

Serbia

Centre for the
Fourth Industrial
Revolution



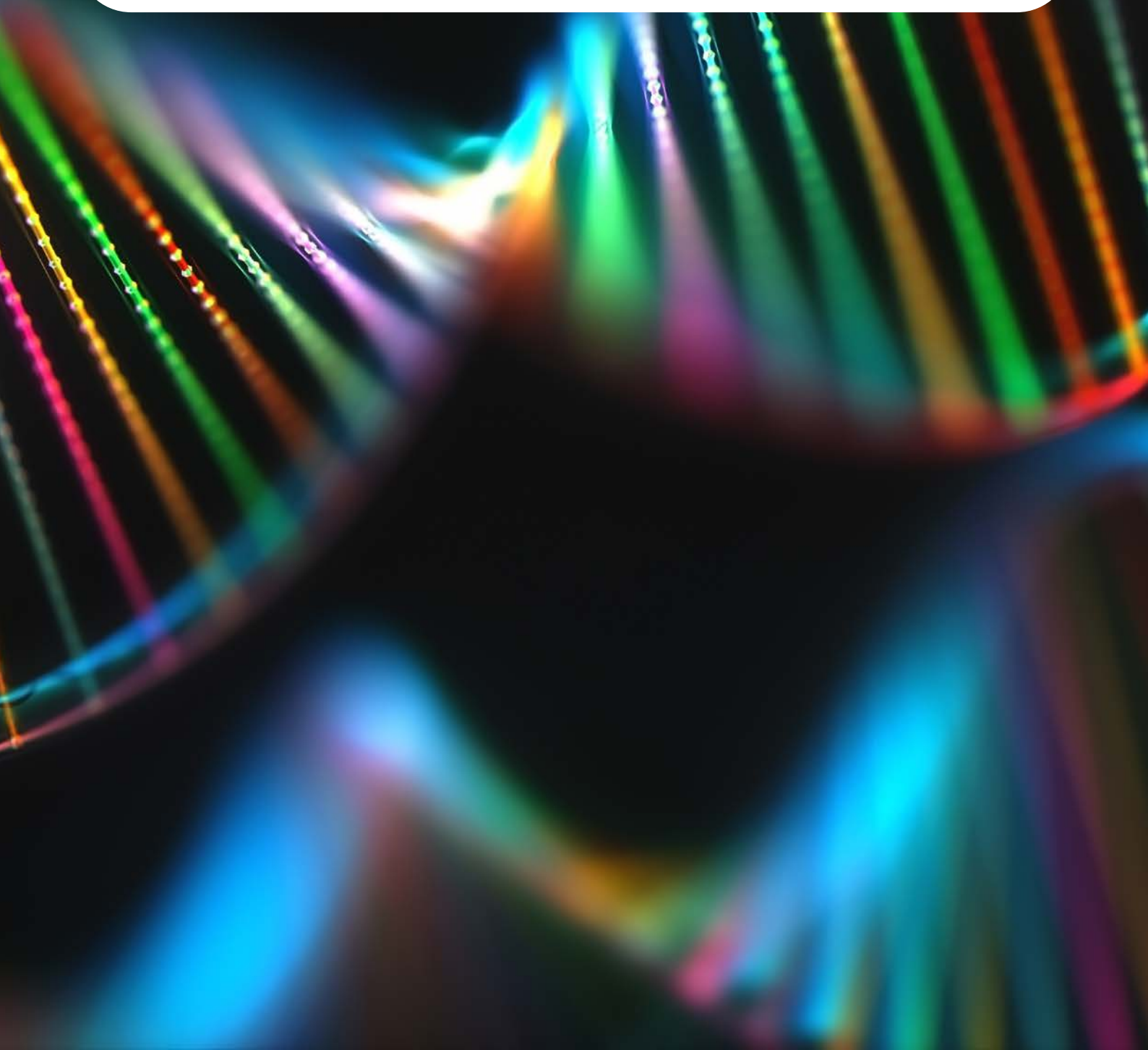
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РЕПУБЛИКА СРБИЈА
МИНИСТАРСТВО ЗДРАВЉА

White Paper June 2023

The Genomic Ecosystem of Serbia Situation Analysis



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Foreword

C4IR in Serbia was founded as a platform for cooperation among different social actors to enhance the conditions for the application of the Fourth Industrial Revolution technologies.



Jelena Bojović
Director of the C4IR Serbia

The Fourth Industrial Revolution is a global transformation of economic and social relations under the influence of new technologies, including the physical, digital and living world. We are witnessing transformations that lead to fundamental changes in the way we live, work and interact with each other. These changes open possibilities for significant improvements of the quality of life, accelerated economic development and response to numerous global crises. However, the changes, are happening extremely quickly and have significant scale, creating great challenges for institutions expected to protect society against risks and imbalances.

In this context, the World Economic Forum founded in 2017 the first Centre for the Fourth Industrial Revolution (C4IR) in San Francisco, USA, with the objective to support the smart application of new technologies worldwide. In February 2022, Serbia joined the global network – which consists of 18 national C4IR centres throughout the world – and is the first such centre in the region and third in Europe. The Government of the Republic of Serbia, together with the Forum, founded the C4IR in Serbia as a platform for interaction and cooperation among different social actors in order to enhance the conditions for the application of Fourth Industrial Revolution technologies.

