Microarray long oligo probe designing for *Escherichia coli*: an *in-silico* DNA marker extraction

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Introduction Urinary tract infections are predominant diseases which may be caused by different pathogenic microorganisms, particularly *Escherichia coli* (*E.coli*). DNA microarray technology is an accurate, rapid, sensitive, and specific diagnostic tool which may lead to definite diagnosis and treatment of several infectious diseases. DNA microarray is a multi-process method in which probe designing plays an important. Therefore, the authors of the present study have tried to design a range of effective and proper long oligo microarray probes for detection and identification of different strains of pathogenic *E.coli* and in particular, uropathogenic *E.coli* (UPEC).

Material and methods *E.coli* O26 H11 11368 uid41021 was selected as the standard strain for probe designing. This strain encompasses the largest nucleotide sequence and the most number of genes among other pathogenic strains of *E.coli*. For performing this *in silico* survey, NCBI database, GReview Server, PanSeq Server, Oligoanalyzer tool, and AlleleID 7.7 were used to design accurate, appropriate, effective, and flexible long oligo microarray probes. Moreover, the genome of *E.coli* and its closely related microorganisms were compared.

Results In this study, 15 long oligo microarray probes were designed for detecting and identifying different strains of *E.coli* such as UPEC. These probes possessed the best physico-chemical characteristics. The functional and structural properties of the designed probes were recognized by practical tools and softwares.

Conclusions The use of reliable advanced technologies and methodologies for probe designing guarantees the high quality of microarray probes and makes DNA microarray technology more flexible and an effective diagnostic technique.

Key Words: urinary tract infection → *E.coli* → microarray → probe designing

INTRODUCTION

*Escherichia coli* (*E.coli*) is a potential uropathogenic bacterium which may cause a wide range of urinary tract infections (UTIs) including asymptomatic and/or symptomatic bacteriuria, cystis, and pyelonephritis both in children and adults. UTIs caused by UPEC are the most spread infections in the world. According to previous surveys, community acquired UTIs caused by UPEC ranks first in comparison with other pathogenic agents and UPEC related nosocomial UTIs are in second place, worldwide [1–8].

