Indian J. Anim. Hlth. (2020), 59(2)-Special Issue: 89-99
DOI: 10.36062/ijah.59.2SPL.2020.89-99

Invited Review Article

COMPARISON OF FOUR HIGH THROUGHPUT SEQUENCING PLATFORMS IN A MEDICAL LABORATORY FOR GUT MICROBIOME RESEARCH[#]

P. DAS[1], R. DADWAL[1], V. RADHAKRISHNAN[2], M. PARIHAR[3], S. BHATTACHARYA[1*], D. K. MISHRA[4], AND M. CHANDY[2]

[1]Department of Microbiology, Tata Medical Center, Kolkata- 700 160, West Bengal, India
[2]Department of Clinical Hematology, Tata Medical Center, Kolkata- 700 160, West Bengal, India
[3]Department of Cytogenetics, Tata Medical Center, Kolkata- 700 160, West Bengal, India
[4]Department of Molecular Genetics, Tata Medical Center, Kolkata- 700 160, West Bengal, India

Abstract

Next Generation Sequencing (NGS) systems, which are more appropriately called High Throughput Sequencing (HTS) systems have revolutionized the way microbiome of a given environment may be studied in considerable details. The reach of HTS is significantly more than that of the standards of Sanger sequencing, and the results from the study of gut microbiome and other metagenomic research has shown that HTS is going to remain as an indispensable tool for such studies for some time to come. Microbiology laboratories working in the field of human medicine, veterinary medicine, agriculture or environmental sciences would need to know and understand the intricacies of HTS methods. This would entail not only a theoretical understanding of the principles of HTS, but also a practical know how about processes, consumables, pitfalls and costs associated with HTS. The current review attempts to explain this important but complex topic with the help of a comparative description of various HTS/NGS systems available in a cancer hospital in eastern India, which is engaged in the use of such technologies for patient care and basic research.

Key words: Gut microbiome, HTS, Microbiome, Microbiota, NGS

Introduction

The human body is made up of more than 10 trillion cells and approximately 30,000 genes (Deloukas *et al.*, 1998; Sender *et al.*, 2016). The human microbiome consists of nearly 100 trillion microbes (Johnson and Versalovic, 2012). These microorganisms help the human body in digestion, vitamin synthesis, immune regulation and various other functions regulated by the immense microbial diversity (Rowland *et al.*, 2018). Until about two decades back, there was no feasible way to study the microbiome of the human gut or any other natural/artificial environment. Culture-based methods are insensitive and not suitable for studying the vast diversity of microbes in the human or animal environment. The advent and rapid development of NGS technology in the last couple of decades has now enabled us to

[*]Corresponding Author
[#] Article submitted by the ALTITUDE Study Investigators group, Tata Precision Oncology program

study the microbiome in considerable details. The terms Next Generation Sequencing (NGS) and High Throughput Sequencing (HTS) are interchangeably used; however, HTS is the technically more appropriate terminology. In this review we have used the terms as appeared in the primary reference.

The difference between Sanger sequencing and Next generation sequencing (NGS)

It is of utmost importance to understand the significant difference between Sanger sequencing and NGS. Sanger sequencing is a low throughput method to determine the nucleotide sequence of a target. It is also known by the name of di-deoxy chain termination sequencing. It is performed with capillary electrophoresis, followed by the computerized detection of the fluorescence emitted by di-deoxy nucleotides (Karger and Guttman, 2009). It is known to be one of the first generation sequencing methods and commercialized by Applied Biosystems initially. Sanger sequencing works optimally for 750bp-1000bp genomic fragments. However, it can sequence only a single DNA fragment at a given time. Sanger sequencing is less sensitive than the NGS with a limit of detection (LOD) of 15%-20% for a mixture of DNA fragments. Sanger sequencing is relatively fast and cheaper than NGS (Tsiatis *et al.*, 2010). The advancements in the field have enabled Sanger sequencing to sequence up to 20 samples at a time. Instead of the limitations in terms of throughput and LOD, Sanger sequencing remains the gold standard for clinical research and medical diagnostics. Sanger sequencing has been used in a variety of applications such as DNA fragment analysis, microbial identification (16S rRNA for bacteria and internal transcribed spacer, ITS for fungi), short tandem repeat (STR) analysis and even confirming the findings of NGS.

