ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Selection of Highly Efficient (siRNA) Targeting Membrane Genes in *Helicobacter pylori 26695*

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Manuscript Info

Manuscript History:

Received: 28 June 2014
Final Accepted: 29 July 2014
Published Online: August 2014

Key words:
Computational Methods, siRNA, Target design, and Helicobacter pylori

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Abstract

Helicobacter pylori a liable influence risk of carcinogenesis that is a reason of most of mortality worldwide which has developed resistance to drugs. The present study was carried out to identify potential drug targets in *H. pylori* by design of potent siRNA. To reach the goal, the collection membrane essential gene for *H. pylori* from DEG, then a variety of bioinformatics databases studying STRING v9.1 for selection non-homologous genes, then the software to siRNA Sfold and MEGA6 to determine similarity between siRNA as groups. Further studies were carried out to list out essential protein of the *H. pylori*, approximately six (6) membrane proteins become drug target, than synthesized siRNA which are shorter than 21 nt about 9027 siRNA, 66 siRNA (following three rules: Ui-Tei, Amarzguioui and Reynolds), 29 siRNA groups 17 of them have many similar siRNA other are singe siRNA (MEGA6), 9 siRNA(cleavage sites), 5 siRNA Drug target (cleavage sites stability rule), this steps respectively. Conclusion: five (5) siRNA which could use as potential drug target for resistant of *Helicobacter pylori 26695*.

Introduction

Helicobacter pylori is a primary pathogenic factor in benign and malignant gastro duodenal disease [1, 2] the complete sequence of the circular genome of *H. pylori* concluded that genetic effects influence the acquisition of *H. pylori* infection that colonizes the gastric mucosa of approximately 50% of the world's population [3].The essential genes crucial for the survival of pathogen and nonexistent in the host are also identified using the subtractive genomics approach [4], and the increase in accuracy can be seen proteins form a variety of functional connections with each other including metabolic pathways and a bewildering array of direct and indirect regulatory interactions [5, 6]. This is because functional interactions between proteins can span a wide ring of mechanisms and specificities, and may depend on biological context such as environmental condition or tissue type [7, 8]. As a result, considerable information is needed to describe the various aspects of a given protein–protein association and a number of standards have been developed for this purpose with distinct levels. Moreover , a number of algorithms have been devised that allow prediction of functional links between proteins [9,10] by used STRING v9.1 database [11] that view network on a genome is increasingly being taken in many areas of applied biology protein networks are used in human genetics to aid in drug discovery [12,13]. For find the chances of cross – reactivity and side- effects to genes and their products which can be used as potential drug targets are also identified by analyzing these genes with the STRING v9.1 pathway database [11]. Looking like this approach will ensure that the drug target is available only in the pathogen and not in the human through the use of successfully for various pathogens [14].In most cases the infection established during childhood and persists lifelong. this chronic infection leads to inflammation continuous, which can lead to peptic ulcer disease or gastric cancer [15].These reasons need to effective drug to counter to the evolution of pathogenic bacteria thus the mechanism double-stranded RNA (dsRNA) is used in eukaryotes the

phenomenon, in which is critical for the suppression of the target gene, RNA interference (RNAi) is a powerful tool for knocking down the expression of target genes by short interference RNAs (siRNAs) of (21-19) nucleotides[16], have to be used for gene silencing in mammalian cells in order to prevent the activation of an unspecific bacteria response [17]. For the first time discovered in Caenorhabditis elegans and was described subsequently in insects and in other animals [18]. After that their introduction became a promising approach for RNAi in eukaryotes cells This approach was found to be successful in targeting bovine prion gene PRNP in livestock [19] also RNAi approach is successfully exploited in various cases such as hepatitis infection[20] carcinoma of the breast [21] RNAi utilized in HIV-1 infection in human peripheral blood mononuclear cells via best env-specific siRNAs [22]. In experimental brain cancer pegylated immunoliposomes (PIL) [23] also siRNA used in plants [24].This technique was also used for silencing of capsid genes of Flavivirus using computational methods [25]. As that consider synthetic siRNAs are becoming cost effective and the sequences of many bacteria genes are becoming available from databases, wedon't know whether siRNAs designed using a commercial guideline fitted for mammalian genes are also effective in bacteria in host cells. [26, 27]. Aim of study, designing better and effective drugs need a faster method for resistance of H. pylori by using small interfering RNA (siRNA).

