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Designing probe from E6 genome region of human *Papillomavirus* 16 for sensing applications

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ABSTRACT

Human Papillomavirus (HPV) is a standout amongst the most commonly reported over 100 types, among them genotypes 16, 18, 31 and 45 are the high-risk HPV. Herein, we designed the oligonucleotide probe for the detection of predominant HPV type 16 for the sensing applications. Conserved amino acid sequences within E6 region of the open reading frame in the HPV genome was used as the basis to design oligonucleotide probe to detect cervical cancer. Analyses of E6 amino acid sequences from the high-risk HPVs were done to check the percentage of similarity and consensus regions that cause different cancers, including cervical cancer. Basic local alignment search tools (BLAST) have given extra statistical parameters, for example, desire values (E-values) and score bits. The probe, 'GGG GTC GGT GGA CCG GTC GAT GTA' was designed with 66.7% GC content. This oligonucleotide probe is designed with the length of 24 mer, GC percent is between 40 and 70, and the melting point (Tm) is above 50 °C. The probe needed an acceptable length between 22 and 31 mer. The choice of region is identified here can be used as a probe, has implications for HPV detection techniques in biosensor especially for clinical determination of cervical cancer.

1. Introduction

Over the last decade, amazing progress has been made to understand the pathogenesis of Human Papillomavirus (HPV). Examining the natural history and the study of disease transmission of HPV is complicated, because there are more than 100 strains of HPVs found [1–4]. Since all HPV sorts are immovably related, tests can be intended to target the conserved region of the genome or areas where sequences can best be used to isolate assorted HPV strains [5]. High-risk types are those like the sorts most of the time, were found in anogenital malignancies; by and large generally the low-risk types are those like the types related in condylomata [6–8]. There are several approaches to distinguish different types of HPV strains in clinical specimens. One of the basic ways is by utilizing primers coordinated to the generally conserved regions of HPV genome and after that, the consensus amplicon is recognized by the proper strategy, for example, dot blot hybridization with particular probe(s), restriction fragment length polymorphism examination, or sequencing [9–15].

Generally, cervical cancer is created by the high-risk types of HPV [16–18] and the genome association of HPV strain 16, is one of the subtypes with a specific genome is known to cause cervical cancer predominantly (Fig. 1). This HPV genome includes an upstream regulatory region (URR), or long control region (LCR), has early genes (E1, E2, E6, and E7), and late genes (L1 and L2). It ought to be noted, about overlapping adjacent regions: e.g., E6, which is more prominent than E7, covers a huge segment of E7 [19–23]. Integration is essential, goes before cancer growth development, and could be incorporated into the malignant change [24]. In cervical carcinoma cell lines, the viral deoxyribonucleic acid (DNA) is typically incorporated into the human cell genome [25–27]. Previously, integration form had been found as much as 44%, episomal had 44% and both forms had 11% for cervical carcinomas containing HPV 16 [16,28,29]. A technique including real-time PCR has been utilized to screen for mixing status by determination of the E2/E6 proportion and might be appropriate for checking disease progression. Combination includes breakage of the HPV DNA and loss of parts in genome. Early perceptions of cervical carcinoma cell lines uncovered erasure of 2–3 kb that incorporated the E2 to L2 area [30–32]. The most crucial part is E6–E7 transcripts could be recognized in the cells. Open reading frame (ORFs) of E1, E2, E4, and E5

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0141-8130/

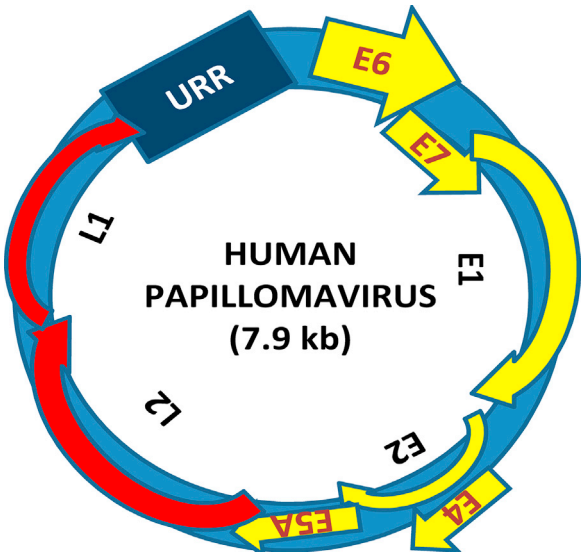

Fig. 1. Genome organization of human papillomavirus type 16. This subtype is known to cause cervical cancer. It has E1-E7 early genes, L1-L2 late genes: and capsid region.

