Received: 22 June 2021 | Accepted: 30 July 2021

DOI: 10.1002/bab.2239

ORIGINAL ARTICLE

WILEY

# MicroRNA of N-region from SARS-CoV-2: Potential sensing components for biosensor development

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DNA probe designing based on SARS-CoV-2 microRNA.

**Abstract**

An oligonucleotide DNA probe has been developed for the application in the DNA electrochemical biosensor for the early diagnosis of coronavirus disease (COVID-19). Here, the virus microRNA from the N-gene of severe acute respiratory syndrome-2 (SARS-CoV-2) was used for the first time as a specific target for detecting the virus and became a framework for developing the complementary DNA probe. The sequence analysis of the virus microRNA was carried out using bioinformatics tools including basic local alignment search tools, multiple sequence alignment from CLUSTLW, microRNA database (miRbase), microRNA target database, and gene analysis. Cross-validation of distinct strains of coronavirus and human microRNA sequences was completed to validate the percentage of identical and consent regions. The percent identity parameter from the bioinformatics tools revealed the virus microRNAs' sequence has a 100% match with the genome of SARS-CoV-2 compared with other coronavirus strains, hence improving the selectivity of the complementary DNA probe. The 30 mer with 53.0% GC content of complementary DNA probe 5′ GCC TGA GTT GAG TCA GCA CTG CTC ATG GAT 3′ was designed and could be used as a bioreceptor for the biosensor development in the clinical and environmental diagnosis of COVID-19.

**KEYWORDS**

In silco analysis, biosensor, DNA probe, Biomarker, microRNA, SARS-CoV-2

**Abbreviations:** APTES, 3-aminopropyl triethoxysilane; BLAST, basic local alignment search tool; CLUSTLW, multiple sequence alignments; COVID-19, coronavirus disease; CQDs, carbon quantum dots; IDE, interdigitated electrode; MERS-CoV, middle east respiratory syndrome; miRbase, microRNA database; miRDB, microRNA target database; N-gene, nucleocapsid gene; PANTHER, gene analysis tool; qRT-PCR, real-time quantitative reverse transcriptions polymerase chain reactions; SARS-CoV, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome 2.

## 1 | INTRODUCTION

The emergingcoronavirus disease (COVID-19) is an infectious caused by severe acute respiratory syndrome-2 (SARS-CoCV-2), initially identified in Wuhan, China, in December 2019.[1,2] Since the Middle East Respiratory Syndrome coronavirus (MERS-CoV) in 2012 and SARS-CoV in 2003, this new virus has already been recognized as the seventh coronavirus family that could theoretically spread across individuals.[3] The major routes of SARS-CoV-2 dissemination are primarily by inhalation, aerosols, or droplets to individuals and spread by immediate contact or virus-infected surfaces.[4] The infected individuals encountered respiratory disorders from minor respiratory symptoms such as cough, flu, fever, and persistent respiratory failure such as pneumonia, contributing to mortality.[5,6] Besides, the virus' asymptomatic carriers have also been reported and pose a significant public health risk.

The correlation study of genome studies with multiple coronaviruses demonstrates that the SARS-CoV-2 virus genome was consistent with the already studied coronavirus genome.[7] The diameter of SARS-CoV-2 ranges between 50 and 200 nm, containing single-stranded RNA and about 29,891 nucleotides in length that encoded for ~9860 amino acids. Structurally, the SARS-CoV-2 genome includes 6–11 open reading frames that encode 16 nonstructural proteins and four main structural proteins, including spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein that are aligned in the order of the 5′ untranslated region (UTR)-replicase complex (ORF1ab) (S)–Spike (E)–Envelope (M)–Membrane (N)–Nucleocapsid 3' UTR and accessory genes such as 3a, 6, 7a, 7b, and 8.[1,2,8]

The diagnosis of COVID-19 for infected humans consists of two main ways: real-time quantitative reverse polymerase chain reaction (qRT-PCR) and serology test. The qRT-PCR is a gold method for detecting RNA SARS-CoV-2 on clinical samples. Although this assay exhibited intensively specific and sensitive detection, some constraints existed, which were time consuming, crucial for trained personnel, and included longer duration of data management and analysis.[9] The serology test, such as enzyme-linked immunosorbent assay, detects the antibody towards the SARS-CoV-2 antigen through the blood test. However, serology tests only indirectly detect the resolving or past virus infection by quantifying the human immune response to the virus.[10] Therefore, this diagnosis does not typically replace the direct detection methods as the primary and early tools for diagnosing active SARS-CoV-2 RNA virus.

When it comes to the early diagnosis of the infectious disease, virus microRNA (miRNA) is one of the potentials and ideal biomarkers that could be detected for the particular virus disease. This is due to its presence in the latency period of the infection stage, stable and existing in a long period in extracellular environments, and can be accessible by noninvasive approaches.[11,12] A few studies have proved that virus miRNA can be discovered early at the beginning of the disease before a pathogen can be detected and seroconversion starts.[12] Besides, miRNA diagnosis can also detect asymptomatic infections that become a significant challenge in the pandemic spread and disease monitoring.[13]

miRNAs are part of a class with small noncoding endogenous RNA molecules consisting of 18–24 nucleotides long and considered one central part of post-transcriptional regulators of gene expression.[11,14,15] Current findings have shown that the miRNAs are transported between various cellular components to regulate the translation rate and even transcription.[16] This mechanism is performed by binding miRNA to the 3′ UTR of target mRNA by base-pairing and then triggering whether the translational repression or mRNA destruction thus inhibit expression. The interactions between miRNAs and other regions, including 5′ UTR, coding sequence, and gene promoters, have also been documented.[17]

Viruses, specifically DNA and RNA viruses, can encode miRNA, also known as viral miRNA (v-miRNAs). Pfeffer[17] revealed the first study of DNA viral encoded miRNAs to classify the cloning of viral miRNAs from Epstein–Barr virus-infected cells. Several known viruses were discovered, including herpervirus,[16] poly-omavirus,[18] retrovirus,[19] West-Nile virus,[20] as well as bovine leukemia virus[21] that encode miRNAs have DNA and RNA genomic sequence that can utilize the cellular machinery in the nucleus where they replicate to encode miRNA.[22] Shi et al.[23] reported that hepatitis A virus, a cytoplasmic RNA virus, could encode two miRNAs within its coding region, including hav-miR-1-5p and hav-miR-2 5p. The SARS-CoV virus has also been reported to encode miRNA contributing to the infection associated with lung pathology.[24] This revealed that the cytoplasmic RNA viruses could express small regulatory RNAs, for instance, miRNA.

