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ENI-CROSS BORDER COOPERATION



JOINT PLATFORMS

validation for biomolecular research



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A project implemented by
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i. Project identification data	
<i>Title of the Action:</i>	Changes in human colonic microbiome in antibiotic generated stress
<i>Project acronym:</i>	COLONSTRESS
<i>Lead Beneficiary:</i>	PMSI Institute of Oncology, Republic of Moldova
<i>EMS - ENI:</i>	2 SOFT/1.2/105
<i>Priority:</i>	1.2 Promotion and support for research and innovation
<i>Total implementation period:</i>	24 months
<i>Start Date:</i>	10.10.2020
ii. Information concerning the cross border partnership	
<i>Beneficiary no.1:</i>	Regional Institute of Oncology Iasi



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INTRODUCTION

The cross-border cooperation between the PMSI Institute of Oncology, Chisinau and the Regional Oncology Institute Iasi has a huge advantage. The main long-term benefit of this cooperation is the development of two superior molecular diagnostics and research laboratories, capable of further training staff in metagenomic DNA sequencing techniques and accumulating theoretical and practical knowledge of NGS Ion Torrent și Illumina technologies .

Based on the new technology, we aimed to produce a cross-border group collaboration of excellence for parallel DNA sequencing. Both medical institutions (partners) will use DNA sequencing equipment, so that we increase the level and quality of expertise. As a result, the creation of a common platform for synergistic work and with compatible protocols.

The aim of the project is to compare microbiome differences in two populations that are exposed to different antibiotic protocols and to better understand the impact of broad-spectrum antibiotics as a driving force for the development of resistance.

The project, through its objectives and results, will develop two superior molecular diagnostic and research laboratories, dedicated to oncological diagnosis and treatment.

The expected results of this project include the validation of a common platform for biomolecular research, the qualification of the training of the personnel involved in it, a new protocol for prophylactic antibiotic treatment, including a report on antibiotic-induced changes and the response of the human colonic microbiome to different stress situations .

The project "Changes in human colonic microbiome in antibiotic generated stress " will improve the condition of the innovation framework for cross-border research actions in the field of the human microbiome.



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THE LABORATORY OF PMSI INSTITUTE OF ONCOLOGY, CHISINAU

The Laboratory of Molecular Immunology and Genetics (LIGM) is a unit organized within PMSI IO, in which laboratory investigations with immunological and molecular genetic profile are carried out, which come to complete all investigations aimed at ensuring the process of specialized medical care, curative, preventive and recovery for patients.

The subdivision actively participates in the implementation / development of the managerial control system, the implementation / development of the operational procedures regarding the quality system, as well as of the norms of labor protection, safety and health at work;

In its activity, the Laboratory of Immunology and Molecular Genetics complies with the provisions of the Labor Code of the Republic of Moldova; law no. 411/1995 on health care; Law no. 264/2005 regarding the exercise of the medical profession; Law no. 263/2005 On the rights and responsibilities of the patient; instructions, orders of the founder and of the PMSI IO, which is annexed to this Regulation.

The subdivision of "Cancer Genetics Research" is a new branch of the Immunogenetic Laboratory which is, in turn, part of the Institute of Oncology of Republic of Moldova (PMSI IO). The creation of this subdivision in 2015 was supported by the Moldo-Japanese project JICA (with the involvement of the Japan International Cooperation Agency). In the middle of 2015, all the equipment was installed by specialists of the Thermo Fisher Scientific Company.

THE LABORATORY OF REGIONAL INSTITUTE OF ONCOLOGY IASI

Regional Institute of Oncology (IRO) in Iasi, Romania is a new public health institution (active since 2012) with 300 beds, committed for cancer diagnostic and treatment, and covering the North-East part of the country (roughly 44 000 Km² and 5 million inhabitants). Main clinical services are: surgery, medical oncology, hematology, radiotherapy, palliative care, medical imaging department, pathology, and clinical laboratory. Starting from 2016 two new functional components are operational in the frame of IRO configuration: A Stem Cell Transplantation Service connected to the Hematology Clinic and a Center of Fundamental Research and Experimental Development in Translational Medicine (TRANSCEND).

All TRANSCEND laboratories dedicated for research are included in a separate self-contained building. As a consequence, two interconnected research levels will make TRANSCEND a unique translational hub: molecular medicine and nuclear medicine.

