*Review Article*

Journal of Microbiology and Related Research
Volume 6 Number 2, July - December 2020
DOI: http://dx.doi.org/10.21088/jmrr.2395.6623.6120.1

# Next generation sequencing technologies: Methods and applications in animal virology

**Koushlesh Ranjan[1], Basanti Brar[2], Aman Kumar[3] , Minakshi Prasad[4]**

**Author Affiliation:** [1]Department of Veterinary Physiology and Biochemistry, SVP University of Agriculture and Technology, Meerut, Uttar Pradesh
[2]LLR University of Veterinary and Animal Sciences, Hisar, Haryana
**Corresponding Author:** Koushlesh Ranjan1 Assistant professor Department of Veterinary Physiology and Biochemistry, SVP University of Agriculture and Technology(Sardar Vallabhbhai Patel University of Agriculture and Technology) Meerut, Uttar Pradesh-250110
**E-mail:** drkoushleshranjan@gmail.com

**Abstract**

Recent advancement in next-generation sequencing (NGS) technologies hasrevolutionized the sequencing technology for research and diagnostic applications by virtue of its high throughput and accuracy of data generation. In animal virology, NGS has been successfully used formetagenomicsbased discovery of previously unknown viruses. Moreover, NGS technologies are alsoemployed for study of viral dynamics and genetic characterization of viral genomes.The absenceof proof reading during genome replication inseveral viruses along with high replication rate, results in formation of several genetically related viral variants known as quasi-species which in turn, got selected and established itself as new virus variant by antiviral drugs or immune system itself. The identification ofviral quasi species havingbiological significance is difficult using conventional sequencing approach. However, NGS may provide exact sequence information about these virus clones. NGS is a powerful tool to investigate deeper insights ofvirus activities such as viral genetic diversity, vaccine candidate selection, identification of viral reservoirs in nature, re-emergence of viral disease after treatment interruptions, development of drug resistance etc.

**Keywords:** Virus, NGS, Ion Torrent, Illumina sequencing

**How to cite this article:**

Koushlesh Ranjan, Basanti Brar, Aman Kumar, et al. / Next generation sequencing technologies: Methods and applications in animal virology. J Microbiol Relat Res. 2020;6(2):5–8

## Introduction

Viruses are omnipresentcreatures and responsible for severe diseasesin humans, animals and plants. Several animal viruses are of zoonoticin nature and important frompublic health point of view. Livestock is directly or indirectly remain in contact with humans and they are also part of food sourcesfor humans. Therefore, the specificdiagnoses of the viral diseases and their etiological agents especially in livestock are of prime importance(Lambe et al., 2016). The early diagnosis ofthe viral pathogen at the primary level of infectionthrough molecular diagnostics techniques such as Real-Time PCR and Next-generation sequencing (NGS) techniques, etc.is essential to control the infection before the maximum population is affected which may result in decreased losses to human health and livestock industry (Vishwaradhya et al., 2013; Stephen et al., 2015).

Recent advancement in sequencing technologies hasinitiated a revolution and new perspectives for research and diagnostic in field of virology (Minakshi et al., 2012). The NGS technologies have hallmark features of high throughput sequencing at a modest cost, lesser time, and huge amount of sequence data (in Gigabases)generation in a single run of reaction. The first commercially NGS platform was made available by 454 Life Sciencesin 2005. Subsequently, several NGS technologies such as HiSeq sequencers (Illumina, USA), Genome Sequencer (GS) FLX (454 Life Sciences,

USA),Heliscope platform (HelicosBioSciences, USA),Ion Torrent (Applied Biosystems),SOLiD technology (Applied Biosystems, USA), and PacBio RS (Pacific Biosciences, USA) came in the market for commercial use (Capobianchi et al., 2013). All these NGS platforms have their specific advantages and disadvantages. However, with the decreasing costs of sequencing, the NGS techniques have allowed several achievements in virology researchsuch as diagnosis of emerging viral infections, the study of molecular epidemiology of viruses, the studyof viral drug-resistance, and basic and clinical research (Radford et al., 2012). In the current review paper, we have discussed the various NGS techniques and their applications in the field of animal virology.

### *Virus asa potent pathogen*

Viruses constitute serious form of pathogens found in diverse form of ecosystems and hosts such as human, animal, birds, plant and marine ecosystems. Several metagenomics studies have shown that viruses are the dominant species of our living system (Vibin et al., 2018; Schulz et al., 2020). The deep sequencing of natural samples shows that approximately 90% of the sequences obtaineddid not encode any known proteins, which are already reported in other organisms, including viruses that have been characterizedrecently. Thisclearly indicates that the actual viral diversity has not been explored so far (Chalkias et al., 2018). Recently, the major emphasis has been given to study on economic as well aszoonoticimportant viral infections.

