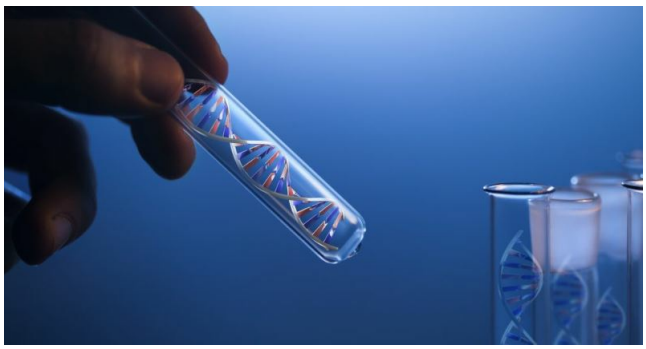

Genetic Analysis S8-044 A1 Genomic DNA Extraction System Installation Guide

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GA-map® | **Dysbiosis Test Lx v2**

INSTALLATION GUIDE

for GA-map® Dysbiosis Test Lx v2

Genetic Analysis

Contents

OVERVIEW

This guide provides the information required to prepare a laboratory for performing the GA-map® Dysbiosis Test Lx v2 assay. Requirements for instruments, materials, and reagents are described.

INSTALLATION CHECK LIST

The following installation checklist summarizes the elements that must be completed during the installation phase. Mark each item as it is completed. Once these prerequisites have been met, please contact Genetic Analysis AS to schedule the training.

- Instruments and consumable items for Step 1 Genomic DNA extraction (see tables on page 4-6) must be acquired
- Instruments and consumable items for Step 2 Amplification of the bacterial 16S rRNA gene – Step 6 Hybridization and Signal Detection (see tables on page 6-9) must be acquired
- All instruments, including the Luminex detection system, must be installed and ready for use
- Operators must have been trained in use and maintenance of the Luminex detection system

RECOMMENDED SAFETY EQUIPMENT

Fecal samples should be treated as potentially infectious material until inactivation and require the use of BSL-2 grade laboratory equipment and precautions. This involves the use of appropriate PPE, biological safety cabinet, proper waste disposal and risk-minimizing routines for sample handling. Make sure to follow laboratory and local guidelines for EHS.

COMPUTER REQUIREMENTS

The GA-map® Analyzer is a cloud-based software compatible with most web browsers. See the GA-map® Analyzer Manual for details.

LABORATORY REQUIREMENTS

The GA-map® Dysbiosis Test Lx v2 requires a typical molecular laboratory set-up. It is recommended that the laboratory contains, at a minimum, dedicated pre-PCR, template-free, and PCR/post-PCR zones. The zones should be separated to prevent contamination.

The table below contains an overview of the different laboratory procedures that are performed in the individual zones, in addition to instruments and equipment needed in the different workstations. Please refer to the Equipment, materials, and reagents required section for equipment specifications. To equip the lab with appropriate pipettes, please refer to the IFU for volumes to be pipetted.

Zone	Lab procedures	Workstation
Pre-PCR	Sample preparation and gDNA extraction. Addition of template for the amplification step.	Biological safety cabinet equipped with: • Vortex mixer • Decapper for Lysing Matrix-E tubes • Appropriate single- and 8-channel pipettes w/ wide orifice tips Open bench equipped with: • Bead beater • Plate centrifuge for Lysing Matrix-E tubes • Water bath • DNA extraction robot • Vortex mixer • Microcentrifuge • Dispenser pipette w/ tips • Single- and 8-channel pipettes with tips • Ice or cooling elements for other reagents and plate
Template-free	Mastermix preparation for the amplification, clean-up, and End-Labeling steps.	PCR cabinet with available equipment: • Vortex mixer • Microcentrifuge • Dispenser pipette w/ tips • Single channel pipettes w/ tips • Freezing block for enzymes • Ice or cooling elements for reagents and plate
PCR/Post-PCR	Amplification reaction. The quantification step. Addition of template for the End-labeling step and the End-labeling reaction. The Hybridization and signal detection step.	Open bench equipped with: • Plate centrifuge for quick spin • Thermal cycler • DNA quantification system • Ring magnet plate • Luminex detection platform • Vortex mixer • Microcentrifuge • Dispenser pipette w/ tips • Single- and 8-channel pipettes w/ tips • Ice or cooling elements for reagents and plate • Freezing block for plates

EQUIPMENT, MATERIALS, AND REAGENTS REQUIRED

The lists below describe the required equipment, materials, and reagents for the GA-map® Dysbiosis Test Lx v2.