Materialsand Methods

Data collection:

Twelve essential cell membrane genes of Helicobacter pylori26695 have no homology with human essential genes (118 proteins) were recovered in previous study [28]were retrieved from Database of Essential Genes (DEG), available at http://tubic.tju.edu.cn/deg//[29].

Protein-protein interactions (PPI)Network [30]

To determine genes that have interactions to human genes was used Search Tool for the Retrieval of Interacting Genes/ProteinsVersion 9.1(STRING v9.1) database [11]available at (http://string-db.org/). STRING v9.1[11] aims to detection the entire proteins of 'possible' interactions for any fully sequenced organism as well as between two proteins that shows available 3D structure information for a protein in the context of its domain, with this extensive of protein–protein similarity data is imported from and cross-linked with the Similarity Matrix of Proteins and their identity for alignment and score protein similarities. It is likely that only a subset of these interactions will be realized in any given cell**[30]**.

Target identification siRNA molecule by computationally algorithms:

Software for Statistical Folding of Nucleic Acids and Studies of Regulatory RNAs (Sfold) (http://sfold.wadsworth.org) tool[31], was presented algorithm to calculation of equilibrium partition functions and base-pairing probabilities from the structural RNAs. This statistical sampling algorithm guarantees the generation of a statistically representative sample of structures rigorously and that may have unique structures. In addition, this algorithm enables the development of unique tools for the rational design of RNA-targeting nucleic acids.

Filter siRNA

Filtered all siRNA that we get from Sflod according mixed Empirical rules approach of Ui-Tei, Amarzguioui and Reynolds rules [32, 33].

Similarity groups:

Molecular Evolutionary Genetics Analysis Version 6.0 (MEGA6)[34]software was used identify any target sequence similarity and appeared them as phylogenetic tree that estimated trees using multiple sequence alignments by applying statistically significant sequence similarity was inferred using the Neighbor-Joining method [35]. Branch-length was mean evolutionary distances were computed using the Maximum Likelihood method [36].

Importance of complementarity within positions 9–11 for target effects: [37]

We was chooses only siRNA that containing combinations do correlate in positions 9–11 binding sites located in the 3′ UTR (canonical siRNA cleavage sites) [37] with host protein the accessibility to the target. In Sfold [31] tools can run module Srna [31] to show diagrams of representative structures and associated sampling frequencies from this module are helpful in providing a confidence assessment about the degree of correct cleavage sites to siRNA.

Rules of Stability Cleavage Sites

The effective drug siRNAs are chosen according to relative stability at the cleavage site their silencing efficiency to three criteria: Average Internal Stability at the cleavage site (AIS > -8.6, in kcal/mol) [38] and Average unpaired Probability Stability for cleavage site nucleotides (APS ≥ 0.5)[39].

Resultand Discussion

After choosing the candidate targets (Table 1) by subtractive genomics approach [28], a first step towards a more reliable way to assess the durability of proteins. We must check interaction proteins are required to evaluate the biological significance of individual interactions where revealed many studies there are many bacterial physiological processes and host pathogen induced pathological processes some of these can be cause or prevented availability for antibacterial drug targeting.

