



DNA-Extraction-ClostridiaProject V.2

Tobias Wandt¹

¹Universität Leipzig

Version 2 ▾

Oct 24, 2020

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Works for me

This protocol is published without a DOI.

Tobias Wandt

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

Tobias Wandt 2020. DNA-Extraction-ClostridiaProject. **protocols.io**
<https://protocols.io/view/dna-extraction-clostridia-project-bnw3mfgn>
 Version created by Tobias Wandt

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CREATED

Oct 24, 2020

LAST MODIFIED

Oct 24, 2020

PROTOCOL INTEGER ID

43707

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

15m

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

- 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 continuously 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 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 go to next step 2m
place in a 4°C fridge overnight, tipmix 10 times next day
 **100 µl distilled water**  **00:02:00 room temperature**  **Overnight at 4°C**
- 11 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