were hindered by flanking the host cell DNA, indicating as sites of combination [33]. HPV mRNAs present hybridized probes for the whole E6 and E7 ORF and a minor part of the E1 ORF, implying that these were the main portions of the HPV genome present [34,35]. Investigation of six cervical carcinoma tests demonstrated that the E6/E7 region was observed to be held on joining, yet the E2 was lost. Viral genome integrated into human chromosomal DNA of cancer cells generally erases E2 ORF, which results in the loss of expression of the E2 gene, however, is connected with the continuation of high expression of E6 and E7 [36–42]. Whatever the rest of the genome may have a part in transmission of the infection to another host cell, yet it is the proteins encoded by E6 and E7 that cause the malignancy. Joining of HPV DNA happens early in cancer development and is vital as an enactment mechanism for movement from the precancer to cancer [43–46]. E6 and E7 regions demonstrate the best contrasts in nucleotide sequence between various HPV types and is constantly held by cells that are contaminated compared to other regions in HPV 16 [47–49].

DNA has a role as biomarker of HPV. Investigation of potential biomarker helps to identify a new pathway involved in the HPV pathogenesis to prevent cervical cancer. DNA probes are stretches of single-stranded used to detect the presence of complementary nucleic acid sequences; in other word, is a target sequence by hybridization process. HPV type-specific probes provide a rapid and cost-effective method to simultaneously detect HPV genotypes. Suitable DNA probe synthesize based on E6 area of the different types of HPV able to answer whether a patient is infected with a high-risk HPV, can be integrated and promptly fused into the sensing purposes [50,51]. The use of E6 DNA probes make the detection is considered to be applicable for early discovery of cervical cancer and expenses will be lower. The general principle of DNA biosensor involves the immobilization of a DNA or DNA-analog probe onto the electrode surface. The importance of DNA probe in the sensing application with biosensor has been shown to give a higher sensitivity and specificity, fast hybridization kinetic strength rather than ionic strength and requires only a shorter probe length [52]. Fig. 2 showed a research overview in the development of highly sensitive and selective nanoparticles based biosensor for early detection of cervical cancer.

To analyse the region of interest, BLAST programs are widely used as tools for looking protein and DNA databases for grouping similarities. Table 1 showed Basic Local Alignment Search Tool (BLAST) results of Oligonucleotide A for the identification of HPV 16 target. BLAST have been figured to differentiate protein or DNA queries with databases, with DNA sequences frequently experiencing reasonable interpretation before any experimental procedures is performed. The aim of this research was to analyze the importance and usage of probe resides in E6 genome region of HPV 16 for sensing purposes through bioinformatics analysis such as BLAST and CLUSTALW. This type of probe identification can be used as a biomarker for biosensing receptor element in biosensor application for early detection of cervical cancer (Fig. 3). Fig. 3 is overall figure revealing the sensing application for the detection of HPV. DNA biomarker have been selected from the gene profiling and involved 4 strains of HPV that cause cervical cancer, HPV 16 (AAA46939.1), HPV 18(P06463.1), HPV 31(AGM34418.1), and HPV 45 (AGM34429.1). The biosensor device requires the efficient immobilization of nucleic acids over the surface of a transducer, which is responsible for the analyte recognition and executed a response signal proportional that can be measured the analyte concentration. The challenge for biosensor device relies on the importance of preserving the biological activity of probe as well as the transducing activities for measurable electrical biosensor.

In this research work, designing a probe from E6 genome region of HPV 16 for sensing applications was developed using HPV DNA probe complementary to a 24 bases region unique to E6 region gene of HPV type 16. Gravitt et al. [53] conclude that a high viral load is associated with prevalent cervical cancer precursors for the most HR-HPV genotypes, but only HPV 16 load predicts the development of cervical cancer disease.

