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IHMS – QUALITY PROTOCOL

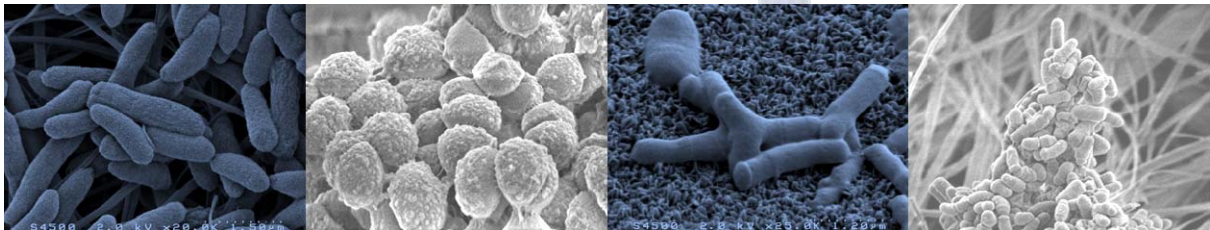
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IHMS SOP 06 V1: STANDARD OPERATING PROTOCOL FOR FECAL SAMPLES

DNA EXTRACTION

Protocol Q



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Introduction

IHMS seeks to coordinate development of standard operating procedures (SOPs) and protocols that will optimize data comparisons in the human microbiome field. The IHMS project concentrates on following objectives:

- Coordinate standardization of procedures and protocols within the existing Human Microbiome research programs and those yet to come,
- Gather and compare the protocols used to collect, identify and process human samples and aid to develop the standard operating procedures for sample collection, identification and processing,
- Compare sequences of genes and genomes of human-associated microorganisms generated by various methodologies and approaches, and to develop standards to define sequence quality and recommend procedures to reach the standards,
- Assess the approaches and procedures used to analyze the sequence data and the associated metadata and recommend standards for data analysis.

Beside these objectives, IHMS project aims at ensuring the optimal public access and use of the data generated by various microbiome projects. The project is supported by the European Commission under the 7th Framework Programme. The consortium gathers 8 partners and 15 contributors across 12 different countries. Its total cost has been evaluated at 2,3 million €, the funding from the European Commission has been set with an upper limit of almost 2 million € and a duration of 4 years, beginning in February 1st, 2011.

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1. OBJECTIVE:

Optimize data comparisons in the human microbiome field by the standardization of the protocol for fecal samples DNA extraction.

This SOP is of first interest for good fecal samples DNA extraction practice in order to characterize the fecal microbiota by metagenomic profiling.

2. PRINCIPLE:

This SOP aims to standardize the fecal samples DNA extraction by giving a step-by-step description of the protocol.

3. RELATED DOCUMENTS:

Titles	Codes	Localization
IHMS quality procedure template	IHMS_INS 01 V1	INRA MGP

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4. Persons entitled to use the protocol:

This SOP applies to any person involved in fecal samples DNA extraction, for optimal fecal sample DNA extraction.

This person can be a trainee, fellow, technician or engineer in charge of fecal samples DNA extraction.

5. Preliminary steps, specificities:

Protocol should be approved by an ethics committee according to national regulations.
Protocol should be declared on international database (e. g. <https://clinicaltrials.gov>).

Volunteers and patients should have signed an informed consent according to approved protocol.

As preliminary steps for this SOP, an inventory of available protocols with IHMS partners and associated laboratories was undertaken. For the preparation of the nucleic acids, aliquots were prepared under anaerobic conditions from appropriately identified and collected samples, with respect to IHMS SOP 01 V1 and IHMS SOP 02, respectively.

Consideration was given to the requirement for high throughput treatment of large sample sets. Thus obtained DNA was analyzed with respect to sequencing standards (IHMS SOP 08, 09 & 10 V1) and sequence data further analyzed with respect to data analysis standards (IHMS SOPs 11, 12, 13 & 14 V1).

Finally, a subset of 3 satisfactory protocols was selected for the assessment of inter-laboratory reproducibility, and only two were validated (IHMS SOPs 06 & 07 V1).

Results of data analysis and comparison between protocols are not yet available at this time.

Moreover, it must be kept in mind that the specific area of nucleic acids preparation does see constant evolutions and improvements, such that it is hardly conceivable to definitely "freeze" a protocol that will be considered as optimal in the long term.

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6. Step by step procedure:

Fecal DNA extraction with the use of Qiagen QIAamp DNA stool kit

1. Homogenize the 150 to 200mg frozen feces with 1.0mL ASL lysis buffer of the kit by vortexing for 2min in a 2mL tube containing 0.3g of sterile zirconia beads Ø 0,1mm zirconia (BioSpec, Cat. No. 11079101z). [if buffer shows precipitate, heat at 70°C before use]
2. Incubate for 15min at 95°C.
3. Cells are mechanically lysed by running the Fastprep™ Instrument for 8min15sec (series of beating 1 min and resting 5 min are preferable).
4. Samples are allowed to cool down on ice for 2min.
5. Samples are centrifuged at 16000 x g, 4°C, for 5min.
6. Supernatant is transferred to a new 2mL tube.
7. The pellet is mixed with 300µL ASL lysis buffer of the kit, and steps 2-5 are repeated.
8. Supernatants are pooled in the new 2mL tube.
9. Add 260µl of 10M ammonium acetate to each lysate tube, mix well, and incubate on ice for 5 min.
10. Centrifuge at 16000 g, 4°C, for 10min.
11. Transfer the supernatant to two 1.5mL Eppendorf tubes, add one volume of isopropanol, mix well, and incubate on ice for 30 min.
12. Centrifuge at 16000 g, 4°C, 15min, remove the supernatant using aspiration, wash nucleic acids pellet with 70 % EtOH (0,5mL) and dry the pellet under vacuum for 3min.

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13. Dissolve the nucleic acid pellet in 100µL of TE (Tris-EDTA) buffer and pool the two aliquots.
14. Add 2µL of DNase-free RNase (10mg/mL) and incubate at 37°C, 15 min.
15. Add 15µL proteinase K and 200µL AL buffer to the supernatant, vortex for 15sec and incubate at 70°C for 10 min.
16. Add 200µL of ethanol (96-100%) to the lysate, and mix by vortexing.
17. Transfer to a QIAamp spin column and centrifuge at 16000 g for 1min, at Room Temperature (RT).
18. Discard flow through, add 500µL buffer AW1 (Qiagen) and centrifuge at 16000 g for 1min, at RT.
19. Discard flow through, add 500µL buffer AW2 (Qiagen) and centrifuge at 16000 g for 1min, at RT
20. Dry the column by centrifugation at RT for 1min.
21. Add 200µL Buffer AE (Qiagen), incubate for 1min at RT
22. Centrifuge for 1min at 16000 g to elute DNA.

Quality control: use 1% agarose gel
Sample concentration: use Nanodrop or Qubit

7. Contacts:

If you have any question regarding this SOP please contact us at:
contact@human-microbiome.org

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