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# Recent insilco advancements in genome analysis and characteristics of SARS-Cov2

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**Abstract**
Coronavirus disease (COVID-19) is caused by SARS-CoV-2 (severe acute respiratory syndrome 2) is a newly discovered virus that belongs near the subdivision of Coronavirinae, a family *Coronaviridae* of the order Nidovirales, and is classified as SARS-like despite belonging to different clusters. It has been studied for many years. Bioinformatics tools developed specifically against rapidly for understanding and treatment of COVID-19. It poses one of the greatest global threats in modern history, and it has already caused severe social and economic costs. This review will focus on the recent updates in the genome, viral proteins characterization, potentially therapeutic, bioinformatics tools for the routine detection of SARS-CoV-2 infection, vaccine method, and genome analysis. As a result of this pandemic, social and economic problems will have a profound impact happening, public health our lives in the coming years.

**Keywords:** bioinformatics, severe acute respiratory syndrome coronavirus 2, tools, vaccine

## Introduction

Coronavirus is the sub-family of Coronaviridae which causes diseases such as in humans as well as in many animals. In January 2020 a newly identified beta coronavirus, which was designated as a viral disease known as severe acute respiratory syndrome coronavirus 2, causative factor of a cluster of pneumonia and a complete genomic sequence of the novel beta coronavirus was determined by using a meta transcriptomics approach, supplemented by PCR and Sanger sequencing (Poon *et al.*, 2020; Zhou *et al.*, 2020) [40]. The real-time PCR is based on a development test to diagnose the availability of a reference genome assembly (Corman *et al.*, 2020) [3]. In mid-2020, after a December 2019 flare-up in China, the World Health Organization recognized SARS-CoV-2 as another sort of Covid. The episode immediately spread throughout the planet. Coronavirus disease 2019 is transmitted through infection with this newly discovered pathogen, which spreads quickly and causes severe acute respiratory syndrome-CoV-2 that is most commonly transmitted among humans through airborne droplets and direct contact (Dong *et al.*, 2020) [14]. According to the incubation time, it occurs between two to fourteen days, and however, longer intervals have been observed and symptoms such as fever. SARS-Cov has been recognized by the International Committee on Taxonomy of Viruses (ICTV). There are many studies to develop molecular characteristics, conclusively natural sampling and Meta transcriptomic sequencing is needed to resolve these issues (Zhang *et al.*, 2020) [40].

The DNA and phosphatized nucleocapsids (N) form a spiral nucleocapsid which is found within the envelope. The diameter up to 80 to 160 millimeters, enveloped surface coated with a spike that is represented by (S) protein, membrane (M) protein, and envelope proteins were located with S proteins (Jin *et al.*, 2020). Coronaviruses are RNA viruses with genome sizes ranging from 26 to 32 kilobases, coding for positive-stranded, single-stranded RNA. There are five caps, three polyadenylated tails, and six open reading frames (ORFs) in this genome (Lu *et al.*, 2020). SARS-CoV-2 genome structure consists of 2 large parallel open reading frames ORF1a and ORF1b (Sola *et al.*, *2015*) [23]. The genome at the 5′ end. ORF1a is translated into polyprotein 1a and polyprotein 1b which is generated by −1 ribosomal frameshifting. There are sixteen non-structural proteins (NSPS) that are used for replication and transcription (Plant E. *et al.*,2008). NGS technologies are powerful tools for detecting the cause viral diseases e.g., influenza pandemic, the Middle-East Respiratory Syndrome coronavirus (MERS-CoV) as well as for observing the evolution of the pathogens (quick *et al.*, 2016) [19]

