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International Human Microbiome Standards
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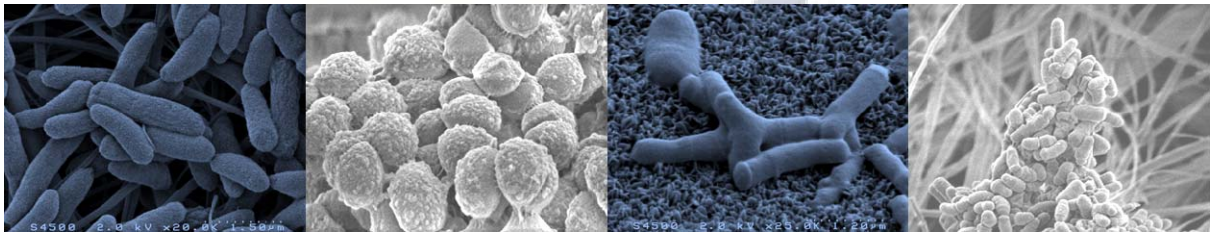
IHMS SOP 07 V1: STANDARD OPERATING PROTOCOL FOR FECAL SAMPLES

DNA EXTRACTION

Protocol H



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© Dore, J., Ehrlich, S.D., Levenez, F., Pelletier, E., Alberti, A., Bertrand, L., Bork, P., Costea, P.I., Sunagawa, S., Guarner, F., Manichanh, C., Santiago, A., Zhao, L., Shen, J., Zhang, C., Versalovic, J., Luna, R.A., Petrosino, J., Yang, H., Li, S., Wang, J., Allen-Vercoe, E., Gloor, G., Singh, B. and IHMS Consortium (2015). *IHMS_SOP 07 V1: Standard operating procedure for fecal samples DNA extraction, Protocol H.* International Human Microbiome Standards. http://www.microbiome-standards.org

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.

AUTHORS/CREDITS: IHMS CONSORTIUM

No	Name	Short Name	Country	Authors
1	INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE	INRA	France	Joël Doré, Florence Levenez, Christel Béra-Maillet, Fabienne Béguet-Crespel, Patricia Lepage, Maxime Rochet, Angélique Doré, Thierry Vanduyvenboden, Sebastian Burz, Charlene Fouquéray
2	INSTITUT CATALA DE LA SALUT	HUVH	Spain	Francisco Guarner, Chaysavanh Manichanh, Alba Santiago et al.
3	BAYLOR COLLEGE OF MEDICINE	BCM	United States	James Versalovic, Ruth Ann Luna, Joseph Petrosino et al.
4	SHANGHAI JIAO TONG UNIVERSITY	SJTU	China (People's Republic of)	Liping Zhao, Jian Shen, Chenhong Zhang et al.
5	COMMISSARIAT A L'ENERGIE ATOMIQUE ET AUX ENERGIES ALTERNATIVES	CEA Genoscope	France	Eric Pelletier, Adriana Alberti, Laurie Bertrand, Karine Labadie, Céline Orvain
6	BEIJING GENOMICS INSTITUTE AT SHENZHEN	BGI Shenzhen	China (People's Republic of)	Huanming Yang, Songgang Li, Jun Wang et al.
7	EUROPEAN MOLECULAR BIOLOGY LABORATORY	EMBL	Germany	Peer Bork, Paul I. Costea, Shinichi Sunagawa, Sean Powell, Julien Tap, Tomas Larsson, Alison Waller, Kalliopi Trachana, Pablo Minguez Paniagua, Jaime Huerta Cepas, Samy Deghou, Tobias Doerks
8	THE UNIVERSITY OF WESTERN ONTARIO	Western	Canada	Emma Allen-Vercoe, Greg Gloor, Bhagirath Singh et al.