No.	Gene Name	No.	Gene Name
1	DEG10080047(rfaC/waaC), 340 aa, lip polysaccharide heptosyltransferase-1 (rfaC)	7	DEG10080223(hetA), 578 aa, multidrug resistance protein (hetA)
2	DEG10080052(dppA), 549 aa, dipeptide ABC transporter, periplasmic dipeptide-binding protein (dppA)	8	DEG10080224 (HP1216), 660 aa, conserved hypothetical secreted protein
3	DEG10080054(dppD), 287 aa, dipeptide ABC transporter, ATP- binding protein (dppD)	9	DEG10080230(HP1234), 298 aa, hypothetical protein
4	DEG10080062(ycf5), 936 aa, cytochrome c biogenesis protein (ycf5)	10	DEG10080247(HP1289), 161 aa, hypothetical protein
5	DEG10080093(HP0586) , 977 aa, hypothetical protein	11	DEG10080290 (HP1450), 547 aa, putative inner membrane protein translocase component YidC
6	DEG10080124(HP0746), 419 aa, hypothetical protein	12	DEG10080323(HP1580), 220 aa, hypothetical protein

Table 1: Nonhomology essential membrane genes of *H.pylori* with human essential by database Essential Gene [28]

Moreover, to reduce the likelihood of resistance development, targeting maintain centers in bacterial or pathogen – host interactomes is consistent with the recent trends in antibacterial drug discovery[30] so are required to evaluate our drug membrane target with STRONGv9.1 database[11] to find pathogen for *H.pylori* and host protein – protein interactions, in general the first drug target in our study (Table 1)that has many interactions with other host proteins is protein HP_0302 (DEG10080054) (Figure 1)show that output of using STRING v9.1[11] database for estimation interactome of revealed that protein HP0301 (dipeptide ABC transporter, ATP-binding protein (DppD)) have many interactions with other host proteins (Homo sapiens, Pan troglodytes, and others) represented that homology as phylogntictree, where our study is interested on interactions of *H.pylori* with host proteins so that protein HP_0301 (DEG10080054) homology with human proteins (HP_0301, HP_0302, obg ,HP_1366 and rpmA) that display as network with colored nodes are to each host protien, and (Figure 1) shown clearly the alignment between DppD (HP0301, *Helicobacter pylori* 26695, 287 aa) homology with human protein ObgE (*Homo sapiens*, 406 aa) at position 163-170 with identity: 52.4% and score 252.1 bits, protein-ligand interactions usually take place at preferred sites on the protein surface known [37],whereas revealed the crystal structures of proteins in complex with their ligands since proteins participate in protein – protein interactions shown in homology model (1udxA),whereas revealed the crystal structures of proteins in complex with their ligands since proteins participate in protein – protein interactions , even if they do a small molecule binding site, so modulating these interactions may be of a highly effective means of modulating a target [30].

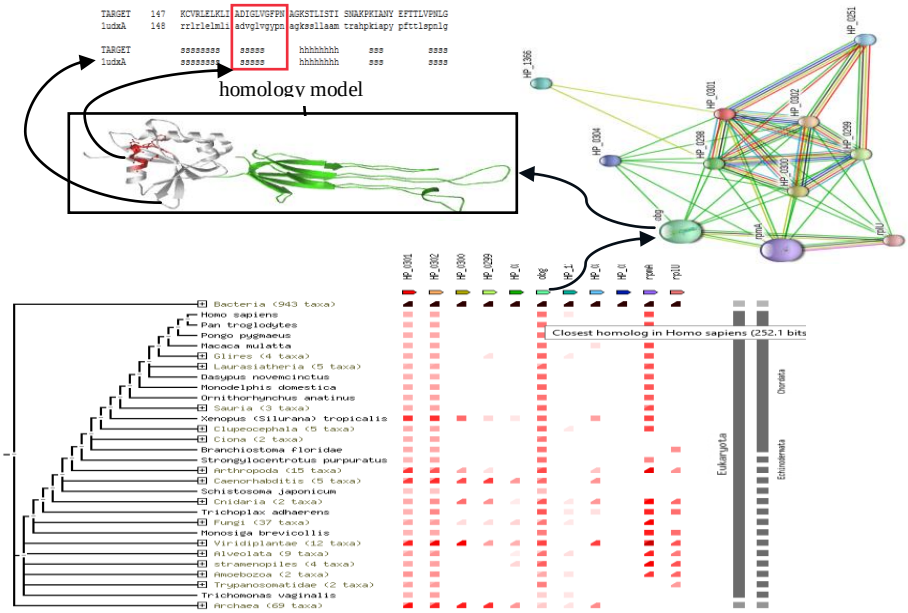

Figure 1: protein – protein interactions from DppD (HP0301, *H. pylori 26695*, DEG10080054) with host ObgE (HP_0301, HP_0302, obg, HP_1366 and rpmA) using STRING v9.1[11]

