nature protocols

https://doi.org/10.1038/s41596-023-00949-x

Protocol

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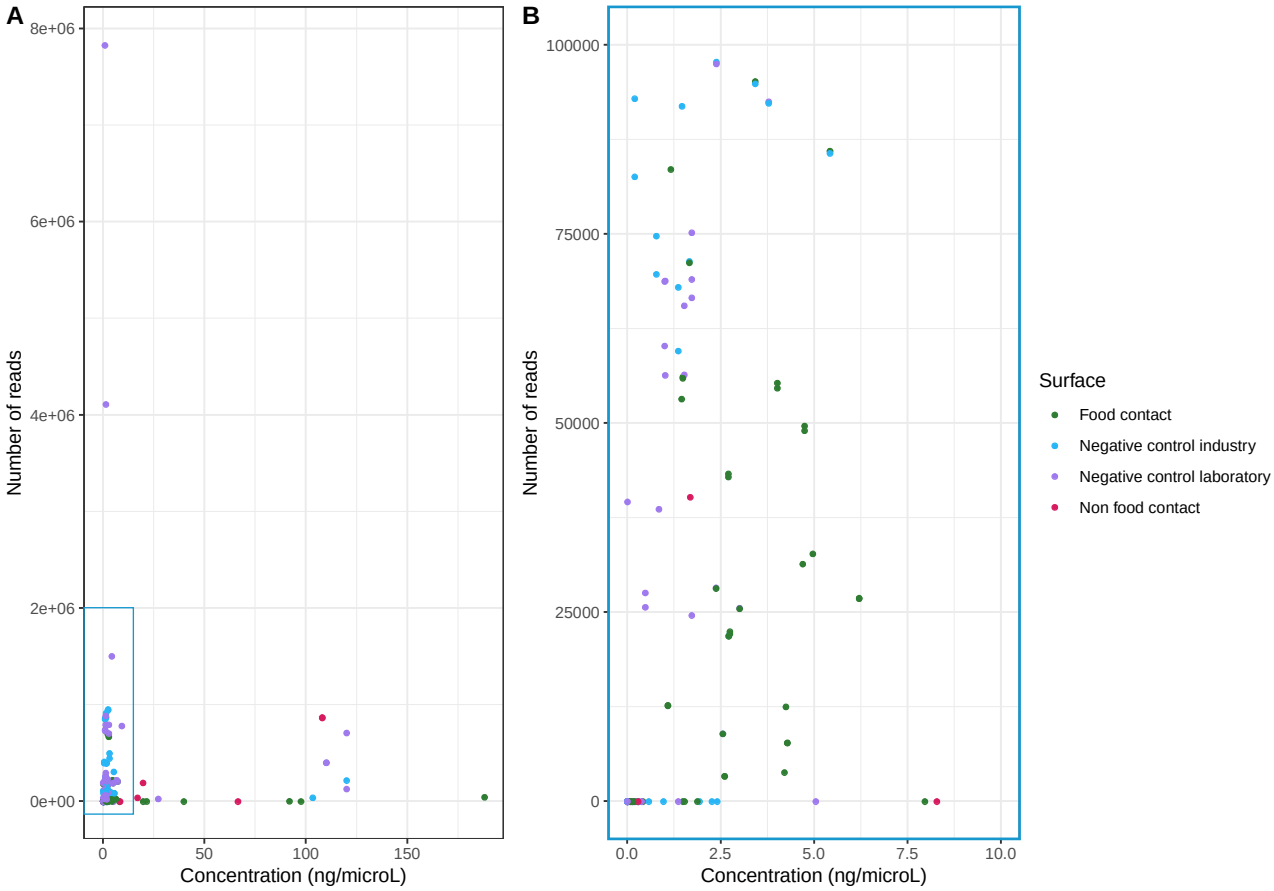
Improved sampling and DNA extraction procedures for microbiome analysis in food-processing environments

In the format provided by the authors and unedited

Supplementary information:



Supplementary Figure 1: Number of reads compared to DNA concentration on those samples from the MASTER program failing sequencing. Dot color indicates the surface where the sample was taken (food contact surfaces, non-food contact surfaces, negative control samples taking in food companies, or negative control samples taken in the lab where sample pre-processing took place). Those samples with 0 reads were not successful on library preparation. B) Zoom overview of the blue rectangle in A).

Supplementary Video: Microbiome mapping in the food industry: detailed visual procedure on how to prepare the materials and take the samples at a food facility environment. Also, the steps that should be followed in the laboratory for sample pre-processing are shown.

Supplementary Note: Detailed information related to the Supplementary Video.

