MEDGENET

Medical Genomics and Epigenomics Network

H2020-TWINN-2015-692298

D1.8 2nd on-line book of abstracts WP1

Work Package:	WP1
Task:	T1.1
Deliverable due date:	31/12/2017
Responsible partner:	EMBL
Editors:	Vladimir Benes, Daria Kucharova
Deliverable number:	D1.8
Deliverable type:	R
Dissemination level:	PU
First Created:	10/02/2017
Last Updated:	19/12/2017
Version:	1.0

Table of Contents

1 Introduction

The "Medical Genomics and Epigenomics Network" (MEDGENET) project aims to create a well-educated taskforce of biomedical researchers, who will contribute to the development of new genomics and bioinformatics tools and their application in clinical practice, and enable establishing best practices for performing innovative and high quality applied research.

In order to achieve the stated aim various workshops are organized in the project. In order to share the ideas discussed in these workshops, and therefore, widen their impact beyond the workshop participants ***books of abstracts*** are be published on the MEDGENET website in Section Study Materials. The ***2nd online book of abstracts*** is following the 1st book and includes abstracts from the workshops that took place in the Work Package 1 "Knowledge transfer in medical genomics, gene expression control and bioinformatics" in year 2017.

The Work Package 1 focuses on educating and training new generation of genomics and bioinformatics scientists as well as transfer of methodological proficiency to sustain technological platform for genomic research at CEITEC MU. It aims to help to develop expertise for gene expression regulation studies and performing functional studies on important signalling pathways in disease development, microenvironment interactions and resistance.

Two workshops were organized in the Work Package 1 in year 2017:

- The Workshop on Next-Gen Sequencing Technologies and Applications at CEITEC MU, the Czech Republic (January 18th – 20th, 2017)
- Talking Colors: Advanced Microscopy at CEITEC MU, the Czech Republic (October 24th – 25th, 2017)[1]

Abstracts form these workshops are presented in the next chapters.

[1] The workshop was originally planned under working title "Workshop on advanced microscopy and spectroscopy methods in quantitative analysis of biomolecular interactions". During the preparation it was decided to change the title for a shorter and more attractive version "Talking Colors: Advanced Microscopy". The focus of the workshop hasn't changed.

2 The Workshop on Next-Gen Sequencing Technologies and Applications

The Workshop on Next-Gen Sequencing Technologies and Applications took place from 18th to 20th January 2017 at CEITEC, Masaryk University. The focused on both theoretical and practical aspects of next-generation sequencing including but not limited to applications of NGS in medicine (e.g. sequencing of somatic genomes, rare variant screening).

This three-day event included invited lectures of experts from MEDGENET partner institutions as well as practical exercises on in next-generation sequencing technology and space for individual discussions with invited speakers.

2.1 DAY I – THEORY AND PRACTICE IN NEXT-GENERATION SEQUENCING TECHNOLOGY

Boris Tichy, CEITEC MU – theoretical overview and practical exercises

The Genomics Core Facility members organized a hands-on workshop that focused on NGS library preparation. Due to laboratory capacity, the number of participants in the practical part was limited to 16. PhD students and young researchers were first instructed on the basic terminology of NGS sequencing, different applications and technology, by the head of Genomics Core Facility, Boris Tichy. Participants were then split into groups. Each group followed a variant of protocol, which used a library preparation kit from KAPA, to generate a high quality genomic DNA library. This process is considered to be a crucial skill in any kind of NGS application by the Core Facility staff. For each group, they tweaked the protocol a little, so that libraries leading to different applications or sequencing lengths could be generated and assessed during the final step of preparation - Library quality control. This was the program for the first day of the workshop.

In the afternoon of the second day, participants evaluated the result of their respective library preparations. By the end of the workshop, participants had the opportunity to learn about and to consider in their future work:

- The effect of different DNA fragmentation methods;
- The importance of SPRI bead size selection and optimization strategies;
- Methods for the effective quality control of NGS libs and interpretation of the metrics.