2. Materials and methods

2.1. Analysis of HPV sequence from high-risk HPVs

Different strains of HPV sequences were searched in GenBank by using FASTA. Nucleotide sequences of HPV in GenBank were identified. A sequence was viewed as a match if it had more than 70% nucleotide comparability [54]. The reliability of this method for generating other probes, rather than HPV with no less than 80% comparability to the relating HPV sequences in GenBank were thought to be available. If a sequence search produced 95% similitude to HPV type 16 (HPV 16) and 85% closeness to HPV 18, the sample was viewed as positive for both types. HPV16 oligonucleotide probe sequence can be utilized to cross-examine a grouping database utilizing a homology search by utilizing BLAST programming. Primer-BLAST programming [55] allow quick homology finding of a sequence within a constantly updated sequence database.

2.2. Identification of HPV 16 target DNA

HPV16 oligonucleotide probe sequence can be utilized to investigate a sequence database utilizing a homology search by BLAST programming. Broad databases are accessible on the web and can be openly obtained (http://www.ncbi.nlm.nih.gov). Primer-BLAST software [55] allows quick homology finding of a sequence inside a consistently redesigned sequence database. At the point when the query is submitted, either as a sequence in FASTA group or as an arrangement identifier, e.g. GenBank accession form, the inquiry is sent to the BLAST server and a 'Request Identifier' (RID) is returned. The inquiry and results are put away in an organized arrangement for up to 24 h after a RID is issued. The RID distinguishes the query and permits the outcome to be seen in a few organizations, which incorporate the natural BLAST report, an improved 'hittable', XML and ASN.1. [56]. Single-stranded carboxylate 24 mer synthetic

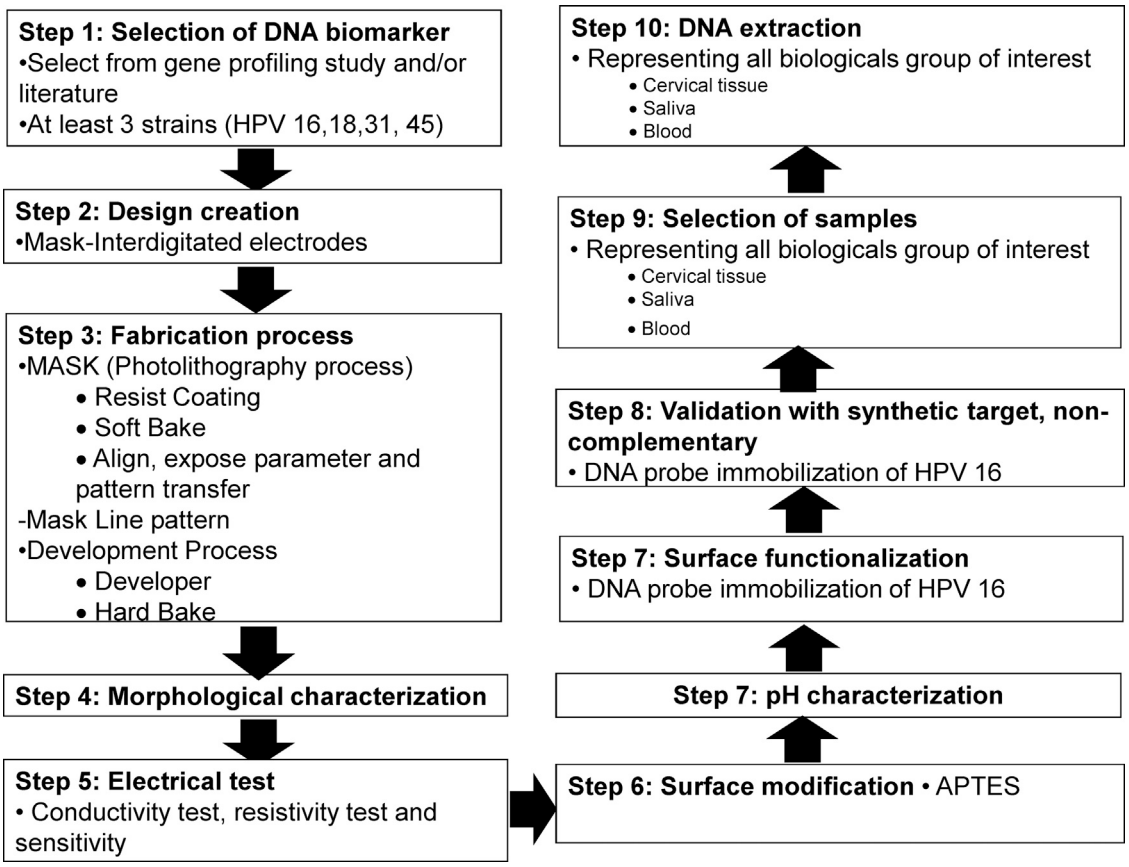

Fig. 2. Research overview in development of highly sensitive and selective nanoparticles based biosensor for early detection of cervical cancer.