CONTRIBUTORS/CREDITS: IHMS WP2 PARTICIPANTS

Name	Short Name	Country	Authors
Laboratory of Microbiology Wageningen University	WUR	The Netherlands	Erwin Zoetendal, Hans Heilig, Ineke Heikamp-de Jong, Willem de Vos et al
University College Cork	UCC	Ireland	Paul O'Toole, Jenifer Deane, Eibhlis O'Connor et al
Merieux NutriSciences, Nantes	Biofortis	France	Françoise Le Vacon, Thomas Carton et al
University of Helsinki, Faculty of veterinary Medicine	UH	Finland	Anne Salonen, Outi Immonen, Laura Huuskonen et al
University of North Carolina, Kannapolis	UNC	USA	Ryan Balfour Sartor, Melanie Spencer et al
University of North Carolina, Chapel Hill	CGIBD UNC	USA	Ryan Balfour Sartor, Ian Carroll et al
Statens Serum Institute, Copenhagen	SSI	Denmark	Soren Persson, Joan N Jensen et al
University of Florida, Gainesville	UF	USA	Volker Mai, Maria Ukhnova et al
INRA Micalis Jouy-en-Josas	INRA	France	Philippe Langella, Jean-Pierre Furet et al
University of Tokyo & Azabu University	UniT	Japan	Masahira Hatori, Hidetoshi Morita et al
Medical University Graz	MUG	Austria	Ingeborg Klymiuk et al
Maastricht University Medical Center	EPID	The Netherlands	John Penders et al
University fo British Columbia, Vancouver	UBC	Canada	Brett Finlay, Marie Claire Arrieta, Lisa Thorson et al
Rowett Institute of Nutrition and Health, University of Aberdeen	RINH	Scotland, UK	Harry Flint, Karen Scott, Petra Louis et al
Danone Research, Palaiseau	Danone	France	Johan van Hylkama Vlieg, Tamara Smokvina, Anne Druesne et al
German Institute of Human Nutrition, Potsdam Rehbruecke	DIfE	Germany	Michael Blaut, Kathleen Slezak, Delphine Saulnier et al



Summary

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.

3. RELATED DOCUMENTS:

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

4. Persons entitled to use the protocol:

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

This person can be a technician or the 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.

6. Step by step procedure:

Fecal DNA extraction IHMS Protocol H
(see annex for preparation of solutions and suggested suppliers)

Day 1:

1. Add 250µL Guanidine Thiocyanate to each tube containing frozen faeces (~200mg)
2. Add 40µL of N-lauroyl sarcosine 10% and thaw.
3. Then 500µL of N-lauroyl sarcosine 5% and vortex to mix well.
4. Incubate 70 °C in dry bath for 1 hour.
5. At the end of incubation, add 750mg of glass beads (0.1 mm) in each tube and vortex vigorously.
6. Shake for mechanical disruption:
   - with Bead Beater™ :
     - Turned on (medium speed) for 5min.
     - Stoped for 10min, and again
     - Turned on (medium speed) for 5min.
   - with MixerMill MM400 (Retsch) :
     - Balancing tubes for symmetry
     - Run on Program 1, 10min at 25 agitations per second
7. Add 15mg of PVPP (powder) per sample and vortex vigorously.
8. Centrifuge at 14000rpm (18000 g) for 5min, 4°C.
9. Recover the supernatant in a sterile tube graduated 2mL and set aside.
10. Add 500µL of TENP (resuspend before use) to the pellet and vortex vigorously.

11. Centrifuge at 14000rpm (18000 g) for 5min, 4°C.
12. Recover the supernatant and pool with the first.

13. Repeat washing operation of the pellet with TENP twice (3 washes in total)

14. Centrifuge the tube graduated 2ml containing supernatants at 14000rpm (18000 g) for 5min, 4°C

15. Dispense evenly supernatant in 2 Eppendorf tubes graduated 2mL (making duplicates ; i.e. 2 tubes / sample).

16. Add 1 mL of isopropanol (propanol-2) in all tubes and mix gently by inversion.

17. Leave for 15 minutes at room temperature and centrifuge 14000rpm (18000 g) for 10min, 4°C.
18. Remove the supernatant
19. Dry the tube by carfully inverting on a paper towel (careful not to lose the pellet).

20. Add 450µL of phosphate buffer and 50µL of potassium acetate in one of the duplicates, and transfer the pellet and the solution into the second duplicate.