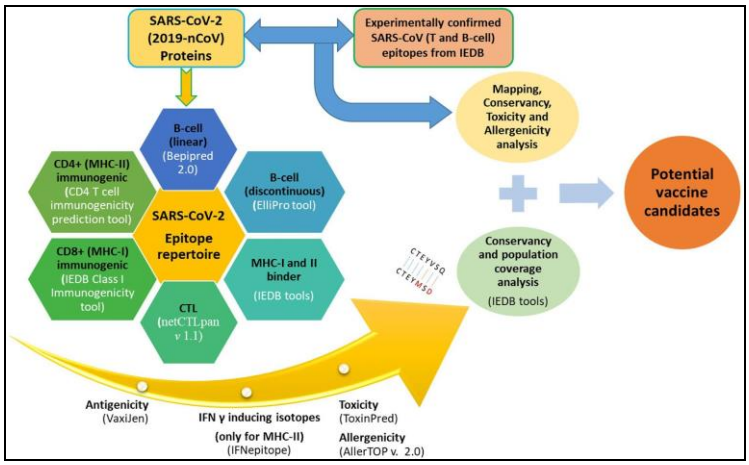


**Fig 1**

**Table 1:** List of the non-structural proteins (nsps) of SARS-CoV-2 their amino acid lengths, and their functions.

| Non-Structural proteins (NSPs) | Amino Acid diameter and posture | How it works | References |
|---|---|---|---|
| Nonstructural proteins-1 | One eighty aa(residues 1–180) | Inhibits the host's innate immunity. | (Jauregui *et al.*, 2013) |
| proteins-2 Nonstructural | 638 aa (residues 181–818) | The cellular component that inhibits host factors 1 and 2. | (Cornillez *et al*., 2009) |
| Nonstructural proteins-3 | residues A819-G2763 | The Coronavirus sequence contains its largest protein. This large membrane-bound protein is a papain-like protease that processes viral polyproteins encoded by genomic RNA into individual proteins. It was also shown to antagonize host innate immunity. | (Shanker *et al*., 2020) |
| Nonstructural proteins-4 | residues K2764 – Q3263 | In double-membrane vesicles, NMSP6 are involved. The formation between viral replication complexes depends on this interaction. | (Sakai *et al*., 2017) [21] |
| Nonstructural proteins-5 | 306 aa (residues S3264 – Q3569) | Mpro, a chymotrypsin-like protease is the main enzyme responsible for digesting | Jin *et al*.,2020) |
| Nonstructural proteins-6 | 290 aa (residues S3570- Q3859 | Transmembrane scaffold protein interacts with nsp3 and nsp4 | Oostra *et al*.,2008) [16] |
| Nonstructural proteins-7 | 83 aa (residues S3860 – Q3942) | The cofactor attaches to nsp8, forming hexadecameric complexes and acting as a processivity clamp | Zhai *et al*.,2005) |
| Nonstructural proteins-8 | 198 aa (residues A3943 – Q4140) | It forms a hexagonal complex with NSP7 and acts as a processivity clamp for RNA polymerase and primase | Zhai *et al*.,2005) |
| Nonstructural proteins-9 | 113 aa (residues N4141 – Q4253) | It binds ssRNA. The protein helps to protect nascent nucleic acids from nucleases during replication or transcription. | (Egloff *et al*.,2004) [6] |
| Nonstructural proteins-10 | 139 aa (residues A4254 – Q4392) | This protein interacts with nsp14, which is involved in replication fidelity | (Fehr *et al*., 2015) [8] |
| Nonstructural proteins-11 | 11-23 aa (residues S4393 – V4405) | An ORF1a frameshift line is represented by this short peptide at its end. | (Fehr *et al*., 2015) [8] |
| Nonstructural proteins-12 | 932 aa (residues S4393 – Q5324) | The RNA-dependent RNA polymerase (RdRp) is responsible for both replication and transcription of the viral genome | (Posthuma *et al*., 2017) |
| Nonstructural proteins-13 | 601 aa (residues A5325 – Q5925) | Helicases are characterized by metal-binding domain and a helicase conserved domain. Through its capacity to unwind double-stranded RNA and DNA, it is crucial for viral replication. | (Jang *et al*.,2020) |
| Non structural proteins-14 | 527 aa (residues A5926 – Q6452 | The guanine methyl transferase activity which is involved in mrna capping | (Snijder *etal*., 2016) [23] |
| Nonstructural proteins-15 | 346 aa (residues S6453 – Q6798) | Poly (U)-specific endoribonuclease that cleaves RNA at the 3′ end of purines. Loss of nsp15 was found to affect viral replication and virulence. | (Snijder *et al*., 2016) [23] |
| Nonstructural proteins-16 | 298 aa (residues S6799 –N7096 | For viral mRNA to avoid host detection, it has 2-O methyltransferase activity | (Totura *et al*., 2012) |