Through the activities of the C4IR Serbia will join global efforts in shaping the applications of emerging technologies for the development of

knowledge-based society, enabling cooperation of the government, academia, companies and civil society. The C4IR started its operations in March 2022, under the auspices of Serbia's Office for IT and eGovernment (OITeG), and is focused on two central priorities: biotechnology and artificial intelligence in the health sector. The Centre is currently coordinating several pilot projects, the most prominent of these being the Genetic and Biomedical Data Registry. The role of the Centre in this project covers the establishment of the informational system and introduction of standards for storing and exchange of genetic data, including technical and data protection aspects. The Registry will be available to scientific and healthcare institutions, and the use of data will be focused on research and development in the fields of biotechnology, bioinformatics, bioeconomy, genetics and medicine. This important national resource will be housed in the safe environment of the Serbia's National Data Centre.

C4IR Serbia, together with partners, has conducted a situation analysis of the country's genomic ecosystem, identifying key stakeholders and mapping available sequencing systems. This national inventory, created at the beginning of the work on the Genetic and Biomedical Data Registry, will enable progress monitoring during the forthcoming year, by means of a series of factors: increasing capacities and applications of high-throughput sequencing, increasing number of studies, improving the IT environment for genomics and other indicators.

Summary

Centre for the Fourth Industrial Revolution in Serbia put the focus of its activities in 2022 on establishing the Genetic and Biomedical Data Registry. The first stage encompassed the situation analysis, including an overview of capacities for next-generation sequencing, as well as resources for data storage and processing. The analysis additionally looked at the needs, projects and plans of three focus groups for which genomic data are of particular significance: academia, the health sector and businesses. As the Registry will link health and genetic data, overview presented here is limited to humans and their pathogens. The report is compiled based on publicly available data, questionnaires and interviews with stakeholders.

The results in summary:

- In Serbia, 13 organizations have equipment for next generation sequencing.
- A total of 21 second-, third- and fourth-generation systems are available manufactured by Illumina, BGI, PacBio and Oxford Nanopore.
- The assessed current maximum sequencing capacity is 4493 whole human genomes per year.
- The key missing components identified are IT resources for storage and processing of large amounts of data.



List of Terms



Genome – a complete set of genetic material contained in a cell. The humane genome consists of 22 autosomal chromosomes and sex chromosomes X/Y in the cell nucleus and the mitochondrial DNA.



Exome – the set of all coding regions (exomes) of genes in the body. The exome makes up ~1-2% of the genome.



Clinical exome – targeted exome sequencing limited to genes linked to human diseases.



Transcriptome – set of all transcripts present in the cell. Transcripts are made by translating the DNA sequence into RNA. Research of transcriptome helps understand the genes activities in different tissues



Sequencing – laboratory method for determining the primary structure of biopolymers (nucleic acids and proteins).



High-throughput sequencing – term encompassing the sequencing technologies of second, third and fourth generations. It denotes the possibility of massive parallel sequencing.



Targeted sequencing – sequencing of a selected set of genes or genomic regions.



DNA locus – DNA sequence or gene with a known physical location within the genome.



Mutation – change in DNA sequence which is permanent and is transferred to the next generation of cells.



Read – a raw sequence produced by the sequenator, which may be consisted of several fragments.



Read depth – the number of reads which include a given nucleotide at the same location during sequencing. Greater read depth means more reliable results.



Reference genome – a digital set of chromosomes, non-localized sequences and alternative loci which represent the haploid genome of a certain organism.

List of Abbreviations

CES – Clinical exome sequencing

NGS – Next Generation Sequencing

TES – Target exome sequencing

WGS – Whole genome sequencing

WES – Whole exome sequencing

Introduction

The Center for the Fourth Industrial Revolution in Serbia leads initiatives to position Serbia as a global force in biotechnology.

Sequence of the nuclear and mitochondrial genome is the carrier of information on hereditary and biochemical characteristics of living organisms.

The process of determining the sequence of building blocks, the nucleotides, in the DNA chain is called the DNA sequencing. The first sequencing technique was developed in 1977 by Frederick Sanger's team and it is still routinely used for determining the sequence of DNA fragments. Sanger's sequencing was used to determine the sequence of complete genomes of different organisms and it was the basis for the development of second, third and fourth generations of sequencing, jointly referred to as next-generation sequencing (NGS). This method was originally named "massive parallel sequencing", as it enables sequencing of several DNA chains at the same time, instead of one by one, which is the case with the conventional Sanger's sequencing by capillary electrophoresis. The human genome sequencing project was finalized in 2003, and the first commercial NGS platform was presented in 2005.

After it came into broad use in 2000s, NGS

became a valuable tool for researchers engaged in developing new drugs, precise diagnostics and personalized therapy. Continuous sequencing-related costs reduction, along with permanent technological advancements and automation, have led to more widespread use of NGS in clinical practice. Although routine DNA sequencing in healthcare institutions is still far away, bigger medical centers have started using sequencing for detection and treatment of some diseases. In cases of cancers, for example, doctors are increasingly able to use sequencing data to establish the diagnosis and identify the type of cancer that the patient is suffering from. This enables the doctor to make an adequate choice of therapy and monitor the patient's response. NGS is also a valuable tool in metagenomic studies and is used for diagnostics, monitoring and management of infectious diseases. During 2020, NGS methods were crucial in characterizing the genome of the SARS-CoV-2, and to this day are contributing to monitoring the COVID-19 pandemic.