NGS, on the other hand, is a high throughput method of DNA sequencing, which is also known by the name of massively parallel sequencing or second-generation sequencing. NGS works on the principle of sequencing by ligation (SOLiD), sequencing by synthesis (Illumina) or pyrosequencing (Ambardar *et al.*, 2016). The manufacturers such as Illumina, Thermo Fisher Scientific and Oxford Nanopore Technologies are the key players in the field of NGS. NGS is a method that can sequence millions of nucleic acid fragments simultaneously at a time. NGS technology has a LOD of about 1% for a mixture of sequences (hence known as the deep sequencing method). However, the technique of NGS is time-consuming and not cost-effective, if the sample numbers to be processed for NGS are <20 (Sanger sequencing is preferred in these cases with smaller sample numbers). NGS is being applied increasingly in the clinical and research labs for whole-genome sequencing (WGS), transcriptome studies and the detection of genetic variants (Behjati and Tarpey, 2013).

NGS platforms and their applications

NGS platforms such as Ion Torrent has been applied for a variety of clinical and laboratory usage. In cancer research, it is used in liquid biopsy, targeted detection and sequencing of the oncogenes; in infectious disease and microbial research, it is used in pathogen typing, metagenomic studies, whole genome sequencing (WGS) of microorganisms and de-novo sequencing. NGS has also been used in RNA sequencing, exome sequencing and targeted RNA sequencing. NGS has also been widely employed in agriculture and veterinary fields (Ambardar *et al.*, 2016).

The Ion Torrent sequencing machines have various versions and models, which are differentiated based on the number of reads, read length, assay time and the number of sensors. The template preparation time, genetic barcode type, sequencing output, number of sequencing reads also differ between equipment

version and NGS equipment manufacturers. Consequently, the sequencing cost of the equipment per run and the cost of sequencing per specimen varies. Comparison of MiSeq from Illumina and Ion Torrent PGM shows that the difference in the capital cost could be as much as $50,000. One run cost is close to $1,000 for MiSeq and $700 for the Ion Torrent. The cost of sequencing per sample for MiSeq and Ion Torrent PGM is $60 and $130, respectively. This is likely to be; because the number of sequencing reads in MiSeq is about 30% more compared to that of the Ion Torrent PGM and the sequencing output is 20% more in the case of MiSeq. Sequencing output is 1.23 GB in case of MiSeq, whereas; it is only 1 GB in the case of Ion Torrent PGM, and reads for MiSeq and Ion Torrent are 8.16 million and 6.5 million, respectively. The template preparation time for MiSeq, is approximately 1 hour compared to 5.5 hours in the case of Ion Torrent, whereas the runtime for MiSeq is 27 hours and only 3 hours for Ion Torrent PGM (Quail *et al.*, 2012).

Pyrosequencing

Roche diagnostics developed a system named Roche 454 GS FLX based on pyrosequencing technology. In this technology, the library preparation is performed by fragment emulsion PCR and read length is approximately similar to Sanger sequencing (750bp - 1000bp). However, the data generated per run is only 0.7 GB compared to 600 GB for a HiSeqIllumina system. The system is less often used these days despite its fast runtime and long reads because of the high error rate and high running cost (Liu *et al.*, 2012).

Comparison of various Illumina systems

Illumina NGS machines are of various models such as MiSeq, NextSeq 500, HiSeq 2500 and HiSeqX. These versions of sequencing machines are differentiated based on various characteristics. These sequencing machines are also of various sizes and weights. The MiSeq and NextSeq approximately have dimensions of 2x2x2 feet (WxHxD), whereas the HiSeq is 4x2.5x3 feet in dimensions. The weight of machines may vary from 55 kg for MiSeq to 226 kg for HiSeq. These machines may have a variable number of flow cells, the number of lanes per flow cell, read length, data production per run and number of hours per run. We can observe that MiSeq can take up to 56 hours per run, NextSeq can take up to 30 hours per run, HiSeq can take up to 6 days and HiSeq X can take up to 3 days for a single run. MiSeq, NextSeq, HiSeq and HiSeq X are known to generate 15 GB, 120 GB, 2000 GB and 1800 GB data, respectively. The read length of these machines varies; MiSeq, NextSeq, HiSeq 2500 and HiSeq X have read lengths of 300bp, 150bp, 125bp and 150bp, respectively. The cluster varies from 25 million in MiSeq to 600 million in HiSeq X. In terms of applications; these machines are used for human WGS where they produce 100 GB data per sample, exon sequencing (25 million clusters per sample), bacterial WGS (0.56 GB per sample), gene expression (20 million clusters per sample) and gene panels (2 million clusters per sample).