**Viruses that are different from SARS-CoV-2**

A Coronavirus is classified according to the international commission on viruses which may be conserved regions based like similarities of the amino acid sequences which have the seven regions contained within ORF1ab including ADRP, NSP5, and NSP12-16. Similarly, the S-protein homology between the SARS-CoV and the SARS-CoV-2 is relatively low at 76.5 percent. In seven regions of the amino acid sequence by similarity, however, SARS-CoV-2 is more divergent than its closest relative (Xu *et al.,* 2020) [37]. Several viral genetic revisions recommended that 149 mutagenic sites have appeared in SARS-CoV-2. Presently SARS-CoV-2's genetic code is more resistant to mutation than SARS-CoV and it is further divided into 2 types: L is more variated and spreads fast and S protein in SARS-CoV-2 changes greatly (Tang X *et al*., 2020)

**Methods for sequencing**

In recent years, sequencing techniques, various experiments in virology, biotechnology, and even the characterization of new viruses through meta genomic analyses, complete viral genome sequence, diversity, and evolution (Domingo *et al*., 2012) [4]. The technologies have been successfully applied to several different protocols of preparing library preparation developed independently. It is important to align sample type, viral load, RNA extraction procedure, RNA quality, parallelization, and automation requirements with the experiment's objectives. There are four techniques like (1) shotgun meta transcriptomics (2) hybrid enrichment, (3) DNA amplifying (4) sequence analysis of RNA (Pillay *et al.,* 2020)

**Translational and epigenomic profiling of SARS-CoV-2**

The transcription program SARS-CoV-2 transcriptome studies were mostly based on Oxford Nanopore technologies, with nanotube ball sequencing, which confirmed that transcription in SARS-CoV-2 is a discontinuous and highly controlled process based on a template switch during sub genomic negative-strand RNA synthesis. In infected mouse cells, quantitative sequencing identified an 'AAGAA-like' motif enriched in the 3′ region of this genome, indicating possible post-transcriptional modifications. Post-transcriptional changes may occur more frequently (Kim *et al.,* 2020). Individual SG mRNAs have been identified through RNA-seq reads spanning template switch sites. Epitranscriptome variations include transient changes such as N6-methyladenosine and 5-methylcytosine involved RNA editing and potentially contribute to the interaction between biotin and viruses (Tian *et al.,* 2018).

**Targeting of COVID-19 via in silico and *in vitro* approaches**

This review will discuss how potential drugs for treating SARS-CoV-2 have been identified *in vitro* and silico studies. There are mainly different methods used in producing an anti-CoV-2 drug for SARS. In research,

Ribavirin did not affect the strain, while the new antiviral drug Remdesivir inhibited the virus' replication. The nucleocapsid protein of SARS-CoV-2 also revealed unique drug-targeting sites that can be utilized to design new antiviral drugs (Vickers *et al*., 2017) [35]. A study has also been conducted on the effects of combining three drugs Oseltamivir, Lopinavir, and Ritonavir by using molecular dynamics (Muralidharan *et al.*, 2021). The application of the drug repurposing strategy was tested on chloroquine and hydroxychloroquine (Yao *et al.*, 2020) [38]. *In vitro*, hydroxychloroquine and azithromycin showed synergistic effects against SARS-CoV-2 (Andreani *et al*., 2020) [1]. The effectiveness of hydroxychloroquine as a treatment and its cardiovascular effects have recently focused on clinical studies (Kalra *et al*., 2020) [12].