The molecular medicine field of research will be represented by a flow of chained activities developed in the frame of following units: Molecular Diagnostic Imaging (flow-cytometry and tissue digital cytometry), Cytogenetics and Molecular genetics, Molecular Biology (PCR, sequencing, microarray,



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molecular cloning), Cell culture and cell manipulation (2D and 3D-organ-like cultures), Proteomics, cryogenics and biobanking, and Bioinformatics.

The nuclear medicine level will consist of a radiotracer synthesis and research unit (cyclotron and radiopharmaceutical laboratories) and a PET-CT translational diagnostic unit.

The functional concept of TRANSCEND is equally in favor of independent continuation and subsistence of each unit through dedicated projects and financial capital as well as comprehensive projects that fit into place all units of the center for the design of a biomarker with diagnostic value and ultimately linked to the application of a specific and tailored therapy. Pairing Diagnostics with Therapeutics is currently framed under the notion of theranostic. TRANSCEND will promote this convention implying that drugs must be paired with diagnostic biomarkers to enable the right drug to be selected for the right patient at the right time.

Central to the integration in such a scheme, which aspire to diagnose, deliver targeted therapy and monitor the response to therapy, is the growth of units of proteomics, bioinformatics and isotopic tracer production for developing a translational biomarker repertoire and a functionally interpretable systems medicine.

ORGANIZATION OF THE LABORATORY

PRESENTATION OF WORKSPACES AND FUNCTIONAL CIRCUITS OF THE LIGM, IMSP IO

The Laboratory of Immunology and Molecular Genetics (LIGM) is a unit organized within PMSI IO, in which laboratory investigations with immunological and molecular genetic profile are carried out, which come to complete all investigations aimed at ensuring the process of specialized medical care, curative, preventive and recovery for patients.

The subdivision is part of the PMSI IO mission: to contribute in maintaining and improving the health of the population by providing specialized and quality medical services for patients with oncological diseases and malignant hematological diseases, at the level of specialized outpatient and hospital care, both in consultative, curative, and scientific methodological plan.

The PMSI IO Immunology and Molecular Genetics Laboratory consists of two subdivisions. Immunology subdivision and Molecular Genetics subdivision with the necessary equipment for Next-generation sequencing (NGS). The rooms are placed in such way that the Molecular Genetics subdivision can ensure both PCR tests and Next Generation Sequencing.



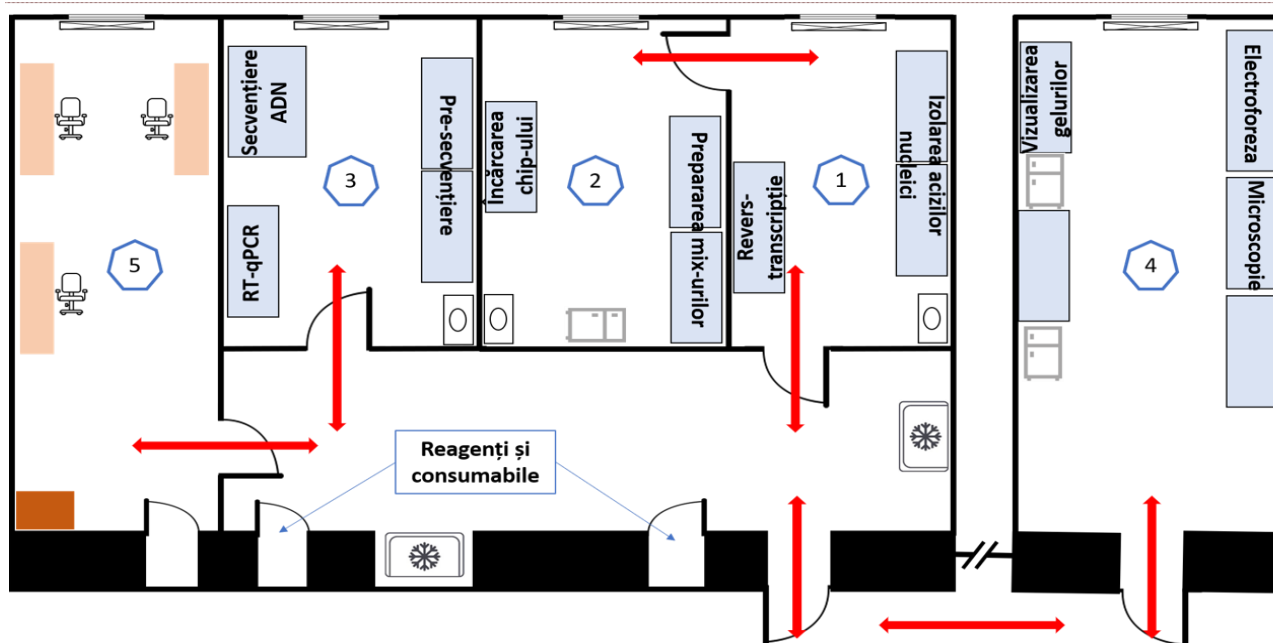
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The Molecular Genetics subdivision consists of five rooms, four rooms are separated by their own hall (separate from the Immunology subdivision), and a room for electrophoretic migration and post PCR processing. For DNA sequencing are used the 4 rooms with their own hall. The first room is used for the isolation of nucleic acids (DNA and RNA) and reverse transcription. The second room is used for the preparation of genomic (metagenomic) libraries. The third room is used for the creation of ISPs, the enrichment of ISPs and Massively Parallel Sequencing and the fourth room is intended for the bioinformatics analysis of genomic and metagenomic data.