SARS-CoV-2 is one of the cytoplasm RNA viruses that could encode miRNA.[25,26] However, each step of the full biogenesis and the functional mechanism remains unclear and needs to be explored.[27] Plenty of knowledge concerning miRNA prediction of the SARS-CoV-2 genome has been chiefly focused on computational predictions using bioinformatic tools. The findings from this computational approach provide information about the predictive miRNA encoded by the virus genome (Figure 1), their host gene targets, and also the pathogenesis of SARS-CoV-2.[28] The classical and gold method for detecting miRNA is northern

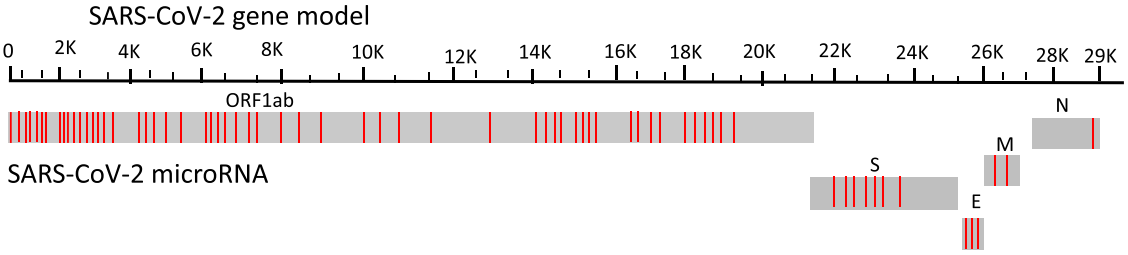


**FIGURE 1** Full genome diagram of SARS-CoV-2 that consists of five genes include ORF1ab, S, E, M, and N. The predictive virus microRNA (red) that encoded by the SARS-CoV-2 RNA virus using bio informatics tools[28]

blotting, which involved molecular hybridization and gel electrophoresis. However, some limitations exist, needed for the vast sample amount that will consume a lot of time and materials, using the carcinogenic risk of chemicals, low sensitivity, and qualitative measurements.[29] Microarray is the standard hybridization technology that has been used for the detection of miRNA in high throughput detection. However, in the long run, it is not cost-effective. Besides, cross-hybridization may be a problematic issue that could lead to mismatched sequences and false-positive outcomes.[30]

Compared with conventional miRNAs detection technologies, DNA electrochemical biosensors have advantages in inexpensive instruments, simple operation, fast response, and high sensitivity. miRNA constitutes only a small fraction of the overall total RNA in biological materials, which are approximately 0.01%, and their level of expression can differ up to a factor of $10^5$.[31] Therefore, the practical and sensible miRNA biosensor detection technique is needed by detecting low detection limits and dynamic ranges. Most of the biosensors built up to detect SARS-CoV-2 focused only on the RNA genome and antibody presence towards the antigen. To date, no study described the detection of virus miRNA for SARS-CoV-2 using a biosensor. Therefore, this paper describes the first designing DNA probe based on the virus miRNA of SARS-CoV-2 as a target for developing a DNA electrochemical biosensor for the COVID-19 diagnosis.

DNA electrochemical biosensor applied the hybridization principle between the DNA probe (single-stranded nucleic acid) and the target (complementary nucleic acid) includes RNA, DNA, and miRNA. The use of a DNA probe that complements the miRNA target of SARS-CoV-2 makes the detection is considered applicable for early diagnosis of COVID-19, and the following precautions action could be taken. The primary foundation of the DNA electrochemical biosensor comprises the immobilization of the DNA probe on the surface of the electrode. The features of the DNA probe in the biosensing approach as it produces higher sensitivity and selectivity, rapid hybridization, highest kinetic strength, and is vital for shorter probe length.[32]

This study aimed to analyze the virus miRNA-targeted nucleotide sequence and design the complementary DNA probe for sensing purposes through bioinformatics tools, for instance, basic local alignment search tool (BLAST), multiple sequence alignments (CLUSTLW), miRbase, microRNA target database (miRDB), and gene analysis tool (PANTHER). The complementary DNA probe identification could be employed as a biorecognition element in biosensor applications for the early detection of COVID-19. The biosensor system involves the competent immobilization of the bioreceptor throughout the transducer surface, which subsequently identifies the analyte and adopts an output signal is proportional to the analyte concentration. In this study, designing a complementary DNA probe from the virus miRNA-targeted sequence analysis for sensing application was developed based on the conserved N-region of the miRNA target.

## 2 | MATERIALS AND METHODS

### 2.1 | Gene profiling of SARS-CoV-2 miRNA target sequences

Distinct sequences were searched and extracted from the literature's computational prediction.[28,33–40] The gene profiling was done to all the extracted miRNA sequences for gene identifying purposes using the database in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/). miRNA nucleotide sequences of the SARS-CoV-2 N-region were identified as 5′ AUCCAUGAGCAGUGCUGACUCAACUCAGGC 3′.

### 2.2 | Analysis of the SARS-CoV-2 miRNA target sequence

NCBI BLAST programming (https://blast.ncbi.nlm.nih.gov/) was utilized to identify the sequence as it matches more than 70% nucleotide comparability the other related SARS-CoV-2 sequences in the Genbank. If a sequence

search produced 95% similarity, the sample was positive for the target sequence of SARS-Cov-2 miRNA. NCBI-BLAST programming enables a sequence to be identified easily within a continuously updating sequence database. The query was submitted as a FASTA sequence, sent to the BLAST server, and a Request Identifier appears. The results were obtained in a few parameters related to the query, including E-value, percent identity, and score bits.

### 2.3 | Multiple sequence alignment using CLUSTLW

The multiple sequence alignment was carried out using CLUSTLW (https://www.ebi.ac.uk/) of default parameter between the miRNA target sequence with other Malaysia strains of SARS-CoV-2 sequences (MT372480.1, MT372481, MT372482, MT372483) and different miRNA coronavirus strains, MERS-CoV isolate KSA_1722, complete genome (MH259485.1), SARS coronavirus Urbani isolate icSARS-C7-MA, complete genome (MK062184) with genome sequence SARS-CoV-2 (accession number NC_045512.2) as a reference sequence. The output of the multiple sequence alignment appeared as a percentage correlation between the analyzed sequences in the matrix formed.

### 2.4 | Sequence alignment correlation between human miRNA

The miRNA target sequence was searched for the nucleotide correlation with all human miRNA by employing the BLAST/SEARCH menu in the miRbase database using the default parameters (http://www.mirbase.org/). The complete sequences of human miRNA were retrieved from miRbase and aligned to the CLUSTLW for multiple sequence alignments with the miRNA target. Human miRNA sequences are available in the miRbase database and were dependent on the average length of miRNA. The information obtained in the miRbase database was the percentage query coverage and types of human miRNA.[36,41]

### 2.5 | Predicted gene target

Human target genes of a novel miRNA target were investigating using the miRNA prediction method, miRDB custom with target score ≥95 option (http://mirdb.org/custom). The expected target genes obtained from this tool were used for subsequent analysis. The gene ontology (GO) was analyzed using PANTHER tools, and the results were obtained in the form of a pie chart that comprises various clusters with percentage values. Once the analysis of the miRNA target was complete, the single-stranded carboxylate 30 mer synthetic oligonucleotide probe was developed as complementary to the miRNA target from the N-region of the SARS-CoV-2 (Accession No: NC_045512.2).