Oxford Nanopore

The HTS instruments from Oxford Nanopore Technologies come in the following varieties: MinION and PromethION. The average read length for these machines is 900 kb. Its output is 5 GB and it can produce 1 million reads. The instrument price is considerably less for MinION ($1000) and the cost per run has been estimated to be $500-$700. In nanopore sequencing, a membrane separates two chambers containing electrolyte solution. Nanopores are present on these membranes and when a small voltage is applied across the nanopore, the current through the part can be measured. When a molecule such as a nucleic acid base passes through the nanopore, it results

in the disruption of the ionic current. By measuring the disruption of the ionic current, the molecule is identified (Patel *et al.*, 2018).

Qiagen Gene Reader

Qiagen Gene Reader has a high degree of automation and is used like other NGS sequencers, bioinformatics pipelines for the identification of mutations and potentially the best drugs for treatment. The steps involved in the Gene Reader system include, like others, nucleic acid extraction using kits such as Gene Reader FFPE kits, QiaSymphony and clonal amplification. NGS is performed using Gene Sequencing kits and the data analysis and interpretation are achieved by using Qiagen Clinical Insight (QCI) bioinformatics pipeline (Koitzsch *et al.*, 2017). Quality control is performed using the DNA quantification, quality analysis kits prior to NGS.

Difference between the microbiome and microbiota

Before applying NGS in gut-microbiome studies, we need to understand the difference between microbiome and microbiota clearly. Microbiota mainly focuses on microorganisms in a particular habitat, whereas the microbiome implies the genetic makeup of the microorganisms in a specific habitat. In the broader sense, the microbiome refers to the biotic and abiotic factors within a particular environment, whereas the term microbiota is only concerned with the biotic factors (bacteriome, fungome or virome). Microbiome would be associated with genes and metabolome (Mohajeri *et al.*, 2018).

Difference between prebiotic, probiotic and postbiotic

Prebiotics are foods, food supplements, or chemical compunds that stimulates the growth and/or activity of endogenous microbiota. Postbiotics, on the other hand, are bacterial metabolic products from endogenous microbiota that have biologic activity on the host. Probiotics are bacteria or yeasts which are said to be beneficial to the host due to its effect on attenuation of inflammation, regulation of apoptosis, promotion of intestinal barrier function. Prebiotics support probiotic growth, whereas, post-biotics mimic probiotic function. Dietary fibres are said to be rich sources of prebiotics, whereas, examples of postbiotics include short chain fatty acids. Probiotics are live micro-organisms. Synbiotics are a combination of prebiotics and probiotics. The post-biotics generated by the gut microbes are influenced by prebiotics, probiotics, synbiotics and antibiotics. Garlic, onion, honey, apples, coconut, whole grains, asparagus, leak, tomatoes are considered to be rich sources of prebiotics. Besides short chain fatty acids, vitamins, oligosachharides, polysachharides, certain proteins, enzymes and amino acids are known to be produced from the gut microbiota and act as postbiotics (Pandey *et al.*, 2015).

Human microbiome

The human microbiome is present or divided into various body compartments such as the nose, mouth, skin, genital tracts, gastrointestinal tract etc. There are many factors which affect the microbiota and microbiome those include genetic, environmental, hormonal, drugs (antibiotics), diseases and chemicals. Many diseases cause changes or perturbation of the human microbiome and microbiota, e.g. intestinal obstruction, *Clostridium difficile* associated diarrhea and bacterial vaginosis etc. (Muszer *et al.*, 2015).

Microbiome in animal health

The gastro-intestinal (GI) tract of domesticated animals such as cats and dogs have been investigated by the College of Veterinary Medicine (Texas, USA). The researchers have shown the effect of nutrition on the GI microbiome of cats and dogs. The study