The flow in the laboratory is circular.

PRESENTATION OF WORKSPACES AND FUNCTIONAL CIRCUITS OF THE MBL, IRO IASI

The Laboratory of Molecular Biology is a unit organized within IRO Iasi in the Center for Fundamental Research and Experimental Development in Translational Medicine/ TRANSCEND financed under SOP IEC Axis 2 Competitiveness through RDI, Operation 2.2.1 - "Development of existing R&D infrastructure and creation of new infrastructures", SMIS Code 48871.

The translational research promoted by the TRANSCEND center aims at an adequate approach to the complexity of neoplastic disease and its diversity of perception in order to identify a useful, personalized medical approach.



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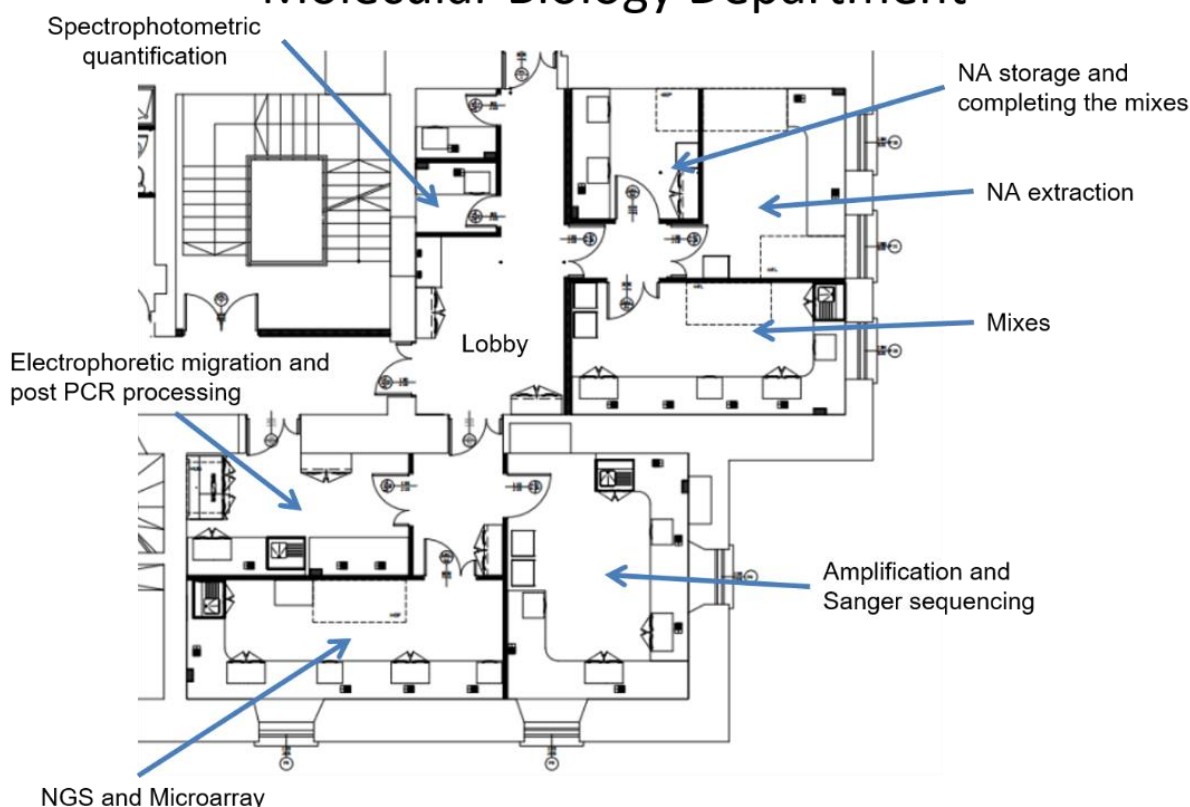


