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Editorial

Capabilities, prospects and applications of the NGS platform of the National Infrastructure OMIC-Engine in agro-biotechnology

By Prof. M. Grigoriou

In the past 10 years biological sciences have been transformed by the rapid development of next-generation sequencing (NGS). NGS involves parallel sequencing of millions of DNA fragments quickly and at an affordable cost. As a result, a multitude of NGS methods, that enable scientists to study complex biological systems at a level never before possible but also to design and develop novel applications, services and products, are now available.

The global next-generation sequencing market from USD 5.70 billion in 2018 is expected to increase to USD 16.35 billion by 2024 at a Compound Annual Growth Rate (CAGR) of approximately 20%¹. So far most of the applications and services have been clinically oriented, however agro-biotechnology market is also significantly growing and a rise in demand for commercial applications, especially for microbial screening in food and chemical industry, is anticipated to accelerate market growth in the coming years².

A major goal of the National Infrastructure OMIC-Engine is to serve the agro-biotechnology sector. To this end one of the approaches that OMIC-Engine has undertaken is the development, at the Department of Molecular Biology & Genetics of Democritus University of Thrace, of a midsize NGS Platform that can be used for the most widely used NGS methods, namely microbiome sequencing, targeted sequencing, small genomes/organelle sequencing and RNA-sequencing.

The OMIC-Engine NGS Platform allows for the development of a plethora of applications and services for researchers and for companies operating in crops and livestock production, in food (dairy, oil etc.) and feed production, in food safety, as well as in the design of dietary additives and functional foods. As the breadth of applications is very wide, a number of representative applications in the agro-biotechnology, some of which are already part of the implementation of OMIC-Engine,

are presented here.

For instance using rDNA microbiome sequencing facility-specific “house” microorganisms that have key roles in shaping specific characteristics of fermented products can be identified, providing therefore a unique “house signature” that adds value to the final commodity³. The analysis of the grape microbiome of several varieties on the other hand, has unravelled the existence of regional microbiome patterns that along with “core microbiome” of each variety affect wine chemical composition, pointing out that microbiota are an important aspect of the *terroir*⁴ and providing an economic incentive to develop oenological practices that identify, maintain and exploit indigenous microbial biodiversity. This approach can also be used to follow changes in the microbial composition during fermentation along with changes in the expression patterns of key genes related to different stages of this process⁵. Microbiome sequencing approaches can also be applied to accurately assess hygiene and maintenance of the sanitary standards at all stages of food manufacture and for the identification or the presence of specific pathogenic or spoilage microorganisms^{6,7}. Microbiome analysis of the soil can be used to assess the risk for specific pathogens, the potential to support the growth of the plant⁸ but also to develop novel strategies to improve plant growth through microbiome engineering⁹.

NGS targeted sequencing applications have key roles in the identification and validation of biomarkers linked with a specific disease or an infectious agent affecting the health of livestock^{10,11}. Moreover, NGS based approaches are used in breeding programs for marker-assisted selection or marker-assisted breeding in order to accelerate plant and animal breeding respectively¹², while NGS-DNA barcoding is increasingly used for biodiversity studies to identify species, to assess population structure and size¹³ as well as in food traceability applications^{14,15}. Transcriptomic analyses and metatranscriptomic approaches contribute to the functional characterization of the complex interactions and to the identification of gene products that are essential for the integration of different processes and may be used in synthetic biology approaches especially in the field of environmental ecology¹⁶.

Agri-food complex has an integral role in Greek economy; it is the second largest employer providing 15% of the employment and supports approximately 20% of the Greek exports thus, it is

considered as one of the 7 specific key sectors that will enhance productivity and competitiveness in the post crisis era^{17,18}. To this end the modernization of Agri-food sector by investing in novel technologies and applications, in this instance in NGS may contribute significantly to the development of this vital sector of the economy.

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¹ DNA Next Generation Sequencing Market to 2025 - Global Analysis and Forecasts By Product, Application, End User and Geography, ID: 4614537, August 2018, Research and Markets2

² DNA Next Generation Sequencing Market to 2025 - Global Analysis and Forecasts By Product, Application, End User and Geography, ID: 4614537, August 2018, Research and Markets

³ Bokulich, N. A., and Mills, D. A. (2013) Facility-specific "house" microbiome drives microbial landscapes of artisan cheesemaking plants. *Appl. Environ. Microbiol.* 79:5214.