The presence of several virulence factors enables UPEC to cause different types of UTIs in human hosts. Adhesins, different types of fimbrial and afimbrial structures, haemolysins, and cytotoxic necrotizing factors are the most important and well-known virulence factors in UPEC strains. Although identification of virulence factor genes is possible through Polymerase Chain Reaction (PCR) based molecular techniques in simple levels, for fine detection and
| E. coli strains | Complete genome/RefSeq accession no | Sequence length (bp) | Total genes |
|----------------|-----------------------------------|----------------------|-------------|
| E. coli 042 uid161985 | NC_017626 | 5,241,977 | 5,392 |
| E. coli 536 uid58531 | NC_008253 | 4,938,920 | 4,816 |
| E. coli 55989 uid59383 Removed from NCBI RefSeq | NC_011748 | 5,154,862 | Not mentioned |
| E. coli ABU 83972 uid161975 | NC_017631 | 5,131,397 | 5,083 |
| E. coli APEC O1 uid58531 | NC_008563 | 5,082,025 | 5,572 |
| E. coli APEC O78 uid187277 | NC_020163 | 4,974,435 | 4,810 |
| E. coli ATCC 8739 uid58783 | NC_012971 | 4,974,218 | 4,644 |
| E. coli BL21 DE3 uid161947 | NC_012971 | 4,558,953 | 4,530 |
| E. coli BL21 DE3 uid161949 | NC_012892 | 4,558,947 | 4,530 |
| E. coli BW2952 uid59391 | NC_011745 | 5,209,548 | Not mentioned |
| E. coli B REL606 uid161975 | NC_017625 | 4,630,707 | 4,578 |
| E. coli B REL606 uid162051 | NC_012971 | 4,621,430 | 4,573 |
| E. coli CFT073 uid57915 Removed from NCBI RefSeq | NC_004431 | 5,231,428 | Not mentioned |
| E. coli DH1 uid161951 | NC_017631 | 4,630,707 | 4,578 |
| E. coli DH1 uid162051 | NC_017638 | 4,621,430 | 4,573 |
| E. coli E24377A uid58395 | NC_009801 | 4,979,619 | 5,301 |
| E. coli ED1a uid59379 Removed from NCBI RefSeq | NC_011745 | 5,209,548 | Not mentioned |
| E. coli ETEC H10407 uid161993 | NC_017633 | 5,153,435 | 5,411 |
| E. coli H5 uid58393 Removed from NCBI RefSeq | NC_009800 | 4,643,538 | Not mentioned |
| E. coli IA1 uid59377 Removed from NCBI RefSeq | NC_011741 | 4,700,560 | Not mentioned |
| E. coli IA39 uid59381 | NC_011750 | 5,132,068 | Not mentioned |
| E. coli IHE3034 uid162007 | NC_017628 | 5,108,383 | 5,132 |
| E. coli JJ1886 uid226103 | NC_009801 | 5,129,938 | 5,397 |
| E. coli K011FL uid162099 | NC_017660 | 5,021,812 | 4,963 |
| E. coli K011FL uid2593 | NC_016902 | 4,920,168 | 5,037 |
| E. coli K12 substr DH10B uid58979 Removed from NCBI RefSeq | NC_010473 | 4,686,137 | Not mentioned |
| E. coli K12 substr MD542 uid193705 | NC_017638 | 3,976,195 | 3,872 |
| E. coli K12 substr MG1655 uid57779 | NC_009133 | 4,641,652 | Not mentioned |
| E. coli K12 substr W3110 uid161931 Removed from NCBI RefSeq | NC_007779 | 4,646,332 | Not mentioned |
| E. coli LF82 uid161965 Removed from NCBI RefSeq | NC_011993 | 4,773,108 | Not mentioned |
| E. coli LY180 uid219461 | NC_017644 | 4,835,601 | 4,789 |
| E. coli NA114 uid162139 | NC_017644 | 4,971,461 | 5,039 |
| E. coli O103 H2 12009 uid41013 | NC_013353 | 5,449,314 | 5,689 |
| E. coli O104 H4 2009EL 2050 uid175905 | NC_018650 | 5,253,138 | 5,529 |
| E. coli O104 H4 2009EL 2071 uid176128 | NC_018661 | 5,312,586 | 5,475 |
| E. coli O104 H4 2011C 3493 uid176127 | NC_018658 | 5,273,097 | Not mentioned |
| E. coli O111 H 11128 uid41023 | NC_013364 | 5,371,077 | 6,034 |
| E. coli O127 H6 EE3486 69 uid59343 Removed from NCBI RefSeq | NC_011601 | 4,965,553 | Not mentioned |
| E. coli O157 H7 EC4115 uid59091 | NC_011353 | 5,572,075 | 5,924 |
| E. coli O157 H7 EDL933 uid57831 Removed from NCBI RefSeq | NC_002655 | 5,528,445 | Not mentioned |
| E. coli O157 H7 TW14359 uid59235 | NC_013008 | 5,528,136 | 5,820 |
| E. coli O157 H7 uid57781 | NC_002695 | 5,498,450 | Not mentioned |
| E. coli O26 H1 11368 uid41021 | NC_013361 | 5,697,240 | 6,125 |
| E. coli O55 H7 CB9615 uid46655 | NC_013941 | 5,386,352 | 5,526 |
| E. coli O55 H7 RM12579 uid162153 | NC_017656 | 5,263,980 | 5,495 |
| E. coli O7 K1 CE10 uid162115 | NC_017646 | 5,313,531 | 5,443 |
| E. coli O83 H1 NRG 857C uid161987 | NC_017634 | 4,747,819 | Not mentioned |
identification of diverse E. coli strains in genomic scale, high-throughput techniques, including microarray as a next generation sequencing (NGS) technology, are needed. Therefore, pan-genomics and comparative genomics are appropriate means to gain this goal [6–15]. Despite knowing the fact that E. coli is a natural member of human gastro-intestinal tract microflora, the extraintestinal pathogenic E. coli (ExPEC) strains may cause different infections, such as UTIs, in their human hosts. The use of rapid, accurate, cost effective, sensitive, specific, and advanced diagnostic methods enables us to have a reliable diagnosis and definite treatment [5, 10–18].

Due to the importance of E. coli in association with different infectious diseases such as UTIs, several genomes relating to different strains of E. coli are identified and reported to genome databases. Currently there are 61 recorded whole genomes pertaining to E. coli strains which are reported by the Genomes OnLine Database (GOLD, http://www.genomesonline.org/) and deposited in NCBI FTP site (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/). These strains are indicated in Table 1 [11, 12, 24]. One of the most important purposes of comparative genomics is to detect and identify the unique genomic regions which may be used for appropriate microarray probe designing. In accordance with several recorded investigations, there are close relationships between E. coli, Salmonella enterica, and Shigella (Sh.) sonnei, Sh. flexneri, Sh. dysenteriae and Sh. boydii [22, 23].

The main goal of this original article is to design several effective and proper long oligo microarray probes for detection and identification a diversity of E. coli strains, such as UPEC pathotypes, from the other close related bacterial genera.

