Romania-Republic of Moldova
ENI-CROSS BORDER COOPERATION

# TRAINING PROGRAM

## FOR SPECIALIST IN MOLECULAR BIOLOGY

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A project implemented by
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the European Union

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| **1. Project identification data** | |
| *Title of the Action:* | Changes in human colonic microbiome in antibiotic generated stress |
| *Project acromym:* | COLONSTRESS |
| *Lead Beneficiary:* | PMSI Institute of Oncology, Republic of Moldova |
| *EMS - ENI:* | 2 SOFT/1.2/105 |
| *Priority:* | 1.2 Promotion and support for research and innovation |
| *Total implementation period:* | 18 months |
| *Start Date:* | 10.10.2020 |
| **2. Information concerning the cross border partnership** | |
| *Beneficiary no.1:* | Regional Institute of Oncology Iasi |
| **3. Work plan by group of activities** | |
| *GA 3: Activity 3.2.* | Generating a complex multilevel training program for all young specialist in molecular biology |

# CONTENT



# LIST OF ABBREVIATIONS

DNA - deoxyribonucleic acid

ES - Enrichment System

ISPs - Ion Sphere Particles

LIGM - The Laboratory of Molecular Immunology and Genetics

NGS - Next-generation sequencing

PCR - Polymerase chain reaction

PGM - Personal Genome Machine

RNA - Ribonucleic acid

PMSI IO - Public Medical Sanitary Institution Institute of Oncology

# CONTEXT

The training program is a material made within the project "COLONSTRESS - Changes in human colonic microbiome in antibiotic generated stress".

Project main objective is to set up a joint CBC network in molecular research focused on establishing the impact of antibiotic treatment on human colonic microbiom in order to create a measurable proof of antibiotic induced changes and promote a new restrictive protocol for antibiotic usage as a way to reduce costs and limit the development of multi resistant germs.

For development of two research laboratories and higher molecular diagnosis, given that there are two medical institutions, dedicated to diagnosis and oncological treatment, capable of further training staff in the field of molecular biology, including for developing and testing a joint research platform in molecular biology for characterization of human microbiome focused on establishing the impact of antibiotic treatment on human colonic microbiome in order to create a measurable proof of antibiotic induced changes the needs for the development of laboratory staff skills were defined.

# CURRICULA FOR MULTILEVEL TRAINING

Human resource in molecular biology is a major problem across border and highly trained personal is among most wanted specialists in western laboratories. The current project foresee a training of medical doctors and biologists in molecular biology and sequencing technologies.

## TRAINING GOAL

Instruction of researchers and medical doctors in metagenomic DNA sequencing techniques and accumulation of theoretical and practical knowledge regarding *Ion Torrent* and Illumina MiSeq NGS technology.

## TRAINING OBJECTIVES:

1. Studying the stages and methods of collecting and processing human microbiome samples. Metagenomic DNA extraction.

2. Mastering the work process and the stages of creating fragment libraries according to *Ion Torrent* technology.

3. Mastering the work process and the stages of creating fragment libraries according to *Illumina* technology.

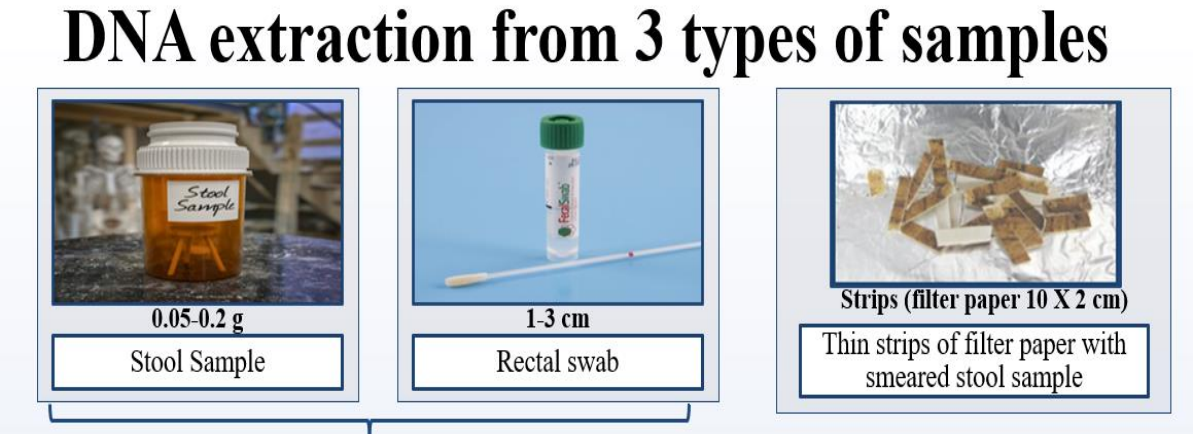
4. Obtaining the skills to perform sequencing experiments on the *Ion PGM* instrument. Bioinformatic data analysis.