The video shows the steps to follow during the first part of the protocol, from sampling at the food industry (steps 1-5) until the pellet is obtained and ready for DNA purification (steps 6-10). A cheese-making facility is shown, however, the protocol for processing environments has been also applied with some minor modifications for sample manipulation to other food chain samples (raw material or end products), which will be highlighted in this Supplementary Note.

0:10-0:42 - Wear the correct PPE: disposable/lab coat, mop cap, shoe covers, etc. Wash your hands before entering the food processing environment.

0:47-1:09 - All the materials needed for the sampling should be prepared in advance.

1:10-2:11 – Swabbing non food contact samples. For further details about the surface swabbing Supplementary Method is available.

2:12-2:15 – Changing gloves from sample to sample is essential to avoid cross contamination.

2:16-2:58 - Swabbing food contact samples. For further details about the surface swabbing Supplementary Method is available.

2:59-3:04 – Change gloves between sample types.

3:05-3:12 - Liquid samples (such as raw milk, brine and whey in a cheese-making facility) have to be taken in sterile containers. Approximately 200 mL are needed. Other examples of liquid samples could be vegetable soups, beverages. Raw materials and final products should be taken into consideration.

3:13-3:19 - Solid samples (such as cheese in a cheese-making facility) have to be taken in sterile stomacher bags. Other examples of solid samples could be meat batter in a sausage production facility or fruits in a beverage facility. When the sample/food is difficult to carry to the laboratory (e.g., meat carcass) swab the surface of it and process as a surface sample pooling 5 swabs.

3:20-3:35 – Swabbing PPE from operators.

3:36-3:39 – Change gloves between sample types.

3:40-3:51 – A pool of 5 swabs exposed in the bag opened to the air at the industry will be considered as negative control. Each swab included in the pool should be taken evenly from all the rooms inside the facility for a better representative negative sample. The same should be done in the laboratory where samples are processed until DNA extraction.

3:52-4:03 – Keep all the samples refrigerated until the processing (<24 h after the sampling) in the laboratory.

4:04-4:13 – Prepare all the materials for the sample pre-processing.

4:14-5:05 – Add 10 mL of sterile PBS to the pool bag with 5 swabs. Homogenate in a stomacher at 175 rpm for 2 minutes. Recover 10 mL into a plastic tube.

5:06-5:57 - Centrifuge at 5,000 x g for 5 min and discard the supernatant. Keep the pellet.

6:01- 6:18 - Pre-processing of a solid sample (applicable to any type of solid sample). Add 90 mL of sterile PBS to 10 g of sample. Note that the sample need to be aseptically weighted and cut.

6:19-6:47 – Homogenize the sample in a stomacher at 175 rpm for 2 min and transfer the homogenate to 50 mL tubes.

6:48-7:14 – Centrifuge at 5,000 x g for 5 min. Discard the supernatant and keep the pellet.

7:15-7:30 - Wash the pellet by adding 10 mL of sterile PBS, resuspending the pellet and centrifuging again at 5,000 x g for 5 min. Discard the supernatant and repeat untril three serial washes are completed.

7:31-7:53 - Store at -80ºC until DNA extraction.

Note: The video shows the pre-processing of swabs and solid samples. For liquid samples, the liquid would be poured into 50 mL tubes and centrifuged at 5,000 x g for 15 minutes. In case of any compound, such as a fat layer on top, that is not of interest, remove carefully and discard the supernatant. Wash the pellet as following: resuspend the cell pellet with 10 mL sterile PBS and centrifuge again at 5,000 x g for 5 min, discard the supernatant and keep the pellet. Wash the pellet three times. Store at -80ºC until DNA extraction.

Supplementary Methods. Methodology followed for the 16S V4 qPCR.

For 16S V4 qPCR quantification, the following reagents were used:

- UCP PCR Water (QIAGEN: 1109005)
- UCP SYBR Green Real-Time PCR Master Mix 2x (QIAGEN:1116244)
- Primer 515f - 5'-GTGYCAGCMGCCGCGGTAA-3'
- Primer 806r - 5'-GGACTACNVGGGTWTCTAAT-3'm

Each reaction was prepared by mixing:

- 7 µL UCP water
- 10 µL Master Mix (2x)
- 0.5 µL primer F
- 0.5 µL primer R
- 2 µL sample template (previously diluted 1:100 in UCP water).

Genomic DNA from *Escherichia coli* ATCC 100128 serotype O15:H7 was used as control for absolute quantification, prepared with 6 fresh serial dilutions in UCP water prepared from frozen stock, corresponding to $2x10^6$, $2x10^5$, $2x10^4$, $2x10^3$, $2x10^2$ and 10^2 *E. coli* copies (being 7 x *E. coli* copies = 16S copies).

The qPCR program used was:

- Step1 of 95ºC for 2 minutes
- Step2 (x45) of 95ºC for 10 seconds
- 50ºC for 20 seconds
- 72ºC for 20 seconds