demonstrated that the presence of various proportions of carbohydrates, proteins, fats, prebiotics and probiotics affect the composition or function of the microbiome. Pet food contains substances such as beet pulp, starch, cellulose, which may differ in solubility and fermentability (de Godoy *et al*., 2013; Kröger *et al*., 2017). These differences in composition and consequently, solubilization and fermentability can affect the microbiome composition or function. Studies on pets have also demonstrated the impact of various postbiotics on pet health (Wernimont *et al.*, 2020). Important examples include short-chain fatty acid (SCFA) acetate which acts on the neuroendocrine system; SCFA propionate affects the systemic energy availability; SCFA butyrate affects the colon and indole sulphate affects the colon and neuroendocrine system. Polyamines viz. spermidine, putrescine and cadaverine delay the intestinal epithelial senescence; hydrogen sulfide has an effect on ulcerative colitis; indoxyl-sulfate reduces kidney functions. A study from the agricultural university of Gujrat, India, characterized the microbiome of cattle using the Ion Torrent PGM platform. The taxonomic study showed that the microbiome of cattle was dominated by *Bacteroidetes* followed by *Firmicutes*, and *Proteobacteria* (Patel *et al.*, 2014). *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Fusobacteria*, *Spirochaetes*, *Actinobacteria* are the commonly found microbes from the gut of human, cat, cattle and dog (Wernimont *et al.*, 2020).

Technologies for nucleotide sequencing and comparative sequence analysis has been in use in veterinary medicine for some time (Granberg *et al*, 2016). Viral diversity in swine intestinal mucus has been analysed by HTS (Dumarest *et al.*, 2015). A study from France found *Circoviridae* and *Parvoviridae* members as the most prevalent viruses in swine intestinal mucus, together with viruses from the *Picornaviridae*, *Astroviridae*, *Reoviridae*, *Caliciviridae*, *Adenoviridae*, *Birnaviridae*, and *Anelloviridae* families. Some putative new viral species were also identified by HTS (Dumarest *et al.*, 2015). Similarly,NGS has been applied to fish ecotoxicogenomics (Mehinto *et al.*, 2012).

Table 1 shows the various HTS platforms available at Tata Medical Centre, Kolkata. Various other HTS platforms are available from Thermo Fisher Scientific (Ion Proton, Ion PGM, Ion X5 Xl), Illumina (HiSeq 2500/3000/4000, HiSeq X, MiSeq, NextSeq and NovoSeq 5000/ 3000), Qiagen (Gene Reader) and Oxford Nanopore Technologies (MinION, Prometh ION) (Table 2). The GeneStudio S5 series machines employ various versions of Ion Chips; these versions of IonChips differ in the type of kit used, applicability in microbiome studies, targeted RNA sequencing, micro and small RNA profiling (Table 3). When comparing the limitations of the Gene Reader platform of Qiagen to the Illumina NGS system, both of them are NGS, but certain functions can be performed by only the Illumina NGS platform, e.g. Whole genome sequencing, Gene Expression analysis, micro-RNA analysis and targeted RNA sequencing and not the Gene Reader (Table 4). The NGS platforms differ in the applications, specifications, cost, read length, accuracy, dimensions, consumables, requirements, user-friendliness, runtime, weight, software used and various other parameters. The platforms iSeq, MiSeq, MiniSeq and NextSeq can be differentiated based on applications like microbial WGS, 16S metagenomic sequencing, transcriptomic analysis and shotgun metagenomic sequencing (Table 5). Some of the smallest NGS platforms are available from Oxford Nanopore Technologies named MinION, PromethION and others (Table 6). These sequencers weigh less than 100gms (Flongle and MinION) and they may be powered by a laptop. The run time may be varying from 1 minute to 72 hours, depending upon the application.

Table 1. Four major NGS manufacturers in the market with their range of NGS equipment models

Equipment manufacturer	Platform/ Model/Version
Thermo Fisher Scientific	▷ Ion S5 XL ▷ Ion Proton ▷ Ion PGM
Illumina	▷ HiSeq 2500/3000/4000 ▷ HiSeq X ▷ MiniSeq ▷ MiSeqDX/MiSeq FGX ▷ NextSeq 500/550 ▷ NovoSeq 5000/6000
Qiagen	▷ Gene Reader
Oxford Nanopore Technologies	▷ MinION; PromethION

Table 2. Comparisons of 4 different NGS technologies

	QIAGEN Gene Reader	Illumina	Ion GeneStudio S5 System	Nanopore
Company contact	QIAGEN	Illumina	Thermo Fisher Scientific	Oxford Nanopore Technologies
Name of instrument	QIAGEN, Gene Reader NGS System	Illumina, MiSeqDx System	Thermo Fisher Scientific, Ion GeneStudio S5 System	MinION Mk1C
The country where designed/ manufactured/ FDA cleared or approved	Germany	U.S./U.S./yes	U.S./U.S./—	UK/USA
First year sold in U.S./Outside U.S./ First year installed	2016/2016/2015	2013/2013/2013	2018/2018/2018	2014/2015/ 2015
Equipment supplied with system/ automation for library preparation	GeneRead QIAcube for clonal library amplification, PC work station for Gene Reader, power station server for data analysis, QIAcube for sample extraction and library preparation/yes	Single unit inclusive of amplification, paired-end sequencing/yes	Ion GeneStudio S5 sequencer/yes	MinION Mk1C with VolTRAX forportable sample preparation, PC work station

Table 2. Cont.