World Health Organization reported that communicable diseases (including major culprit as viral diseases) is approximately 15 million annually (Dye et al., 2013).

Viruses play a central role among infectious diseases due to its smallest size, short generation time, large population sizeand high mutation rates. Moreover, variation in nature of genome (DNA/ RNA),genome size, assembly of virion particles make viruses an ideal subject for evolutionary study of living system (Koonin and Dolja, 2013). It is well known that viruses use all the known replication and expression strategies dynamically to adapt the continuously changing environments. Viruses possess several molecular mechanisms to escape from host defense mechanismwhich can be deciphered throughNGS based genome sequencing and subsequent bioinformaticsanalyses.

### *Viralgene and genome sequencing*

#### *Sanger sequencing*

Sanger sequencing is first-generation DNA sequencing protocol. This technique is based on principle ofselective incorporation of chain-terminating dideoxynucleotides (ddNTPs) during in vitro DNA replication in a DNA strand (Sanger et al., 1977a). Once these ddNTPsare incorporated in DNA, the chain elongation gets termination. The radio-labeled(32P)nucleotide bases and addition of one out of four modified nucleotide (ddNTP) bases per reaction allows the determination of nucleic acid sequence on polyacrylamide gel by X-ray films based autoradiography. The introduction of thermostable DNA polymerases (Saiki et al., 1985) in molecular biology, florescent labeleddideoxynucleotidesfollowed by capillary sequencing technique (Marsh et al., 1997) allowed the automation of Sanger's method of sequencing. This led to major breakthrough in unraveling of genomes of several model organisms. Later on it was commercialized by Applied Biosystems(ABI) in 1986 (Adams, 2008).The ABI system (3030xL genetic analyzer) was used for molecular characterization several Bluetongue virus serotypes in India (Ranjan et al., 2013; 2014; 2015a;Dadawala et al., 2013; Kumar et al., 2013).

#### *Next-Generation Sequencing*

The story of complete genome sequencing of viruses starts from complete genome sequencing of bacteriophage MS2 having RNA genome of 3,569 nucleotides long (Fiers et al., 1976). Next year complete genome sequence of a DNA bacteriophage ΦX174 of approximately 5,375 nucleotides was sequenced using Sanger's shotgun sequencing technique (Sanger et al., 1977b).The major objective of early days genome sequencing was to characterize the genomic content of an organism in terms of its amino acid coding. In last decades, the sequencing technologies havegrown tremendously and apart from viral genome several other eukaryotic species genomes are also sequenced. Later on, several platforms of next generation sequencing technologies such as Ion torrent, Illumina etc. have been developed to generate complete genome sequence data of any organisms. The huge scale of generating the genome sequence data became a reality today, which was unimaginable previously. The major advantages of NGS technique over the conventional capillary sequencing are the rapid generation of complete genome sequencing data on a very massive scale and at relatively lower cost.NGS also provides tools for several types of molecular studies includingsingle-nucleotide polymorphism, RNA profiling, transcriptomics, gene expression and regulation etc.Viral genome

sequencing has important role in development of newer vaccines, to understand and predict the spread of viral epidemics (Kasibhatla et al., 2016). Several NGS based techniques such as Ion Torrent (https://www.lifetechnologies.com), Illumina(http://www.illumina.com/),Roche 454 (http://www.454.com/), and recently developed fourth-generation sequencing methodologies such as single-cell sequencing, including Oxford Nanopore (https://www.nanoporetech.com/) and Pacific Biosciences (http://www.pacificbiosciences.com/) are available for complete genome sequencing.

### *Different platforms for NGS technology*

Although chemistry of different NGS technologies varies but their basic workflow is similar (Figure 1). Based on chemistry involved, NGS technologies can be classified into sequencing by ligation, sequencing by synthesis and single molecule sequencing. The basic principles, advantages and disadvantages of various sequencing platforms are summarized in Table 1.

**Table 1:** Comparisons of different sequencing platforms (Minakshi et al., 2014; https://nanoporetech.com/products/comparison)