The basic characteristics of NGS technologies



include: (1) generating millions of short nucleotide sequences simultaneously, which are called reads, (2) great speed, (3) relatively low costs, and (4) computationally demanding data processing.

Whole genome sequencing (WGS) refers to the analysis of the entire nucleotide sequence of genome. On the other hand, the whole exome sequencing (WES) is a form of targeted sequencing related only to parts of the genome which code for proteins. In humans, the exome makes up about 2% of the genome and it is therefore a more cost-effective option, still adequate for analyses in targeted regions requiring greater sequencing depth. However, sequencing only parts of the genome may miss vitally important information, and the potential for new discoveries is reduced. Despite the high, although rapidly decreasing costs and the related data analysis challenges, WGS provides a more complete picture and more powerful analyses. In addition to studying the genetic factors related to human and animal diseases, WGS is used also to determine the characteristics of microbial and agricultural populations. Currently, WGS is used for monitoring antimicrobial resistance, one of the greatest global health challenges. As the costs are constantly decreasing, WGS is used more frequently for a repeated whole human genome sequencing of clinical samples and could become routine in clinical practice. Target exome sequencing (TES) and WES are more focused analyses and are therefore attractive options for population and clinical studies. Despite the limitations, TES and WES are currently important clinical tools in the field of personalized medicine.

In addition to many applications that NGS has in DNA sequencing, it can be used also for RNA analysis. For instance, it makes it possible to

determine the genomes of RNA viruses, such as SARS and the flu. RNA sequencing is often used in quantitative studies, facilitating not only the

identification of transcribed genes in the genome, but also the level of transcription based on the relative quantity of RNA transcripts. The potential rearrangements of DNA sequences can also be detected by identifying new transcripts.

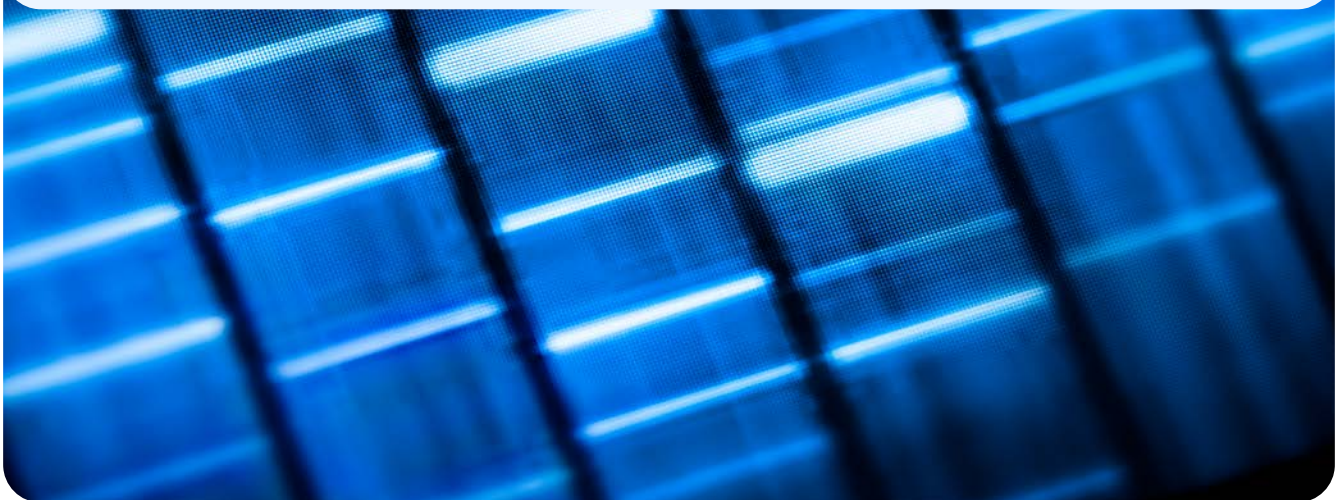
There are two main approaches to NGS technology, sequencing of short and long reads, each with its own advantages and limitations. Each NGS technology generates a significant amounts of output data. The complexity of interpreting results pointed to the bottlenecks in managing, analyzing and storing resulting sets of sequencing data. One of the key challenges globally and in our country is the computational resources needed for collection, mapping and analysis of NGS data. The whole community is committed to developing strategies to increase efficiency, reduce errors and achieve maximum reproductivity. Data centers worldwide are continually trying to address the increasing requirements for data storage and data processing capacities. From March to June 2022, the Centre for the Fourth Industrial Revolution in Serbia conducted a situation analysis in the country of capacities for sequencing and processing and application of genetic data. A series of surveys and interviews was conducted among the academic and health professionals and businesses representatives. The survey included 37 respondents from 16 organizations, all in managing positions and/or directly in charge of sequencing or sequencing related activities, sales of sequencers and customer support. The results of these analyses represent an overview at the national level, concluding with June 2022.



01

Elements of the Genomic Ecosystem in Serbia

NGS data is utilized in diagnostics, research and software development across academic institutions, healthcare and IT companies in Serbia.