⁴ Bokulich NA, Collins TS, Masarweh C, Allen G, Heymann H, Ebeler SE, Mills DA (2015) Associations among wine grape microbiome, metabolome, and fermentation Behavior Suggest Microbial Contribution to Regional Wine Characteristics DOI: 10.1128/mBio.00631-16.

⁵ Polka, J., Rebecchi, A., Pisacane, V., Morelli, L., and Puglisi, E. (2014) Bacterial diversity in typical Italian salami at different ripening stages as revealed by high-throughput sequencing of 16S rRNA amplicons. *Food Microbiol.* 46: 342.

⁶ De Filippis, F., Parente, E., and Ercolini, D. (2017). Metagenomics insights into food fermentations. *Microb. Biotechnol.* 10:91.

⁷ Pothakos, V., Stellato, G., Ercolini, D., and Devlieghere, F. (2015) Processing environment and ingredients are both sources of *Leuconostoc gelidum*, which emerges as a major spoiler in ready-to-eat meals. *Appl. Environ. Microbiol.* 81: 3529.

⁸ Babu AN, Jogaiah S, Ito SI, Nagaraj AK, Tran L-SP. (2015) Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant Sci.* 231:62.

⁹ Orozco-Mosqueda MC, Rocha-Granados MC, Glick BR, Santoyo B. (2018) Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. *Microbiological Research* 208 25.

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¹¹ Raszek M M., Guan L L., Plastow G S. (2016) Use of Genomic Tools to Improve Cattle Health in the Context of Infectious Diseases *Front Genet.* 7: 30 .

¹² Soham R & S Pratik (2015) NGS technologies for next generation plant breeding *Front. Plant Sci.* 5: 367.

¹³ Eimanifar A, Kimball RT, Braun EL& Ellis JD Mitochondrial genome diversity and population structure of two western honey bee subspecies in the Republic of South Africa (2018) *Sci. Rep.* 8: 1333.

¹⁴ Dubrulle, N & Giraud N. DNA Metabarcoding As a Tool to Trace Plants of Interest in Ingredients or Cosmetics. (2017) *IFSCC Mag* 2: 51.

¹⁵ Utzeri V., Schiavo G., Ribani A., Tinarelli S, et al. (2018) Entomological signatures in honey: an environmental DNA meta-barcoding approach can disclose information on plantsucking insects in agricultural and forest landscapes. *Sci. Rep.*8:9996

¹⁶ Rodríguez Amor D & Dal Bello M. Bottom-Up Approaches to Synthetic Cooperation in Microbial Communities *Life* 2019, 9: 22.

¹⁷ *The Greek economy: Hellenic Republic , Hellenic Statistical Authority, June 2016 report .*

¹⁸ *A Holistic Growth Strategy for Greece, General Secreteriat of Media & Communication Report.*

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Interviewing Researchers of OMIC-Engine



Dr Theodora Tsirka holds a degree in Molecular Biology & Genetics from Democritus University of Thrace, a Master by Research in Molecular Oncology from the University of Oxford and a PhD from the

Democritus University of Thrace. She has received scholarships from the Bodossaki Foundation, the Onassis Foundation and the Greek State Scholarships Foundation. She is currently working as a post-doctoral scientist in the OMIC -Engine infrastructure. Her work focuses on the molecular identification of the microbiome of grape vine (*V. vinifera*) and she investigates the microbial flora impact on wine quality, using next generation sequencing.

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Question: Theodora describe briefly your research work

We now know, from work published in the last 5 years, that part of what makes a wine-growing region special is its resident microbiome. Different vineyards have distinct microbiome that provides the finished wine with a unique group of flavor metabolites. However, for the past decades industrial winemaking is almost entirely based on the use of commercially available starter cultures, thus most wines produced from one variety, for instance Cabernet Sauvignon, lack a unique character. My work at the OMIC-ENGINE hub in MBG focuses on the development of molecular tools that will be used to profile the indigenous microbiome of grapes, to understand the microbial interactions that occur but also to analyze the impact of these interactions on the quality of the final product. I am using next generation sequencing of the 16S rRNA region and of the ITS regions, to identify the bacterial and fungal communities from three Greek varieties, namely Asyrtiko, Mavroudi and Xinomavro from vineyards located in Eastern Macedonia – Thrace. We believe that our analysis of the microbiome will result in the identification of “microbial fingerprints” for the viticultural areas analyzed in Thrace as well as in the characterization and the isolation of indigenous strains that may be further exploited to be used as starter cultures.

Question: Which opportunities did your secondment offer you in terms of training, networking and personal growth?