## 3 | RESULTS DAN DISCUSSIONS

### 3.1 | Gene profiling of SARS-CoV-2 miRNA target sequences

The detection of miRNA is nuanced and demands an interdisciplinary approach. The bioinformatic approaches have become the crucial technology and contribute novel amounts of evidence regarding identifying, predicting, and conserving miRNA sequence within the viral genomes.[42] The computational studies of miRNA SARS-CoV-2 from an earlier study[28] showed that the SARS-CoV-2 genome encoded 170 mature miRNA. These miRNAs were present in the various genomic region: noncoding regions of the intronic and comparatively few protein-coding gene exons.[34,43,44] The gene identification of all mature miRNA sequences was made using databases from GenBank, and miRNA sequence (29,500–29,529) from the N-gene (protein-coding gene exons) was utilized as a target for the biosensing application (Figure 2). The choice of miRNA from the N-gene of SARS-CoV-2 as the target in the biosensing approach, due to the N gene is more conserved, expressed abundantly during infection, and has fewer mutations than another genes. Thus, provide more stable detection that lead to the positive results.[45,46]

### 3.2 | BLAST for SARS-CoV-2 miRNA target sequence

Further analysis by employing the BLAST tool contributes the added variables parameter, for instance, expectation value (e-values), score bits, query coverage, and percent identity (Figure 3). The bit score (maximum score) evaluates the correlation of the sequence regardless of the length of the query sequence and the database scale. It is standardized depending on the raw pair alignment score.[47] The total score is the sum of the alignment score for all segments from the same database sequence that match the query sequence. The percentage of the query length included in the aligned segments and its similarity can be observed through the percent query coverage and percent identity score. *E* value is the leading statistical parameter in BLAST measure the likeliness that sequence similarity happened by chance.[48] The analysis from the BLAST of miRNA target sequence exhibits an enormous hit with *E*

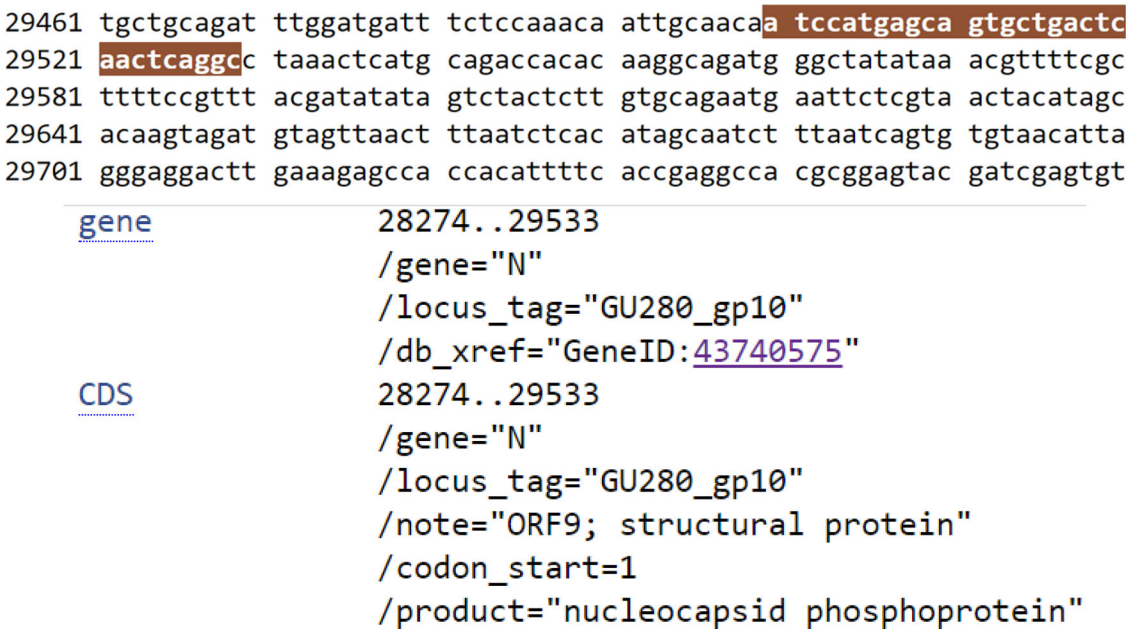


**FIGURE 2** Genome browser of GenBank database shows the virus microRNA target sequence in N-region of SARS-CoV-2 genome

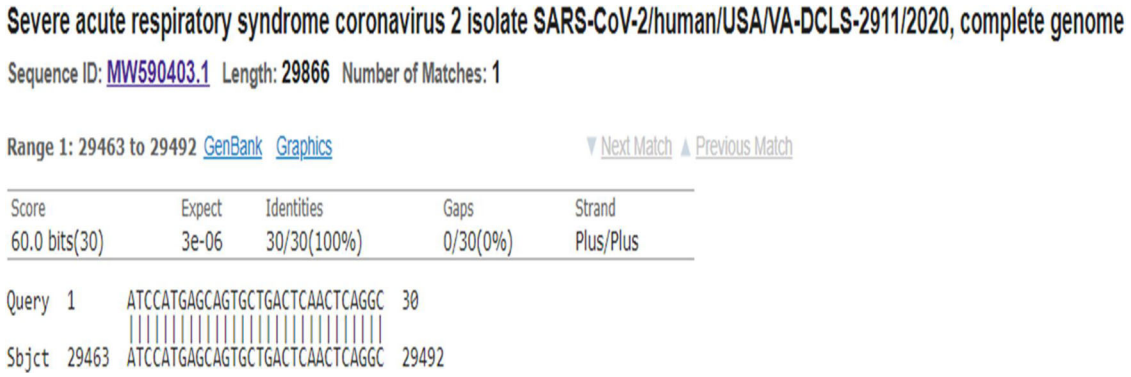


**FIGURE 3** Alignment statistics for a match of virus microRNA target with the sequence from the N-region of SARS-CoV-2 genome

values as $3 \times 10^{-6}$% and the highest identity of 100% and 60.0 score bits based on the N-gene of SARS-CoV-2 genome sequences (Table 1). The higher value of the bits score indicates a significant match. In contrast, the lower *E* values or values near zero imply the highest similarity between the match leads to the sequences homologous.[49] The values given by the parameters revealed that the selectivity of the DNA probe that is complementary to the miRNA target sequence could be enhanced.