| Sequencing Platforms Parameters | Ion Torrent (Ion semi-conductor) | Roche 454 (Pyrosequencing) | Illumina (Sequencing by synthesis) | SOLiD (Sequencing by ligation) | Pacific Bio (Single-molecule real-time sequencing) | Helicos (True Single Molecule Sequencing) | Oxford Nanopore Technology (Real-time sequencing) | Sanger method (Chain termination) |
|---|---|---|---|---|---|---|---|---|
| Sequencing chemistry | Detection of released H+ | Pyrosequencing | Reversible terminators | Ligation | Fluorescently labelled dNTPs | Reversible terminators | Nanopore sequencing | Di- deoxy Chain termination |
| Amplification method | Emulsion PCR | Emulsion PCR | Bridge ampli-fication in situ | Emulsion PCR | Linear amplification | No amplification | Amplification free approach | Sequencing PCR |
| Separation method | Ion Spheres and high density array | Microbeads and 'picotitre' plate | Glass slide hybridization | Beads on glass slide | Captured by DNA polymerase in microcell | Flow-cell hybridization | Changes to electrical current as nucleic acids passed through protein nanopore | Electro-phoresis |
| Read length | 200 -400bp | 700 bp | 50 to 250 bp | 50-75 bp | 1000 bp | 25bp | Up to 2Mb | 400 to 900 bp |
| Reads per run | up to 5 million | 1 million | up to 3 billion | 1.2 to 1.4 billion | 35-75 thousand | 1 billion | 7-12 million | Not available |
| Maximum data output per run | 1 Gb | 700 Mb | 600 Gb | 20 Gb | Not available | 35 Gb | 2 Gb to 5.2 Tb | Not available |
| Accuracy | 98% | 99.9% | 98% | 99.9% | 99% | 99% | 98-87% | 99.9% |
| Advantages | • Equipment relatively less expensive<br>•Fast reaction | •Long read size.<br>• Fast reaction. | •High sequence yield | •Low cost per base of sequencin-g | •Longest read length.<br>•Less time consuming | •No PCR induced bias and errors<br>•Tolerates degraded samples | •Portable machine<br>•Less time consuming<br>•Real-time result | •Long individual reads.<br>•Applied in many sequence based research. |
| Disadvantages | Homopolymer error. | •Homopolymer error.<br>•Runs relatively expensive. | . •High DNA concentratio-n required<br>•Very expensive equipment. | • Slower than other sequencin-g methods. | •Low yield at high accuracy.<br>• Equipment very expensive. | •More time to sequence a single nucleotide<br>•High error rate | •High error rate | •Higher cost per base of sequencing.<br>•Impractical inwhole genome sequencing |

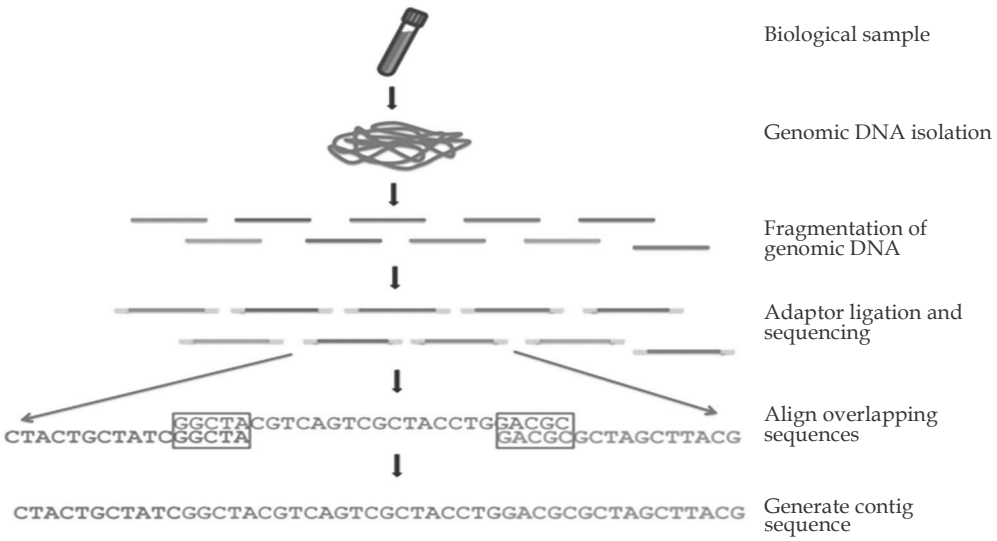


Fig. 1: Basic workflow of next generation sequencing technologies (Ranjan et al., 2015b)

***Sequencing by ligation***

This method of DNA sequencing uses DNA ligase enzyme to identify the position of a particular nucleotide in a DNA sequence. It does not require DNA polymerase enzyme to create a second strand. The target DNA sequence is determined by the mismatch sensitivity of DNA ligase enzyme.The SOLiD (Sequencing by Oligonucleotide Ligation and Detection) sequencing platform is based on the principle of sequencing by ligation (Valouev et al., 2008).

In this method of sequencing, clonal magnetic bead based library of DNA fragments is prepared from the sample in such a way that only one fragment will be present on surface of each bead. Emulsion PCR is allowed to run using primers against the P1 adopter attached to terminal end of each fragment on beads. The resulting PCR products attached to the beads are allowed to covalently bind to glass slide.

