M., Glawe, A. J., Lee, H., Huttelmaier, S., Hellgeth, N., . . . Hartmann, E. M. (2021). Toothbrush microbiomes feature a meeting ground for human oral and environmental microbiota. *Microbiome*, 9(1), 32. doi:10.1186/s40168-020-00983-x.