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Protocol for metagenomics sampling, storage, and sequencing of human stool

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We use this protocol and it's working

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Abstract

This SOP provides guidelines for optimal human fecal sample collection and conservation practice with the scope of fecal microbiota characterization by shotgun sequencing.

General guidelines for sample processing for shotgun sequencing

1



The protocols provided below are routinely applied by Prof. Nicola Segata Lab and are based on results by the International Human Microbiome Standards (IHMS) consortium.

Before starting a metagenomic project/experiment, a list of considerations and controls must be taken into account:

- Work in a clean environment, meaning cleaning regularly surfaces and equipment with 1% (vol/vol) sodium hypochlorite solution, and/or UV exposure in addition to cleaning with 70% (vol/vol) ethanol.
- When possible, DNA/RNA isolation, PCR amplification/library preparation, and analysis should occur in physically separate areas. Most important is to keep separate the handling of high microbial mass biomass samples (e.g. fecal, sewage, soil) and DNA/RNA isolation to avoid cross-contamination.
- To avoid batch effects, all samples should be extracted around the same time, using DNA/RNA isolation kits and supplies purchased around the same time.
- For each extracted sample, record the date, lot number of the DNA/RNA isolation kit, name of individual who performed the DNA/RNA isolation, and any other aspects that may impact on inferred microbial community composition.
- Perform DNA/RNA-extraction controls, such as i) a DNA/RNA extraction (blank/negative) control in each batch of DNA/RNA-extractions; ii) when using swabs, filters and/or other devices for collecting samples check the DNA/RNA content from unused swabs, filters, or other.

Stool sampling (self-collection) and storage

2

This SOP provides guidelines for optimal human fecal sample collection and conservation practice with the scope of fecal microbiota characterization by shotgun sequencing.

The protocol is based on storing the stool in a stabilizing solution. Our suggested buffer is the DNA/RNA Shield from Zymo research (See below in Storage section for instructions)

Stool sampling (self-collection)

Materials



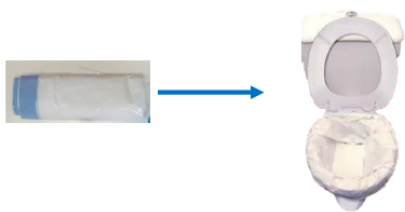
Example of the kit

Kit contains:

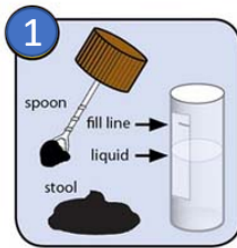
- A stool collection system for toilet bowl
- A tube containing a stabilizer medium and a spoon
- A pair of gloves
- A plastic bag to put the tubes
- An extra brown bag (optional)

2.1 Step 1: Label tubes with the date and time of collection and put on disposable gloves

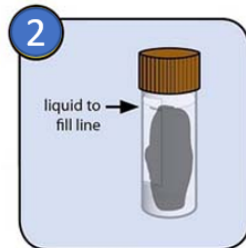
2.2 Step 2: Carefully place the stool collection system on the toilet bowl (instruction on the paper).



2.3 Step 3 : Sample the stool using the sterile scoop and place it into the stool container. The sample should fill up to the label line in a tube with buffer and approximately to half of the tube without buffer (an abundant scoop should be sufficient in both cases).



Collect on plastic wrap and transfer to vial until liquid reaches fill line.



Replace cap on vial tightly and shake for a minute. Place vial in refrigerator until ready to ship.

2.4 Step 4 : Close the tubes tightly and place the tube with buffer in the bag.



2.5 Step 5. Keep samples at -20°C (or at the temperature suggested by the researcher) until delivery.

Storage

- 3 In an appropriate tube for stool collection with an integrated spoon, add 9 ml of the DNA/RNA Shield™. The tube is then ready for use.
- 4 When adding the sample, do not exceed 10% (v/v). Example: 9 ml buffer + 10gr stool (a spoonful of stool is normally enough).
- 5 Samples in the Zymo Research DNA/RNA Shield solution can be stored at different conditions, according to the processing time.
- 6 Here is the sample stability:
RNA: Ambient temperature (4°C- 25°C) >1 month
DNA: Ambient temperature (4°C- 25°C) >2 years
DNA & RNA: Frozen (<-20°C): Indefinitely



DNA Extraction from stool

- 7 The protocol is based on the DNeasy PowerSoil Pro Kit (Qiagen Cat No./ID: 47014, Cat No./ID: 47016). Two main modifications of the kit protocol: i) a quick stool pre-treatment and ii) a smaller volume of the final elution buffer in order to increase DNA concentration (see below).

Materials

- Microcentrifuge (up to 16,000 x g)
- Pipettes (100–1000 µl)
- Vortex Genie
- Vortex Genie Adapter for 1.5 or 2 ml tubes

- 8 Unfreeze the Zymo buffer + Stool (if stored in the freezer).

- 9 Stabilize samples at room temperature.

- 10 Vortex for 30 seconds (Ideally stool must be dissolved).

- 11 Withdraw 600 µl of sample and transfer it to the PowerSoil pro tube (with beads).

- 12 Follow the Qiagen PowerSoil pro kit instructions
(<https://www.qiagen.com/it/resources/resourcedetail?id=11075370-15f5-467a-983a-dc18bacbe542&lang=en>)

- 13 NB:in step 4, transfer all the supernatant and add 200ul of CD2 solution

- 14 IMPORTANT! Final elution volume of C6 is 100 µl + 10 min of incubation before centrifugation

- 15 Quality control of the extracted DNA: use 1% agarose gel

- 16 Quantify the isolated DNA amount using Qubit 1xdsDNA BR Assay kit.
<https://www.thermofisher.com/order/catalog/product/Q33266>



- 17 Nanodrop can be used to determine DNA purity. Bioanalyzer can be used to determine DNA quantity and quality.
- 18 The extracted DNA can be stored at -20°C or -80°C until further use.

DNA Library and sequencing (NovaSeq)

- 19 This SOP aims to standardize the library preparation for Illumina NovaSeq sequencing of metagenomic DNA from stool samples. It is based on Illumina DNA Illumina® DNA Prep (M) Tagmentation (cod. 20060059) Prep reference guide, without any modification. Here is the link to the complete Illumina documentation (<https://emea.support.illumina.com/downloads/illumina-dna-prep-reference-guide-1000000025416.html>).

It might be useful to fill out the Illumina DNA Library Prep Checklist:

<https://emea.support.illumina.com/downloads/illumina-dna-prep-checklist-1000000033561.html>

It is important before sequencing to check the quality of the library with (as recommended in the Illumina DNA Prep reference guide):

- an Advanced Analytical Fragment Analyzer or
- the Agilent 2100 Bioanalyzer with a High Sensitivity DNA kit.

Libraries dilutions should follow the recommended loading concentration defined in the Illumina DNA Prep reference guide (pg 15) and in the NovaSeq 6000 Sequencing System Guide ()

https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/illumina_prep/illumina-dna-prep-reference-guide-1000000025416-09.pdf