5. Obtaining the skills to perform sequencing experiments on the *MiSeq* instrument

# STUDYING THE STAGES AND METHODS OF COLLECTING AND PROCESSING HUMAN MICROBIOME SAMPLES. METAGENOMIC DNA EXTRACTION (4 HOURS)

1.1. Methods of collection and short therm storage of biological samples

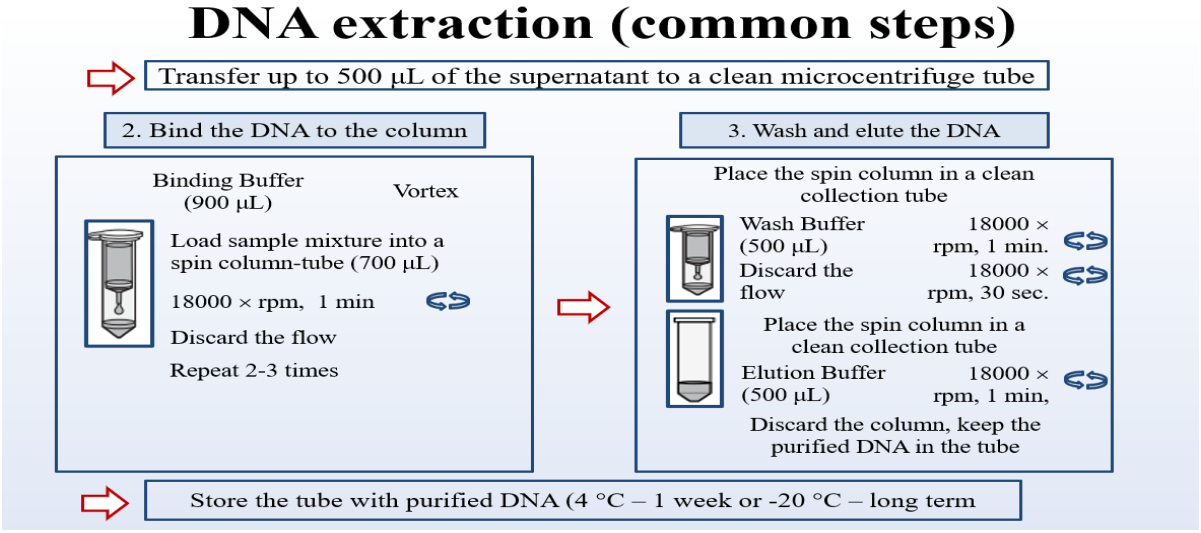
1.1.1. Stool samples collected in containers
1.1.2. Stool samples smeared on filter paper strips
1.1.3. Rectal swabs




1.2. DNA extraction from different types of samples

1.2.1. Methods of extraction DNA from bacteria
1.2.2. Features of the PureLink Microbiome DNA Purification Kit
1.2.3. Quality of purified nucleic acids (elimination of inhibitors)

*Provides hands-on instructions regarding methods of DNA extraction from bacteria. Several criteria will be established to evaluate: DNA yield, DNA quality/stability, reliability, and time. The students also will identify the optimum conditions for each stage of the protocol (lysis, purification, elution), each investigated separately.*