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Here, laboratory investigations with immunological and molecular genetic profile are carried out, which come to complete all investigations aimed at ensuring the process of specialized medical care, curative, preventive and recovery for patients.

The TRANSCEND center consists of three levels: ground floor, 1st floor and 2nd floor which are divided in a series of departments. The Molecular Biology department is situated at the 1st floor of the building having a circuit as shown in the building plan below.

Molecular Biology Department



The Molecular Biology department consists of seven rooms divided as follows: pre-PCR with a room for nucleic acid (NA) extraction, a room for PCR mixes, a room for NA storage and completing the mixes, a room for spectrophotometric quantification, and post PCR with electrophoretic migration and post PCR processing, a room for amplification and Sanger sequencing and a room for NGS and microarray.



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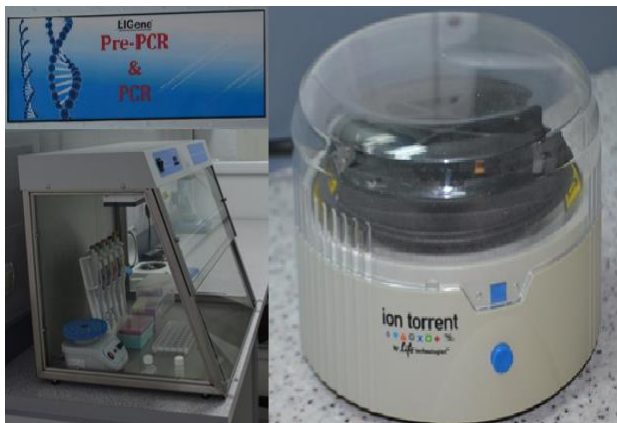
LABORATORY EQUIPMENT

LABORATORY EQUIPMENT OF THE LIGM, IMSP IO

The nucleic acid isolation room: DNA/RNA UV-cleaner box (Biosan) in which takes place the DNA/RNA isolation process, a PCR thermocycler (ThermoFisher) for performing RNA reverse transcription, and a Qubit 3.0 fluorometer (ThermoFisher) for DNA, RNA, genomic libraries and ISPs quantification.



Room for mixes: DNA/RNA UV-cleaner box (Biosan) in which are prepared metagenomic libraries and one Ion Chip MiniFuge (ThermoFisher) for effective and efficient sample loading.



Sequencing room: Ion OneTouch 2 System for performing template amplification, Ion OneTouch ES (Enrichment System) for ISPs enrichment, and the Ion PGM System used for high-quality next-generation sequencing.



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The electrophoresis and gel visualization room: Consort Electrophoresis System (Consort) and E-Gel iBase Electrophoresis System (ThermoFisher) for DNA electrophoresis, and Gel documentation System Doc-Print VX5 (Consort) for archiving the results.



The Bioinformatics data analysis room: two laptops for genomic and metagenomic data processing, folders with archived documents, and software (R language, maftools library, etc.) for data analysis.





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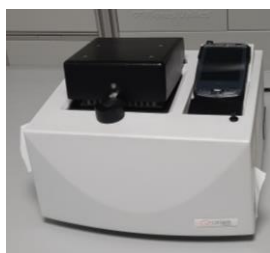
There are and other auxiliary equipment for sample processing in the laboratory (centrifuges, laboratory vortexes, Thermo-Shakers, and others).

LABORATORY EQUIPMENT OF THE MBL, IRO IASI

The nucleic acid isolation room: Fume hood with laminar air flow, Bimek FXP laboratory workstation (Bekman Coulter), DNA automatic Extractor Magnesia (Anatolia Geneworks), mechanical pipettes, cooling centrifuges, thermoshaker, vortex (Biosan MSV-3500), refrigerator.