Later on, primers hybridize to P1 adapter in library template. A set of four fluorescentdyeslabeled di-base probes compete for ligation to sequencing primer. Specificity of the di-base probe is achieved by searching every 1st and 2nd nucleotide base in each ligation reaction. For complete sequencing of nucleic acid strand, multiple cycles of ligation, detection and cleavage are required. In subsequent ligation cycle, the extension product is removed and the nucleic acid template is reset with primer complementary to the n-1 position and second round of ligation cycle starts. Although this technique got popularity in early days but later on short read length of 75 bp only reduces its use over other NGS techniques.

***Sequencing by synthesis***

In this technique library is prepared form nucleic acid (DNA) fragment by fragmentation of DNA strand,adopter ligation and clonal amplification called as library preparation. Later on, clonally amplified products are purified and allowed for sequencing. During sequencing reaction,newnucleotides are added by the polymerase enzyme and generate signals which are detected and read by the NGS machine.

Sequencing by synthesis can be of two type viz., singlenucleotide addition and cyclic reversible termination. Single nucleotide addition approach is used inIon Torrent and 454 pyrosequencingtechniques. The Ion Torrent is a unique technique for sequencing because it is based on detection of pH change during newer dNTP incorporation which releases of H+ ions (Rothberg et al., 2011). It utilizesanion semiconductor sensor to identify the H+ ions released.The Ion-Torrent platform was used for complete genome sequencing of Bluetongue virus (BTV) serotype 16 from India (Minakshi et al., 2012). However, thePyrosequencing method is based on detection of bioluminescence signal which isgenerated due to release of pyrophosphate upon fresh nucleotide incorporation (Margulies et al., 2005).

In cyclic reversible termination method cleavable fluorescent terminator molecule blocks the chain elongation (Guo et al., 2008). GeneReaderand Illuminaplatform utilize this technique with certain modifications. In this technique the mixture of all the four nucleotide bases along with di-deoxydNTP's are added each time of reaction. The unbound dNTP's are removed by washing.

The detection of fluorescent signal determines the specificity incorporated dNTP.

### *Single-molecule sequencing*

The recent advancement in sequencing technology allows real-time sequencing of single molecule of nucleic acid. The Oxford Nanopore Technology and Pacific Biosciences utilizethis technique. Both the sequencing platforms can read 10 to 100 kb of ssDNA (single-stranded DNA) strand. The single-stranded DNA molecule is made to pass through a protein pore in presence of electric current. Nanopore sequencing technique does not utilizethe labeleddNTP. The DNA strand translocation into the pore causes a significantchange in voltage which can be measured. The change in voltage is characteristic of specific DNA sequence. This technique can be used for sequencing of about 70 bp per second. The latest modification to nanopore sequencing is CsgG bacterial amyloid secretion pore based sequencer which can achieve DNA translocation rate upto 250 bases per second (Carter and Hussain, 2017) with much higher sequencing accuracy (Brown and Clarke, 2016). Nanopore platform was successfully used in monitoring of Ebola hemorrhagic fever outbreaks (Quick et al., 2016). Oxford Nanopore Technologies Limited Company has developed MinION machine (a portable DNA sequencer) for the direct analysis of single DNA molecules even in spacecraft and space (Spaceref, 2016).However, the Pacific Biosciences platform uses thephospho-linked fluorescent nucleotides. The signal produced by incorporation of such nucleotide is monitored by a zeromodewaveguide detector (Eid et al., 2009). Although the sequencing errors are higher than othertechniques, the nanopore sequencer has several additional benefits such asits low cost of equipment, portablesizeand real-time data generation.

### *Application of NGS in Animal Virology*

The high-throughput sequencing methods can be used for sequencing of all the organisms available in a sample. It can assists in metagenomics level of study. It has several applications in animal virology which are mentioned below.

### *Diagnosis of viral diseases*

Conventionally, diagnosis of viral disease is done by symptomatic study of disease, virological assays or immunological assays etc. Compared to conventional methods, molecular assays have higher sensitivity and specificity. However, the knowledge of complete genome sequence of the virus is a prerequisite for molecular assays such as PCR, RT-PCR or nucleic acid sequencing etc. Moreover, conventional diagnosis depends on availability of agent-specific diagnostics reagents whereas, NGS can be used for metagenomics study of a biological sample and can identify several types of infectious agentssuch asbacteria, viruses, fungus etc. NGS can also be used diagnosis of mixed infections especially of those that are of immunosuppressive in nature with no clear clinical symptoms. Pyrosequencingtechnique was used to identify torque teno virus and a novel boca-like parvovirus along with causative agent porcine circovirus2 in pig lymph node (Blomstrom et al., 2010). Furthermore, NGS can also be used for molecular identification andepidemiological characterization of non-cultivable viral infectious agents such as retrovirus infection leading to Jaagsiektein sheep (Spencer and Palmarini, 2012).