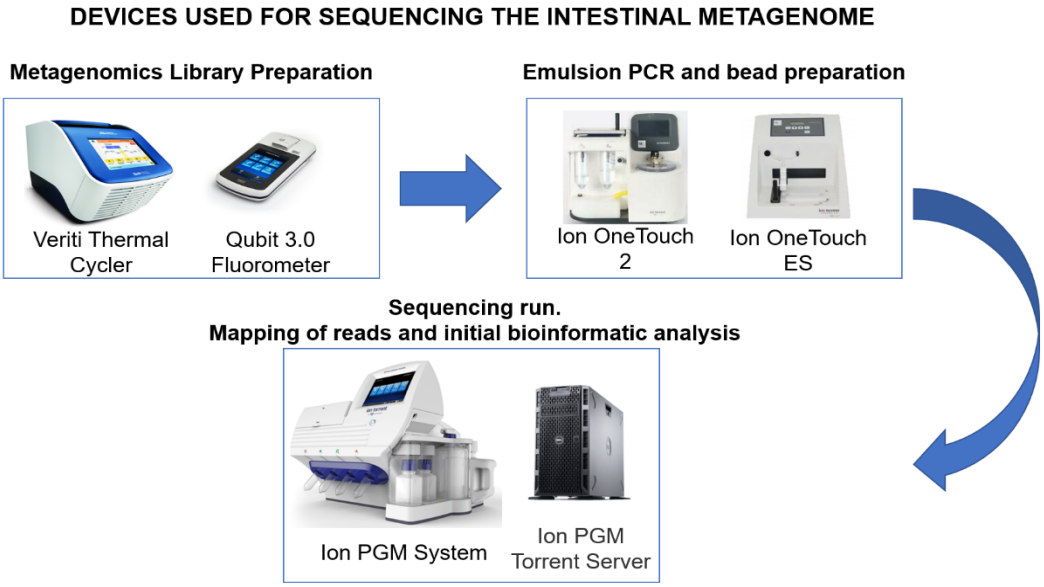
**a. Ion Torrent Next Generation Sequencing**

*Definition, principle, advantages and disadvantages comparing with other technologies*

i. Ion Torrent Semiconductor Sequencing

*The students will learn how Ion Torrent technology combines massively parallel semiconductor chip sequencing with natural chemistry to directly translate chemical information into digital data.*

ii. Devices used for sequencing of the intestinal metagenome




iii. Ion Torrent Workflow

*This topic includes a summary of library construction, template preparation, sequence generation, and data analysis steps.*

**b. Library preparation types**

*The students will discover the methods of library preparation such as nebulisation, sonication, restriction enzyme digest, or PCR amplification. Also depending on application, they will learn about fragment library types.*

i. 16S Metagenomics Library Construction

*Researchers will learn about designing polybacterial libraries focusing on rapid, comprehensive, and broad-range research analyses of mixed microbial populations.*

ii. Sample Multiplexing Guidline for 16S RNA profiling

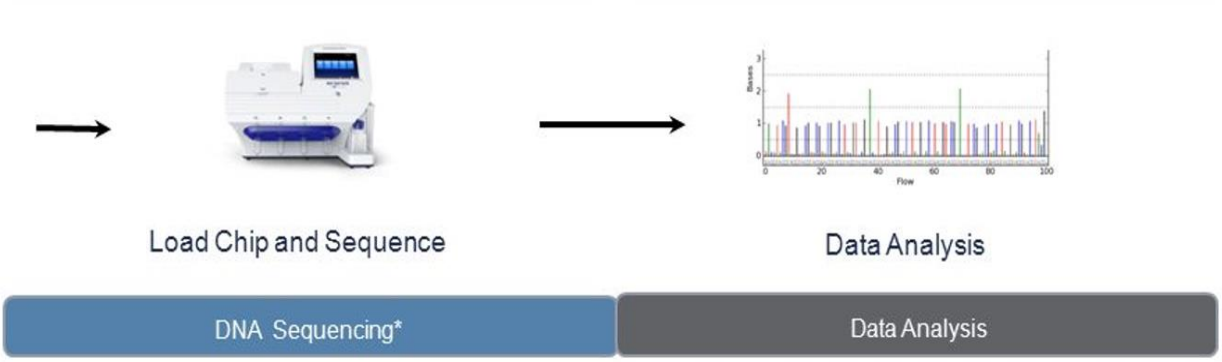
*This topic includes the best practices of multiplexing samples and lower prices for metagenomics research.*