All items are required, unless otherwise specified. Genetic Analysis must be informed of any deviations from these requirements and a separate validation might be necessary.

The quantity for all equipment is based on the set-up described in the Laboratory Requirements section above. It should be noted that different laboratory set-ups (e.g. with additional zones) might affect the quantity.

FOR STEP 1 – GENOMIC DNA EXTRACTION
List of equipment when extracting DNA according to STEP 1 of the IFU – need of equipment may vary with choice of extraction kit and instruments.

	Equipment type	Specifications	Recommended options
☐	Decapper for Lysing Matrix-E tubes	Capacity: 8-12-channel capper/decapper for matrix tubes	8-Channel Screw Cap Decapper (4105MAT), Thermo Scientific
			AlteCap™ Switch (404000) with Cassette 12/96 - Internal thread Matrix screw caps (404014), AltemisLab
☐	Bead beater	Capacity: 96-well rack Force: 1600-1800 RPM	FastPrep-96™ Homogenizer w/96-well plate insert (116010500), MP Biomedicals
☐	Plate centrifuge	Capacity: 96 well plate with min 6 cm height Force: 1300 rcf	Any
☐	Water bath	Capacity: 96 well plate Temperature: 65°C	Any
☐	DNA extraction robot	96 well plate format Magnetic particle processor	KingFisher Flex – 96 deep well head (540 0630) w/magnetic micro-plate Separator, installed with KF Flex 96 KF heating block (2407 5420), Thermo Scientific
			Bioer GenePure (NAP-32P)
			Contact Genetic Analysis for approved instruments. Or request our standardized extraction validation procedure.
☐	Vortex mixer	Speed: ~2800 rpm	Any
☐	Micro Centrifuge	Capacity: 1.5ml/ 2ml tubes (spin down only)	Any

☐	Dispenser pipette w/tips	Volumes to be dispensed: 20, 200, 250, 270 and 720 µl	Any Multipette®E3/E3x (4987000371/4987000380), Eppendorf
☐	Pipettes single channel w/tips	Volumes: 10 – 1200 µl	Wide orifice tips recommended for pipetting of fecal samples prior to gDNA extraction
☐	Pipettes 8-channel w/tips	Volumes: 100 – 1200 µl	Wide orifice tips recommended for pipetting of fecal samples prior to gDNA extraction
☐	Ice or cooling blocks	For keeping reagents/sample intermediates cold during handling	Any

List of materials

	Material	Specifications	Recommended options
☐	Lysing Matrix-E tubes	Capacity: 2 ml Bead type: 1.4 mm Ceramic Spheres, 0.1 mm Silica Spheres, and 4 mm Glass Bead.	Lysing Matrix E, 96-tube rack, barcoded tubes, 1 Rack (116984001B), (note: individual Matrix tubes with screw cap), MP Biomedicals
☐	Tube for Lysis mix	Volume: 5, 15 or 50 ml	Any
☐	Deep well plate for DNA extraction robot	DeepWell 96 Plate, V-bottom	KingFisher Deepwell 96 Plate, V-bottom, (95040450), Thermo Scientific 96 Well Plate SW 2ml with V-Bottom PP (BAKR43001-0504), VWR
☐	Deep well Tip Combs for DNA extraction robot	96 tip comb for DeepWell magnets	KingFisher 96 tip comb for DW magnets, (97002534), Thermo Scientific 96 Tip Comb PP (BAKR43001-0505), VWR
☐	Elution plate for DNA extraction robot	96 well microplate, ≥0.2 ml	KingFisher 96 KF microplate (200 µl), (97002540), Thermo Scientific Well Plate 96 SW 0.2ml with V-Bottom PP (BAKR43001-0506), VWR
☐	Adhesive PCR plate seal	Suitable for deep well plate for DNA extraction robot	Adhesive PCR Plate Seals (AB0558), Thermo Scientific
☐	Microtiter sealing tape	Suitable for plates for DNA extraction robot	Adhesive Plate Seals (AB0580), Thermo Scientific
☐	Microtiter plate w/seal for gDNA dilution	Capacity: 96-well, ≥250 µl	Any