My recruitment in the OMIC-Engine National Research infrastructure has offered me the opportunity to be trained and acquire expertise in next generation sequencing, a technology which enables our hub to develop a powerful set of methods that can be used in several areas of basic research but also to provide services for the private or the public sector. So through my work in OMIC-ENGINE I believe that I have enhanced not only my theoretical background and research skills but in general I have become a more mature scientist with broader scientific horizons. In addition, the OMIC-Engine network has given me the chance to interact with other scientists at different stages of their career who all share similar interests and have the vision of achieving scientific excellency and advancing our research work forward; this networking is quite inspiring and, as our research work progresses I believe it will become more active. In a more personal note, the OMIC-Engine infrastructure offered me the chance to make a strong and full feeling come-back in the research field after a 2-year break due to the birth of my twin children. Overall, through this secondment experience I feel that I have bettered myself as a scientist.

Question: What do you think will be the impact in your future career?

The experience that I gain through the developing of the NGS facility as well as the new field of the study of the microbiome that I now pursue, will be very important for my next career move in which I will be more mature. Plus, the networking will be valuable even after the completion of my work at OMIC-ENGINE as these collaborators will still be there to support my efforts but also to work with me in different projects. For these reasons I believe that my work in OMIC-ENGINE is an asset for my future career and I think that I will have a broad spectrum of choices along the way.

Question: Has this secondment experience matched your expectations so far?

So far, the OMIC-Engine has met with most of my

expectations. Of course, there is always room for improvement, especially when it comes to bureaucracy – which unfortunately is a general problem that we face everywhere, but overall, the OMIC-Engine has offered me a good working environment, a chance to develop my skills on hot-trend topics and the opportunity to interact with scientists from other places of Greece which, living in the border area of Alexandroupolis, is not always easy.

Question: Three words that sum up your experience within the OMIC-Engine infrastructure.

I am really happy to be a part of OMIC-ENGINE of this multidisciplinary network made up of experts in the different areas of biosciences that all share the vision to advance the exciting field of Synthetic Biology!

Meeting the OMIC-Engine Research Groups – Democritus University of Thrace (DUTH) Hub @ the department of Molecular Biology & Genetics (OMIC-Engine MBG)

In this section we will present each time a different hub of OMIC-Engine. The second one presented will be the research groups of the Hub of Democritus University of Thrace participating in OMIC-Engine

Democritus University of Thrace is one of the four main Hubs of the OMIC-Engine Research Infrastructure (RI) and is located in the region of East Macedonia and Thrace, in North-eastern Greece.



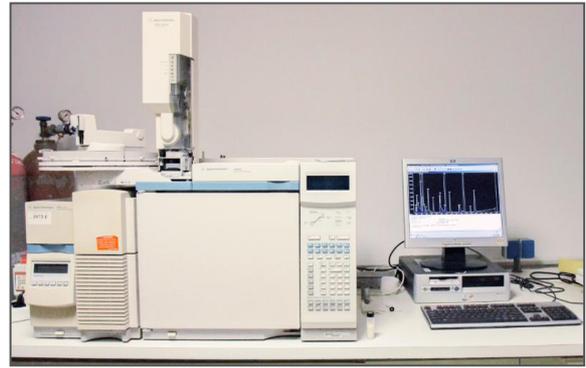
Five Principal Investigators of the Department of Molecular Biology and Genetics (MBG, www.mbg.duth.gr) collaborate closely in the DUTH Hub (OMIC-ENGINE-MBG, see Figure on the left, clockwise): Professor [M. Grigoriou](#), Associate Professor [I. Kourkoutas](#), Assistant Professor [A. Paleologou](#), Associate Professor [G. Skavdis](#), Professor [R. Sandaltzopoulos](#).

The research infrastructure of the OMIC-ENGINE-MBG hub has been largely funded by EU and National competitive programs which allowed for the procurement of state-of-the-art equipment, organized in technology platforms that were further developed through funding from the OMIC-Engine RI.

Basic research at OMIC-ENGINE-MBG focuses on the analysis of biological phenomena and mechanisms at the molecular level, while applied research projects revolve around three pillars: *Biotechnology* with emphasis on applied microbiology and functional foods, *Molecular Agrobiology* with emphasis on insects of economic importance and *Health* with emphasis on human and animal diseases. The projects are frequently aligned with core strategic priorities identified both by the National Strategy for the European Research Area (ERA) as well as by the Research and Innovation Strategy for Smart Specialization (RIS3) of the Region of East Macedonia & Thrace.