2.2 DAY II – GUEST LECTURES

Vladimir Benes (EMBL Heidelberg, Germany) - *"NGS today and in the future"*

Lesley Ann Sutton (Uppsala University, Sweden) - *"Applications of NGS technologies in functional genomics: The CLL story"*

Andreas Agathangelidis (CERTH, Greece) - *"High throughput immunogenetics in Chronic lymphocytic leukemia"*

Nikos Papakonstantinou (CERTH, Greece) - *"Dissecting the role of the histone methyltransferase EZH2 in CLL"*

Filip Pardy (CEITEC, the Czech Republic) - *"Introduction to MinION technology"*

Jan Provaznik (EMBL Heidelberg, Germany) - *"Genome assembly – Size matters"*

Jonathan Landry (EMBL Heidelberg, Germany) - *"RNA-seq data analysis – Practical assessment of the best practices"*

During the second day lectures of invited speakers focused on emerging NGS technologies, basic bioinformatics approaches and practical insights into the cancer biology of leukaemia. The lectures were open to everyone interested in the topics. More than 70 listeners participated in this open part of the workshop.

Vladimir Benes (EMBL Heidelberg, Germany) - *"NGS today and in the future"*

In only 40 years, the sequencing has taken great strides: from the development of first Sanger sequencing in 1977 to the Nanopore, a tool smaller than a hand, which allows carrying out your own sequencing almost everywhere. Over the past 20 years, next-generation DNA sequencing technologies have increased the quality and amount of data, cutting drastically down costs and reducing time. Dr Vladimir Benes recapitulated the findings of those years highlighting the difference between the platforms, their principles and countless applications.

Although next generation sequencing technologies are expanding continually, there are still unresolved challenges, such as sample control, analysis of massive number of data or the error rate. Massive Parallel Sequencing can be used in a widespread range of applications and every step is crucial. From library preparation to data analysis, the lecture provided the knowledge to be aware of potential of this instrument and the best way to use it in "routine".

Lesley Ann Sutton (Uppsala University, Sweden) - *"Applications of NGS technologies in functional genomics: The CLL story"*

Next-Generation Sequencing technologies are spreading faster and faster, and become useful not only in research, but in clinical routine as well. NGS accelerated diagnostic time, which allows for the analysis of clinical markers and recognition of patients suitable for appropriate therapy, or at early relapse. During the lecture, Dr Sutton showed, through several works, how NGS has changed and is changing the clinical world, focusing in particular on chronic lymphocytic leukemia (CLL) and lymphoma. CLL is still a hard challenge, but through sequencing, a range of mutations with relevant prognostic impact, such as in IGHV, TP53, ATM, NOTCH1, SF3B1, have been identified. NGS is replacing Sanger sequencing in clinical routine and in this way it is possible to analyse more patients and more target genes simultaneously, providing a fast and specific diagnosis and a prompt and targeted therapy.

Andreas Agathangelidis (CERTH, Greece) - *"High throughput immunogenetics in Chronic lymphocytic leukemia"*

In recent years, high-throughput BcR IG repertoire analyses have been applied in studies of different B cell populations to reveal the existence of gene repertoire and rearrangement biases. Reasonably, the great advance in analysis depth offers unpreceded detail in determining the clonal population, studying clonal diversity and dynamics and monitoring the disease course, using the BcR as a molecular marker. In all, applying high-throughput sequencing to immunogenetics can increase our ability to sensitively monitor the disease course, improve patient management and understand the biology of CLL.

Sequence analyses of the TCR have been pivotal for elucidating critical mechanisms that underlie CLL pathogenesis. In our recent NGS study of the T-cell repertoire in CLL we reported the existence of T-cell clones that were shared by different patients. These clones persisted and further expanded over time. Notably, these shared expanded clonotypes were CLL-specific, as they were not found in healthy controls. Altogether, these findings indicate that antigen drive likely underlies T-cell expansions in CLL and may be acting in a CLL-specific context.

Nikos Papakonstantinou (CERTH, Greece) - *"Dissecting the role of the histone methyltransferase EZH2 in CLL"*

In the wide applications of Next-Generation-Sequencing (NGS), Dr Nikos Papakonstantinou provided evidence about how ChIP-Seq and RNA-seq can be useful to understand the molecular mechanisms of action in different subgroups of the same disease, in this case Chronic Lymphocytic Leukemia (CLL). CLL is a clinically heterogeneous disease; over the years low-throughput and recently NGS studies have highlighted the potential role of several genes implicated along various B cell signaling/activation pathways and assisted in the compartmentalization of the patient cohort. One of the existing categorizations concerns that of "stereotypy" subsets i.e. subgroups of patients carrying quasi-identical B cell receptors. In particular, his talk enabled miRNA profiling in the study of CLL pathogenesis and the role of the histone methyltransferase Enhancer of zeste homolog 2 (EZH2). He showed how the expression of miRNA-101 changes in the different immunogenetic subset of CLL, leading a differential expression levels of EZH2 between CLL subsets. EZH2 participates in DNA methylation inducing gene silencing in broad cell processes. EZH2 is overexpressed in different types of cancer, including lymphomas and aggressive CLL. ChIP-seq provides accurate information about protein-DNA binding, showing targeted genes and pathways' involvements that could be studied for targeted therapy in the different CLL subsets.