Table 1
Basic Local Alignment Search Tool (BLAST) results of Oligonucleotide A for the identification of HPV 16 target DNA.

Accession	Description	Max Score	Total score	Query coverage	E value	Max identity
KF700140.1	Human papillomavirus type 16 isolate 85 E6 protein (E6) gene, complete CDS	48.1	48.1	100%	0.001	100%
KF700139.1	Human papillomavirus type 16 isolate 67 E6 protein (E6) gene, complete CDS	48.1	48.1	100%	0.001	100%
KF700134.1	Human papillomavirus type 16 isolate 28 E6 protein (E6) gene, complete CDS	48.1	48.1	100%	0.001	100%
KM030565.1	Alphapapillomavirus 9 isolate Afr4 E6 oncoprotein (E6) gene, complete CDS	48.1	48.1	100%	0.001	100%
KM030564.1	Alphapapillomavirus 9 isolate Eur18 E6 oncoprotein (E6) gene, complete CDS	48.1	48.1	100%	0.001	100%
KM030563.1	Alphapapillomavirus 9 isolate Eur14 E6 oncoprotein (E6) gene, complete CDS	48.1	48.1	100%	0.001	100%
KJ869425.1	Human papillomavirus type 16 isolate HPV BHU 3 E6 protein (E6) and E7 protein (E7) genes, partial CDS	48.1	48.1	100%	0.001	100%
KJ922765.1	Human papillomavirus type 16 strain 7 isolate salem-2 transforming protein (E6) gene, complete CDS	48.1	48.1	100%	0.001	100%
KJ922764.1	Human papillomavirus type 16 strain 7 isolate salem-1 transforming protein (E6) gene, complete CDS	48.1	48.1	100%	0.001	100%

oligonucleotide probe designed from HPV 16 for recognizable probe of cervical cancer (Accession No.: A18875.1).

3. Results and discussion

3.1. Computational biology tools for analysing HPV sequences

Computational science apparatuses have opened new bits of knowledge concerning the portrayal and protection of biological molecule sequences among the particular viral genomes [57]. By utilizing different sequence alignments (CLUSTALW), the target of present study is to perform the relative analysis of E6 protein sections among the high-risk HPV genotypes that cause cervical cancer. For these analyses, 4 types of E6 protein segments present in HPV high-risk genotypes that cause cervical cancer, namely; 16, 18, 31 and 45 were considered. At first, amino acid sequences of E6 protein from these fourth genotypes of high-risk HPV were searched utilizing GenBank database (http://www.ncbi.nlm.nih.gov/protein). Recognizable proof of amino acid sequences comparing to E6 protein was saved in Biology Workbench database (http://workbench.sdsc.edu) with accession numbers: AIQ82781.1, P06463.1, AGM34418.1 and AGM34429.1 for HPV16, 18, 31 and HPV 45, individually. The numerous alignment sequences of the HPV high-risk amino acid residues in the E6 viral protein among unmistakable HPV genotypes were created utilizing the CLUSTALW database apparatuses (Fig. 4), giving significant factual parameters, for example, similarity and identity. Multiple sequence alignments of the nucleotide residues in the E6 in a complete genome of HPV 16 by using CLUSTALW were displayed in Fig. 5. Columns showing indistinguishable nucleotide similarly situated in numerous types 'i' were analyzed exclusively highlighted by an asterisk (*) mark which showed fully conserved residues. Symbol (–) showed no consensus of the groups. Specific HPV E6 region as oligonucleotide probe for the detection of HPV 16 is indicated in red, which prove