**c. Ion Sphere Particles (ISPs) preparation**

*At this point trained people will understand how to prepare the final products of the clonal amplification – Ion Sphere Particles (ISPs) and how to enrich them.*

i. Emulsion PCR/Clonal amplification
ii. Ion Sphere Quality Control
iii. Ion Sphere Enrichment

## OBTAINING THE SKILLS TO PERFORM SEQUENCING EXPERIMENTS ON THE *ION PGM* INSTRUMENT. BIOINFORMATIC DATA ANALYSIS




ii. Clean and initialize
iii. Load the chip and start the sequencing run

e. Data analysis
i. Torrent Suite Data Analysis Flow – PGM
ii. Primary Data Analysis Workflow
iii. Torrent Variant Caller

## MASTERING THE WORK PROCESS AND THE STAGES OF CREATING FRAGMENT LIBRARIES ACCORDING TO *ION TORRENT* TECHNOLOGY

Workflow Summary:

1. Amplicon primers – there are two primer pools to amplify seven hypervariable regions (V2, V3, V4, V6, V7, V8, and V9) of bacterial 16S rRNA. One primer set amplify V2-4-8 and another primer set amplify V3-6, 7-9. First primer pool generates amplicon fragments of ~250 base pairs (bp), ~288 bp, and ~295 bp, respectively. In a second single tube, a multiplex PCR reaction generates amplicon fragments of ~215 bp, ~260 bp, and ~209 bp, respectively.

2. Prepare library–The protocol describes the steps to amplify the V2, V3, V4, V6, V7, V8, and V9 region, add IonXpress sequencing adapters and barcodes to the amplicon target.

3. ISP preparation and enrichment - The PCR then denatures the library fragment leading two separate strands, one of which (the reverse strand) anneals to the bead. The annealed DNA is amplified by polymerase starting from the bead towards the primer site. The Ion OneTouch ES uses magnetic bead technology to isolate template-positive Ion Sphere particles that can be loaded directly onto the Ion semiconductor chip.

4. Sequence - following library construction and template preparation, sequencing runs are completed in as little as 4 hours. The Ion 318 Chip can generate up to 2.0 Gb of sequence data and, assuming 4 indexed samples, can generate ~ 500 Mb per sample.

5. Analyze - BAM files produced with Torrent Suite Software are automatically analyzed, annotated, and taxonomically assigned in the Ion Reporter Software 16S metagenomics workflow. Ion Reporter Software enables the rapid identification (at genus or species level) of microbes present in complex polybacterial research samples, using both curated Greengenes and premium curated MicroSEQ ID 16S rRNA reference databases. The Ion Reporter metagenomics workflow also provides primer information, classification information, percent ID, and mapping information. It's easy to interpret population diversity for your research at any taxonomic level with the interactive display.

## MASTERING THE WORK PROCESS AND THE STAGES OF CREATING FRAGMENT LIBRARIES ACCORDING TO *ILLUMINA* TECHNOLOGY (10 HOURS)

