Team Tony Schapira

partner INRAE, Metagenopolis

Protocol for DNA extraction from saliva samples, used for shotgun microbiome analysis

Other SOPs for shotgun microbiome analysis can be accessed at: http://www.microbiome-standards.org/ & http://mgps.eu/standard-operating-procedure/

Summary

1. OBJECTIVE

This protocol is an adaptation of MGP_SOP 001 V1 for saliva samples collected with DNA Genotek's OMNIGene·ORAL. MGP_SOP 001 V1 is a standardized stool DNA extraction protocol, recommended as a replacement for International Human Microbiome Standards (IHMS) SOP 006 as QIAGEN no longer makes QIAamp DNA stool kit commercially available. Similarly to MGP_SOP 001, this protocol requires the QIASymphony DSP Virus/Pathogen Midi Kit and the QIASymphony SP instrument from the same supplier.

This protocol is of first interest for automated and quick saliva samples DNA extraction in order to characterize the oral microbiota by metagenomic profiling.

2. PRINCIPLE

The step-by-step description of this protocol will allow to generate lysates from saliva samples using a triple lysis: thermal, chemical and mechanical. The DNA from these lysates will then be purified using magnetic beads and a custom QIASymphony SP protocol developed by QIAGEN and MGP.

3. PERSONS ENTITLED TO USE THE PROCEDURE

This protocol applies to any person involved in saliva samples DNA extraction. This person can be a trainee, fellow, technician or the engineer in charge of saliva samples DNA extraction.

4. 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 the approved protocol.

For the preparation of the nucleic acids, aliquots are prepared from appropriately identified and collected samples using DNA Genotek's OMNIGene·ORAL. Following collection and delivery to the laboratory, **saliva samples must be heated for 2 hours at 50 °C in an incubator, then aliquoted in 500 µL subsamples into 2 mL tubes and stored at -80 °C**.

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.

5. CONDITIONS AND USAGE CONSTRAINTS TO FOLLOW

1- Observation of hygiene and safety rules

2- Mandatory use of lab coat, gloves, glasses

3- Mandatory use of a Biosafety cabinet (BSC), fume hood when necessary

4- Disposal of biological waste in appropriate containers

5- Disposal of chemical waste in appropriate containers

6. MATERIALS, REAGENTS, PRODUCTS, KITS, SOLUTIONS

Materials:

Materials	Risks	Prevention	Providers Names and references
0.1 mm glass beads			152016 DUTSCHER
Screw cap microtubes, 2 mL, steriles			73.693.005 SARSTEDT
Safelock Eppendorf tubes, 2 mL, steriles			033297 DUTSCHER
Toothpicks, steriles			046003 DUTSCHER
1000, 200, 100 and 10 µL micropipettes			Eppendorf Research
1000 µL filter tips			S1122-1830 STARLAB
200-100 µL filter tips			S1120-8810 STARLAB
100 µL filter tips			S1121-3810 STARLAB
10 µL filter tips			S1121-3810 STARLAB
Sample Prep Cartridges 8-well			997002 QIAGEN

8-Rod Covers			997004 **QIAGEN**
Filter-Tips, 1500 µl, Qsym SP (1024)			997024 **QIAGEN**
Filter-Tips, 200 µl, Qsym SP (1024)			990332 **QIAGEN**
Mixer mill (Retsch MM400)	 Hand crush hazard	Beware of balancing	20750001 **RETSCH**
Benchtop microcentrifuge	 Hand crush hazard		**DUTSCHER**
Benchtop refrigerated centrifuge		Beware of balancing	5409000217 **Eppendorf**
70 °C dry bath	 Burn hazard		060917 **DUTSCHER**
Vortex mixer			250158 **DUTSCHER**
Biosafety cabinet			1046-2614 **FISHER SCIENTIFIC**

Kits:

Materials	Risks	Prevention	Providers Names and references
QIAsymphony DSP Virus/Pathogen Midi Kit, version 1			937055 **QIAGEN**

Products/Reactives:

Materials	Risks	Providers Names and references

Guanidine Thiocyanate		G6639 SIGMA
Tris-HCl 1 M pH 8		15893661 Thermo scientific
N-Lauroyl Sarcosine 20 %		L7414-50mL SIGMA
N-Lauroyl Sarcosine		L9150-50g SIGMA
NaCl		S3041 SIGMA
EDTA 0.5 M pH 8		E7889-100ML SIGMA
PBS 1X pH 7.4		15374875 Thermo scientific
PVPP		77627 SIGMA

7. STEP-BY-STEP PROCEDURE

1. Add 250 µL de Guanidine Thiocyanate 4 M Tris-HCl 0,1 M to each frozen sample.
2. Add 40 µL N-lauroyl sarcosine 10 % and thaw.
3. Add 500 µL N-lauroyl sarcosine 5 % PBS 1X and vortex to mix well.
4. Incubate at 70 °C in a dry bath for 1 hour.
5. Add 750 mg glass beads (0.1 mm) in each tube and vortex vigorously.
6. Shake for mechanical disruption:
    a. with Bead Beater™:
        i. Turned on (medium speed) for 5 min
        ii. Stopped for 10 min
        iii. Turned on again (medium speed) for 5 min
    b. with MixerMill MM400 (Retsch): run Program 1, 10 min at 25 s^{-1} (Hz)
7. Add 15 mg PVPP (powder) per sample and vortex vigorously.

Possible variation: steps 4, 6 and 7 can be replaced by 1 hour, 70 °C incubation at 1400 rpm in a thermoshaker. In this case, it should be performed after step 5.

8. Centrifuge at 18,200 ×g for 5 min, 4 °C.

9. Recover the supernatant in a sterile 2 mL tube and set aside.

10. Add 500 µL TENP (resuspend before use) to the pellet and vortex vigorously. Use a toothpick if necessary.

11. Centrifuge at 18,200 ×g for 5 min, 4 °C.

12. Recover the supernatant and pool with the first.

13. Pipet 800 µL lysate in a 2 mL screw cap Sarstedt tube. The remaining lysate can be stored at -80 °C for up to at least 3 months.

14. Turn on, prepare, and run the QIASymphony according to the manufacturer's instructions and use the protocol **COBL1200_CR23506_ID4502**. Elution will be performed in 110 µL buffer, in 0.5 mL Matrix tubes contained in a 96-well SBS rack (*« Deep well »* then *« TS#3744 2D storage tubes* »).

The QIAGEN client can install the protocol used on demand. One cartridge is enough to extract 110 samples. Users will need to plan a training with QIAGEN to learn how to use the QIASymphony.

15. Measure DNA concentration with a fluorometer (e.g. Qubit) and check for DNA quality with a 1 % Agarose gel or Fragment Analyzer (Agilent).

Annex. Preparation of solutions

TEN (50 mM Tris-HCl, 20 mM EDTA, 10 mM NaCl) / TENP (TEN + 1 % PVPP)

- 150 mL Tris-HCl 1 M pH 8
- 100 mL EDTA 0.5 M pH 8
- 14.6 g NaCl
- H_2O q.s. 2.5 L

Mix until totally dissolved. Filter through 0.2 microns Millipore filter and store at 4 °C protected from light. For TENP, add 0.1 g PVPP powder to 10 mL TEN and mix (it will not dissolve).

N-Lauroyl Sarcosine 10 %

- 50 mL N-Lauroyl Sarcosine 20 %
- H_2O q.s. 100 mL

N-Lauroyl Sarcosine 5 % PBS 1X

- 50 g N-Lauroyl Sarcosine
- 1 L PBS 1X ph 7.4

Guanidine Thiocyanate 4 M Tris-HCl 0,1 M

- 236.32 g Guanidine Thiocyanate
- 50 mL Tris-HCl 1 M pH 8
- H_2O q.s. 500 mL

Mix until totally dissolved, protected from light by aluminum foil. Filter through 0.2 microns Millipore filter and store at 4 °C protected from light.