The genomic ecosystem in Serbia has been recognized as a complexly interrelated set of (1) academic and health institutions that conduct sequencing by next-generation technologies, (2) academic institutions analyzing NGS data even though they do not have experimental units, (3) companies developing data processing software, (4) sponsors organizations, and (5) other national institutions that provide funding, regulate, or support the use of NGS technologies.

Although the use of high-throughput sequencing in Serbia became common only recently, there is a broad coverage of analyses conducted up to now. NGS methods are used to determine the genetic information of viruses, bacteria, animals and humans. Table 1 shows the scope of analyses conducted so far in the country and internationally according to the survey results.

The analysis of funding sources for NGS projects identified the following institutions that provide funding

for these purposes: the Ministry of Education, Science and Technological Development of Serbia, the Serbian Academy of Sciences and Arts, the Science Fund of the Republic of Serbia, as well as businesses and others. Additionally, diagnostics using NGS have been recognized by the Republic Fund of Health Insurance, which covers the costs of a range of diagnostic services and genetic panels, of which the most frequently used one currently is the diagnosing of rare diseases and cancers. In view of the relevance of genomic and NGS analyses for contemporary research, it is not surprising that despite increasing investments in these areas, respondents stated the need to increase the scope of funding and access to sequencing equipment, and the greatest challenge that they pointed to is the lack of IT resources for data storage and processing. In addition, respondents stated the need for greater and better investments in human capital, particularly for bioinformatics.

Table 1: Samples from Serbia sequenced using NGS until June 2022. The table includes data on samples sequenced in Serbia and abroad, which are available to institutions for analysis and research (i.e. institutions having access to raw data).

Institution	Type of sample	Type of sequencing	Completed
Institute of Molecular Genetics and Genetic Engineering, University of Belgrade	human	WGS	50
	human	WES	34
	human	CES	200
	plants	transcript	9
	microorganisms	WGS	6
	viruses	WGS	>900
Institute for Oncology and Radiology of Serbia	human	target	>1000
Faculty of Biology, University of Belgrade	human	WGS	40
	human	WES	120
National Reference Laboratory, Ministry of Agriculture, Forestry and Water Management	microorganisms	WGS	8
Veterinary Specialist Institute "Kraljevo"	microorganisms, viruses	WGS, target	>300
Institute for Microbiology and Immunology, Medical Faculty, University of Belgrade	viruses	WGS, target	>500
Neurology Clinic, University Clinical Centre of Serbia*	human	CES	60
	human	target	200
Institute for Biological Research "Siniša Stanković", University of Belgrade	animals	WGS, target, 16s rRNA, transcript, miRNA	40
	plants	transcript, target	30
	microorganisms		100
"Vinča" Institute of Nuclear Science, University of Belgrade	human	target	20
Vincula Biotech Group	human	WGS	50
	human	target	several hundred

* Medical Faculty, University of Belgrade – ownership of the machine (capital asset of the Ministry of Science)

1.1 The academic sector

In most countries with advanced field of genomics, its development is supported by significant state investments in science and research. Looking at the comparative analysis of total investments in science and research published in the Strategy of Scientific and Technological Development of the Republic of Serbia for the period 2021-2025, "The power of knowledge", it was identified that state investments in scientific research institutions in 2018 were 46% greater in the European Union than in Serbia. Taking into consideration the difference in absolute amounts of GDP in the EU and Serbia, the difference is 7.33 times greater. The same source also states that ambitious countries continually increase their investment in science (South Korea 4.3%, Japan 3.4%, Finland 3.2%, Switzerland 3.2%, Austria 3.1%, Germany 2.9%, USA 2.7%, Slovenia 2.4%, France 2.3%). Based on the understanding of these processes as well as the inherent conditions in Serbia, it is expected that in the above strategic period the appropriations for science and technological development will increase from the current 0.92% to 1.4% in 2025. This lagging behind in investments is also reflected in the field of genomics in Serbia.

However, the situation is improving. In 2021, the Serbian government supported the establishment of the Centre for Genome Sequencing and Bioinformatics within the Institute of Molecular

Genetics and Genetic Engineering (IMGGE), in cooperation with the Beijing Institute for Genomics. The Centre encompasses a sequencing facility, of approximately 450m² of laboratory space, with systems for the second- and fourth-generation sequencing, produced by various manufacturers. The Centre was established with the objective to implement the 4P medicine (predictive, preventive, personalized and participatory), as well as further development of biomedicine and biotechnology. The infrastructure for safe storage of data, as well as the use of supercomputers of the National AI Platform and big data analysis, was provided through cooperation between the IMGGE and Office for IT and eGovernment.

1.2 The healthcare system

In the healthcare sector, the Republic Fund of Health Insurance in its list of services for 2022 includes the identification of genetic mutations by applying the NGS for different panels: clinical exome, hereditary diseases, cardiovascular diseases, hematological malignancies of myeloid strain and the solid tumors panel, as well as pre-implantation genome analysis and genomic profiling of solid tumors. The costs of such analysis can also be covered by individuals.