Bluetongue viruses (BTV) from field samples are regularly genotypedusing conventional cell culture, vp2 gene specific RT-PCR followed by nucleic acid sequencing (Ranjan et al., 2013; 2014; 2015; Dadawala et al., 2013; Kumar et al., 2013). Now a day, NGS techniques are regularly used for typing of newer BTV isolates to identify new serotypes and genomic reassortants (Minakshi et al., 2012). Different NGS platforms had also been used for diagnosis of several animal viruses in India such as Bluetongue virus (Minakshi et al., 2012), foot and mouth disease virus (Mahapatra et al., 2016)etc.

### *Vaccine development*

The NGS data could be of help at different levels in vaccine industry.NGS has shown its importance inselection of vaccine candidate andpreparation as well as testing of viral vaccines. Apart from identification of candidate vaccine stains, NGS can also establish the vaccine contaminating agents. Live attenuated vaccines have major problem with reversion to virulence strains. Nucleic acid based assays including NGS can be used to identify the virulent markers in candidate vaccine stains. The genetic mutations in poliovirus, necessary for attenuation have been identified by complete genome sequence based study using NGS (Victoria et al., 2010). Similarly, NGS can also be used for detection of virulent markers in vaccine viruses. It will improve the safety of viral vaccines.

The vaccine should be devoid of anything other than the vaccine specificantigenic material (Kumar et al., 2012).The vaccine materials must be free from contaminating agents such as bacteria, virus, fungi, rickettsia, protozoa etc. Several contaminating virusesin vaccines have been reported (Table 2).

**Table 2:** Major viral contamination to human and animal vaccines.

| S.n. | Vaccine against | Contamination | Reference(s) |
|---|---|---|---|
| 1 | Canine vaccines | Bluetongue virus | Akita et al., 1994 |
| 2 | Rotavirus | Porcine circovirus 1 | Victoria et al., 2010 |
| 3 | Yellow fever | Avian retroviruses | Hussain et al., 2003 |
| 4 | Lumpy skin disease and sheep pox | Bluetongue virus | Bumbarov et al., 2016 |
| 5 | Marek's disease | Reticuloendotheliosis virus | Takagi et al., 1996 |
| 6 | Measles, mumps and Rubella (MMR) | Bovine viral diarrhea virus | Studer et al., 2002 |
| 7 | Poliovirus and adenovirus | Simian virus-40 | Lewis, 1998 |
| 8 | Poliovirus and MMR | Bacteriophage (φV−1) | Haselkorn et al., 1978 |
| 9 | Measles and mumps | Avian leucosis virus | Tsang et al., 1999 |
| 10 | Swine fever | Bovine viral diarrhoea virus | Wensvoort and Terpstra, 1988 |

### *Identification of exotic viral pathogens*

Many of the animal diseases are spread from territory of one country to another through import of live animals, their products and live attenuated vaccines. To control trans-boundary movement of diseases, various serological and molecular assays along with post-import quarantine measure is being practiced by most of the nations. In India, imported animals are quarantined for 30 days to develop disease symptoms. Several viruses such as RNA virus where, where nucleic acid diversity is high (e.g. BTV), primers designed for diagnosis specific to one territory may not diagnose the viruses from other territory (Kumar et al., 2013; Shafiq etal., 2013). Apart from that, the test needs to be done against pathogens. Despite strict measure of animal import, several exotic viral pathogens have already been entered to India such asBTV (Gollapalli et al., 2012). Introduction of exotic pathogens to previously unexposed population lead to severe disease outbreak, mortality and economic losses. These exotic pathogens can be easily diagnosed by NGS technique and complete genome sequencing based analysis.

### *Transcriptome analysis*

The transcriptome analysis refers to study of complete set of expressed RNA which are produced by a genome of specific cells, microbes etc under specific condition. This technique can be employed to study the loss and gain of specific function of a mutant strain of pathogens, disease diagnosis, functional characterization of genes, detection of molecular pathways inside a cell which may improve the environmental stress tolerance capability, in biomedical research field such as biomarker discovery, risk assessment related to newer medicines etc.Moreover, RNA-Seq analysis can also be used to detect single nucleotide polymorphisms in pathogens and host, allele specific gene expression etc.

In one of the study, RNA-Seq based transcriptomeanalysis using various tools such as Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) in cattle vaccinated with killed Bovine viral diarrhea virus I (BVDV-I) vaccine was performed to identify the immune response related differentially expressed genes (DEGs) (Lopez et al., 2020). The study identified the severalgenes related with immune response, interferon-γ production and MHC class I genes with up and downregulated activitiesindicating the immune response against the BVDV-I in cattle which potentiates the application of RNA-Seqin animal improvement programs by selection of specific animals with improvedefficacy of the vaccine.