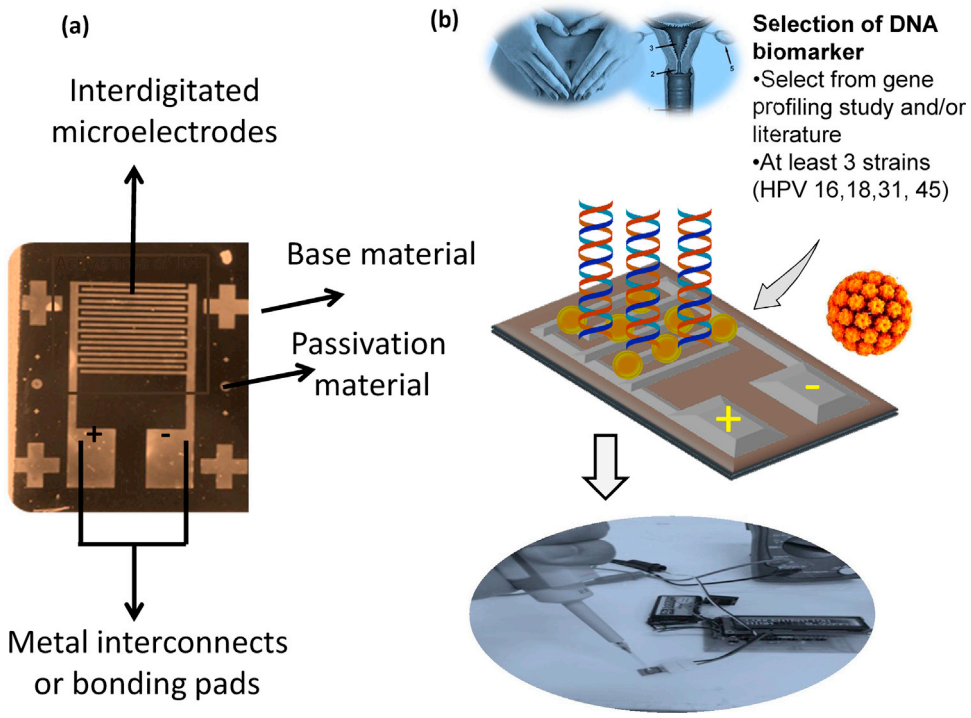

Fig. 3. Overall figure for sensing application for the detection of HPV. DNA biomarker have been select from gene profiling and involved 4 strains of HPV that cause cervical cancer, HPV 16 (AAA46939.1), HPV 18(P06463.1), HPV 31(AGM34418.1), and HPV 45 (AGM34429.1).

```
AGM34429.1_HPV_45_E6      -----MARFDDPKQRPYKLPDLCTELNTSLQDVSIACVYCKATLERTEVY
P06463.1_HPV18_E6         -----MARFEDPTRRPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEVF
AAA46939.1_HPV_16_E6      MHQKRTAMFQDPQERPRKLPQLCTELQTTIHDIILECVYCKQQLLRREVY
AGM34418.1_HPV_31_E6      -------MFKNPAERPRKLHELSSALEIPYDELRLNCVYCKGQLTETEVL
                                 *.:* .** ** :*.: *: . .:: : *****  *   **

AGM34429.1_HPV_45_E6      QFAFKDLCIVYRDCIAYAACHKCIDFYSRIRELRYYSNSVYGETLEKITN
P06463.1_HPV18_E6         EFAFKDLFVVYRDSIPHAACHKCIDFYSRIRELRHYSDSVYGDTLEKLTN
AAA46939.1_HPV_16_E6      DFAFRDLCIVYRDGNPYAVCDKCLKFYSKISEYRHYCYSLYGTTLEQQYN
AGM34418.1_HPV_31_E6      DFAFTDLTIVYRDDTPYGVCTKCLRFYSKVSEFRWYRYSVYGTTLEKLTN
                          :*** ** :****  .:..* **: ***:: * * *  *:** ***:  *

AGM34429.1_HPV_45_E6      TELYNLLIRCLRCQKPLNPAEKRRHLKDKRRFHNIAGQYRGQCNTCCDQA
P06463.1_HPV18_E6         TGLYNLLIRCLRCQKPLNPAEKLRHLNEKRRFHNIAGHYRGQCHSCCNRA
AAA46939.1_HPV_16_E6      KPLCDLLIRCINCQKPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSS
AGM34418.1_HPV_31_E6      KGICDLLIRCITCQRPLCPEEKQRHLDKKKRFHNIGGRWTGRCIVCWR--
                          . : :*****: **:** * ** ***..*:***** *:: *:*  *

AGM34429.1_HPV_45_E6      RQERLRRRRETQV
P06463.1_HPV18_E6         RQERLQRRRETQV
AAA46939.1_HPV_16_E6      R-----TRRETQL
AGM34418.1_HPV_31_E6      R-----PRTETQV
                          *      * ***:
```