### MATERIAL AND METHODS

In the present in silico research, 61 recorded complete genomes belonging to different strains of E. coli were studied via NCBI ftp (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/) and the RefSeq complete genome pertaining to each strain was detected through the related files of GeneMark-2.5m. Then, each RefSeq complete genome was retrieved from NCBI (http://www.ncbi.nlm.nih.gov/nuccore) to study the sequence length and total genes involved in a complete genome. The data is shown in Table 1 [11, 12, 24]. The E. coli strain of O26 H11 11368 uid41021 ranks first for encompassing the most number of genes and nucleotides (Table 1); hence it was presumed as the standard criterion for comparative genomic analysis and long oligo probe designing. Although the aforementioned strain is known as Enterohaemorrhagic E. coli (EHEC), it contains a huge number of virulence factor genes including different types of adhesins, which are common in UPEC and EHEC strains in order to have a successful colonization and infection [5, 7, 11, 13, 25]. The .gbk file regarding E. coli O26 H11 11368 uid41021 and .fna files relating to Sh. sonnei, Sh. flexneri, Sh. dysenteriae, Sh. boydii, and Salmonella enterica were downloaded from the NCBI FTP site.

These files were uploaded to GVView Server (https://server.gview.ca/) to have a schematic view of comparative genomes in association with E. coli O26 H11 11368 uid41021, Sh. sonnei, Sh. flexneri, Sh. dysenteriae, Sh. boydii, and Salmonella enterica. The unique regions of genome as the analysis type and other default parameters represented by the GVView Server (1e-10 for value cutoff, bacteria for genetic code, 100 for

### Table 1. 61 Reported identified Escherichia coli (E. coli) genomes via NCBI FTP site [11, 20, 21]

| E. coli strains | Complete genome/RefSeq accessio no | Sequence length (bp) | Total genes |
|----------------|-----------------------------------|----------------------|-------------|
| E. coli PMV 1 uid219679 | NC_022370 | 4,984,940 | Not mentioned |
| E. coli S88 uid62979 | Removed from NCBI RefSeq | | |
| E. coli SE11 uid59425 | NC_011742 | 5,032,268 | Not mentioned |
| E. coli SE15 uid161939 | NC_013654 | 4,887,515 | 5,207 |
| E. coli SMS 5 5 uid58919 | NC_010498 | 5,068,389 | 5,164 |
| E. coli UM146 uid162043 | NC_017632 | 4,993,013 | 5,082 |
| E. coli UMN026 uid62981 | NC_011751 | 5,202,090 | Not mentioned |
| E. coli UMNK88 uid161991 | NC_017641 | 5,186,416 | 5,863 |
| E. coli UT189 uid58541 | NC_007946 | 5,065,741 | 5,171 |
| E. coli W uid162011 | NC_017635 | 4,900,968 | 5,023 |
| E. coli Xuzhou21 uid163995 | NC_017906 | 5,386,223 | 5,651 |
| E. coli BL21 Gold DE3 lysS AG uid59245 | NC_012947 | 4,570,938 | 4,560 |
| E. coli clone D i14 uid162049 | NC_017652 | 5,038,386 | 4,959 |
| E. coli clone D i2 uid162047 | NC_017651 | 5,038,386 | 4,958 |
alignment length cutoff and 80 for percentage identity cutoff) were applied for comparing *E. coli* O26 H11 11368 uid41021 with other bacterial strains in this research (Figure 1) [24]. Furthermore, the pan-genomic sequence analysis was achieved by PanSeq Server (https://lfz.corefacility.ca/panseq/analyses/#userNovel) [24]. The analysis was done via novel region detector. *E. coli* O26 H11 11368 uid41021 was added to the selected query and other strains including *Salmonella enterica* subsp. enteritidis str. P125109 chromosome complete genome, *Shigella boydii* CDC 3083-94 chromosome complete genome, *Shigella dysenteriae* Sd197 complete genome, *Shigella flexneri* 2a str. 2457T complete genome, and *Shigella sonnei* Ss046 chromosome complete genome were also added to selected reference. The other parameters (including “Minimum novel region size: 500”, “Nucmer values b: involving 200, c: 50, d: 0.12, g: 100, and i: 20”, “Percent Sequence Identity Cutoff: 90”, “Fragmentation Size: 500”, “Core Genome Threshold: 3”, and “Blast Word Size: 20”) were selected as suggested by the server.

The identified unique genomic regions pertaining to *E. coli* O26 H11 11368 uid41021 were then blasted via NCBI BLAST tool software [7].