### *Common tools for NGS data analysis*

The complex genomic research demand deep insight of information which is beyond the capacity of traditional sequencing platforms. The NGS has filled the gap and became a regular research tool to address specific problems. However, NGS machines generates huge amount of data which again needs high computing power and specific dedicated software tools for data analysis. For NGS data analysis several bioinformatics tools are available. Some of these tools are commercially available for various computer operating systems.

## Conclusion

The current revolution in field of virology is primarily driven by advancement in sequencing technology especiallyby development of massive parallel sequencing technology or NGS. NGS

has led to high throughput genome sequencing with accuracy at reduced cost in comparison to conventional Sanger's sequencingtechnology.In Animal virology, NGS may play a crucial role in earlydisease diagnosis and control. However, in current scenario, NGS techniques are too expensive to use in animal disease diagnosis. The high cost of NGS machine and its reagents along with need of skilled molecular biology andbioinformatics staffs limits its application in routine veterinary applications.However, the recent advancement in NGS technologies such as fast and portable Nanoporesequencing platform may replace currently used other molecular diagnostic techniques in animal virology.

*Acknowledgements*

Authors are thankful to SVP University of Agriculture and Technology, Meerut, Uttar Pradesh for providing facility to prepare the manuscript.

**References**

1. Adams J. DNA sequencing technologies. Nature Education, 2008;1(1):193.
2. Akita GY, Ianconescu M, MacLachlan NJ, Osburn BI. Bluetongue disease in dogs associated with contaminated vaccine. Vet. Rec., 1994;134: 283-284.
3. Blomstrom AL, Belak S, Fossum C, Fuxler L, Wallgren P, Berg M. Studies of porcine circovirus type 2, porcine boca-like virus and torque teno virus indicate the presence of multiple viral infections in postweaningmultisystemic wasting syndrome pigs. Virus Res., 2010;152: 59-64.
4. Brown CG, Clarke J. Nanopore development at Oxford Nanopore. Nat. Biotechnol., 2016, 34: 810-811.
5. Bumbarov V, Golender N, Erster O, Khinich Y. Detection and isolation of Bluetongue virus from commercial vaccine batches. Vaccine, 2016;34: 3317-3323.
6. Carter JM, Hussain S. Robust long-read native DNA sequencing using the ONT CsgGNanopore system. Wellcome Open Res., 2017; 2: 23.
7. Chalkias S, Gorham JM, Mazaika E, Parfenov M, Dang X, DePalma S, McKean D, Seidman CE, Seidman JG, Koralnik IJ. ViroFind: A novel target-enrichment deep-sequencing platform reveals a complex JC virus population in the brain of PML patients. PLoS One. 2018;13(1):e0186945.
8. Dadawala AI, Kher HS, Chandel BS, Bhagat AG, Chauhan HC, Ranjan K and Minakshi P. Isolation and molecular characterization of bluetongue virus 16 of goat origin from India. Adv. Anim. Vet. Sci. 2013;1(4S): 24-29.
9. Dye C, Mertens T, Hirnschall G, Mpanju-Shumbusho W, Newman RD, Raviglione MC, Savioli L, Nakatani H. WHO and the future of disease control programmes. Lancet. 2013; 381(9864):413–418.
10. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Korlach J, Turner S. Real-time DNA sequencing from single polymerase molecules. Science, 2009; 323: 133-138.
11. Fiers W, Contreras R, Duerinck F, Haegeman G, Iserentant D, Merregaert J, Min Jou W, Molemans F, Raeymaekers A, Van den Berghe A, Volckaert G, Ysebaert M. Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene. Nature. 1976; 260(5551):500-507.
12. Gollapalli SR, Mallavarapu S, Uma M, Rao PP, Susmitha B, Prasad PU, Chaitanya P, Prasad, G, Hegde NR, Reddy YN. Sequences of genes encoding type-specific and group-specific antigens of an Indian isolate of bluetongue virus serotype 10 (BTV-10) and implications for their origin. TransboundEmerg. Dis., 2012; 59: 165-172.
13. Guo J, Xu N, Li Z, Zhang S, Wu J, Kim DH, Sano Marma M, Meng Q, Cao H, Li X, Shi S, Yu L, Kalachikov S, Russo JJ, Turro NJ, Ju J.. Four-color DNA sequencing with 3'-O-modified nucleotide reversible terminators and chemically cleavable fluorescent dideoxynucleotides. Proc Natl. Acad Sci. USA, 2008; 105: 9145-9150.
14. Haselkorn R, Schichman S, Milstien J, Petricciani J. Characteristics of bacteriophage phiV-1 isolated from live virus vaccines. Proc. Soc. Exp. Biol. Med., 1978;158: 383-387.
15. Hussain AI, Johnson JA, Da Silva Freire M, Heneine W. Identification and characterization of avian retroviruses in chicken embryo-derived yellow fever vaccines: investigation of transmission to vaccine recipients. J. Virol., 2003;77: 1105-1111.
16. Kasibhatla SM, Waman VP, Kale MM, Kulkarni-Kale U. Analysis of Next-generation Sequencing Data in Virology - Opportunities and Challenges; Next Generation Sequencing - Advances, Applications and Challenges, Jerzy K Kulski, IntechOpen, 2016; DOI: 10.5772/61610.
17. Koonin EV, Dolja VV. A virocentric perspective on the evolution of life. CurrOpinVirol. 2013; 3(5):546–57.
18. Kumar D, Beach NM, Meng XJ, Hegde NR. Use of PCR-based assays for the detection of the