A second strategy involved screening molecular databases for compounds that had antiviral activity against SARS-CoV-2. (NSP12) major protein targets are modeling of homologyotein targets in SARS-CoV-2 include papain-like (PLpro), main protease (M pro), RNA-dependent RNA polymerase (RdRp), helicase. Researchers have also conducted experiments by using medicinal plant libraries to identify potential antiviral phytochemicals (Qamar *et al*., 2020).

Thirdly, homology analysis of viral proteins is used to find potential binding sites and determine binding mechanisms. Study designed that identified a mechanism-based inhibitor of SARS-CoV-2 Mpro and tested more than 10,000 compounds for their ability to inhibit this protease (Jin *et al*., 2020). A study by scientists identified compounds that may target Mpro, the main protease of SARS-CoV-2 (Liu *et al*., 2020). Furthermore, scientist designed inhibitors from the protease domain of ACE2 and, using molecular dynamics simulations, determined that the helical peptides were highly specific for SARS-CoV-2 (Han *et al*., 2020) [10].

**There are several types of vaccines being developed**

Currently, different technologies and methods have been investigated to develop a successful CoV-2 vaccine, but so far there have only been types that are in Phase 3 clinical trials. Several technologies are being used to implement novel approaches for the study of COVID-19 infections, allowing for accelerated research and development, and reducing the risk of COVID-19 infected individuals who are elderly, pregnant, or immune-compromised (Thanh Le *et al*., 2020) [33]. Authorizations (EUAs) for Pfizer-BioNTech COVID-19 (BNT162b2) and Moderna COVID-19 (mRNA-1273) vaccines (Oliver *et al*., 2020) [15]. Adenovirus Type 5 (Recombinant Novel Coronavirus Vaccine) developed by CanSino Biological Inc./Beijing Institute of Biotechnology does not require 2 doses in its Phase 3 study (Shrotri *et al*., 2021)

**A subunit vaccine**

It is a group of generally immunogenic antigens capable of stimulating the immune system. While this kind of vaccine is harmless and simpler to produce, and it requires the use of adjuvants to generate a powerful immune response (Zhang *et al*., 2020) [40]. Moreover, these vaccines may require repeated administration, as they do not activate killer T cells or CD8+ T cells. A virus VLPs (Virus-like Particles) consists of subunit vaccines that are composed of several virus-derived proteins that match the structure of wild viruses, but they lack the viral genome (Jeyanathan *et al*., 2020). Due to their role in cell penetration, these proteins provide competent cell entry, making them a suitable option for vaccine production. VLP vaccines combine sound safety profiles with robust immunogenicity, so they are a safe alternative to inactivated viruses. These kinds of expression systems can be created from insect, yeast, or bacterial cells, and they have outstanding adjuvant properties. The formerly developed vaccines activated efficient immune responses. offer a superior platform for the development of vaccines because of these properties (Ghorbani *et al*., 2020) [9]. VLP vaccine COVID-19 particle (CoVLP) by Medicago, a Canadian company, using genetically modified plants. Unpublished studies indicate that it can induce neutralizing antibodies in mice (Wire *et al.*, 2020)

**The mRNA vaccine works in the following way**

Human cells are injected with synthetic mRNA, which instructs them to produce the virus' proteins. Immune cells recognize the viral proteins and mount an immune response against them (Hague *et al.*, 2020). Due to their affordable price, short manufacturing cycles, and high potency, mRNA vaccines can replace traditional vaccines. mRNA vaccines have not yet been approved for human use, but previous studies of influenza, rabies and Zika virus infections in animals support their potential (Pardi *et al*., 2018) [17]. It is not well-suited for use as a prophylactic vaccine since mRNA is not very stable and cannot be taken up easily by cells. Therefore, required lipid nanoparticle carriers (LNP) that are highly effective in stabilizing and packaging the mRNA for injection. In addition, they require cold storage conditions (Wang *et al*., 2020) [20].