The NA storage and completing the mixes room: a PCR thermocycler (Palm Cyclyer, Corbett) for performing RNA reverse transcription, mechanical pipettes, refrigerator and a deep freezer (-80°C) (Thermo Scientific)



The PCR mixes room: Fume hood with laminar air flow, mechanical pipettes, freezer.





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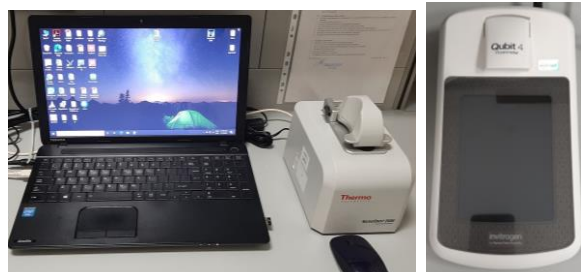
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Room for spectrophotometric quantification:

a Nano Drop 2000 spectrophotometer (Thermo Scientific) and a Qubit 4.0 fluorometer (ThermoFisher) for DNA, RNA and genomic libraries quantification.



The room for amplification and Sanger sequencing: Thermocyclers: SensoQuest Labcycler, GeneTouch (Bioer) SureCycler 8800 (Agilent); Lightcycler 480 II (Roche), Cobas Z 480 (Roche), Stratagene MX3005P (Agilent Technologies) and Sanger sequencer ABI 3500 (Applied Biosystems), ELITE InGenius system (Biomedica), GeneXpert System Cepheid (Biomedica), freezer.





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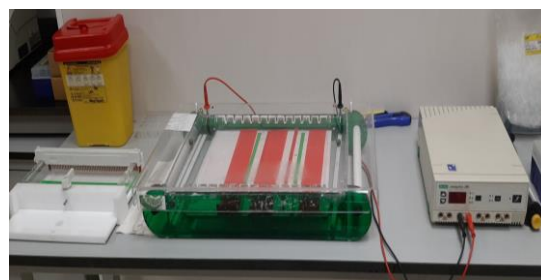
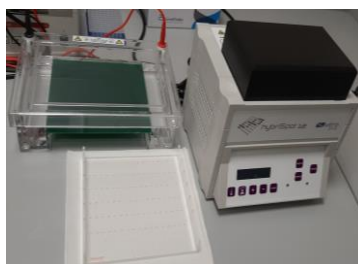


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Electrophoretic migration and post PCR processing room: Bee Robotics B20 automatic system for western blot and hybridisation strips, HybriSpot 12 (Vitro Group), Agarose gel electrophoresis system, UVP BIO DOC-IT Imaging System, fume hood, mechanical pipettes, centrifuges, thermoshaker, vortex (Biosan MSV-3500), water bath (Biosan).



NGS and Microarray room: NextGeneration Sequencing MiSeq (Illumina) platform, Ion Torrent PGM (Thermo Fisher Scientific) platform, Microarray – Agilent platform, UPC equipment, 64-bit computer with 4 core processor (4 processing cores) and at least 16 GB of RAM, hard disk of at least 2 TB/year for storing MiSeq sequencing data.



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Our laboratories have been written guidelines for the collection, storage and processing of biological samples, as well as a common guideline for the purification of nucleic acids. Also, have been written individually, for each sequencing platform, Standard Operating Procedures (SOP) for the metagenomic libraries preparation and DNA sequencing:

- Guideline for sampling human microbiome
- Guide about the technical procedure for colon metagenomics DNA extraction and library preparation
- STANDARD OPERATION PROCEDURE SOP, ION TORRENT NEXT GENERATION SEQUENCING (NGS) METAGENOMICS
- STANDARD OPERATION PROCEDURE ILLUMINA NEXT GENERATION SEQUENCING (NGS) METAGENOMICS

CONCLUSIONS

1. Both laboratories have implemented an appropriate research infrastructure for colonic metagenome sequencing;
2. The sequencing technologies used in the laboratory from Institute of Oncology of Republic of Moldova and that of the Regional Institute of Oncology Iasi have the necessary capacity to obtain reliable metagenome data.
3. Both laboratories have implemented the operational procedures and guidelines necessary to perform metagenome sequencing, including all the guidelines necessary to ensure the functionality of the equipment and its maintenance