Cont. Table 2.

	QIAGEN Gene Reader	Illumina	Ion GeneStudio S5 System	Nanopore
Necessary equipment not included with the system (additional cost)	Thermocycler (~$3,000), Qubit (~$2,000)	NA	Ion Chef System for automated library and template prep, general laboratory supplies	NA
Bioinformatics tools provided/For use by biologist or bioinformatician	GeneRead Link middleware, QIAGEN Clinical Insight Analyze, QIAGEN Clinical Insight Interpret/biologist	Local Run Manager, MiSeq Reporter, BaseSpace/ biologist	Torrent Suite, Ion Reporter, Oncomine Reporter, Torrent Circuit/biologist	EPI2ME Real-time data analysis workflows accessed through the cloud orlocally using MinION Mk1C or MinIT, Min KNOW and Guppy also for base calling
Supplied with UPS/ entire workflow can occur in the same lab	yes (extra charge)/yes	yes (extra charge)/yes	no/yes	no/yes
Cleanroom requirements/ electrical connection	—/100–240 VAC, 50–60 Hz	—/100–240 VAC at 50–60 Hz	none/100–240 VAC, 50–60 Hz, 6.5–14.5 A	Supplied with a 6.3-19.6 VDC power supplyMax rated current 10 A Max rated power 60 W
Purchase options	capital purchase, reagent rental, lease	purchase, reagent rental, lease (financing available)	purchase, trade-in, lease (financing available)	capital purchase, reagent rental, lease
Warranties offered	1 year	first year included with purchase, extended warranty available	first year included with purchase, extended warranty available	first year included with purchase, extended warranty available
Training included/ Total time for a standard installation and basic training	yes/1 week	yes/<1day installation, <1day training	yes/1 day	Online workshop (free)1 day training for Rapid Start Day Training

Table 2. Cont.

Cont. Table 2.

	QIAGEN Gene Reader	Illumina	Ion GeneStudio S5 System	Nanopore
				($6000) 2-day training in Advance Nanopore Training ($15000)
Training location/ Follow-up training available	on-site/yes	on-site/yes (extra charge)	on and off-site/yes (extra charge)	Due to COVID-19, training packages available online
Instrument core performance •Maximum number of libraries amplified in single amplification event	24×3	384 samples (>384 samples with custom barcodes)	384 (with custom barcodes)	Up to 30 Gb per MinION Flow Cell / 2 Gb per Flongle Flow Cell

Table 3. Different application Ion GeneStudio S5 series from Thermo Fisher Scientific with kit specification

	Kit used	Ion 510 Chip	Ion 520 Chip	Ion 530 Chip	Ion 540 Chip	Ion 550 Chip
Max read size		3M	6M	20M	80M	130M
Targeted DNA seq*	Ion Plus Fragment Library Kit	YES	YES	YES	YES	YES
16S metagenomics*	Ion 16S Metagenomic kit	-	YES	YES	-	-
Small genome seq*	Ion Xpress Plus fragment library kit	-	YES	YES	-	-
Exomeseq	Ion AmpliSeq Exomepannel	-	-	-	YES	YES
Targeted RNA seq	Ion AmpliSeq made to order RNA panels	YES	YES	YES	YES	YES
mi RNA/small RNA profile	RNA Seq v2 kit	YES	YES	YES	-	-
Low pass whole genome sequencing (PGS)	Ion reproseq PGS kit	YES	YES	YES	-	
Targated transcriptome seq	AmpliSeq transcriptome human gene expression kit	-	-	-	YES	YES

*Most commonly used protocol for different microbiome studies

Table 4. Limitation of the Gene Reader platform as compared to Illumina NGS systems

	Illumina	Gene Reader NGS System
Whole genome analysis	Yes	No
Gene expression analysis	Yes	No
Small RNA-seq, including miRNA	Yes	No
Targeted RNA panels	Yes	No