List of reagents

	Reagent	Specifications	Recommended options
☐	Extraction control positive (optional)	Fecal sample of known quality	Any
☐	DNA extraction reagent kit	Validated for GA-map® Dysbiosis Test Lx v2	mag™ maxi DNA purification kit, 288 tests (NAP40430), LGC Genomics
☐	Ethanol (for extraction kit)	96-100% ethanol ≥500 ml	Any
☐	Acetone (for extraction kit)	≥99% acetone ≥350 ml	Any
☐	Water for dilution of gDNA and as PCR ctrl neg.	Sterile, Nuclease-free water ≥500 ml	Any

FOR STEP 2 – AMPLIFICATION OF THE BACTERIAL 16S RRNA GENE TO STEP 6 – HYBRIDIZATION AND SIGNAL DETECTION

List of equipment

	Equipment type	Specifications	Recommended options	Used in assay steps
☐	Plate centrifuge	Capacity: 96-well plate (quick spin down only)	Any	*Step 2* *Step 3* *Step 4* *Step 5* *Step 6*
☐	Thermal cycler	Capacity: 96-well Lower maximum volume ≥ 75µl Temperature range: 4-105°C	Veriti™ 96-Well Thermal Cycler (4375786), Applied Biosystems	Step 2 Step 4 Step 5 Step 6
			VeritiPro™ 96-Well Thermal Cycler (A48141), Applied Biosystems	
			T100™ Thermal Cycler (186-1096), Bio-Rad	
☐	DNA quantification system	System for quantifying dsDNA (0-100ng/µl)	Qubit® Fluorometer 3.0 (Q33216) or 4.0 (Q33238) or Flex (Q33327), Invitrogen	Step 3
			FLUOstar OMEGA Microplate Reader with filter-based absorbance (415-103) w/ appropriate filters for the quantification assay, BMG LabTech	
☐	Ring Magnet Plate	Capacity: 96-well Hybridization plate, 100µl	96-Well Ring Magnet Plate (S380), Permagen	Step 6
☐	Luminex detection platform	Luminex xMAP® technology Capacity: 96-well Multiplexing capacity: ≥ 50	MAGPIX® system or NxTAG®-Enabled MAGPIX® system with xPONENT® version 4.2 or higher (MAGPIX-XPON4.1-CEIVD), Luminex*	Step 6

		Laser type: Green and red	Luminex® 200™ system with xPONENT® version 4.2 or higher (LX200-XPON-IVD/RUO), Luminex*	
☐	Vortex mixer	Speed: ~2800 rpm	Any	Step 2 Step 3 Step 4 Step 5 Step 6
☐	Micro Centrifuge	Capacity: 2ml tubes (spin down only)	Any	Step 2 Step 3 Step 4 Step 5 Step 6
☐	Dispenser pipette w/tips	Volumes to be dispensed: 20µl and 40µl	Multipette®E3/E3x (4987000371/4987000380), Eppendorf	Step 2 Step 5 Step 6
☐	Pipettes single channel w/tips	Volumes: 0.5 – 1000µl	Any	Step 2 Step 3 Step 4 Step 5 Step 6
☐	Pipettes 8-channel w/tips	Volumes: 0.5 – 300µl	Any	Step 2 Step 3 Step 4 Step 5 Step 6
☐	Ice or cooling blocks	For keeping reagents/sample intermediates cold during handling	Any	Step 2 Step 3 Step 4 Step 5 Step 6
☐	Freezing block for tubes	Capacity: 1.5/ 2ml reagent tubes For use during handling of enzymes	Benchtop cooler (5115-0012), Thermo Scientific	Step 2 Step 4 Step 5
☐	Freezing block for plates	Capacity: 96-well microtiter plate For use during End-labeling	PCR-Cooler 0.2ml (3881000031), Eppendorf	Step 5