Fig. 4. CLUSTAL W (1.81) multiple sequence alignments of the amino acid residues in the E6 protein sequences among high-risk HPV types cause cervical cancer. Four high-risk genotypes was identified; 16, 18, 31, 45. Columns indicate identical amino acids in the same position in all types analyzed are exclusively indicated by an asterisk (*) which showed fully conserved residues, whereas alignments showing the presence of similar amino acids are represented by other symbols (:) for conservation of strong groups or (.) for conservation of weak groups. Symbol (–) showed no consensus of the groups.

that oligonucleotide probe for the detection of HPV16 was present in the sequences. Nucleotide and amino acid sequences encoded HPV 16 isolate IR-32 E6 (E6) genes; complete coding sequence (CDS) is shown in Fig. 6. Specific HPV E6 region as oligonucleotide probe for the detection of HPV 16 is indicated by underline. RGRWTGRC was identified as a biomarker region for E6 region of HPV 16.

Fig. 5. CLUSTAL W (1.81) multiple sequence alignments of the nucleotide residues in the E6 and E7 in a complete genome of HPV 16. Columns indicating identical nucleotide in the same position in all types analyzed are exclusively indicated by an asterisk (*) which showed fully conserved residues. Symbol (–) showed no consensus of the groups. Specific HPV E6 region as oligonucleotide probe for detection of HPV 16 are indicated by red. Comparative analysis of the E6 viral protein from HPV strains 16, 18, 31 and 45. In order of appearance of E6 region for different HPV strains; HPV 16 (AAA46939.1), HPV 18(P06463.1), HPV 31(AGM34418.1), and HPV 45 (AGM34429.1).

HPV16 was the most frequent genotype in cervical and oropharyngeal cancer [58].

3.2. Comparative analysis of HPV E6 viral region cause cervical cancer

The relative examination of the E6 viral protein demonstrated an 81% comparability between HPV 18 and 45 types, 65% between HPV 16 and 31, 54% between HPV 16 and 45, 53% between HPV 16 and 18, 51% between HPV 18 and 31, 49% between HPV 31 and 45 (Fig. 7). The amino acids alignment between HPV 18 and 45 genotypes revealed the similarity value corresponding 81% and showed the highest similarity among pairwise alignments, even though both strain are too far from each other, followed by 65% between HPV 16 and 31. The relative examination of the E6 viral protein demonstrated 53% comparability between the regular HPV types 16 and 18. The most reduced comparability is between HPV 31 and 45.

3.3. Basic local alignment search tools (BLAST) for HPV 16

Further examination by utilizing basic local alignment search tools (BLAST) gave extra statistical parameters, for example, expectation values (e-values) and score bits [59]. E-values in blast results represent to the probability of the alignment has happened by chance, while the estimation of score bits relies on upon size of alignment, the number of matches/mismatches/gap and matrix utilized for the correlation of sequences and is normalized by the mean for variable statistics [17]. Blast analysis of oligonucleotide A for recognizable proof of HPV 16 target DNA produced huge hits with E-values comparing to 0.001% and the greatest identity of 100% and 48.1 score bits based on HPV 16 isolate 85, E6 protein (E6) gene (Fig. 8). According to Brzeski [60], E-values near zero shows that the match of E6 proteins, is noteworthy in short sequences and subsequently, the discoveries introduced in this study exhibit complemented likenesses in the alignments of the amino acid residues deposits in the E6 protein among high-risk HPV. While Basic Local Alignment Search Tool (BLAST) outflanks accurate techniques through its utilization of heuristics, the pace of the BLAST programming is imperfect for long inquiries or database arrangements. There are a few weaknesses in the User Interface (UI) of the present charge line applications. Notwithstanding performing alignments, BLAST gives an expect value, statistical data about the significance of every arrangement of HPV E6 region.

