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Protocol for DNA Extraction from Urine

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Protocol status: Working
We use this protocol and it's working

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Abstract

Introduction. Recent data suggest the urinary tract hosts a microbial community of varying composition, even in the absence of infection. Culture-independent methodologies, such as next-generation sequencing of conserved ribosomal DNA sequences, provide an expansive look at these communities, identifying both common commensals and fastidious organisms. A fundamental challenge has been the isolation of DNA representative of the entire resident microbial community, including fungi.
Materials and Methods. We evaluated multiple modifications of commonly-used DNA extraction procedures using standardized male and female urine samples, comparing resulting overall, fungal and bacterial DNA yields by quantitative PCR. After identifying protocol modifications that increased DNA yields (lyticase/lysozyme digestion, bead beating, boil/freeze cycles, proteinase K treatment, and carrier DNA use), all modifications were combined for systematic confirmation of optimal protocol conditions. This optimized protocol was tested against commercially available methodologies to compare overall and microbial DNA yields, community representation and diversity by next-generation sequencing (NGS).
Results. Overall and fungal-specific DNA yields from standardized urine samples demonstrated that microbial abundances differed significantly among the eight methods used. Methodologies that included multiple disruption steps, including enzymatic, mechanical, and thermal disruption and proteinase digestion, particularly in combination with small volume processing and pooling steps, provided more comprehensive representation of the range of bacterial and fungal species. Concentration of larger volume urine specimens at low speed centrifugation proved highly effective, increasing resulting DNA levels and providing greater microbial representation and diversity.
Conclusions. Alterations in the methodology of urine storage, preparation, and DNA processing improve microbial community profiling using culture-independent sequencing methods. Our optimized protocol for DNA extraction from urine samples provided improved fungal community representation. Use of this technique resulted in equivalent representation of the bacterial populations as well, making this a useful technique for the concurrent evaluation of bacterial and fungal populations by NGS.

Guidelines

This work is best conducted in a positive pressure hood.

Materials

MATERIALS

- 0.1 mm Zirconia/Silica Beads **Bio Spec Products Inc.** **Catalog #**11079101z
- 0.5 mm Zirconia/Silica Beads **Bio Spec Products Inc.** **Catalog #**11079105z
- 1.5 mL Eppendorf tubes
- Proteinase K **Thermo Fisher Scientific** **Catalog #**EO0491
- Lysozyme **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**12671-19-1
- QIAamp® Fast DNA Stool Mini Kit **Qiagen** **Catalog #**51604
- Lyticase **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**L2524-50KU

Enzyme Buffer: (0.5 M Tris, 1 mM EDTA, 0.2% 2-mercaptoethanol, pH 7.5)

Stratec Stool DNA Stabilizer #1038111100 250 ml

Poly (A) Polyadenylic Acid (Sigma-Aldrich #10108626001 (5ul/ml Buffer AL)

Safety warnings

Gloves, lab coat and eye protection

Before start

1. Set heat blocks at 30 and 70 degrees C
2. Label tubes
3. Get ice and place enzymes on ice

1 Spin 30 ml for 15 minutes at 1500 X G at room temperature

2 Pour off supernatant into a separate, 50 ml tube and store at -80 c.Enzymatic Disruption

3 Resuspend pellets in 500 uL Enzyme buffer (0.5 M Tris, 1 mM EDTA, 0.2% 2-mercaptoethanol, pH 7.5) in a 1.5 ml Eppendorf tube

4 Add Enzymes: 100 uL Lyticase (2000 U/ml) and 100 uL Lysozyme (100 mg/ml)

5 Incubate at 30°C for 30 minutes, inverting every 5-10 minutes

6 Centrifuge at 4000 rcf for 5 minutes

7 Remove supernatantMechanical Disruption

8 Resuspend pellet in 800 uL Stool DNA stabilizer

9 Add Beads: 100 uL 0.1 mm beads and 300 uL 0.5 mm beads

10 Bead beat on high for 1 minute

11 Spin briefly at 17,000 X G

12 Bead beat on high for 1 minuteThermal Disruption

13 Heat at 95°C for 5 minutes

14 Vortex for 5 seconds

15 Heat at 95°C for 5 minutes

16 Vortex for 5 seconds

17 Cool on ice for 5 minutes

18 Centrifuge at 17,000 X G for 1 minuteDNA Extraction

19 For each sample, add 350 ml to each of 2 1.5 ml tubes

20 Add 10 ul proteinase K mixture (Qiagen #1017738) to each sample

21 Add 250 ul Buffer AL+Carrier [Poly (A) Sigma-Aldrich #10108626001 10ng/ml] to each sample

22 Vortex for 15 seconds

23 Incubate at 70°C for 10 minutes

24 Add 250 uL 100% EtOH to each sample

25 Mix by vortexing for 5 seconds

26 Transfer mixture from first tube to QIAamp Mini Spin Column (#1011706) and centrifuge at 17,000 X G for 1 minute

27 Change collection tube, transfer mixture from second tube to spin column and centrifuge at 17,000 X G for 1 minute

28 Change collection tube and add 500 uL Buffer AW1 to column

29 Centrifuge at 17,000 X G for 1 minute

30 Change collection tube and add 500 uL AW2 to column

31 Centrifuge at 17,000 X G for 3 minutes

32 Remove residual EtOH by centrifuging an additional 1 min at max

33 Transfer column to new 1.5 mL Eppendorf tube

34 Add 50 uL Buffer AE to column, incubate at RT for 5 minutes and spin at 17,000 X G for 1 minute

35 Transfer fluid from collection tube back to spin column and repeat last step

36 Measure DNA concentration on Nanodrop 2000 Spectrophotometer