The expertise of the DUTH OMIC-ENGINE hub covers a wide range of methodologies of Molecular Biosciences and offers to academic and industrial users from Greece or neighbouring countries access to technologies and services:

- Identification and validation of Biomarkers.
- DNA analysis and molecular identification of species/varieties in agricultural production.
- Analysis of food quality characteristics (microbiological control, pathogen control, acidity, sugars, organic fatty acids and alcohols, volatile compounds in food, etc.).
- Antimicrobial activity of natural products for use as bio-preservatives in foodstuffs.
- Design and development of functional foods using probiotic microbial strains.



In the context of implementation of the OMIC-ENGINE RI, the OMIC-ENGINE-MBG Hub acquired a midsize Next Generation Sequencing platform (Thermo S5) which greatly enhances its research potential by introducing a broad range of targeted, microbial and transcriptomic NGS applications. OMIC-ENGINE-MBG has developed new NGS based technologies and services:

- Microbiome analysis in various samples.
- Monitoring of the resistance of insects to insecticides.
- Molecular profiling.



In the frame of OMIC-Engine, the Hub Democritus University of Thrace is currently employing three Post-Doctoral Scientists.

Professor M. Grigoriou is the research coordinator of the OMIC-Engine hub at Democritus University of Thrace.

New Projects coming in the OMIC-Engine Network

- **The Emblematic Action of the Hellenic Ministry of Education "*The Routes of the Vineyards*", University of Thessaly & Democritus University of Thrace.**

OMIC-Engine is participating in this Emblematic Action through the HUBS of University of Thessaly (Dr D. Karpouzas & Dr K. Papadopoulou) and of the Democritus University of Thrace (Dr M. Grigoriou, Dr I. Kourkoutas and Dr G. Skavdis). The main tasks of the OMIC-ENGINE groups will be to (i) analyse the core microbiome (leaf and fruit) of Greek emblematic grapevine varieties cultivated in different regions (ii) to determine the dynamics and the metabolic potential of the microbiome of the vinification process through amplicon sequencing and metagenomics-metatranscriptomics respectively and identify strains that are associated with desirable or undesirable features of wines (iii) identify and isolate indigenous strains of yeasts. This project started in April 2019 and will last for 24 months.

- **RESEARCH-CREATE-INNOVATE call, "*Development and implementation of novel biobased methods for the treatment of pesticide-contaminated wastewaters from agro-industries (MINOTAUR)*"**

The project aims to develop and implement a full-scale biological system for the treatment of wastewaters produced by fruit-packaging industry and contain toxic and persistent fungicides. The role of the group of Plant and Environmental Biotechnology is to isolated and develop specialized microbial inocula for the effective biodegradation and detoxification of the pesticides contained in those effluents. In addition the group will monitor the fate of these inocula under practical conditions in the wastewater treatment system, follow microbial succession and the role of plasmidome in the transfer of genetic elements involved in the transformation of pesticides in those systems. The Laboratory of Plant and Environmental Biotechnology, Department of Biochemistry and Biotechnology are participating in this project with Dr D.G. Karpouzas being responsible.

OMIC-Engine Seminars



On Wednesday 6th of March, Dr Kriton Kalantidis visited the Department of Biochemistry and Biotechnology in the University of Thessaly and gave a presentation on The RNA silencing pathways in plants and their interaction with biotic and abiotic stressors. He focused mainly on the silencing pathways involved in the plant responses to infections by the smallest known pathogens

on earth, viroids.

He also presented tobacco plants mutated for each and every plant silencing pathway and their combinations. These plants can be useful tools to study RNA silencing but also as platforms to overexpress exogenous proteins.

K. Kalantidis is an Assoc. Professor at the Dept. of Biology Dept., University of Crete and Affiliated Researcher at IMBB/FoRTH. Email: kriton@imbb.forth.gr.

Networking Day

OMIC-Engine is excited to announce the organization of the first **Networking Day** which will take place at Hotel Mediterranean, Thessaloniki at the **13th of June 2019**.

OMIC-Engine invites entrepreneurs, R&D professionals, academic researchers and other stakeholders active in Agrofood, Environment and Chemical Manufacturing sectors to attend. The main scopes of the networking day will be to:

- Familiarize with new Biotechnological applications and methods used in modern corporations
- Create partnerships between Academia and Industry towards the development of high added value products
- Develop collaborations for future funding calls

Read the Networking Day's Program:

https://drive.google.com/file/d/1_ENNdObSTrZyWIB09k7ZIKexsAE41_Uf/view?usp=sharing

Register:

https://drive.google.com/file/d/1_ENNdObSTrZyWIB09k7ZIKexsAE41_Uf/view?usp=sharing



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