adventitious agent porcine circovirus type 1 (PCV1) in vaccines, and for confirming the identity of cell substrates and viruses used in vaccine production. J. Virol. Methods, 2012; 179: 201-211.

19. Kumar P, Minakshi P, Ranjan K, Dalal R and Prasad G. Evidence of reassortment between eastern and western topotype strains of bluetongue virus serotype 16 (BTV–16) from India. Adv. Anim. Vet. Sci. 2013; 1(4S): 14-19.
20. Lewis AM, Jr.. SV40 in adenovirus vaccines and adenovirus-SV40 recombinants. Dev. Biol. Stand., 1998; 94: 207- 216.
21. Lopez BI, Santiago KG, Lee D, Ha S, Seo K. RNA Sequencing (RNA-Seq) Based Transcriptome Analysis in Immune Response of Holstein Cattle to Killed Vaccine against Bovine Viral Diarrhea Virus Type I. Animals (Basel). 2020; 10(2):344.
22. Mahapatra M, Statham B, Li Y, Hammond J, Paton D, Parida S.Emergence of antigenic variants within serotype A FMDV in the Middle East with antigenically critical amino acid substitutions. Vaccine, 2016; 34: 3199-3206.
23. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, JirageKB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM. Genome sequencing in microfabricated high-density picolitre reactors. Nature, 2005; 437: 376-380.
24. Marsh M, Tu O, Dolnik V, Roach D, Solomon N, Bechtol K, Smietana P, Wang L, Li X, Cartwright P, Marks A, Barker D, Harris D, Bashkin J. High-throughput DNA sequencing on a capillary array electrophoresis system. J. Capillary Electrophor., 1997; 4: 83-89.
25. Minakshi P, Ranjan K, Brar B, Ambawat S, Shafiq M, Alisha A, Kumar P, Ganesharao JV, Jakhar S, Balodi S, Singh A, Prasad G. New approaches for diagnosis of viral diseases in animals. Adv. Anim. Vet. Sci. 2014; 2(4S): 55-63.
26. Minakshi P, Singh R, Ranjan K, Kumar P, Joshi CG, Reddy YK, Prasad G. Complete genome sequence of bluetongue virus serotype 16 of goat origin from India. J Virol. 2012; 86(15):8337-8338.
27. Quick J, Loman NJ, Duraffour S, Simpson JT, Severi E, Cowley L, Bore JA, et al. Real-time, portable genome sequencing for Ebola surveillance. Nature, 2016; 530: 228-232.
28. Ranjan K, Minakshi P, Prasad G. Bluetongue: Indian perspective. ActaVirol. 2015a; 59(4):317-337.
29. Ranjan K, Minakshi P, Prasad G. Application of molecular and serological diagnostics in veterinary parasitology. J. Adv. Parasitol. 2015b; 2(4): 80-99.
30. Ranjan K, Prasad G, Kumar P and Minakshi P. Vp5 gene based molecular characterization of bluetongue virus 9 from South India. Adv. Anim. Vet. Sci. 2013;1(4S): 30-36.
31. Ranjan K, Prasad G, Kumar P and Minakshi P. Molecular characterization of segment 6 of bluetongue serotype 16 of sheep origin from India. Adv. Anim. Vet. Sci. 2014; 2(2): 98-103.
32. Rothberg JM, Hinz W, Rearick TM, Schultz J, Mileski W, Davey M, Leamon JH, Johnson K, Milgrew MJ, Edwards M, Hoon J, Simons JF, Marran D, Myers JW, Davidson JF, Branting A, Nobile JR, Puc BP, Light D, Clark TA, Huber M, BranciforteJT, Stoner IB, Cawley SE, Lyons M, Fu Y, Homer N, Sedova M, Miao X, Reed B, Sabina J, Feierstein E, Schorn M, Alanjary M, Dimalanta E, Dressman D, Kasinskas R, Sokolsky T, Fidanza JA, Namsaraev E, McKernan KJ, Williams A, Roth GT, Bustillo J. An integrated semiconductor device enabling non-optical genome sequencing. Nature, 2011; 475: 348-352.
33. Sanger F, Air GM, Barrell BG, Brown NL, Coulson AR, Fiddes CA, Hutchison CA, Slocombe PM, Smith M. Nucleotide sequence of bacteriophage phi X174 DNA. Nature. 1977b; 265(5596):687-95.
34. Sanger F, Nicklen S, Coulson AR. DNA Sequencing With Chain-Terminating Inhibitors. ProcNatlAcadSci USA. 1977a ;74 (12): 5463-5467
35. Schulz F, Roux S, Paez-Espino D, Jungbluth S, Walsh DA, Denef VJ, McMahon KD, Konstantinidis KT, Eloe-Fadrosh EA, Kyrpides NC, Woyke T. Giant virus diversity and host interactions through global metagenomics. Nature. 2020; 578(7795):432-436.
36. Shafiq M, Minakshi P,Bhateja A, Ranjan K, Prasad G. Evidence of genetic reassortment between Indian isolate of bluetongue virus serotype 21 (BTV-21) and bluetongue virus serotype 16 (BTV-16). Virus Res. 2013; 173(2):336-343.
37. Spaceref. Sequencing DNA in Space. http://spaceref.com/nasa-hack-space/sequencing-dna-in-space.html (Accessed on 10February 2021), 2016.
38. Spencer TE, Palmarini M. Application of next generation sequencing in mammalian embryogenomics: lessons learned from endogenous betaretroviruses of sheep. AnimReprod Sci. 2012;134(1-2):95-103.
39. Studer E, Bertoni G, Candrian U. Detection and characterization of pestivirus contaminations in human live viral vaccines. Biologicals, 2002;30: 289-296.
40. Takagi M, Ishikawa K, Nagai H, Sasaki T, Gotoh K, Koyama H. Detection of contamination of vaccines with the reticuloendotheliosis virus by reverse transcriptase polymerase chain reaction (RT-PCR). Virus Res., 1996;40: 113- 121.