*Additional items for use and maintenance are required: kits for calibration and performance verification, sheath fluid or drive fluid, and an ultrasonic bath (40-60 kHz)

List of materials

	Material	Specifications	Recommended options	Used in assay steps
☐	Microcentrifuge tubes for mixing of reagents	Volume: 1.5, 2 and 5ml	Safe-Lock tubes, Eppendorf	Step 2 Step 4 Step 5 Step 6
☐	Microtiter plate for PCR/End-labeling	Capacity: 96-well PCR grade	96-Well Semi-skirted Flat Deck PCR Plates (AB1400), Thermo Scientific	Step 2 Step 5
			96-Well Semi-skirted, segmented PCR Plate (AB0900), Thermo Scientific	
☐	8-cap sealing strips	Suitable for microtiter plate	Flat PCR Caps, strips of 8 (AB0784), Thermo Scientific	Step 2 Step 5
☐	Microtiter sealing tape	Suitable for microtiter plate	Adhesive Plate Seals (AB0580), Thermo Scientific	Step 2 Step 5
☐	Tubes/plates for DNA quantification	Suitable for the selected DNA quantification system	For use with plate reader: Nunc™ 96-Well Microplate, Black (237108), Thermo Scientific	Step 3
			For use with Qubit® 3.0/4.0 Fluorometer: Qubit™ Assay Tubes (Q32856), Invitrogen	
			For use with Qubit® Flex Fluorometer: Qubit™ Flex Assay Tube Strips (Q33252), Invitrogen	
☐	Hybridization plate	Capacity: 96-well, unskirted, 0.2ml Suitable for Luminex detection platform	96-well Twin.tec™ PCR Plates, Unskirted, Divisible (0030133358), Eppendorf	Step 6
			Thermowell™ 96-Well Polycarbonate PCR Microplates, Model P (6509), Corning	
☐	Sealing film for Hybridization plate	Non-adhesive For 96-well format	Microseal® 'A' (MSA5001), Bio-Rad	Step 6
☐	Reagent reservoir	≥25ml	Any	Step 4 Step 6

List of reagents

	Reagent	Specifications	Recommended options	Used in assay steps
☐	Water for PCR control neg.	Sterile, Nuclease-free water, same as used for dilution of gDNA	Any	Step 2
☐	Assay kit for DNA quantification	For plate reader	Quant-iT™ PicoGreen™ dsDNA Assay Kit (P11496), ThermoFisher	Step 3
			Quant-iT™ 1X dsDNA HS Assay (Q33232), ThermoFisher	

		For Qubit® Fluorometer	Qubit™ dsDNA HS assay kit (Q32854), ThermoFisher	
			Qubit™ 1X dsDNA HS assay kit (Q33231), ThermoFisher	
			Quant-iT™ 1X dsDNA HS Assay (Q33232), ThermoFisher	

TRAINING PLAN

Training will be scheduled following system set-up. Genetic Analysis will train users in performing the process of GA-map® Dysbiosis Test Lx v2 from fecal gDNA extraction (or from PCR, if the extraction method differs from that described in the IFU) to generation of the reports. Other sampling devices, extraction instruments and chemistry can be validated upon request using our standardized validation method. Basic instrument operation and use of the software will be included in the training. Those being trained will be required to have a basic knowledge of Microsoft Windows and use of general laboratory equipment and tools.

Operators must have been trained in use and maintenance of the Luminex system prior to the tech transfer.

CONTACT INFO

We are happy to help you with your inquiries.
Technical Support: support@genetic-analysis.com

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S8-044 A1 v9 Appendix 6

Documents / Resources

	Genetic Analysis S8-044 A1 Genomic DNA Extraction System [pdf] Installation Guide S8-044 A1 Genomic DNA Extraction System, S8-044 A1, Genomic DNA Extraction System, DNA Extraction System, Extraction System

References

- Home - Genetic Analysis
- User Manual