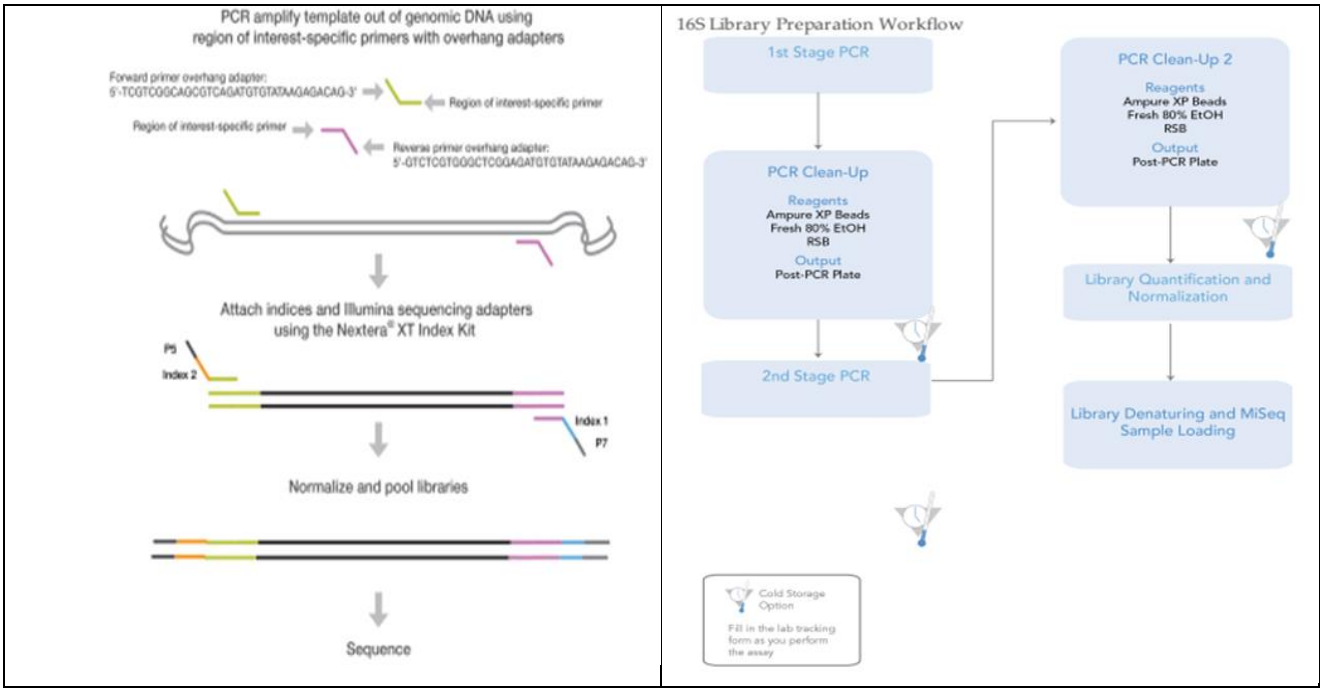
Workflow Summary:

1. Amplicon primers – primer pair sequences for the V3 and V4 region that create a single amplicon of approximately ~460 bp. The protocol also includes overhang adapter sequences that must be appended to the primer pair sequences for compatibility with Illumina index and sequencing adapters.

2. Prepare library–The protocol describes the steps to amplify the V3 and V4 region and using a limited cycle PCR, add Illumina sequencing adapters and dual-index barcodes to the amplicon target. Using the full complement of Nextera XT indices, up to 96 libraries can be pooled together for sequencing.

3. Sequence on MiSeq–Using paired 300-bp reads, and MiSeq v3 reagents, the ends of each read are overlapped to generate high-quality, full-length reads of the V3 and V4 region in a single 65-hour run. The MiSeq run output is approximately > 20 million reads and, assuming 96 indexed samples, can generate > 100,000 reads per sample, commonly recognized as sufficient for metagenomic surveys.

4. Analyze on MSR or BaseSpace–The Metagenomics workflow is a secondary analysis option built into the MiSeq Reporter (on-system software) or available on BaseSpace (cloud-based software). The Metagenomics Workflow performs a taxonomic classification using the Greengenes database showing genus or species level classification in a graphical format. This protocol can be used to sequence alternative regions of the 16S rRNA gene and for other targeted amplicon sequences of interest.




The above curricula will contribute to the familiarization of young lab staff (Junior Scientific Researchers, Resident Doctors) with sequencing molecular biology techniques and metagenomic research.