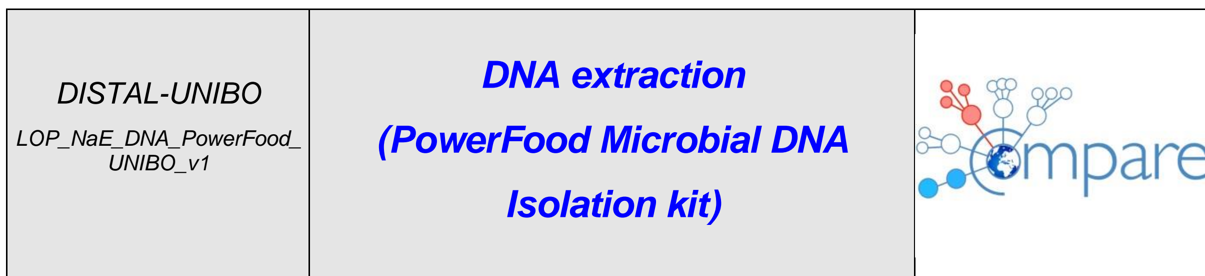

Laboratory Operating Procedure

DNA EXTRACTION PROTOCOL FROM CHICKEN CARCASS WASH-WATER

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Introduction

Whole carcass rinsing (WCR) and neck skin (NS) maceration are the most frequently used sampling methods to detect pathogens from commercially processed broilers in the U.S. and E.U., respectively Becker et al., 2012). In the WCR protocol only 7.5% of the rinsate is often analysed (Becker et al., 2012) and this negatively affects both quantification and detection of foodborne pathogens.
In this protocol all the rinsate is analysed and then DNA is extracted to be further processed by both targeted and shotgun metagenomics analysis.

Sample Material

This step can be performed at the slaughterhouse as well as in the laboratory.

-Aseptically place the carcass in sterile plastic bags and rinse with different volumes of sterile water according to the carcass weight. Carcasses with weights ranging from 2.1 to 2.7 kg must be rinsed with 300 mL of sterile water; carcasses ranging from 1.2 to 1.9 kg must be rinsed with 200 mL of sterile water.
-Vigorously shake the bag containing carcass and sterile water for approximately 1 min.
-Remove the carcass using sterile gloves and aseptically transfer the rinsate into 4 or 6 tubes of 50 ml each according to the total volume of rinsate available. Keep the tubes chilled at 4°C until further processing within 24 hours.

Equipment and Reagents

Successful DNA extraction requires a nuclease-free environment and nuclease-free consumables.

Equipment and consumables

- Refrigerated centrifuge with adaptors for up to 50 ml and up to 2 ml tubes.
- Pipettes 10 µl, 100 µl, 1000 µl
- Sterile and DNA free pipet tips with filters
- Thermal mixer
- Water bath
- Vortex
- Stomacher and/or Pulsifier
- Biospectrometer
- Disposable gloves
- 1.5 and 2 ml sterile tubes
- 15 ml and 50 ml sterile tubes

Reagents

- PowerFood® Microbial DNA Isolation Kit (Mo Bio)

General remarks

The PowerFood® Microbial DNA Isolation Kit (Mo Bio) is designed to process a microbial food culture pelleted by centrifugation and resuspended in lysis buffer. The supernatant is transferred to a bead beating tube containing beads designed for small cell (microbial) lysis and vortex mixed. After the protein and inhibitor removal steps, total genomic DNA is

captured on flat bottom silica spin column and the bound DNA is then washed and eluted from the spin column membrane.

Literature

- https://www.qiagen.com/us/resources/resourcedetail?id=e1b063c8-e0a8-4335-937f-44a9e3e17a6c&lang=en.
- Yang et al., 2016 AEM doi: 10.1128/AEM.00078-16.

Before getting started

- Switch on the thermal mixer at 65°C, the water bath at 55°C and the centrifuge at 4°C.
- Warm the PF1 solution at 55°C for 5-10 minutes.

Procedure

1. Centrifuge the 50 ml tubes containing the rinsate of the same carcass at 6800 rpm for 20 minutes at 4°C.
2. Suspend the pellet of each tube in 5 ml of chilled molecular grade sterile phosphate buffered saline (PBS) and join all the suspensions in one tube corresponding to a single carcass.
3. Centrifuge the tube with the suspensions at 6800 rpm for 20 minutes at 4°C.
4. Suspend the cell pellet in 450 µl of Solution PF1 and vortex the tubes for 30 seconds. Note: Solution PF1 must be warmed to dissolve precipitates prior to use. Solution PF1 should be used while still warm.
5. Incubate the tubes in the thermo-mixer at 65°C for 10 minutes at 300 rpm.
6. Vortex shortly.
7. Transfer the resuspended cells to the MicroBead Tube.
8. Incubate the tubes in the thermos-mixer at 20°C for 10 minutes at 2000 rpm.
9. Centrifuge the tubes at 11900 rpm for 1 minute at room temperature.
10. Transfer the supernatant to a clean 2 ml Collection Tube (provided). Note: Expect approximately 400 µl of supernatant.
11. Add 100 µl of Solution PF2 and vortex briefly to mix.
12. Incubate the tubes at 4°C for 5 minutes and the PF3 solution at 55°C for 5 minutes.
13. Centrifuge the tubes at 11900 rpm for 1 minute at room temperature.
14. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided). Note: Expect approximately 450 µl in volume.
15. Add 900 µl of Solution PF3 and vortex to mix. Note: Check Solution PF3 for precipitation prior to use. Solution PF3 can be used while still warm.
16. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 11900 rpm for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter. Note: A total of two loads for each sample processed is required.
17. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
18. Shake to mix Solution PF4 before use.
19. Add 650 µl of Solution PF4 and centrifuge at 11900 rpm for 1 minute at room temperature.
20. Discard the flow through and add 650 µl of Solution PF5.
21. Centrifuge at 11900 rpm for 1 minute at room temperature.
22. Discard the flow through and centrifuge again at 11900 rpm for 2 minutes to remove residual wash.
23. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
24. Add 100 µl of Solution PF6 to the center of the white filter membrane.
25. Centrifuge at 11900 rpm for 1 minute.
26. Discard the Spin Filter basket. The DNA is now ready for any downstream application. No further steps are required. We recommend storing DNA frozen (-20°C). Solution PF6 contains no EDTA.