The confirmed unique genomic regions were analyzed by alleleID 7.7 software for designing appropriate long oligo microarray probes. In the following, the unique sequences were added to the new sequence page of the microarray tab and the probe designing was done via probe search in the analyze tab. For determining the size of probe, the button of probe length with 55-64 nucleotides was selected, as the software suggested. The produced and designed probe was processed for further analyses [7].

The designed microarray probes were rechecked by NCBI BLAST tool software and their physicochemical and other characteristics such as ΔG, Tm, ΔH, and hairpins were assessed by the online tool of oligoanalyzer 3.1 (https://eu.idtdna.com/calc/analyzer). Other default parameters including target type: DNA, oligo Conc: 0.25 μM, Na+ Conc: 50 mM, Mg++ Conc: 0 mM, dNTPs Conc: 0 mM, Nucleotide type: DNA, Sequence type: linear, Temperature: 25°C, Max foldings: 20, Suboptimality: 50%, Start position: 0, and Stop position: 0 were included in this study. At the end, the accurate and standard long oligo probes were selected to be used in designing diagnostic microarray chip [7].

**RESULTS**

The results from GReview server indicated a close relationship between *E. coli* O26 H11 11368 uid41021 and other selected bacteria such as *Sh. sonnei*, *Sh. flexneri*, *Sh. dysenteriae*, *Sh. boydii*, and *Salmonella enterica* (Figure 1). Figure 1, which was directly taken from the GView server, shows the common sequences in color while the white areas assign the loss of sequences in different studied strains. The PanSeq server identified the unique genomic regions via comparison of the pan-genomes belonging to *E. coli* O26 H11 11368 uid41021, *Salmonella enterica* subsp. enteritidis str. P125109 chromosome complete genome, *Sh. boydii* CDC 3083-94 chromosome complete genome, *Sh. dysenteriae* Sd197 complete genome, *Sh. flexneri* 2a str. 2457T complete genome, and *Sh. sonnei* Ss046 chromosome complete genome. The application of GView and PanSeq servers, NCBI BLAST tool, and AlleleID 7.7 software resulted in 15 appropriate long oligo microarray probes, which are shown in Table 2.

**DISCUSSION**

There are many scientific researches that confirm the wide range of UTIs caused by different strains of ExPEC. ExPEC are important bacterial agents causing up to 90% of community acquired UTIs, over 80% of bacteriuria, more than 60% of recurrent cystitis, over 70% of uncomplicated UTIs, and up to 50% of complicated UTIs [4, 5, 7, 26–31]. *E. coli* is an extraordinary bacterium which possesses a huge number of virulence genes. Therefore, different strains of *E. coli* are considered as potentially pathogenic bacterial agents. Despite several categorizations pertaining to *E. coli*, there are still remark-
able overlaps between virulence factors of *E. coli* strains within different groups in their human hosts. According to previous investigations, UPEC encompasses a collection of virulence genes which may lead to UTIs. At the same time, other strains, such as EHEC, possess the key virulence genes which may also lead to UTIs [5, 6, 7, 25, 32–36].

Table 1 shows that *E. coli* O26 H11 11368 uid41021 (EHEC) bears the highest number of genes (6125 genes) and nucleotide sequences (5,697,240 bps). Hence, this strain was selected as standard sample for designing long oligo microarray probes in the present study.

Annually, governments spend a lot of money treating patients with UTIs. A survey estimates an overall cost of 236€ per patient a year with UTIs [37, 38]. Thereby, an accurate, rapid, sensitive, and specific diagnostic tool results in a definite treatment which may lead to a reduction in the unnecessary medical costs around the world.

DNA microarray is an advanced molecular diagnostic technology which provides a reliable diagnosis for detecting and identifying microbial agents causing different infectious diseases [14, 16, 17, 18, 19, 39, 40, 41]. DNA microarray technology consists of several stages, with probe designing being one of the most important. Today, there is a vast range of databases, servers, tools, and softwares which can be used for designing DNA microarray probes [14, 16, 17, 39, 41].

GViewer Server was used to show clear similarities among *E. coli*, *Shigella spp.* and *Salmonella enterica*. On the other hand, NCBI guaranteed appropriate support for GenBank data and BLAST tools. Finally, PanSeq server provided a suitable result for genomic unique regions. By the help of these technologies, the obtained outcomes were usable for AlleleID 7.7 software to retrieve invaluable raw data in the form of designed DNA microarray probes. In parallel with aforementioned possibilities, the oligoanalyzer tool determined the physico-chemical properties belonging to the designed probes [7, 14].