3.4. Efficiency of E6 region of oligonucleotide probe

Particular probe that coordinated at the E6 region reported to have an especially more prominent probability annealing than consensus probe focusing on the L1 region [61]. Degenerating probe used to distinguish L1 sequences are not 100% dependable, owing to the more sequences succession varieties in this region of the HPV

atgcaccaaaagagaactgcaatgtttcaggacccacaggagcgacccagaaagttacca
M H Q K R T A M F Q D P Q E R P R K L P
cagttatgcacagagctgcaaacaactatacatgatataatattagaatgtgtgtactgc
Q L C T E L Q T T I H D I I L E C V Y C
aagcaacagttactgcgacgtgaggtatatgactttgcttttcgggatttatgcatagta
K Q Q L L R R E V Y D F A F R D L C I V
tatagagatgggaatccatatgctgtatgtgataaatgtttaaagttttattctaaaatt
Y R D G N P Y A V C D K C L K F Y S K I
agtgagtatagacattattgttatagtttgtatggaacaacattagaacagcaatacaac
S E Y R H Y C Y S L Y G T T L E Q Q Y N
aaaccgttgtgtgatttgttaattaggtgtattaactgtcaaaagccactgtgtcctgaa
K P L C D L L I R C I N C Q K P L C P E
Gaaaagcaaagacatctggacaaaaagcaaagattccataatataaggggtcggtggacc
E K Q R H L D K K Q R F H N I R G R W T
ggtcgatgtatgtcttgttgcagatcatcaagaacacgtagagaaacccagctgtaa
G R C M S C C R S S R T R R E T Q L -

Fig. 6. Nucleotide and amino acid sequences encoded HPV type 16 isolate IR-32 E6 (E6) genes, complete coding sequence (CDS). Specific HPV E6 region as oligonucleotide probe for detection of HPV 16 are indicated by underline.

```
Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: AAA46939.1_HPV_16_E6      158 aa
Sequence 2: AGM34429.1_HPV_45_E6      158 aa
Sequence 3: AGM34418.1_HPV_31_E6      149 aa
Sequence 4: P06463.1_HPV18_E6         158 aa
Start of Pairwise alignments
Aligning...
Sequences (1:2) Aligned. Score:  54
Sequences (1:3) Aligned. Score:  65
Sequences (1:4) Aligned. Score:  53
Sequences (2:3) Aligned. Score:  49
Sequences (2:4) Aligned. Score:  81
Sequences (3:4) Aligned. Score:  1
Time for pairwise alignment: 0.038811
```

Fig. 7. Comparative analysis of the E6 viral protein from HPV strains 16, 18, 31 and 45.In order of appearance of E6 region for different HPV strains, for HPV 16 (AAA46939.1), HPV 18(P06463.1), HPV 31(AGM34418.1), and HPV 45 (AGM34429.1).

genome. Contrasted with the E6 region, L1 probe may have higher chances for disappointment in hybridizing and may prompt mispriming. Sequence closeness seeking is an essential undertaking in bioinformatics apparatuses [62]. Comparability searches are a key part and they form the bases of basic structural motif identification, gene identification, and bits of knowledge into useful affiliations. With the fast increment in the accessible genetic data information through a wide assortment of databases, closeness searches are a crucial apparatus down getting to this information in an instructive and beneficial way.

A few studies have demonstrated a noteworthy part of the E6 protein in the induction and maintenance of cell change, acting basically as an oncoprotein by fortifying the decimation of numerous host cell key administrative proteins, coming full circle in the cervical cancer side effects [63]. Steady proof proposes that the genome of papillomaviruses is static, and changes in its sequence by mutation or recombination are exceptionally uncommon occasions [64]. Diverse virus species within the same genus offer around 60% to 70% similarity [65]. Thus, the discoveries introduced in this study, exhibiting the presence of a highly conserved region with critical comparability of the amino acid sequences in the E6 protein among distinct HPV regions that cause cervical cancer uncovered more grounded proof in regards to the molecular characterization and developmental protection of viral genomes utilizing compar-