When, we subjected our proteins (Table 1) to STRING v9.1 database [11].We found that following proteins in (Table 2) have many interactions with host proteins. In order not to miss anything, it has been found that both protein promiscuity and ligand promiscuity are common in nature [12]. Also to that STRINGv9.1 has a very low score-cutoff for reporting protein similarities that means a significant number of the reported hits are expected to be spurious for all these reasons we detecting links for homologs of a specific protein in a particular species bit scores below 60[11] may indicate spurious hits. That make sure the above protein – protein interactions found it relevant.

NO.	Helicobacter pylori 26695	Homology proteins in Homo sapiens	Scores (bits)
1	HP_0301 (HP0301, 287 aa)(DEG10080054)	HP_0301, HP_0302, obg,HP_1366, rpmA	112.8, 116.7, 252.1,51, 82.1
2	HP_0586 (HP0586, 977 aa) (DEG10080093)	HP_0586, HP_0585	55.2, 94.3
3	HP_0746 (HP0746, 419 aa) (DEG10080124)	HP_0746, HP_0745, HP_0750, HP_0748, fliD	75.8, 87.3, 73, 101.3, 53.8
4	HP_1206 HP1206(DEG10080223)	HP_1206,sotB, hefC, gale, HP_1082, hefA, neuB	230.5, 69.9, 54.9, 191.4, 298.6, 58.7, 153.3
5	HP_1234 (HP1234, 298 aa) (DEG10080230)	HP_1234, carA, secY, babA	94.7, 219.7, 66.7, 50.3
6	oxaA (HP1450, 547 aa) (DEG10080290)	oxaA, mrmE, dnaJ, ruoD, rmaY	94, 206.4, 206.4, 389.7, 58

Table2: protein– protein interactions *H.pylori26695* with host and their score respectively by STRING v9.1[11]

After using STRING v9.1 database,we concluded proteins (DEG10080047, DEG10080052, DEG10080062, DEG10080224, DEG10080247, DEG10080323) don't have any interactions and homology with other human proteins so that our candidate targets goals are considered for this study, from these drug targets we get a nine thousand twenty seven siRNA targets were identified for essential membrane gene of H.pylori by online Sfold package[31] where that most widely used algorithms for siRNA probability prediction and potential siRNA molecules, these targets were obtained using mixed rule approach Ui-Tei, Amarzguioui and Reynolds rule.[32,33] Figure 2

