Apr 23, 2024

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

DOI
dx.doi.org/10.17504/protocols.io.e6nvw15dwlmk/v1

Federica Pinto[1], Nicola Segata[1]

[1]University of Trento

ASAP Collaborative Rese…

Federica Pinto
University of Trento

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.e6nvw15dwlmk/v1

Protocol Citation: Federica Pinto, Nicola Segata 2024. Protocol for metagenomics sampling, storage, and sequencing of rodent stool. **protocols.io https://dx.doi.org/10.17504/protocols.io.e6nvw15dwlmk/v1**

Protocol status: Working
We use this protocol and it's working

Created: April 23, 2024

Last Modified: April 23, 2024

Protocol Integer ID: 98662

Keywords: ASAPCRN

Abstract

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

General guidelines for samples 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 control 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. Mostly important is to keep separated 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.

Rodent Stool sampling

2 This SOP provides guidelines for optimal rodent fecal sample collection and conservation practice with the scope of fecal microbiota characterisation 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 instruction)

Materials

Kit contains:

- A tube containing a 500 µl of stabilizer medium
- A pair of gloves
- A plastic bag to put the tubes

Step by step procedure
Step 1: Label tubes with date and time of collection and put on disposable gloves
Step 2: Take the rodent stool (3-5 pieces) and put in the tube containing the medium.
Step 3: Short vortex the tube with the sample
Step 4: Store the tube at -20C or -80C.

Storage

3 Materials

- DNA/RNA Shield (Zymo Research R1100-50 / R1100-250 / R1200-25 / R1200-125)
- Tubes for stool collection with integrated spoon
- Or DNA/RNA Shield Fecal Collection Tube (Zymo Research R1101)

Step by step procedure for preparing the tubes with the buffer

- 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.
- When adding the sample, do not exceed 10% (v/v). Example: 9 ml buffer + 1gr stool (a spoonful of stool is normally enough).
- Samples in the Zymo Research DNA/RNA Shield solution can be stored at different conditions, according to the processing time.
- 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

4 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)
- Pipettor (50–1000 µl)
- Vortex
- Vortex Adapter for 1.5 or 2 ml tubes

Step by step procedure

- Unfreeze the Zymo buffer + Stool (if stored in the freezer).
- Stabilize samples at room temperature.
- Vortex for 30 seconds (Ideally stool must be dissolved).

- Withdraw 600 µl of sample and transfer it in the PowerSoil pro tube (with beads).
- Follow the Qiagen PowerSoil pro kit instruction (https://www.qiagen.com/it/resources/resourcedetail?id=11075370-15f5-467a-983a-dc18bacbe542&lang=en)
- NB:in step 4, transfer all the supernatant and add 200ul of CD2 solution
- IMPORTANT! Final elution volume of C6 is 50 µl + 10 min of incubation before centrifugation
- Quality control of the extracted DNA: use 1% agarose gel
- Quantify the isolated DNA amount using Qubit 1xdsDNA BR Assay kit. https://www.thermofisher.com/order/catalog/product/Q33266
- Nanodrop can be used to determine DNA purity. Bioanalyzer can be used to determine DNA quantity and quality.
- The extracted DNA can be stored at -20°C or -80°C until further use.

DNA Library and sequencing (NovaSeq)

5 This SOP aims to standardise 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 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