Table 5. Applications of different Illumina platforms

	iSeq 100	MiniSeq	MiSeq Series	NextSeq 550	NextSeq 2000
Small whole-genome sequencing (microbe, virus)	Yes	Yes	Yes	Yes	Yes
Targeted gene sequencing (amplicon-based, gene panel)	Yes	Yes	Yes	Yes	Yes
Exome and large panel sequencing (enrichment-based)	-	-	-	Yes	Yes
Targeted gene expression Profiling	-	Yes	Yes	Yes	Yes
16S Metagenomic sequencing	-	Yes	Yes	Yes	Yes
Metagenomic profiling (shotgun metagenomics, metatranscriptomics)	-	-	-	Yes	Yes

Table 6. Different format for Nanopore technologies with its utility and requirements

	Flongle	MiniION Mk1B	MiniION Mk1C	GridION Mk1	PromethION 24/48
Power	Powered by MinION / GridION	Powered by Laptop / MinIT	25 W	800 W	2 kW
Weight	20 g	87 g	450 g	11 kg	Sequencer: 28 kg Data Acquisition Unit: 25 kg
Run time	1 min -16 hours	1 min - 48 hours	1 min - 48 hours	1 min - 48 hours	1 min - 72 hours
Number of flow cells / device	1	1	1	5	24/48
Software License and Warranty annual charge	-	-	$300	$12500	$20000
Suitable applications include	AmpliconsPanels/ targeted sequencing Quality testing Small sequencing tests	WGS/exomes Metagenomics Targeted sequencingWhole transcriptome (cDNA) Smaller transcriptomes (direct RNA) Multiplexing for smaller samples	Whole genomes/exomes Metagenomics Targeted sequencing Whole transcriptome (cDNA) Smaller transcriptomes (direct RNA) Multiplexing for smaller samples Particularly suitable for field use	Larger genomes or projects Whole transcriptomes (direct RNA or cDNA) Large numbers of samples	Very large genomes or projects Population-scale human Whole transcriptomes Very large numbers of samples

Conclusion

HTS platform needs to be selected after careful consideration not only because of the cost of capital and consumables but also for the compatibility with various applications, data quality and ease of use. There are many factors that need an appraisal before selecting an HTS platform. The factors that need to be considered include those related to the machine, people, material, methods and environment. Within machine factors, the laboratory needs to be very clear about the objective of HTS, sample load, budget, space availability, specific applications etc. Other important factors to be considered include the chemistry of detection, read length, number of reads per minute, throughput requirement, additional equipment required. HTS systems cannot be run without appropriate human resources who are skilled in HTS technique, data analysis and bioinformatics. With regards to the material, the laboratory needs to consider the packaging and shelf-life of the kits, pricing and vendor. Suitable kits for research use or diagnostic applications, quality control, financial arrangements such as reagent rentals, supply chain management, compatibility with EQAS (external quality assurance scheme) and specimen compatibility must be ensured before any HTS run.With regard to the methods, one needs to identify the appropriate reference data and reference methods. The SOPs (standard operating procedures) need to determine coverage, error rates, time to result, validation, experiments, data analysis software and DNA library preparation methods.

The environment for HTS experiments needs a well defined IT (information technology) setup, data storage policy, power backup, ultra-pure water supply, sample storage policy, safety and security systems. The machine itself should be protected from temperature changes, vibrations and humidity changes. It is clear from this review that HTS is a potent tool, but the system needs to be selected after considerable review of literature, and discussion with stake holders.

REFERENCES

Ambardar S, Gupta R, Trakroo D, Lal R and Vakhlu J, 2016. High throughput sequencing: An overview of sequencing chemistry. Indian J Microbiol, 56(4): 394-404, doi: 10.1007/s12088-016-0606-4

Behjati S and Tarpey PS, 2013. What is next generation sequencing? Arch Dis Child Educ Prac Ed, 98(6): 236-238, doi: 10.1136/archdischild-2013-304340

Deloukas P, Schuler GD, Gyapay G, Beasley EM, Soderlund C *et al*., 1998. A physical map of 30,000 human genes. Science, 282(5389): 744-746, doi: 10.1126/science.282.5389.744

de Godoy MRC, Kerr KR and Fahey Jr GC, 2013. Alternative dietary fiber sources in companion animal nutrition. Nutrients, 5(8): 3099-3117, doi: 10.3390/nu5083099