Microarray probe designing is a multi-science process with a wide range of facilities and potencies. Long oligo microarray probes are suitable choices

| Microorganism | Long oligo Microarray Probe | length | oligoanalyzer | Location and gene product |
|---------------|-----------------------------|-------|---------------|--------------------------|
| *E. coli*     | GATCAGTCGATGCTACGATGACACCTCCCTATCTCACTGATTGTAGCTTCTGGTT      | 57    | ✓             | 4854732-4854788 Na+/H+   |
| *E. coli*     | CTGAACTCATGGCTTTGGTATTAATATAACCTCCAGAAGCATGATTACAGAGCATGACACATCC | 57    | ✓             | 5629045-5629101 hypothetical protein |
| *E. coli*     | TGTTCCAGTTAATGAGGGTTGGAATATACCAAAATACATTACACTTATGCTGAGTGT        | 58    | ✓             | 4827729-4827786 type I restriction modification DNA specificity domain protein |
| *E. coli*     | AAAACACTTTTTTTTAGGAAACAAATGCCAACCTGCTGATTGACCTGATCCTCCTT      | 58    | ✓             | 3820187-3820244 hypothetical protein |
| *E. coli*     | CTAAATGTGCACTGACTGACGAGGATTTTACATTTACCTCGAATGAAAACGCACATGTTG    | 58    | ✓             | 692085-692142 HNH endonuclease |
| *E. coli*     | ATCAATAAAAGGTCGAGGACATTGAGAGAAAGAAGCTGTTATTAATTGCGG          | 57    | ✓             | 2460848-2460904          |
| *E. coli*     | CATCTGACATAAATACAATACGAAACGCCCATTACACAAACTGCTGATATTGGCG        | 57    | ✓             | 4847093-4847149 hypothetrical protein |
| *E. coli*     | ATTACTCTCTTTAGCTTACTTCTGGCAAATCTTTCTCTATCTCCTGATGACTTCTTT     | 57    | ✓             | 2278419-2278475 hypothetrical protein |
| *E. coli*     | CACTCTGAGATGTTGACATTTGAGCTTACATGTCGAAGACAGTTGTTAATTCGCCGCTTAC | 57    | ✓             | 4763454 to 4763510 hypothetrical protein |
| *E. coli*     | CTCTCTTAAAGATATCTCGTGTGAGATAAACCTCGCTGTGATTGGAACCGCATGAGTGACTGATC | 57    | ✓             | 4368609-4368665 hypothetrical protein |
| *E. coli*     | CGATGTTTGTAGATCTCAGAGAAAGACGATTTATCCCGTATGTTGTTGTTGATAAGCAC  | 57    | ✓             | 623791-623847 inner membrane protein |
| *E. coli*     | ATCGCGCTATCCGTTATGATCGTTAACAGGAGATTATTTTATGAGTATTTATGAGAA   | 58    | ✓             | 4808962-4809019 fimbrial assembly proteinfimbrial protein |
| *E. coli*     | AACAGGAAAGGCAATACAGAAATATCAGTTACGTGATATAATCCCGGAAATTATC      | 57    | ✓             | 5060219-5060275 predicted transporter |
| *E. coli*     | CTCTTATTATGAGCAGAATTTTCAGTGAATTACTATGCTGATGAGTGAATCGCCGCTGTT | 60    | ✓             | 4607597-4607656 putative outer membrane protein |
| *E. coli*     | CGCGCCCTGGGTGCGCATATTTTTATGTCAGGTGTATTTATCCTGATTAATGAC        | 57    | ✓             | 4763030 to 4763086 membrane protein |
for an appropriate and proper diagnosis and definite treatment; thus, this group of probes was designed in the present survey. Probes can also be designed in general or specific forms. As the presence of multi-drug resistant microorganisms is a complicated and considerable problem in traditional medicine and environmental microbiology, the microarray technology may open a new way to incredible approaches for detecting and identifying normal virulence or multi-drug resistance genes [7, 14, 16, 41, 42, 43].

CONCLUSIONS

The use of progressive and advanced technologies enables us to design and produce tens and hundreds of different microarray probes with a wide range of diversity and quality. Simultaneously, the application of practical methodologies has an important role in the physico-chemical characteristics belonging to designed DNA microarray probes. Therefore, in this investigation, we tried to design 15 long oligo microarray probes with the best functional and structural properties. These probes are proper candidates to be used in diagnostic microarray chip for detecting and identifying different strains of E.coli, such as UPEC. All in all, the practical databases, servers, tools, and softwares relating to microarray probe designing give DNA microarray technology a great opportunity to be more flexible, reliable, reproducible, and effective as a pioneer diagnostic technique.

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

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