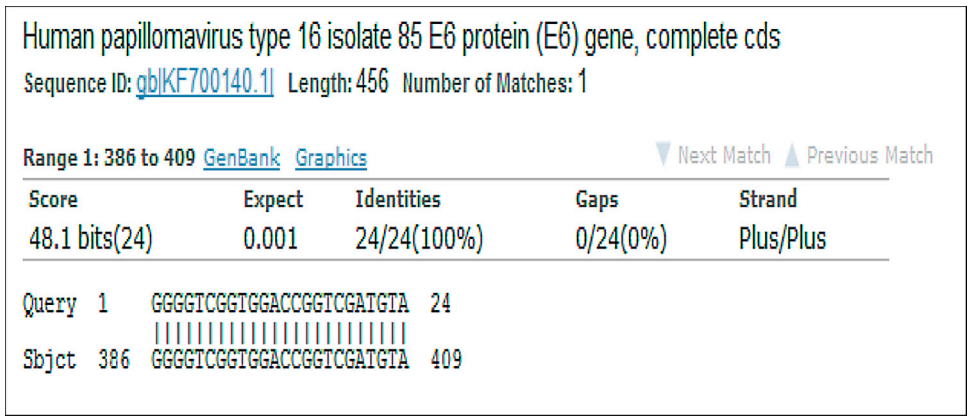

Fig. 8. Alignment statistics for match of Oligonucleotide A for the identification of HPV 16 target DNA and Human *Papillomavirus* (HPV) type 16 isolate 85 E6 proteins (E6) gene.

ative investigation of various sequence alignments. The probe was designed with the sequence "GGG GTC GGT GGA CCG GTC GAT GTA", having 66.7% GC content. The oligonucleotide probe can be used as a biomarker for detection E6 genome region of HPV 16 due to the characteristics; length between 22 and 30 mers, % GC between 40 and 70, and melting point (Tm) is above 50 °C. The probe needed an acceptable length between 22 and 31 mers. Since the higher degeneracy lowers the primer concentration, and affecting the Tm of probe. Besides that, oligonucleotides probe must have a GC content of 40–70% which is known to facilitate stronger binding to target annealing [66].

3.5. Future work of interdigitated electrodes sensor efficiency

High quality of cytology-based method requires a trained personnel and specialized equipment. HPV vaccines may provide the required potential approach for the prevention of cervical cancer. HPV testing mostly required a high technology laboratory-based molecular analysis involved molecular biologist and costly. Currently, several aims of the development of rapid, simple, accurate and affordable HPV tests. Due to rapid results, women can be screened and treated during the same visit to the hospital for examination by the doctor.

Innovative HPV-based biosensor enables the establishment and effective screening detection. The interdigitated electrodes (IDEs) discussed in this study consisted of a two-electrode system prepares as previously described [50,51,67]. We reported the gold nanoparticle mediated method for the spatially resolved deposition of DNA on nano-gapped interdigitated electrodes [50], and its application to the detection of the human Papillomavirus. We also reported a direct detection of human papillomavirus 16 genomic DNA using gold nanoprobes [51]. We summarized the main steps involved in fabrication (wafer cleaning, oxide layer growth, negative photoresist spin coating, photoresist patterning, aluminium (Al) deposition, photoresist removing and wafer dicing) [67]. Briefly, a 4 inch p-type SOI wafer was used as a substrate and cleaned using buffered oxide etchant (BOE) to remove a native oxide layer on SOI surface. After the cleaning process, the SOI wafer was cut into small pieces (2 cm × 2 cm), and followed by EBL process. The IDE nanosensor was fabricated on a wafer with a 145 nm buried oxide layer. Silicon wafer was used as a main substrate in order to form silicon dioxide (SiO_2) as an insulation layer of an electrical device. The wafer was cleaned using BOE to remove the native oxide, which had been naturally grown on it. Growth of SiO_2 using wet oxidation process provides a thicker insulation layer and shorter time consuming compared to dry oxidation process. In order to transfer pattern from a mask on a wafer, photoresist was spin-coated on the growth SiO_2 wafer using spin-coater. Photoresist is a light-sensitive material used to form a patterned coating on a surface. By using deep ultraviolet lithography, a 50 nm silicon layer was patterned and etched when UV-light was exposed on the photoresist; pattern from chrome mask was directly transferred onto the photoresist. After development process, aluminum metal was deposited using sputter-coater and acetone was used to strip the unwanted photoresist. The designed study here can also be implemented in other potential studies have been performed in past for further expansion [68–70].

4. Conclusion

In this research study, designation of oligonucleotides probe designed for the detection of Human Papillomavirus (HPV) 16 towards nanobiosensing. E6 region of HPV16 was considered and revealed the possible specific probe by comparison analyses with other potent subtypes 18, 31 and 45. The probe designed has all appealing characteristics, can be used as a biomarker for detection E6 genome region of HPV 16. By availing the oligonucleotide regions predicted for probe designing, the profoundly sensitive HPV identification by nanobiosensor will significantly overestimate the extent of cervical malignancy patients who have low-quality cytological variations from the norm.

Conflict of interests

None declared.

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