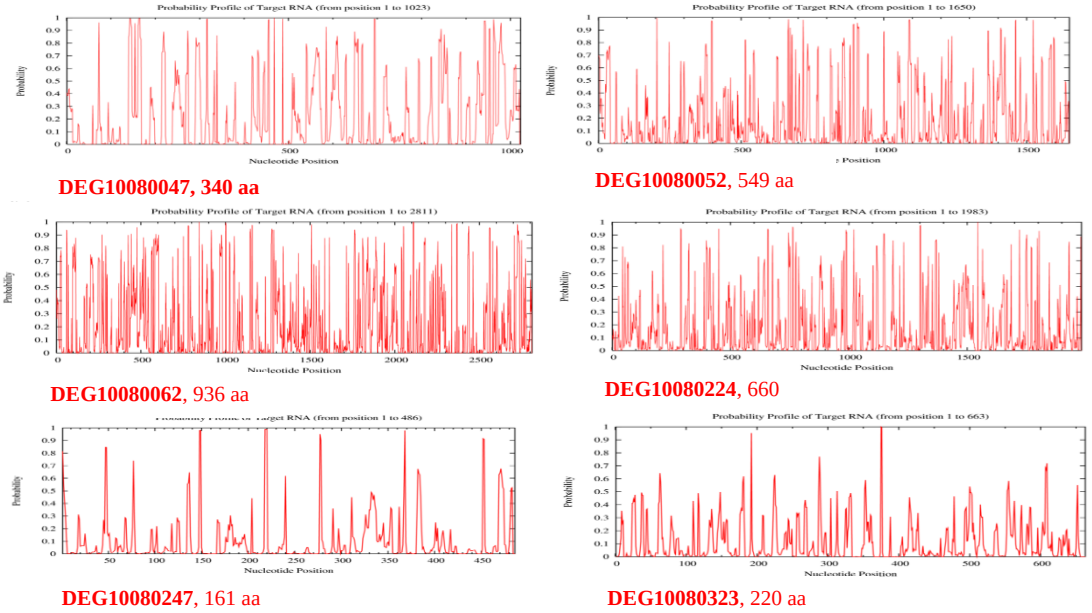

Figure2: (9027) siRNA probability from non-homology and any interactions H.pylorigenes with host by Sflod[31]

Out of nine thousand twenty seven predicted siRNA targets, only sixty six were following all three rules (Ui-Tei, Amarzguioui and Reynolds rule)[32,33]. Hence, these Sixty six targets were subjected to MEGA6 [34] tool for further study and considered potential candidates. Out of theses Sixty six target twenty nine groups were determined on the basis of similarity (Figure 3). All the twenty nine groups effective siRNA selected on the basis of better target

similarity were assessed for target site convenience that can be suitable to knockdown the activity of *H. pylori* of these selected twenty nine groups siRNA targets were depicted that seventeen targets groups have precise similarity, while other twelve groups single sequences.