## 3.3 | Multiple sequence alignment of CLUSTLW

Multiple sequence alignments' fundamental goal is to establish correlations between sequences and create evolutionary correlations between these sequences. Throughout evolution, specific sequences stay conserved. The motif, domain, and catalytic sites of proteins/DNA could be identified by specifying these retained regions.[50] Therefore, this study aimed to analyze the miRNA target's correlation analysis with the other N-region of SARS-CoV-2 Malaysia's strains and other N-region of coronavirus strains. For this analysis, four types of SARS-CoV-2 Malaysia's strains with accession numbers MT372483.1, MT 372480.1, MT372481.1, and MT372482.1 were considered.[51] While for the latter, two types of strains were employed, which were MERS-CoV isolate KSA_1722, complete genome (MH259485), SARS coronavirus Urbani isolate icSARS-C7-MA, complete genome (MK062184), and genome sequence SARS-CoV-2 (accession number NC_045512.2) as a reference sequence. The nucleotide sequences of the N-region for all SARS-CoV-2 and coronavirus strains were searched using the GenBank database. Various multiple

**TABLE 1** Basic local alignment search tool (BLAST) results of SARS-CoV-2 microRNA target sequences

| Accession | Descriptions | Max score | Total score | Query coverage | *e* Value | Max identity |
|---|---|---|---|---|---|---|
| MW362246.1 | Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/RUS/MOS-CRIE-4924360-D168L0195/2020 nucleocapsid phosphoprotein (N) gene, complete cds | 60.0 | 60.0 | 100% | $3 \times 10^{-6}$ | 100% |
| MW362247.1 | Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/RUS/MOS-CRIE-4907775-D168L0199/2020 nucleocapsid phosphoprotein (N) gene, complete cds | 60.0 | 60.0 | 100% | $3 \times 10^{-6}$ | 100% |
| MW362248.1 | Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/RUS/MOS-CRIE-5022647-D168L0269/2020 nucleocapsid phosphoprotein (N) gene, complete cds | 60.0 | 60.0 | 100% | $3 \times 10^{-6}$ | 100% |
| MW362249.1 | Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/RUS/MOS-CRIE-4969973-D168L0286/2020 nucleocapsid phosphoprotein (N) gene, complete cds | 60.0 | 60.0 | 100% | $3 \times 10^{-6}$ | 100% |
| MW590345.1 | Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/OH-UHTL-13/2020, complete genome | 60.0 | 60.0 | 100% | $3 \times 10^{-6}$ | 100% |
| MW589594.1 | Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/4464154/2020 | 60.0 | 60.0 | 100% | $3 \times 10^{-6}$ | 100% |

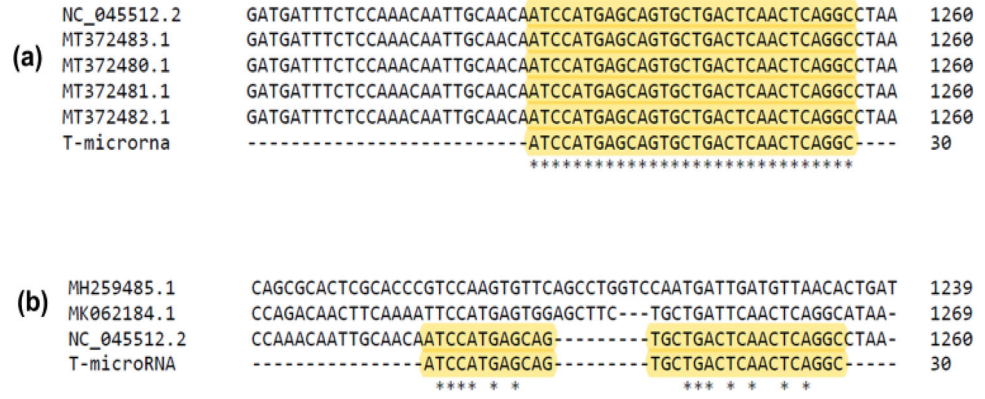


**FIGURE 4** (A) Genome browser of CLUSTLW shows the multiple sequence alignment of microRNA target with Malaysia's strain SARS CoV-2 and Wuhan strains (NC_045512.2) as reference sequence. (B) Multiple sequence alignments of virus microRNA target with other coronavirus strains (MERS-CoV and SARS-CoV) and Wuhan strains (NC_045512.2) as reference sequence using CLUSTLW

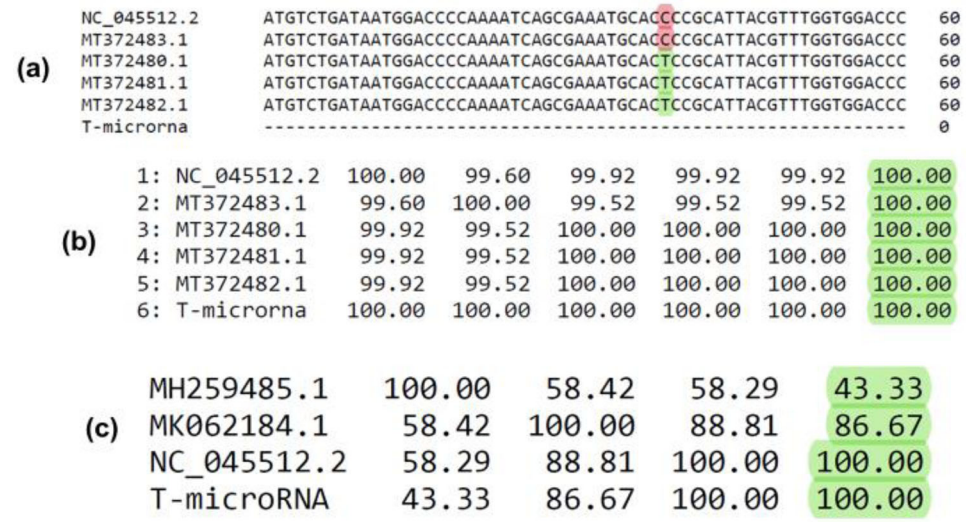


**FIGURE 5** (A) Genome browser of CLUSTLW shows the mutation site of the Malaysian strains with Wuhan strain (NC_045512.2) as a reference sequence. (B) Percent correlation of Malaysia strains and virus microRNA target sequence with Wuhan strain as the reference sequence (NC_045512.2) in the matrix form retrieved from CLUSTLW analysis. (C) Percent correlation of virus microRNA target sequence and other Coronavirus strains (MERS CoV: MH259485.1, SARS-CoV: MK062184.1) with Wuhan strain (NC_045512.2) as a reference sequence in the matrix form retrieved from CLUSTLW analysis

alignment sequences of the SARS-CoV-2 nucleotides in the N region were formed with miRNA target by applying the CLUSTLW tool, revealing the particular variables, such as percent identity and correlation. Figures 4(A) and 4(B) display multiple sequence alignments data. The column indicates identical sequences in the same position in all types analyzed were exclusively marked by an asterisk (*), which meant fully conserved residues, while symbol (-) showed no consensus of the groups. The specific miRNA target of SARS-CoV-2 was designated in yellow, which validated the target for detecting SARS-CoV-2 displayed in the sequences, demonstrating that the target is suitable to utilize as the primary target for all aspects of SARS-CoV-2 strains in Malaysia.