**Analyzing and detecting**

This virus is routinely detected using real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). It detects two distinct sequences, the virus envelope (E) gene and the RNA-dependent RNA polymerase (RdRp) gene (Corman *et al*., 2020) [3]. Using the ARTIC platform, the SARS-CoV-2 nanopore sequencing protocol has been developed and optimized (J. Quick; 2020). This platform provides extensive experience and expertise in using this technology in the sequencing and surveillance of outbreaks, including Zika and Ebola (Quick *et al*., 2017) [18]. The SARS-CoV-2 genomes are sequenced using nanopores, allowing researchers to monitor and the evolution of the viruses (pore Cov). To amplicon-based methods, metagenomic, transcriptomic sequencing can identify the primary pathogen and additional infections that may be present (Moore *et al*., 2020) [14]. Coronaviruses can be detected in environmental samples e.g., from human bronchoalveolar lavage fluid. The presence of SARS-CoV-2 genomic traces in human fecal meta genomes before the outbreak of the current pandemic supports the hypothesis that an inactive, non-virulent form of the virus was present in human populations prior to the outbreak (S. Rampelli *et al*., 2020) [20].

**Toolkit for genome analysis by the viral bioinformatics research center**

Viral Bioinformatics Research Centre (VBRC) has been developed. Several tools are available for users to analyze Gen Bank files by using VBRC information. An organized database can be accessed through Virus Orthologous Groups

(Ehlers *et al*., 2002) [7]. A multiple sequence alignment is generated, visualized, and edited using Base-By-Base (Brodie *et al*., 2004) [2]. By aligning and plotting genomes, genes, and proteins, allows for comparison. Users can add comments to sequences and save alignments locally. The Viral Genome Organizer (Upton *et al*., 2000) [34]. has been designed to enable users to visualize and compare genes in multiple viral genomes. Besides exporting protein or DNA sequences, the tool also displays open reading frames, STARTs, STOPs, and other user-defined results. It can display shared orthologs if genomes are loaded from the database. This tool, created by scientists before in (2006) allows genomes to be annotated using a reference genome (Tcherepanov *et al*., 2006).

## Conclusion

High transmission rate and rapid spread of Covid-19 effects our daily lives badly. In this article, researchers explore the latest advances related to clinical features, characterization of genome, DNA fingerprinting, and disease control by modern tools and techniques. Despite this, many questions remain, which include the patient susceptibility variation, disease progression, and possible outcome of SARS-CoV-2 infection, which, depends on a highly complex interaction between the vulnerability of body, environment, and pathogenic virus. Furthermore, till to date none of the effective and proven clinical method developed,

Mutations have played a crucial role in the virus's evolution. Therefore, the high-throughput and cost-effective methods for reconstructing genetic sequences mutating pathogens shows these are effective and useful tools for detecting and control of the infectious disease spread in humans.

NGS tools and approaches proved very effective and quickly adapted in SARC Cov 2 case for the answering the associated biological questions. Only a few years ago, data production and analysis would have been inconceivable. As of the 17th of June 2020, SARS-CoV-2 confirmed cases have been reported in 216 countries, areas, or territories. Researchers are now focusing efforts on finding a comprehensive understanding of the virus and developing vaccines as preventive measure. Here, on the base of classification and protein structure SARS CoV and SARS Cov2 are compared.

