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DNA-Extraction-ClostridiaProject

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Protocol used for DNA-Extraction of Clostridia in my project.
DNA-Kit used is GenFind V3 by Beckmann/Coulter.

Some steps used from:
Sim JH, Anikst V, Lohith A, Pourmand N, Banaei N. Optimized Protocol for Simple Extraction of High-Quality Genomic DNA from Clostridium difficile for Whole-Genome Sequencing. J Clin Microbiol. 2015;53(7):2329-2331. doi:10.1128/JCM.00956-15

PROTOCOL CITATION

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Growing and Preparing Clostridia 2d

1 Inoculate Clostridia in 10ml TSB-Broth, incubating at least 48h in anaerobe atmosphere at 37°C 2d

10 mL TSB broth **48:00:00 at 37°C anaerobic**

2 Take 1ml of Clostridia TSB-Broth and centrifuge for 15min at 3,000g, after that remove supernatant 15m

1 mL Clostridia TSB broth **00:15:00 centrifuge at 3000g**

DNA Extraction 1h 32m

3 Add 500ul Lysis-Buffer(LBB) and 30ul Proteinase K, tipmixing or vortexing to resuspend the pellet

500 µl LBB **30 µl Proteinase K**

4 Incubate at 57°C for 1h while continously shaking at ~500/min 1h

01:00:00 at 57°C + shaking 500/min

5 Add 300ul vortexed Black Beads(BBB) and tipmix(at least 10 times), incubate for 5 min at room temperature, place on a magnetic tube stand until no or few beads are left in suspension ~10-15min, then carefully remove supernatant without disturbing the beads-formation 20m

300 µl Black Beads **00:05:00 at room temperature** **00:15:00 magnetic tube stand**

6 Add 800ul WBB and tipmix(at least 10 times), place on a magnetic tube stand until no or few beads are left in suspension ~5min, remove supernatant 5m

800 µl WBB **00:05:00 magnetic tube stand**

7 **go to step #6 for a total of 2 WBB washes**

8 Add 800ul WBC and tipmix (at least 10 times), than add 600ul WBC and tipmix, place on a magnetic tube stand until no or few beads left in suspension ~5min , remove supernatant 5m

1400 µl WBC **00:05:00 magnetic tube stand**

9 **go to step #8 for a total of 2 WBC washes**

10 Add 100ul distilled water, tipmix 10 times, incubate for 2min at roomtemp, tipmix 10 times again and place in a 4°C fridge overnight 2m

100 µl distilled water **00:02:00 room temperature** **Overnight at 4°C**

11 Tipmix 10times and place on a magnetic tube stand until no beads are left in suspension

12 Take the supernatant with your DNA and put it in a fresh tube