Currently, IMGGE is using the target exome sequencing method for diagnosing rare diseases on the samples provided by the centers for rare

5 types of sequencing systems are used in Serbia, manufactured by:

Illumina

BGI

Oxford Nanopore Technologies

ThermoFisher Scientific

PacBio

👁️ Table 1 shows numbers of samples from Serbia sequenced by applying NGS: WGS, WES, CES and targeted sequencing

3 sectors of Serbian society are involved in genomic ecosystem, academia sector, healthcare system and private sector

10 institutions from these sectors obtained sequencing data until June 2022

13 institutions in Serbia have 21 sequencing systems



diseases. Additionally, this Institute has recently been accredited to perform non-invasive prenatal screening testing based on NGS methods. For cancer diagnostics, and the monitoring of disease progress and response to therapy, deep sequencing by high-throughput technologies is conducted by the Institute for Oncology and Radiology of Serbia (IORS). Although there is still no accreditation obligation for laboratories conducting sequencing as a health service, the Department for Genetic Counselling for Hereditary Cancers within the Institute has been certified for genotyping and interpretation of gene variants linked to the hereditary breast and ovarian cancer (HBOC), as well as for the hereditary non-polyposis colorectal cancer (Lynch syndrome). It has become the only certified center in Serbia to conduct genetic laboratory tests and full clinical interpretations of results for hereditary breast, ovarian and colon cancer. The certificate is awarded by the European Molecular Genetics Quality Network (EMQN), which has been establishing the best practices and providing accreditation (ISO 17043) for external quality assessment (EQA) to laboratories worldwide. The Department for Genetic Counselling for Hereditary Cancers continually conducts validation of protocols of NGS methods used in its practice. In addition to the stated tests, the Genetics Laboratory of the IORS also conducts somatic mutations testing for BRCA1/2 genes to select appropriate therapy for patients affected by ovarian cancer.

The scope of analyses that can be conducted within the public healthcare system has been extended by private laboratories, which offer NGS panels for breast and ovarian cancer, prostate cancer, Fanconi anemia, etc. They also offer prenatal tests, and exome and genome sequencing.

It is important to mention the International Atomic Agency's Zoonotic Disease Integrated Action (ZODIAC) initiative, which aims to prepare member states for future pandemics. For research and development activities, reference veterinary institutions and public health institutions may use

the jointly developed technical guidelines, as well as technical, scientific and laboratory support of the Agency and its partners. There are plans within ZODIAC to enable member states, including Serbia, to sequence the samples free of charge. The Serbian representative in this project is the Veterinary Specialist Institute "Kraljevo".

1.3 Business

Serbia has been experiencing an expansion of its IT sector, nevertheless there is still a low number of companies developing solutions for genomics and biological data. According to the exploration by the Centre for the Fourth Industrial Revolution, the company Seven Bridges stands out in this community. It has been present in Serbia since 2011; and in 2021 it had approximately 200 employees. The company's most relevant software product is a platform for genomic data processing and analysis. The headquarters of the company are in Boston, USA, while in Serbia there are teams in Belgrade and Novi Sad.

Centogene, headquartered in Germany, is a company developing genetic diagnostic analyses for rare diseases. It has a development unit in Serbia, which in 2021 had 10 employees, mostly programmers.

Apis Assay Technologies, a UK company, focuses on biomarkers development, services for developing new diagnostic tests, and bioinformatic processing and analysis. In Serbia, Apis Assay Technologies established a company Persida for the development of specialized software solutions. According to the Business Registers Agency, in 2021 it had 10 employees in Serbia, on average.

An important component of the genomic ecosystem are distributors of equipment for sequencing Elta90, representing Illumina, EastDiagnostics representing PacBio, and Vivogen distributing equipment by ThermoFisher Scientific.

02

Sequencing Capacities in Serbia

NGS systems of leading global providers are available in Serbia. IT capacities for genomic data storage and processing are recognized as a main deficient resource.

2.1 Sequencing platforms

Sequencers of all generations exist in Serbia, the most widespread being second-generation sequencers. NGS analyses are being conducted for research purposes, as well as for diagnostics, primarily for rare diseases, selecting therapy and monitoring the progress of disease, but also for diagnosing and treatment of cancers. Table 2 shows the list of organizations having sequencing systems, along with the available platforms. 13 institutions in total, including faculties, institutes and healthcare institutions, have 21 sequencing systems.

Serbia's sequencing capacities were assessed

relative to the maximum number of human genomes that can be sequenced within one year. The assessment methodology was taken from Narayanasamy. Front Genet. 2020, and the sequencing capacity is expressed as the theoretically maximum number of sequencing in gigabases (Table 3). Thus, the presently available capacities in Serbia can sequence annually a maximum of 4,493 human genomes. To come up with a realistic assessment, the maximum capacity should be reduced by 20-30% since the systems rarely operate at full capacity, due to work organization, procurement procedures, system maintenance and other reasons.

A survey of users and equipment suppliers indicated that there are serious plans to extend the capacity by systems of all generations.