## 3.4 | Comparative analysis of SARS-CoV-2 strains and miRNA target sequence

Compared with Wuhan strain N-region nucleotides sequences (NC_045512.2), Malaysia strains demonstrated only one nucleotide mutation within the MT372480.1, MT372481.1, and MT372482.1 sequence except for the MT372483.1 (Figure 5(A)). The percent correlation of the

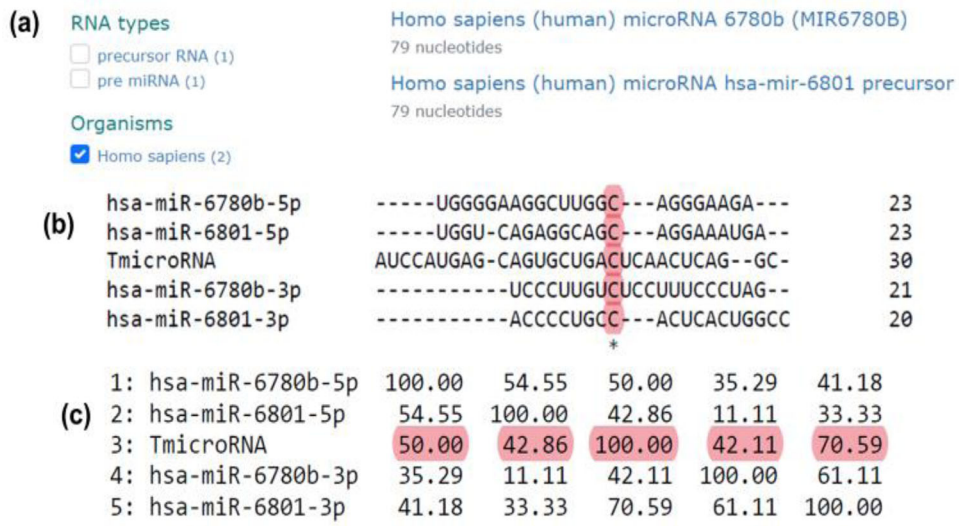


**FIGURE 6** (A) List of similarity of virus microRNA target sequence with human microRNA retrieved from BLAST in the miRDB database. (B) Multiple sequence alignment of human microRNA with virus microRNA target retrieved from CLUSTLW analysis. (C) Percent identity of human microRNA and microRNA target sequences in the matric form retrieved from the CLUSTLW analysis

Wuhan strain with MT372483.1 was 99.60%, while the MT372480.1, 372481.1, and MT372482.1 exhibit 99.92%, respectively. The correlation identification of the miRNA target demonstrated as 100% identical with all four of Malaysia's strains of SARS-CoV-2 (Figure 5(B)). The nucleotide sequence alignment between the miRNA target and SARS-CoV-2 (NC_045512) exhibited an identical value of 100%. It demonstrated the highest similarity among pairwise alignments, followed by 86.67% with (SARS-CoV) and 43.33% between (MERS-CoV) (Figure 5(C)). The value of percent identity of miRNA target analysis revealed that the specificity of the complementary DNA probe to the miRNA target was reinforced to detect SARS-CoV-2 only, without having any cross-reactivity hybridization with other coronavirus strains. Thus, improve the detection selectivity of the biosensors' sensing elements.

## 3.5 | The correlation of miRNA target with human miRNA

Human miRNA is one of the biomarkers that always circulate in the human body in the form of a vesicle.[52] Therefore, the purpose of this analysis was to investigate and cross-check whether the miRNA target sequence has similarities with the human miRNA sequences that could affect the selectivity of the DNA probe as it might be cross hybridized with other targets, especially human miRNA. The list of human miRNAs that shows similarity with miRNA target was searched using the BLAST menu in the miRbase database. The findings showed that two types of human miRNA have the highest similarity with the miRNA target sequence that was *Homo sapiens* (human) miRNA 6780b (MIR6780B) and *Homo sapiens* (human) miRNA hsa-mir-6801 precursor with target coverage 18.99% and 27.85%, respectively (Figure 6(A)). The full nucleotide sequences of these human miRNA were retrieved and aligned in the CLUSTLW tool for the sequence alignments with the default parameter. The results from the multiple sequence alignment revealed that only a single nucleotide shows similarity with the miRNA target (Figure 6(B)). Target miRNA and has-miR-6780b-5p and 3p shows percent identical about 50.0% and 42.11%, respectively. While percent identical between miRNA and has-miR-6801-5p and 3p demonstrated 42.86% and 70.59%, respectively (Figure 6(C)). The percent correlation value for miRNA target and human miRNA sequences validates that the complementary DNA probe designed would selectively interact with the miRNA target, thus increasing the device's selectivity.

## 3.6 | Predicted genes for miRNA target

The miRNAs derived from the SARS-CoV-2 virus could be expressed in host cells and engage in the infection's life-cycle and cause cellular infections. SARS-CoV-2 releases its genomic RNA after entering the host/human cell, converted further into proteins required for its RNA synthesis. Simultaneously, some RNAs are altered to miRNAs for certain host mRNAs, thus distorting the level of