41. Tsang SX, Switzer WM, Shanmugam V, Johnson JA, Goldsmith C, Wright A, Fadly A, Thea D, Jaffe H, Folks TM, Heneine W. Evidence of avian leucosis virus subgroup E and endogenous avian virus in measles and mumps vaccines derived from chicken cells: investigation of transmission to vaccine recipients. J. Virol., 1999;73: 5843-5851.

42. Valouev A, Ichikawa J, Tonthat T, Stuart J, Ranade S, Peckham H, Zeng K, Malek JA, Costa G, McKernanK, Sidow A, Fire A, Johnson SM. A highresolution, nucleosome position map of C. elegans reveals a lack of universal sequence-dictated positioning. Genome Res., 2008; 18: 1051-1063.

43. Vibin J, Chamings A, Collier F, Klaassen M, Nelson TM, Alexandersen S. Metagenomics detection and characterisation of viruses in faecal samples from Australian wild birds. Sci Rep. 2018; 8(1):8686.

44. Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S, Delwart EL. Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. J. Virol., 2010; 84: 6033-6040.

45. Wensvoort G, Terpstra C. Bovine viral diarrhoea virus infections in piglets born to sows vaccinated against swine fever with contaminated vaccine. Res. Vet. Sci., 1988; 45: 143-148.

46. Lambe U, Minakshi P, Bar B, Guray M, Ikbal, Ranjan K, Bansal N, Khurana SK, Manimegalai J. Nanodiagnostics: A new frontier for veterinary and medical sciences. J. Experiment Biol. Agri. Sci., 2016; 4(3S): 308-320. DOI: http://dx.doi.org/10.18006/2016.4(3S).307.320

47. Stephen BJ, Singh SV, Datta M, Jain N, Jayaraman S, Chaubey KK, Gupta S, Singh M, Aseri GK, Khare N, Yadav P, Dhama K, Sohal JS. Nanotechnological Approaches for the Detection of Mycobacteria with Special References to Mycobacterium avium Subspecies Paratuberculosis (MAP). Asian J. Anim. Vet. Adv., 2015; 10: 518-526. doi:10.3923/ajava.2015.518.526.

48. Vishwaradhya TM, Minakshi P, Ranjan K, Supriya, Kumar P and Prasad G. Sensitive detection of novel Indian isolate of BTV 21 using ns1 gene based real-time PCR assay. Vet. World, 2013; 6(8): 554-557.doi:10.5455/vetworld.2013.554-557

49. Capobianchi MR, Giombini E, Rozera G. Next-generation sequencing technology in clinical virology. Clin.Microbiol. Infect. 2013; 19(1):15-22.

50. Radford AD, Chapman D, Dixon L, Chantrey J, Darby AC, Hall N. Application of next-generation sequencing technologies in virology. J. Gen. Virol. 2012; 93(9): 1853-1868.