21. dissolve the pellets by gentle aspirations with a P200 pipette

22. Leave at least 1h30’ on ice.
23. Centrifuge at 14000rpm (18000 g) for 30min, 4°C.

24. Recover the supernatant containing the DNA in a 2mL Eppendorf tube.

25. Add 2µL of RNase (10mg/ml) / Vortex / give a quick spin.
26. Incubate 30min at 37°C.

27. Add 50µL sodium acetate and 1 mL of 100% fresh ethanol (stored at -20°C). Mix gently by inversion.

Leave overnight at -20°C.

Day 2:

28. Centrifuge at 14000rpm (18000 g) for 30min, 4°C and remove ethanol.
29. Make a first wash with 1mL 70% ethanol ; free pellet by flicking the tube a few times
30. Centrifuge at 14000rpm (18000 g) for 5min, 4°C and remove 70% ethanol.
31. Make a second wash with 500µL 70% ethanol ; free pellet by flicking the tube a few times
32. Centrifuge at 14000rpm (18000 g) for 5min, 4°C and remove ethanol.
33. Remove residual ethanol by inverting the tube on a paper towel.
34. Dry the DNA pellet 10min under laminar flow hood.
35. Dissolve the pellet with 200µL of TE.

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

Annex. Preparation of solutions

Phosphate buffer pH 8, 1M

- 9,32 mL Na2HPO4 : 14,2 g for 100mL H_2O (dissolve on heating stirrer)
- 0,68 mL NaH2PO4 (1M) : 12 g for 100mL H_2O
- 90 mL H_2O

Check pH = 8 with pH paper

EDTA, 2 H2O pH 8, 0.5M

9,305g qs for 50 mL H_2O (dissolve by heating)
Adjust to pH 8 with NaOH pellet (approximately one) using a pH meter

Tris-HCL (pH 7.5, 1M or pH 8.0, 1M)

6,05 g Trizma base qs for 50mL H_2O.
Adjust to pH 7.5 or 8.0 with concentrated HCL using a pH meter

TENP(50 mM Tris pH8, 20 mM EDTA pH8, 100 mM NaCl, 1% of PVPP)

- 1,5 mL Tris-HCL pH 8, 1M
- 1,2 mL EDTA pH 8, 0.5M
- 0,6 mL NaCl, 5M
- 0,3 g PVPP (attention, will not dissolve)
- qs for 30ml H_2O.

Guanidine Thiocyanate 4M

-12,37g guanidine thiocyanate in a Falcon tube, manipulation under hood (careful with toxicity)
- 13,5 mL H_2O
- 2,6 mL Tris-HCL 1M pH7.5

Shake overnight on a rocking agitator: in closed flacon, protected from light by aluminum foil
Completing to 26.1 mL of H_2O
Heat in Dry bath or in an oven at 60-70 ° C for 10min (if not totally dissolved)
Filter through 0.2 microns Millipore filter
Store at 4°C protected from light

NaCl 5M

14.6g qs for 50mL H_2O (in a Falcon tube)

N-Lauroyl Sarcosine 10%

2g for 20mL H_2O (in a Falcon tube)

N-Lauroyl Sarcosine 5%

1g for 20mL phosphate buffer pH8, 0,1M (in a Falcon tube)

TE pH 8 (10mM Tris.HCl pH8, 1mM EDTA pH8)

200µl Tris HCL 1M pH8
40µl EDTA 0,5M pH8
qs for 20mL H_2O (in a Falcon tube)

Potassium acetate (5M for Acetate , 3M for potassium)

29,44g Potassium acetate
+ 11,5 mL Glacial acetate
+ 28,5 mL H_2O
qs for 100 mL with H_2O

Sodium acetate 3M

12,304g in 40mL d' H_2O (in a Falcon tube)
Adjust pH at 5,2 with Glacial acetate
qs for 50 mL with H_2O

Rnase (Ribonuclease) 10mg/mL

7. Contacts:

If you have any question regarding this SOP please contact us at:

contact@human-microbiome.org