Dumarest M, Muth E, Cheval J, Gratigny M, Hebert C *et al.*, 2015. Viral diversity in swine intestinal mucus used for the manufacture of heparin as analyzed by high-throughput sequencing. Biologicals, 43(1): 31-36, doi: 10.1016/j.biologicals.2014 10.004

Granberg F, Baìlint Aì and Belaìk S, 2016. Novel technologies applied to the nucleotide sequencing and comparative sequence analysis of the genomes of infectious agents in veterinary medicine. Rev Sci Tech, 35(1): 25-42, doi: 10.20506/rst.35.1.2415

Johnson CL and Versalovic J, 2012. The human microbiome and its potential importance to pediatrics. Pediatrics, 129(5): 950-960, doi: 10.1542/peds.2011-2736

Karger BL and Guttman A, 2009. DNA sequencing by capillary electrophoresis. Electrophoresis, 30(S1): S196-S202, doi: 10.1002/elps. 200900218

Koitzsch U, Heydt C, Attig H, Immerschitt I, Merkelbach-Bruse S *et al*., 2017. Use of the

GeneReader NGS system in a clinical pathology laboratory: A comparative study. J Clin Pathol, 70(8): 725-728, doi: 10.1136/jclinpath-2017-204342

Kröger S, Vahjen W and Zentek J, 2017. Influence of lignocellulose and low or high levels of sugar beet pulp on nutrient digestibility and the fecal microbiota in dogs. J Anim Sci, 95(4): 1598-1605, doi: 10.2527/jas.2016.0873

Liu L, Li Y, Li S, Hu N, He Y *et al*., 2012. Comparison of next-generation sequencing systems. J Biomed Biotechnol, 2012: 251364: doi: 10.1155/2012/251364

Mehinto AC, Martyniuk CJ, Spade DJ and Denslow ND, 2012. Applications of next-generation sequencing in fish ecotoxicogenomics. Front Genet, 3: 1-10, doi: 10.3389/fgene.2012.00062

Mohajeri MH, Brummer RJM, Rastall RA, Weersma RK, Harmsen HJM *et al*., 2018. The role of the microbiome for human health: from basic science to clinical applications. European J Nutr, 57(S1): 1-14, doi: 10.1007/s00394-018-1703-4

Muszer M, Noszczyñska M, Kasperkiewicz K and Skurnik M, 2015. Human microbiome: when a friend becomes an enemy. Arch Immunol Ther Exp, 63(4): 287-298, doi: 10.1007/s00005-015-0332-3

Pandey K, Naik SR and Vakil B, 2015. Probiotics, prebiotics and synbiotics- A review. J Food Sci Technol, 52(12), doi: 10.1007/s13197-015-1921-1

Patel A, Belykh E, Miller EJ, George LL, Martirosyan NL *et al*., 2018. MinION rapid sequencing: review of potential applications in neurosurgery. Surg Neurol Int, 9(1): 157, doi: 10.4103/sni.sni_55_18

Patel V, Patel AK, Parmar NR, Patel AB, Reddy B *et al*., 2014. Characterization of the rumen microbiome of Indian Kankrej cattle (*Bos indicus*) adapted to different forage diet. Appl Microbiol Biotechnol, 98(23): 9749-9761, doi: 10.1007/s00253-014-6153-1

Quail MA, Smith M, Coupland P, Otto TD, Harris SR *et al*., 2012. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. BMC Genom, 13: 341, doi: 10.1186/1471-2164-13-341

Rowland I, Gibson G, Heinken A, Scott K, Swann J *et al*., 2018. Gut microbiota functions: metabolism of nutrients and other food components. European J Nutr, 57: 1-24, doi: 10.1007/s00394-017-1445-8

Sender R, Fuchs S and Milo R, 2016. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol, 14(8): e1002533, doi: 10.1371/journal.pbio. 1002533

Tsiatis AC, Norris-Kirby A, Rich RG, Hafez MJ, Gocke CD *et al*., 2010. Comparison of Sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: diagnostic and clinical implications. J Mol Diagn, 12(4): 425-432, doi: 10.2353/jmoldx.2010.090188

Wernimont SM, Radosevich J, Jackson MI, Ephraim E, Badri DV *et al*., 2020. The effects of nutrition on the gastrointestinal microbiome of cats and dogs: impact on health and disease. Front Microbiol, 11: 1266, doi: 10.3389/fmicb.2020.01266

Received-21.10.2020, Accepted -25.11.20 Published -01.12.2020