Filip Pardy (CEITEC, the Czech Republic) - *"Introduction to MinlON technology"*

In a rapidly evolving environment of NGS technology, we are witnessing a multitude of emerging sequencing platforms becoming available to the scientific community. One such platforms is being developed by Oxford Nanopore company. MinION, a hand-held sequencing device is becoming a potentially widespread device that could change the sequencing paradigm and make us rethink existing workflows and areas of NGS usage. In his talk, Filip Pardy discussed the key advantages and specifics of this sequencer, as well as an outlook to emerging and potential areas of application.

Jan Provaznik (EMBL Heidelberg, Germany) - *"Genome assembly – Size matters"*

Next and third generation sequencing enables discovery and description of novel genomes or genome variants. This information can be used to open up a new model organism to well-developed molecular biology methods, discover specific genomic variants in interesting samples and many, many more. Assembly is a crucial process that places together shattered pieces of sequencing reads into contiguous information. This process is heavily reliant on the quality of input material, software used and fine tuning of parameters during computational analysis step. In this talk, basics of current sequencing technologies are discussed, different error types are mentioned. Then a simplified overview of graph theory is given. De Bruijn and overlap graphs are presented and different usage is discussed. Best practices in quality control

and importance of such is mentioned. Software resources are also discussed. Finally, a test case of de novo assembly is presented.

Jonathan Landry (EMBL Heidelberg, Germany) - *"RNA-seq data analysis – Practical assessment of the best practices"*

Next generation sequencing is becoming a standard tool and is used in a large variety of applications. Transcriptomics analysis by RNA sequencing is one of them and various questions could be addressed. The talk was mainly focus on gene expression quantification and covers all principle steps of the data analysis which include experimental design, quality control, read alignment, quantification of gene and transcript levels, visualization, differential gene expression.

In a first part, the experimental design has to be considered depending on the biological question, the organism studied and other constraints. As an example the library type can vary a lot (single-end, paired-end, strand specificity, long or short reads). The sequencing depth was also discussed depending on the application. The number of replicates as well as sequencing design (usage of spike-ins, biases) were also introduced and resources given. Tools to handle these specific format were also introduced. Standard formats as well as the quality control methods were presented for the raw reads and the alignment files. Alignment visualization programs were also shown.

In a second part, a workflow from reads to gene expression and differentially expressed genes was given. A set of different tools used for the various tasks were listed and discussed. Tools and resources for functional characterization of these differentially-expressed genes were finally discussed.

3 Talking Colors: Advanced Microscopy Workshop

The imaging workshop ***Talking Colors: Advanced Microscopy*** was held on October 24th and 25th, 2017 at CEITEC MU in Brno, the Czech Republic. The workshop started with the opening remarks of its organiser Ctirad Hofr which were followed by the lectures of invited experts Michael Stone and Mark Hink on the mechanism of dynamics and interaction of various molecules at single molecular level. The lectures covered various advanced fluorescence microscopic methods used to study the single molecules. Both lecturers explained the concepts and applications to the heterogeneous group of audience consisting new comers to the field and experts who use the techniques. The lectures were open for a broad audience and took place at Mendel Museum in Brno.
The second part of the workshop was devoted to practical experiments. The practical part started with general training for high-end microscopy instrumentation operation. Further, the participants of the practical part of the workshop were divided into 3 groups for 3 practical sessions.

3.1 PART I – THEORETICAL LECTURES

Michael Stone (University of California, Santa Cruz, USA) – *"Investigating Nucleic Acid Structure and Dynamics using Single Molecule FRET"*

Mark Hink (University of Amsterdam, the Netherlands) – *"Measuring molecular interactions using Fluorescence Lifetime and Correlation Microscopy"*

Michael Stone (University of California, Santa Cruz, USA) – *"Investigating Nucleic Acid Structure and Dynamics using Single Molecule FRET"*

The lecture on "Investigating nucleic acid structure and dynamic using single molecule FRET" by Dr. Michael Stone from University of California, Santa Cruz described how to use the FRET technique to study in vivo dynamics of a molecule. This was explained by elaborating his study on "the folding of DNA as a G-quadruplex structure in the telomere region". A broad range of various tools which are applicable for studying single molecule dynamics including x-ray crystallography, computational modeling, single molecule biophysics and test tube biochemistry was explained. Many classical examples single molecule fluorescence experiments such as patch covered, rotation gamma subunit of ATP synthase F1 complex, kinesin walking, protein synthesis was also shown to familiarize the audience with techniques.