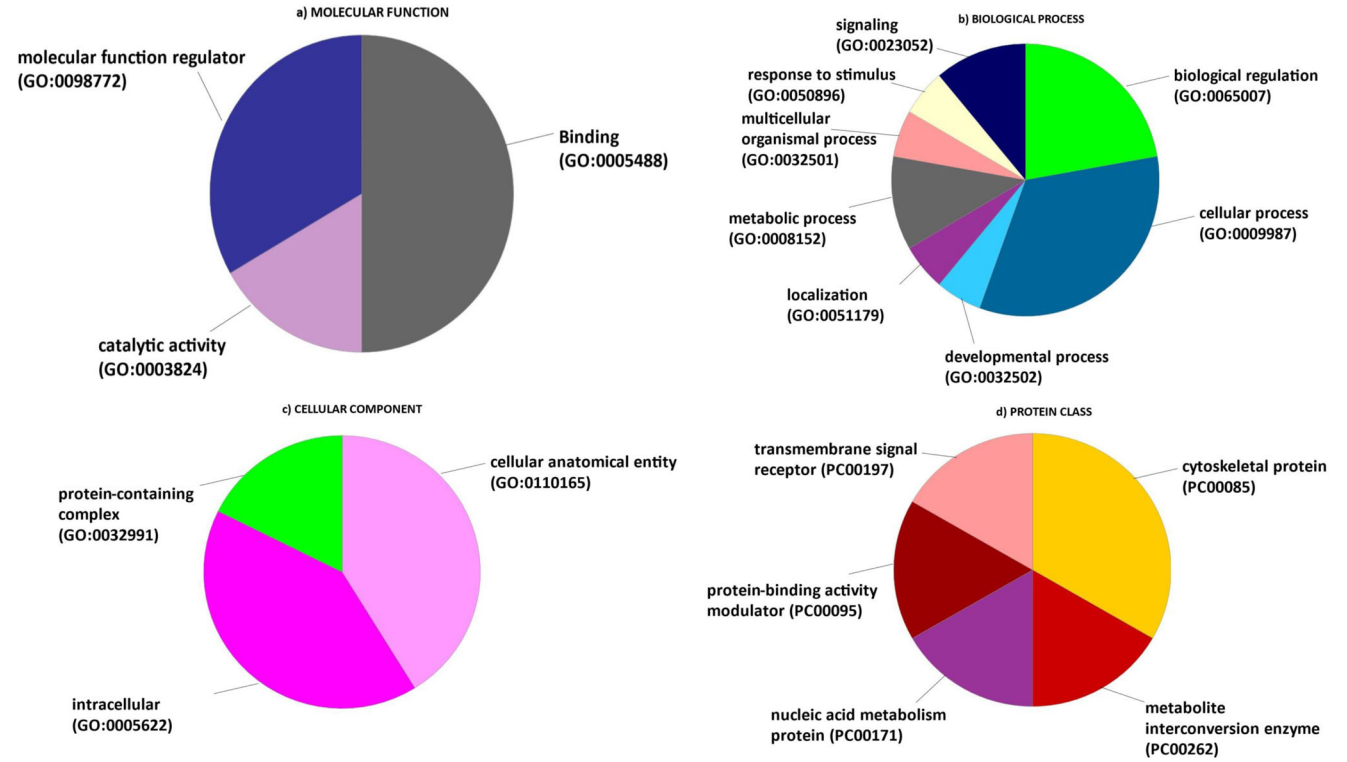


**FIGURE 7** Cluster of gene ontology that consists of molecular function (A), biological process (B), cellular component (C), and protein class (D) retrieved from the PANTHER analysis

expression of the genes and their associated pathways, leading to viral pathogenesis.[36] Besides, many host genes responsible for controlling cell proliferation, apoptosis, and immune response have been discovered as targets for viral miRNA.[40] In this study, the genes were predicted using the miRDB database as the custom prediction (Table 2). The gene predicted with a value of more than 95% target score was retrieved and classified according to their biological process, protein class, molecular function, and cellular compartment by employing PANTHER and GO analysis. The biological process cluster comprises the gene that was taking part in the cellular process (33.3%), biological regulation (22.2%), and metabolic process (11.1%) (Figure 7(A)). The molecular function cluster covered binding (50.0%), molecular function regulator (33.3%), and catalytic activity (16.7%) (Figure 7(B)). The cellular component cluster included cellular, anatomical entity, intracellular (41.2%) respectively, and protein-containing complex (17.6%) (Figure 7(C)). The protein class cluster consists of cytoskeletal protein (33.3%) and nucleic acid metabolism protein (16.7%) (Figure 7(D)).

The predicted gene revealed that the miRNA target could interact with gene ABLIM1 and NR3C1(nuclear receptor subfamily 3 group C member 1) genes involved in the cellular component organization and inflammatory responses. ABLIM1 (an actin-binding cytoskeletal protein) is vital for viral replication at many stages of the viral life cycle. Virus can suppress the force-generating and macromolecular scaffolding properties of the actin cytoskeleton to push viral surfing, internalization, and migration within the cells. This observation suggested the possible role of SARS-CoV-2 miRNA in causing increased inflammation in the lung and facilitating the virus invasion, thus improved viral pathogenesis.[35] miRNA target might also target genes that are associated with brain development. TMOD2 (tropomodulin 2, neuronal-specific member of the tropomodulin family of actin-regulatory proteins) might explain neurological signs like headaches, vomiting, and nausea.[28,41] DIDO1, a gene related to apoptosis, a powerful mechanism to curtail viral spread, also became the target gene for the SARS-CoV-2 miRNA. The suppressive role of the miRNA on this gene suggested the possible role in reducing host cell apoptotic to subvert host defence.[35] Also, miRNAs can target different essential organ-specific cellular functions and pathways, for instance, HS3ST3A1, a gene that is related to the liver, TSPAN33 and CMC1, genes that are related to the kidney. It has been reported that a substantial proportion of COVID-19 patients showed signs of various degrees of liver and kidney damage.[28] The HS3ST3A1 gene is highly expressed in the liver, implicated a possible role of miRNA on the liver damage of COVID-19 patients.[35]

**TABLE 2** Predicted genes target with the gene ontology retrieved from the PANTHER analysis tool

| Gene symbol | Gene ID | Descriptions | PANTHER protein class | Target score (%) | Gene ontology |
|---|---|---|---|---|---|
| DIDO1 | ENSG00000101191 | Death inducer-obliterator 1 (related to the apoptosis) | General transcription factor | 98 | Protein class |
| TSPAN33 | ENSG00000158457 | Tetraspanin 33 (Broad expression in the kidney) | – | 98 | Cellular compartment |
| ABLIM1 | ENSG00000099204 | Actin-binding LIM protein 1 | Actin or actin-binding cytoskeletal protein | 98 | Molecular, biological, cellular, and protein class |
| HS3ST31 | ENSG00000153976 | Heparan sulfate-glucosamine 3-sulfotransferase 3A1 ( most abundant expression in liver) | Transferase | 97 | Protein class |
| VN1R1 | ENSG00000178201 | Vomeronasal 1 receptor 1 ( primarily localized to the olfactory mucosa) | Transmembrane signal receptor | 96 | Protein class |
| NR3C1 | ENSG00000113580 | Nuclear receptor subfamily 3 group C member 1, Ubiquitous expression lung (It is involved in inflammatory responses, cellular proliferation, and differentiation in target tissues) | – | 96 | Molecular, biological, cellular |
| TMOD2 | ENSG00000128872 | Tropomodulin 2, related to the neuronal-specific member. Ubiquitous expression in brain | Actin or actin-binding cytoskeletal protein | 96 | Biological, cellular, protein class |
| PRKAR2B | ENSG00000005249 | Protein kinase cAMP-dependent type II regulatory subunit beta (signaling molecule) | Kinase modulator | 96 | Molecular, biological, cellular, and protein class |
| JPH4 | ENSG00000092051 | Junctophilin 4 (Ubiquitous expression in the brain) | – | 96 | Biological, cellular |
| CMC1 | ENSG00000187118 | C-X9-C motif containing 1 (ubiquitous expression in the kidney) | – | 96 | cellular |
| AEBP2 | ENSG00000139154 | AE binding protein 2 | – | 95 | Biological cellular |

