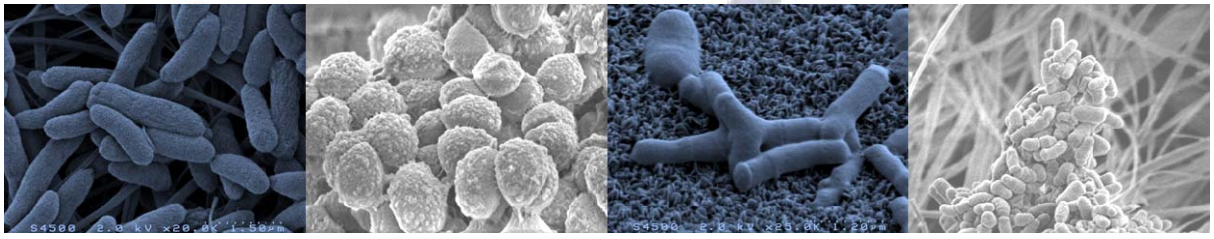
IHMS – QUALITY PROCEDURE

International Human Microbiome Standards

http://www.microbiome-standards.org

IHMS SOP 08 V1: STANDARD OPERATING PROCEDURE FOR DNA SAMPLE QUALITY CONTROL



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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.

SEVENTH FRAMEWORK PROGRAMME

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Summary

1. OBJECTIVE:

Optimize data comparisons in the human microbiome field by the standardization of the procedure for sequencing metagenomic fecal samples.

This SOP is of first interest for efficient and reproducible library preparation for Illumina sequencing of metagenomic samples.

2. PRINCIPLE:

This SOP aims to standardize quality control of DNA extracted from fecal samples.

3. RELATED DOCUMENTS:

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

4. Persons entitled to use the procedure:

This SOP applies to any laboratory personel involved in evaluation of the quality of DNA extractions issued from fecal samples.

5. Conditions and usage constraints to follow:

1-Avoid Cross-Contamination- Pipettes and work surfaces must be cleaned before and after the procedure

2- Handling Liquids - Ensure that pipettes are correctly calibrated. Prepare reagents for multiple samples simultaneously to minimize pipetting errors

6. Materials, products, kits, solutions:

Materials:

Materials	Risks	Prevention	Suppliers Names and references
Mini horizontal device 15-wells combs	NA*		Biorad, ref :1704465
Mini horizontal Gel electrophoresis device with 7x10 cm tray	NA*		Biorad, ref : 1704466
Gel imager system	NA*		General lab suppliers
Qubit™ fluorometer 2.0	NA*		Life Technologies, ref. Q32866
1000 µl single chanel pipette	NA*		General lab suppliers
200 µl single chanel pipette	NA*		General lab suppliers
100 µl single chanel pipette	NA*		General lab suppliers
20 µl single chanel pipette	NA*		General lab suppliers

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10 µl single chanel pipette	NA*	General lab suppliers
Vortexer	NA*	General lab suppliers
Microcentrifuge for individual 1.5 ml and 0.2 ml tubes	NA*	General lab supplier

Risk and Safety statements

Kits:

Materials	Risks	Prevention	Suppliers Names and references
Quant-iT™ dsDNA BR assay kit	 H227		Invitrogen, ref : Q32850
Quant-iT™ dsDNA HS assay kit	S26 S36/39		Invitrogen, ref : Q32851

Risk and Safety statements: H227: Flammable liquids - Category 4

S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S36/39 - Wear suitable protective clothing and eye/face protection

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Products :

Products	Risks	Prevention	Suppliers Names and references
Seakem Agarose	NA*		LONZA, ref : 50074
5x TBE buffer	NA*		SIGMA, ref : T6400
SYBR® Safe DNA gel stain (10,000X concentrate in DMSO)	 H302 H315 H320 H340 H341	 Specific chemical waste	Invitrogen, ref : S33102
5x loading dye	NA*		General lab supplier

RNAse A 100 mg/ml	 Xn Harmful R 42/43		Qiagen, ref: 19101
0.1x TE buffer			General lab supplier
Resuspension buffer (10mM TrisHCl, pH 7,5)	NA*		General lab supplier
DNA molecular weight marker II (0,1 – 23 kb)	NA*		Roche, ref : 10236250001

Risk and Safety statements: H302 - Harmful if swallowed
H315 - Causes skin irritation
H320 - Causes eye irritation
H340 - May cause genetic defects
H341 - Suspected of causing genetic defects
R 42/43 May cause sensitisation by inhalation and skin contact

7. Step by step procedure:

Overview

This protocol describes how to evaluate the quality and quantity of genomic DNA samples using a standard agarose gel as well as Qubit™ fluorometer.