Mark Hink (University of Amsterdam, the Netherlands) – *"Measuring molecular interactions using Fluorescence Lifetime and Correlation Microscopy"*

The second lecture by Dr Mark Hink from the University of Amsterdam was focused on fluorescent techniques and how study of molecular interactions is important for understanding the basis of living cells. In the beginning of his lecture Mark Hink focused on quantitative fluorescence microscopy. The study of signalling cascades at the level of physiological conditions through developing of new non-harmful markers and the progress of molecular techniques provides not only quality pictures but also quantitative analyses on how the living cells work. The second part of the lecture was devoted to detailed examples of fluorescence techniques, such as FCS, FCCS, FRET, FLIM or FFS. By using one of the techniques separately or by their combination one can receive a variety of useful results, including detailed picture of the protein and its interactions as well as receive information on single molecule, its concentration in solution and mobility. Mark Hink also commented on the most common mistakes during observation of samples.

3.2 PART II – PRACTICAL SESSIONS

FLIM microscopy practical

Instructors: Mark Hink

In this practical, participants measured the Förster Resonance Energy Transfer (FRET) of a biosensor via the donor fluorescence lifetime using FLIM.

Förster Resonance Energy Transfer (FRET) is one of the most powerful methods to image molecular activities or interactions in living cells and can be detected using various designs of biosensors. In FRET, excited state energy is transferred between two fluorophores through dipole-dipole interactions. The occurrence of FRET between fluorophores has a number of consequences that can used for its detection and quantification with a microscope: (i) the donor fluorophore will lose its energy, quenching its emission yield, whereas (ii) the acceptor emits photons (sensitized emission) without having been directly excited by light, and (iii) the

duration of the excited state of the energy-donating fluorophore, i.e. the donor lifetime, is shortened.

The aims of the practical were:

- To learn how to set up, perform and analyse quantitative FRET experiments using Fluorescence Lifetime Imaging Microscopy;
- To become aware of possible artefacts and learn about the correct interpretation of FLIM-FRET data.

Workflow Fluorescence Lifetime Imaging Microscopy:

1. Set the conditions for imaging of CFP/mTurquoise2 (donor) and YFP/Venus (acceptor) fluorophores.
2. Check the laser frequency of the FLIM setup and select a proper frequency (if option available). Select a FLIM-compatible detector.
3. Make a FLIM image of the Negative sample. Check the peak intensity and analyse the data.
4. Make FLIM images and analyse the Positive, treated and non-treated EPAC samples.

Telomere DNA Dynamics Lab Practical

Instructor: Michael Stone

The goal of this short lab practical was to expose students to the technique of single-molecule FRET, as implemented using Total Internal Reflection Fluorescence (TIRF) microscopy. The power of the technique was demonstrated by observing structural dynamics in a human telomere DNA primer. Students prepared sample chambers, deposit molecules on the surface, image single molecules using a TIRF microscope, collect movie files, and analyse the data using custom written Matlab scripts. The data generated by the Matlab programs represent the starting point for various downstream analyses that can be applied to smFRET data. The participants took each step together and determined how the experiment was running.

The practical workflow:

1. Molecule preparation (this was prepared by the lecturer);
2. Sample Chamber Preparation (chambers have been prepared by the lecturer);
3. Molecule Deposition onto prepared surface;
4. Data collection;
5. Bead slide;
6. Data analysis.

Fluorescence Correlation Spectroscopy (FCS) practical

Instructors: Ctirad Hofr, Tomáš Janovič, Martin Stojaspal

The session was devoted to exploring Fluorescence Correlation Spectroscopy (FCS) and learning how to perform it practically.

The aims of the practical were:

- To learn general information about FCS and how to apply it for the description of molecular interactions;
- To demonstrate how to perform FCS measurement on standard confocal laser scanning microscope;
- To analyse obtained data by software QuickFit 3.0.

The practical workflow:

1. Sample preparation;
2. Microscope setup;
3. Focus into the sample;
4. FCS setup;
5. Loading the data into QuickFit 3.0;
6. FCS (Auto-correlation) evaluation;
7. FCCS (Cross-correlation) evaluation.