### 3.7 | Efficiency of N-region as the primary target for detection of SARS-CoV-2

The potential to investigate the causative agent at the earliest opportunity is the practical critical approach to restrain and the contagious disease epidemic. This can be accomplished by recognizing several biomarkers related to the pathogen that is formed explicitly during infection. For detecting the SARS-CoV-2 virus, some regions among the virus genomes had conserved sequences utilized as target sequences. These sequences are the RdRP gene, E gene, N gene, ORF1ab gene, and S gene.[53,54] N-gene is the most abundantly expressed transcript in SARS-CoV-2-infected cells at the early stage of infection, thus, enhances the efficiency of viral RNA transcription that is essential for viral replication.[55,56,57] This suggests that the N-gene is one of the best targets for high sensitivity detection of SARS-CoV-2 infection.

The comparison studies of ORF1ab, E, and N target genes revealed that the N-gene was the most positive gene detected in the sample. It also exhibits the most sensitive assay target even in the low concentration of the virus in detecting SARS-CoV-2 using RT-PCR and RT-Lamp assay.[45,53] The main factor that N-gene is more preferred as the target for detecting SARS-CoV-2 virus is that the N gene is more conserved, has fewer nucleotide variation, and lower mutation rate compared with the ORF1ab, thus making the detection of the N-gene more stable, which could partially explain why the N-gene was most often detected in the test results.[58] Except for the N gene, due to these variations' potential to interact with both diagnostic tests and antiviral therapies such as Remdesivirir, a gene mutation in the RdRp gene has been well established.[59] Additionally, several S-gene mutations have been found in three new strains of SARS-CoV-2 that in the UK, South Africa, and Japan. S-gene mutations are more frequent, giving the virus a genetic advantage in increased transmissibility.[60]

The choice of miRNA from the N-gene of SARS-CoV-2 as the target in the biosensing approach is due to the main factors that N-gene has. Because of its essential functions, it represents most critical targets for early screening test of the SARS-CoV-2 virus. Based on the bioinformatics analysis on miRNA target of SARS-CoV-2, the 30 mer of 53.3% GC content of complementary DNA probe was developed with the sequence 5′ GCC TGA GTT GAG TCA GCA CTG CTC ATG GAT 3′. Based on the characteristics in the appropriate range, this DNA probe could be used as a bioreceptor to identify miRNA from the N-region of SARS-CoV-2. Besides, the probe's high GC content leads to a more significant binding between the DNA probe and the miRNA target, increasing the sensor's sensitivity.[61]

### 3.8 | Future work of the development of nanomaterials-based biosensor

The gold standard for early detecting SARS-CoV-2 is a qRT-PCR based on the amplification of the RNA of the virus. N-region genomic sequence of SARS-CoV-2 was amplified to increase the specificity of the diagnostic method. Along with the qRT-PCR, the serology test could also diagnose antibodies toward the virus antigen by late detection. Although this assay exhibit intensively specific and sensitive, some constrain exists that were time consuming, crucial for trained personnel and including a longer duration of data management and analysis. The production of biosensor technology for SARS-CoV-2 detection does have the potential to use fast, high screening, and enormous promise for the future as a diagnostic tool. The biosensor is a smart analytical device that utilizes the specific biorecognition elements attached to solid support or gel matrix to detect chemical or biological analyte targets. The interaction of the biorecognition–the target can be translated into the sensitive readout of signals proportional to the concentration of the analyte presence.[62] The main components of the biosensor include biorecognition elements, interface matrix (immobilization techniques)), and physicochemical transducer elements. The biorecognition element is a biological receptor (enzyme/ substrate, antibody/antigen, nucleic acids, whole cells, and biomimetic molecules) that interacts with a specific target analyte or catalyzes a reaction for the analyte to generate a recognizable signal. The binding of biorecognition elements onto the substrates' surface or near closeness to a transducer is crucial to ensure the biosensor operates effectively. Numerous immobilization techniques (adsorption, covalent binding, cross-linking, entrapment, bulk modification, and sol–gel) have been employed to attach biorecognition elements to various substrates, including nanomaterials. The transducer of the biosensor translates the interaction between the biorecognition elements-target analyte into measurable signals for example, such as electrochemical, optical, thermal, piezoelectric, and mass-sensitive sensors.[63]

For future work, the development of DNA electrochemical biosensor for detection of SARS-CoV-2 comprises of the surface modification of the interdigitated electrode (IDE) using carbon quantum dots (CQDs), followed by functionalization of the surface-modified IDE by DNA probe, and detection of the virus miRNA via hybridization event between the DNA and miRNA through the electrochemical method (voltammetry). CQDs are zero-dimensional nanomaterials with less than 10 nm in size and exhibit a high surface-to-volume ratio that benefits the immobilizing significant amounts of biorecognition elements or linkers on their surfaces, thus enhanced the sensitivity and

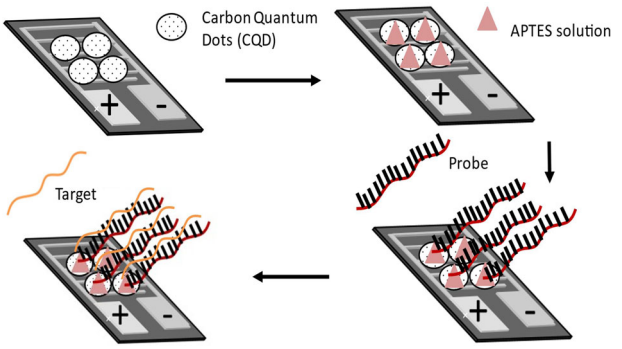


**FIGURE 8** Overview of the sensing approach for the detection of SARS-CoV-2. The modifying of IDE transducer via carbon quantum dots followed by the immobilization of DNA probe using APTES as linker. The hybridization occurs between the target (virus microRNA) and DNA probe

specificity of the biosensor.[64] The modified carboxylate 5′ end of the DNA probe will be immobilized onto the modified surface of IDE via (3-aminopropyl) triethoxysilane (APTES) as a linker. The amide bond formed between the -$NH_2$ group of APTES and -COOH group from the DNA probe could produce the reproducible immobilization, stable and prevent the functional degradation of the DNA probe.[65] The hybridization events of the DNA probe and miRNA target based on the complementary base pairing of Guanine (G) and Cytosine (C), Adenine (A)), and Uracil (U) will be measured by employing the voltammetry measurements conducted that subsequently lead to the concentration of the analyte present in the sample (Figure 8).