## Abbreviations

COVID-19: Coronavirus disease 2019; ORFs: Open reading frames; nsps: Nonstructural proteins; RBD: Receptor binding domain; ACE2: Angiotensin converting enzyme2; WHO: World Health Organization

## References


1. Andreani J, Le Bideau M, Duflot I, Jardot P, Rolland C *et al*. *In vitro* testing of combined hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect. Microbial pathogenesis,2020:145:104228.
2. Brodie R, Smith AJ, Roper RL, Tcherepanov V, Upton C. Base-By-Base: single nucleotide-level analysis of whole viral genome alignments. *BMC bioinformatics*,2004:5(1):1-9.
3. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK *et al*. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance,2020:25(3):2000045.
4. Domingo E, Sheldon J, Perales C. Viral quasispecies evolution. Microbiology and Molecular Biology Reviews,2012:76(2):159-216.
5. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. The Lancet infectious diseases,2020:20(5):533-534.
6. Egloff MP, Ferron F, Campanacci V, Longhi S, Rancurel C, Dutartre H *et al*. The severe acute respiratory syndrome-coronavirus replicative protein nsp9 is a single-stranded RNA-binding subunit unique in the RNA virus world. *Proceedings of the National Academy of Sciences*,2004:101(11):3792-3796.
7. Ehlers A, Osborne J, Slack S, Roper RL, Upton C. Poxvirus orthologous clusters (POCs). *Bioinformatics*,2002:18(11):1544-1545.
8. Fehr AR, Perlman S, Maier HJ, Bickerton E, Britton P. An overview of their replication and pathogenesis; section 2 genomic organization. *Methods in Molecular Biology*,2015:1282:1-23.
9. Ghorbani A, Zare F, Sazegari S, Afsharifar A, Eskandari MH, Pormohammad A. Development of a novel platform of virus-like particle (VLP)-based vaccine against COVID-19 by exposing epitopes: an immunoinformatics approach. *New microbes and new infections*,2020:38:100786.
10. Han Y, Král P. Computational design of ACE2-based peptide inhibitors of SARS-CoV-2. ACS nano,2020:14(4):5143-5147.
11. Haque A, Pant AB. Efforts at COVID-19 vaccine development: challenges and successes. *Vaccines*,2020:8(4):739.
12. Kalra RS, Tomar D, Meena AS, Kandimalla R. SARS-CoV-2, ACE2, and hydroxychloroquine: cardiovascular complications, therapeutics, and clinical readouts in the current settings. Pathogens,2020:9(7):546.
13. Liu S, Zheng Q, Wang Z. Potential covalent drugs targeting the main protease of the SARS-CoV-2 coronavirus. Bioinformatics,2020:36(11):3295-3298.
14. Moore SC, Penrice-Randal R, Alruwaili M, Dong X, Pullan ST, Carter D *et al*. Amplicon based MinION sequencing of SARS-CoV-2 and metagenomic characterisation of nasopharyngeal swabs from patients with COVID-19. *MedRxiv*, 2020.
15. Oliver SE, Gargano JW, Marin M, Wallace M, Curran KG, Chamberland M *et al*. The advisory committee on immunization practices' interim recommendation for use of Pfizer-BioNTech COVID-19 vaccine—United States, December 2020. *Morbidity and Mortality Weekly Report*,2020:69(50):1922.
16. Oostra M, Hagemeijer MC, Van Gent M, Bekker CP, Te Lintelo EG, Rottier PJ *et al*. Topology and membrane anchoring of the coronavirus replication complex: not all hydrophobic domains of nsp3 and nsp6 are membrane spanning. Journal of virology,2008:82(24):12392-12405.
17. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. *Nature reviews Drug discovery*,2018:17(4):261-279.
18. Quick J, Grubaugh ND, Pullan ST, Claro IM, Smith AD, Gangavarapu K *et al*. Multiplex PCR method for MinION and Illumina sequencing of Zika and other virus genomes directly from clinical samples. Nature protocols,2017:12(6):1261-1276.
19. Quick J, Loman NJ, Duraffour S, Simpson JT, Severi E, Cowley L *et al*. Real-time, portable genome sequencing