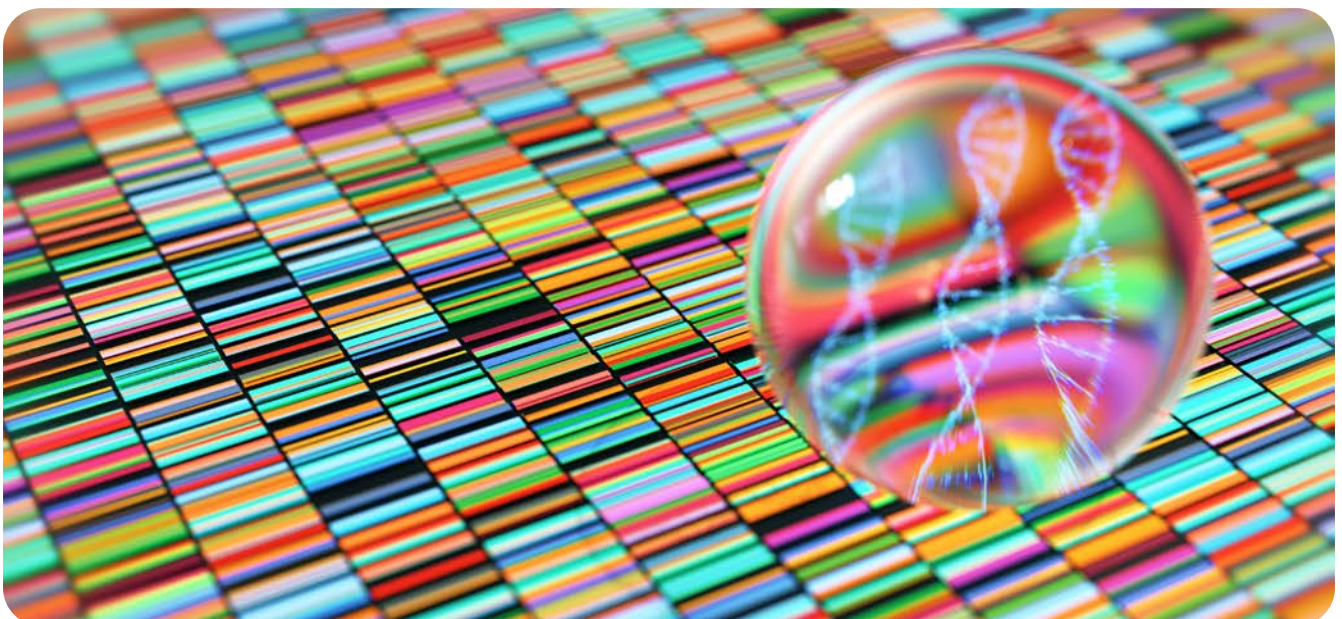


Table 2: Overview of the sequencing systems in Serbia (based on data available by July 2022)

Serial no.	Organization	Manufacturer	Type of device	Number of devices
1	Institute of Molecular Genetics and Genetic Engineering, University of Belgrade	Illumina	MiSeq	1
			NextSeq 550 Dx	1
		BGI	DNBSEQ-G400	3
		Oxford Nanopore Technologies	MinION	1
2	Institute for Microbiology and Immunology, Medical Faculty, University of Belgrade	Oxford Nanopore Technologies	MinION	1
3	Institute for Pathology, Medical Faculty, University of Belgrade	Illumina	NextSeq 550	1
4	The Neurology Clinic, University Clinical Centre of Serbia*	Illumina	MiSeq	1
5	Institute for Oncology and Radiology of Serbia	Illumina	MiSeq	1
			NextSeq 550 Dx	1
6	Veterinary Specialist Institute "Kraljevo"	Oxford Nanopore Technologies	MinION	2
			Illumina	ISEQ100
7	Faculty of Biology, University of Belgrade	ThermoFisher Scientific	Ion S5	1
8	Gynaecological-Obstetrics Clinic "Narodni Front", Belgrade	Illumina	MiSeq	1
9	Institute for Children and Youth Health Protection of Vojvodina	Illumina	MiSeq	1
10	"Vinča" Institute of Nuclear Science, University of Belgrade	Illumina	ISEQ100	1
11	Scientific Veterinary Institute "Novi Sad"	Illumina	MiniSeq	1
12	Institute of Public Health of Vojvodina	Illumina	MiniSeq	1
13	National Reference Laboratory	PacBio	PacBio Sequel	1

* Medical Faculty, University of Belgrade – ownership of the equipment (capital assets of the Ministry of Science)

Table 3: Theoretical maximum sequencing capacity in Serbia (calculated according to Narayanasamy S, et al. Front Genet. 2020)

Platform	Platform specifications		Theoretical maximum annual capacity			Serbia
	Max. Gb by run	Run time (hours)	Number of runs	Max. capacity (Gb)	Human genomes	Theoretical maximum annual capacity (human genomes)
Illumina NextSeq 500/550	120	29	302	36,248	377	1131
Illumina MiSeq	15	56	156	2,346	N/A	N/A
Illumina iSeq 100	1.2	19	461	553	N/A	N/A
BGI DQBSEQ G400	720	107	82	58,946	614	1,842
PacBio Sequel system	500	30	292	146,000	1520	1,520
TFS Ion S5	8	4	2,190	17,520	N/A	N/A
Oxford Nanopore MinION	50	72	122	6,083	N/A	N/A
Ukupno	1,414.2	317	3605	267,697	2,511	4,493
Assumptions:						
<ul style="list-style-type: none"> Human genome sequenced at depth of 30X contains 96 gigabases (Gb) Sequencers operate non-stop (24/7/365 which is 8,760 hours annually) Maximum capacity based on sequenator operating mode yielding the biggest possible result (Gb) 			<ul style="list-style-type: none"> Maximum capacity based on sequenator operating mode with longest possible produced sequence reads Theoretical maximum capacity is calculated based on sequenator specifications Actual capacity will depend on the sequencing institutions 			