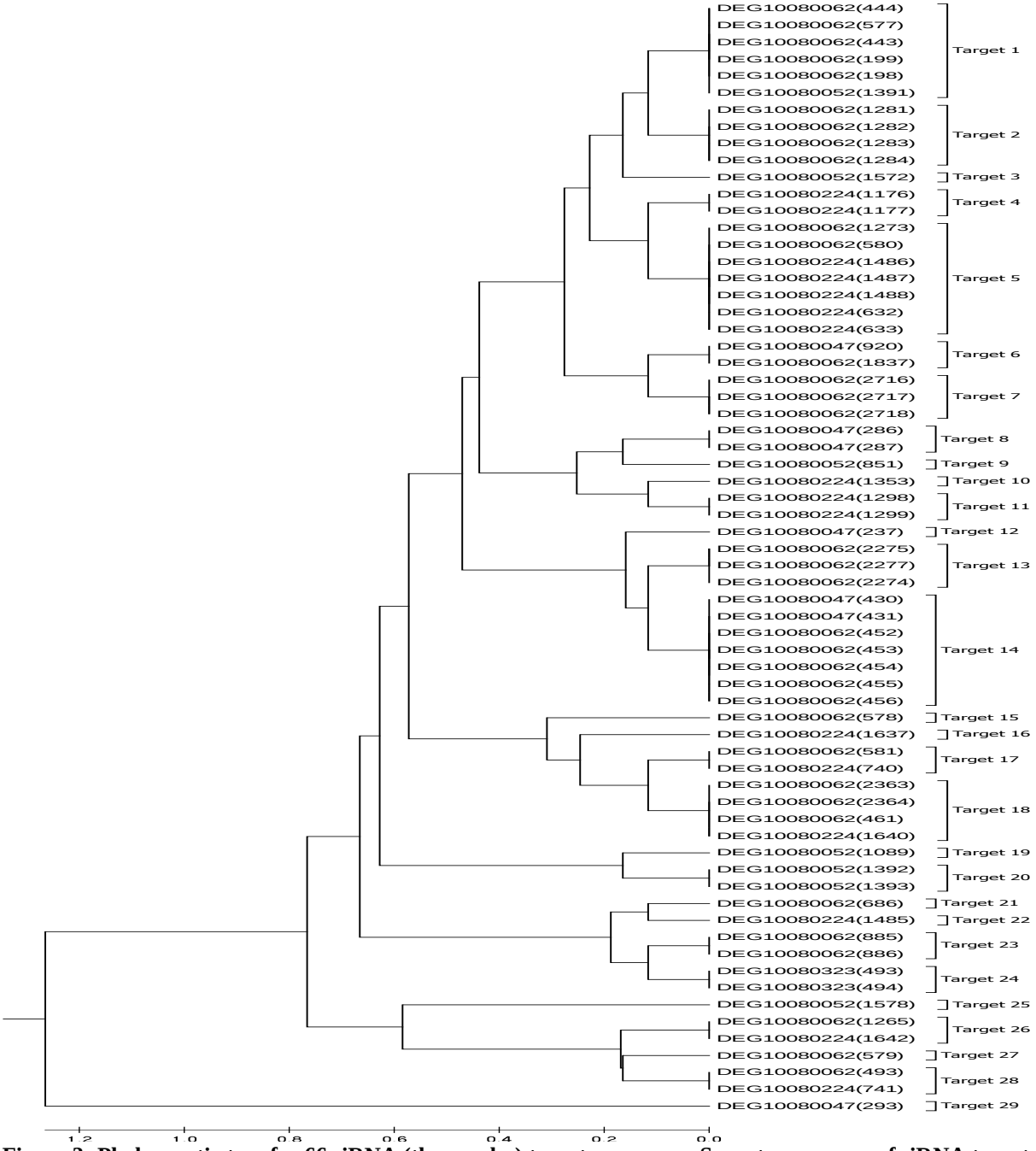

Figure 3: Phylogenetic tree for 66 siRNA (three rules) target sequences. Seventeen groups of siRNA targets showing similarity and a consensus sequence of them. .Twelve target siRNA with dissimilarity (one siRNA each groups).

Then all sequence was executed using online Srna[31] package for these twenty nine targets groups of siRNA where recommend selection of cleavage sites for sequences are at least partially accessible because antisense hybridization is believed to start with nucleation at that site three bases, and elongation then occurs by 'unzipping' the adjacent helix on the target molecules were designed against these consensus target. Therefore every single siRNA might be have one of cleavage sites and for better be at position 11-13 on target siRNA to knock down the inefficient from

twenty nine groups of siRNA[37]. After this analysis only nine siRNA were found have cleavage sites may be suitable for target site have high potential to bind with host and lead to in effective gene silencing (Figure 4)