### 3.9 | Comparison with the alternative sensing system for SARS-CoV-2 detection

The identification of SARS-CoV-2 relies heavily on the selectivity and sensitivity of biosensors' sensing elements. The highest selective of assay techniques is crucial to avoid false-negative or false-positive results. For SARS-CoV-2 identification, numerous molecular diagnostic assays depending on nucleic acid amplification have been developed. As previously stated, one of the significant concerns with the amplification techniques is, it is not designed for the Point of Care (POC) diagnosis and potential of triggering false-positive/negative results owing to the unanticipated cross-contamination.[66] Biosensors exhibit various promising criteria to meet diagnostic fulfilment, and they can detect COVID-19-related biomarkers. Nanomaterials, nano-scale transducers, and portable detection technologies have revolutionized the world of sensors and provided portable biosensing devices for point-of-care diagnostic applications with a low sample consumption.[67] The vital potential for biosensors application is that they can be used for label-free and nucleic acid amplification free detection of DNA/RNA for the sensitive and selective diagnosis of infection. Table 3 shows the features of some previously reported biosensors for SARS-CoV-2 detection that were compared with those of our future work biosensor.

Most of the biosensors develop were label-based assays for detecting RNA and antibodies/antigen toward the virus. Even though this type of sensing detection provided sensitive detection, it suffers from the complexity of the assay and the need for high-cost reagents for signal amplification. Labeled protocols have some adverse drawbacks, such as cost intensive, sophisticated preparation facilities, and time consuming. It can also block the active binding sites, resulting in an alteration of binding properties, thereby affecting the affinity-based interaction between the biorecognition elements and the target molecules, thus limiting its practical application.[31,68] The employment of the CQDs for the electrode modifier allows for the label-free assay as it can enhance the sensitivity of the detection due to the large surface area and has abundant hydroxyl group that benefits the immobilization of the APTES and DNA probe. The target used for most previous biosensors was the RNA of the virus, which needed RNA separation and extraction, limiting the application for quick and in-field sampling for the suspicious COVID-19 patients. For our future biosensor, the utilization of virus miRNA as a target can be achieved in direct detection. It is circulating in the body fluids and allows for noninvasive samples. A few studies have proved that virus miRNA can be discovered early at the beginning of the disease before a pathogen can be detected and seroconversion starts.[12] Besides, virus miRNA diagnosis also can detect asymptomatic infections that become a significant challenge in the pandemic spread and disease monitoring.[13] To the best of our knowledge, the virus miRNA was used for the first time as a biomarker for the COVID-19 diagnosis. Predominantly, our sensing system did not include the use of an antibody or enzyme. The current sensing technique exhibited a simpler necessity in label-free DNA probes than other biosensors that need protein biorecognition elements, including antibodies. The utilization of nucleic acids in biosensors can significantly lower the costs while also improving the chemical and thermal durability due to its stability and longer shelf life than the antibodies and enzymes.

## 4 | CONCLUSIONS

An oligonucleotide DNA probe designation for application in biosensor for detecting virus miRNA of SARS-CoV-2 was done using bioinformatics tools. miRNA from N-region was considered and revealed the possible specific target for virus detection. The BLAST and multiple

**TABLE 3** Comparison of the limitation and advantages of the proposed electrochemical biosensor for SARS-CoV-2 detection with the other biosensors

| Detection methods | Sensing elements | Target | Type of assay | Limitations | Advantages | Ref |
|---|---|---|---|---|---|---|
| Voltammetry | Gold electrode/graphene/ thiol-modified ssDNA-conjugated AuNp | N-gene (RNA) | Label based | Requirements of sample extraction and preparation before testing[69] | Enable the digital detection[70] | 70 |
| Voltammetry | Graphene electrode/ polyimide layer/ Antibodies-HRP label | IgG, IgM against NP, S and C-reactive protein | Label based | Not effective for the early diagnosis of COVID-19[71] | Can be used in during vaccine development to track the antibodies production[71] | 72 |
| Surface plasmons | GC-FP chip/COVID-19 antigen/antibody/anti-IgG/IgM | IgM and IgG against S and N | Label based | | | 73 |
| Chronoamperometry | Electrode chip/dsDNA-ferrocene/antibody | S glycoprotein | Label based | | | 74 |
| Voltammetry | SPCE/modified GO with EDC and NHS label with AuNanostar | S glycoprotein | Label based | High risk of cross detection with other coronavirus family (SARS-CoV and MERS-CoV)[75] | Do not require extraction or biological marker for the detection[76] | 76 |
| Voltammetry | IDE/CQD/APTES/ssDNA | MicroRNA of N-gene | Label free | NIL | Enable the direct and label free detection[15] | This future work |

AuNP, gold nanoparticle; N-gene, nucleocapsid gene; ssDNA, single -stranded deoxyribonucleic acid; HRP, horseradish peroxidase; NP, nucleocapsid protein; S-protein, spike glycoprotein; IgG, immunoglobulin G; IgM, immunoglobulin M; GC-FP, gold coated grating-coupled fluorescent plasmonic; dsDNA, double-stranded deoxyribonucleic acid; SPCE, screen- printed carbon electrode; GO, graphene oxide; EDC, 1-ethyl-3-(3-dimethylamino- propyl)carbodiimide; NHS, N-hydroxysuccinimide (NHS); IDE, interdigitated electrode; CQD, carbon quantum dots; APTES, (3-Aminopropyl) triethoxysilane.

sequence alignments from CLUSTLW analysis showed that the sequence of virus miRNA target was unique with 100% identical for SARS-CoV-2 compared with the other coronavirus strains and human miRNA. The predicted gene target analysis from miRDB and GO showed that the virus miRNA of SARS-CoV-2 could potentially interact in the inflammatory response and cellular organization in specific genes in lung, brain, kidney, and liver. The development of the DNA probe was complementary to the miRNA target, which can be utilized as a selective biomarker for SARS-CoV-2 detection in biosensing for clinical and environmental implementations.

## ACKNOWLEDGMENTS

This research was supported by the Ministry of Higher Education (MOHE) under Grant Nos. RACER/1/2019/TK04/UNIMAP//2, and the author would like to thank all staff members of the Institute of Nanoelectronic Engineering in Universiti Malaysia Perlis for their technical advice.

## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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**How to cite this article:** Halim FS, Parmin NA, Hashim U, Gopinath SCB, Dahalan FA, Zakaria II, et al. MicroRNA of N-region from SARS-CoV-2: Potential sensing components for biosensor development. *Biotechnology and Applied Biochemistry*. 2022;**69:**1696–1711. https://doi.org/10.1002/bab.2239