2.2 Capacities for storage and processing of genomic data

The respondents in the interviews and survey identified data storage and processing capacities as crucial missing elements for genomic data research. Currently,

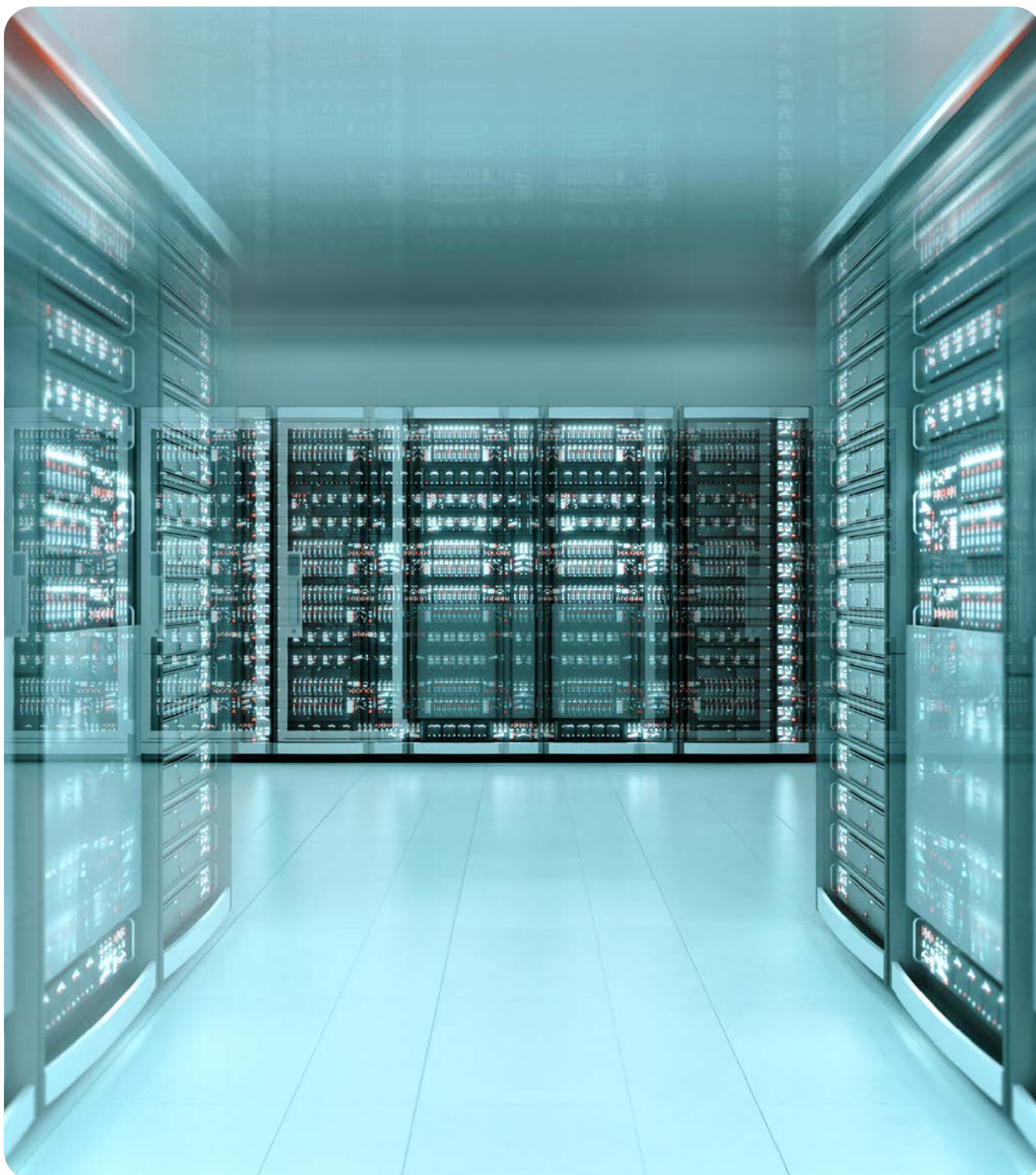
the major state resource for genomic data storage is Serbia's National Data Centre, managed by the OITeG.

The National AI Platform is another resource for the development of advanced solutions based on genomic data and it has a potential to be used for processing of such data, as well as for bioinformatics analyses. The platform consists of four DGX-A100 nVIDIA servers with a total of 32 GPU for processing of DL and HPC simulations with several nodes. This is a universal system for computer tasks in the field of artificial intelligence, including training, analytics

and inference.

The use of both state resources by the academic community has been facilitated by Cooperation Agreements signed by academic institutions and OITeG. So far, such agreements have been signed with the IMGGE in May 2022, and the Faculty of Biology of the University of Belgrade in September 2022. Similar cooperation is planned with other relevant institutions engaged in sequencing and bioinformatics data processing.