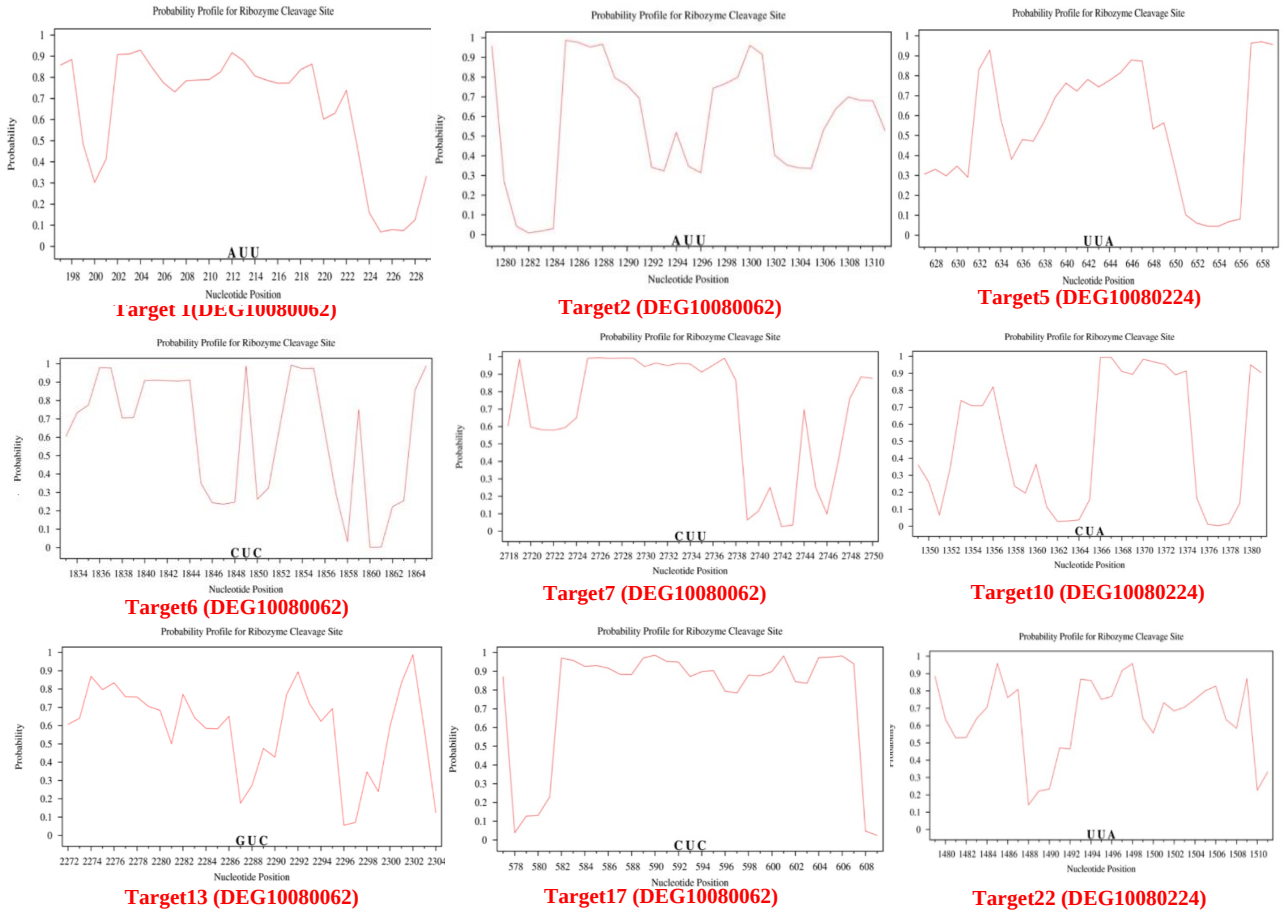

Figure 4: Cleavage sites position for Target siRNA and their Stability Average Probability by online Srna package [31]

Finally, in the present study there are the compatible results concerning the effect of cleavage sites for siRNA must having cleavage site stability rule such as average internal stability at the cleavage site (AIS, in kcal/mol) [38] and average unpaired probability for target site nucleotides [39] for reach to our drug target effective siRNA must take all criteria stability cleavage site rule filter with these we get only five effective target drug siRNA have all criteria show in (Table 3).

No.	No. Target	Ass. gene name	Starting-Ending target position	Antisense siRNA (5p --> 3p)	Cleavage Site	AIS kcal/mol	APS
1	Target 1	DEG10080062 (ycf5)	198- 216	AGGCACAUUCAUUAAUUCUTT	AUU	-7.9	0.773
2	Target 5	DEG10080224 (HP1216)	632- 650	ACGGCUUUAUUUAUUUGCATT	UUA	-7.0	0.682
3	Target 6	DEG10080062 (ycf5)	1837- 1855	CUCUAUUUAUCCUCUUCUATT	CUC	-5.1	0.694
4	Target 7	DEG10080062 (ycf5)	2717- 2735	UCCCUACUUUCUUAUACUUTT	CUU	-7.6	0.834
5	Target 22	DEG10080224 (HP1216)	1485- 1503	CAUCCCUUAUUACACCUUUTT	UUA	-8.0	0.655

Table 3: Drug target siRNA with Cleavage Site and their stability rules [38, 39] by Sfold package [31]

Conclusion:

Using *In Silico* study to RNAi may be designed a number of siRNA molecules for silencing of significant genes for resistance of disease that cause *H. pylori*. Further their interactions with target can also be calculated, computationally. Therefore, in this study five siRNA molecules were predicted from two genes only from twelve membrane essential genes of *H. pylori* as effective candidate using computational approaches .These siRNAs may lead to *H. pylori* therapy. Study outcome would also provide a basis to the researchers and pharma industry persons to develop the antibacterial therapeutics at genomic level, experimentally.

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