for Ebola surveillance. Nature,2016:530(7589):228-232.
20. Rampelli SE, Biagi S Turroni, Candela M. Retrospective search for SARS-CoV-2 in human faecal metagenomes. SSRN Electronic J, 2020.
21. Sakai Y, Kawachi K, TeradaY, Omori H, Matsuura Y, Kamitani W. Two-amino acids change in the nsp4 of SARS coronavirus abolishes viral replication. *Virology*,2017:510:165-174.
22. Snijder EJ, Decroly E, Ziebuhr J. The nonstructural proteins directing coronavirus RNA synthesis and processing. *Advances in virus research*,2016:96:59-126.
23. Sola I, Almazan F, Zuniga S, Enjuanes L. Continuous and discontinuous RNA synthesis in coronaviruses. Annual review of virology,2015:2:265-288.
24. Sudheer Menon. "Preparation and computational analysis of Bisulphite sequencing in Germfree Mice" International Journal for Science and Advance Research In Technology,2020:6(9):(557-565).
25. Sudheer Menon, Shanmughavel Piramanayakam, Gopal Agarwal. "Computational identification of promoter regions in prokaryotes and Eukaryotes" EPRA International Journal of Agriculture and Rural Economic Research (ARER),2021:9(7):21-28.
26. Sudheer Menon. "Bioinformatics approaches to understand gene looping in human genome" EPRA International Journal of Research & Development (IJRD),2021:6(7):170-173.
27. Sudheer Menon. "Insilico analysis of terpenoids in Saccharomyces Cerevisiae"international Journal of Engineering Applied Sciences and Technology,2021:6(1):43-52.
28. Sudheer Menon. "Computational analysis of Histone modification and TFBs that mediates gene looping" Bioinformatics, Pharmaceutical, and Chemical Sciences (RJLBPCS),2021:7(3):53-70.
29. Sudheer Menon, Shanmughavel piramanayakam, Gopal Prasad Agarwal. "FPMD-Fungal promoter motif database: A database for the Promoter motifs regions in fungal genomes" EPRA International Journal of Multidisciplinary research,2021:7(7):620-623.
30. Sudheer Menon, Shanmughavel Piramanayakam, Gopal Agarwal. Computational Identification of promoter regions in fungal genomes, International Journal of Advance Research, Ideas and Innovations in Technology,2021:7(4):908-914.
31. Sudheer Menon, Vincent Chi Hang Lui, Paul Kwong Hang Tam. Bioinformatics methods for identifying hirschsprung disease genes, International Journal for Research in Applied Science & Engineering Technology (IJRASET),2021:9(7):2974-2978.
32. Sudheer Menon. Bioinformatics approaches to understand the role of African genetic diversity in disease, International Journal Of Multidisciplinary Research In Science, Engineering and Technology (IJMRSET),2021:4(8):1707-1713.
33. Thanh Le T, Andreadakis Z, Kumar A. Rom´ an RG, Tollefsen S, Saville M *et al*., The COVID-19 vaccine development landscape, Nat. Rev. Drug Discov,2020:19:305-306.
34. Upton C, Hogg D, Perrin D, Boone M, Harris NL. Viral genome organizer: a system for analyzing complete viral genomes. *Virus research*,2000:70(1-2):55-64.
35. Vickers NJ. Animal communication: when i'm calling you, will you answer too?. Current biology,2017:27(14), R713-R715.
36. Wang F, Kream RM, Stefano GB. An evidence based perspective on mRNA-SARS-CoV-2 vaccine development. *Medical science monitor:* international medical journal of experimental and clinical research,2020:26:e924700-1.
37. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X *et al*. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Science China Life sciences, 2020.
38. Yao X, YeF, Zhang M, Cui C, Huang B, Niu P *et al*. *In vitro* antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clinical infectious diseases,2020:71(15):732-739.
39. Zhang YZ, Holmes EC. A genomic perspective on the origin and emergence of SARS-CoV-2. Cell,2020:181(2):223-227.
40. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang, W *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. nature,2020:579(7798):270-273.