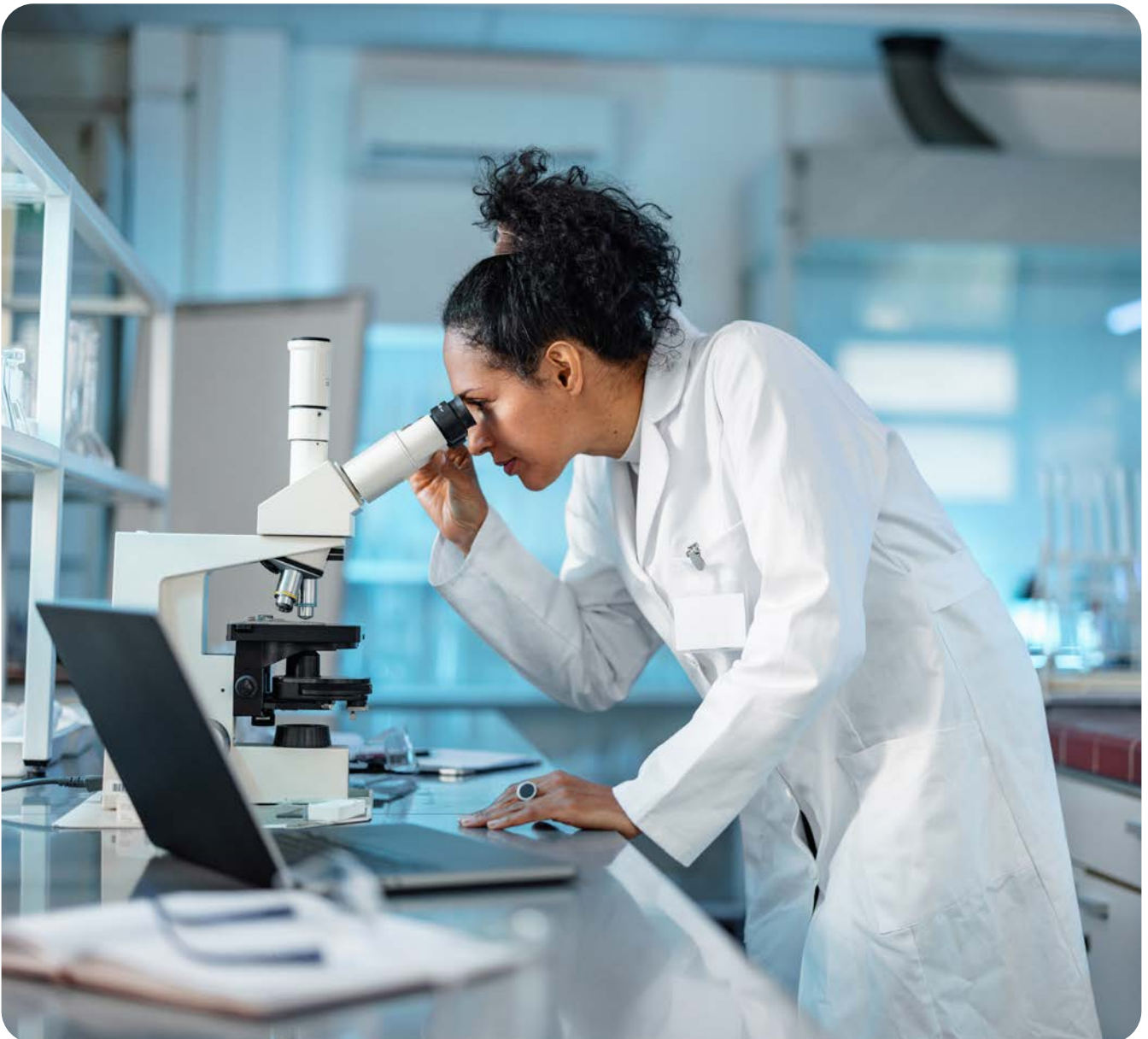
These agreements enable the usage of the National Data Centre for storage of genomic data, for the abovementioned institutions 55 TB have been approved, as well as access and use of the AI Platform for data processing. Additionally, work has been done to increase the infrastructure capacity of the IMGGE by upgrading the interface of the Institute to the Academic Network of the Republic of Serbia and establishing the IPsec crypto tunnel for data transport.



03 | Future Outlook

An accelerated rate of scientific and technological progress in the Fourth Industrial Revolution has major implications for health and medicine. Progress in areas such as genetics, genetic engineering, precision medicine, data science and many others, opens up space for new diagnostics and therapy modalities, offering recovery from disease, reducing suffering, extending life and additional benefits.

The Centre for the Fourth Industrial Revolution of Serbia has prepared this report and made it publicly accessible in order to create a basis for monitoring of progress and improving opportunities for cooperation in the field of genomic research and development of innovative biotechnology products.



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Ministry of Agriculture, Forestry and Water Management, Directorate for National Reference Laboratories

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Waiver

This report has been compiled in accordance with the data received from the respondents in the survey and interviews and public data, and its purpose is to provide information on the situation in the ecosystem to relevant stakeholders. Some institutions are implementing projects with foreign laboratories, within which a certain number of samples from Serbia were sequenced. In such cases, the report includes data on samples which are accessible to institutions for analysis and research purposes (i.e. the institutions have access to raw data). Authors shall not be held accountable for absolute accuracy of this report. In case of doubt regarding any data or obvious error, those concerned can contact directly the Centre for the Fourth Industrial Revolution in Serbia in order to provide relevant evidence and/or corrections in this report.

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