The protocol includes two steps:

- **Quantity evaluation**: quantification by two independent measures by Quant-iT dsDNA BR Assay kit is performed. A mean concentration is calculated. For library preparation protocol, 250 ng input DNA are required. If total DNA quantity is less than 500 ng (2 fold the minimal quantity), the sample is not valid and the quality control (QC) ends at this stage.

- **Quality evaluation**: samples are loaded on a 0,4 % agarose gel and migration is performed at 100V during one hour. A photo is taken and quality of DNA is visually checked. If RNA contamination is present, an RNAse treatment is applied to the sample, after which the sample repeats the QC from the beginning. DNA integrity is visually checked. DNA passes the QC, if the majority of the DNA is located on a tight band at high molecular weight.

Procedure

Upon arrival, store the sample at –20 °C until use.

STEP 1: gDNA quantitation using Qubit™ fluorometer

Use the Quant-iT™ dsDNA BR assay kit following the manufacturer instructions for use of the kit and the Qubit fluorometer.

1.1) Perform two independent measurements using 1 µl of the DNA sample for each measure.

1.2) Calculate the mean concentration in ng/µl.

STEP2: gDNA integrity check by agarose gel electrophoresis

All reagents and stock solution should be prepared prior to the start of the procedure.

2.1) Gel & Sample Preparation

2.1.1) Cast a ~40ml 0,6% Seakem agarose gel with 1X TBE and 10 µl SYBR® Safe DNA gel stain (10,000X concentrate in DMSO). Use a narrow well comb.

2.2.2) For each sample to be tested prepare two clean labeled tubes

Tube 1: transfer 1µl DNA and complete with 5 µl H_2O and 2µl 5x loading dye

Tube 2: prepare a 1:10 dilution of the initial sample in TE buffer and use 1 µl of the dilution. Complete with 5 µl H_2O + 2µl 5x loading dye

2.2) Gel Electrophoresis

2.2.1) Load the gel by leaving an empty well between two samples. Load 100-150 ng of the DNA molecular weight marker II in the two wells located on the left and right edges of the gel

2.2.2) Run gel for 30 min at ~100V in 1X TBE buffer.

2.2.3) Remove gel from gel box and image.

This first image capture allows to better evaluate the presence of RNA contamination

2.2.4) Return gel to gel box and run again for 30 min at 100V

2.2.5) Remove gel from gel box and image

2.3) DNA QC Gel Analysis

Evaluate genomic DNA integrity and RNA contamination

a) **RNA contamination**

If RNA is massively present in the sample (visible as a cloud at < 1 kb and /or two bands at at ~ 5kb and 1,8 kb corresponding to rRNA), treat the initial sample with RNAse A

1) Add 1 µl RNAse A for each 100 µl sample
2) Incubate 90 min at 37 °C
3) Reload 1µl of the treated sample on the gel.
4) If RNA has disappeared, perform a new quantification by Qubit fluorometer as previously described. If RNA is still present, retreat sample with RNAse A.

b) **DNA integrity**

How to evaluate DNA integrity?

The majority of DNA should appear as a tight band > 23 kb. If a smear is present, this means that DNA is partially degraded. If no tight high molecular weight band is visible and DNA is present only in the smear, the degradation is massive and DNA is not suitable for sequencing.

If a quantification software system is available, refer to the software instructions for analyzing DNA quality on gels.

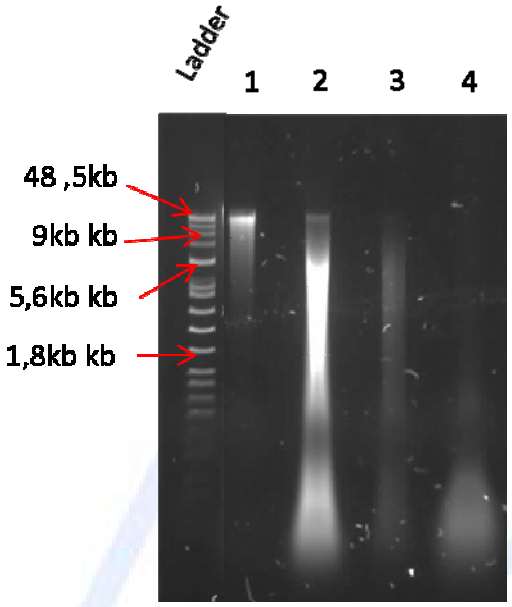

Figure 1: examples of different levels of DNA integrity. Lane 1: high molecular weight genomic DNA; lane 2: partially degraded DNA, but with sufficient quality for sequencing; lanes 3 and 4: almost totally degraded DNA not suitable for sequencing.

8. Contacts:

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

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