Prospective gene therapy design against coronavirus COVID-19 by recruiting CRISPR Cas9 approach

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Abstract. A novel Coronavirus disease (COVID-19) has being originated from animals and causing to running outbreak of viral pneumonia in human all over the world. The World Health Organization (WHO) has considered COVID-19 infection as Epidemic disease in March 2020. The high percentages of death rate among people lead the researchers and scientists in different fields of medicine in order to find solution for this threating problem. In this regard, an editing gene technique was employed in this study, in order to deter the viability of coronavirus genetically. The main objective in this paper first has been determined and obtained the essential proteins of COVID-19 coronavirus proliferation by using NCBI website which included *S, E, N, M, ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF10* proteins. The second objective is to use a very precise technique of editing gene called CRISPR-Cas9 to make changes to the virus's RNA, through designing single-guide RNA for the essential each protein of COVID-19, in order to inactivate an effective certain protein. These techniques will be provided for each patient and healthy person by injection of all genes components using the Gene-gun machine or spray aerosol to make ensure reach it to the target cell.

Keywords. CRIPR Cas9, Coronavirus, Gene Therapy, WHO.

1. Introduction

The A novel Coronavirus disease (COVID-19) has being originated from animals and causing to running outbreak of viral pneumonia in human all over the world [1, 2, 3]. Coronavirus (Covid-19) is a considerable aggressive comprehensive public health threat. It is a large family of enveloped RNA viruses [4]. There are four groups of coronaviruses, which originated from animals. Alpha and beta presence at bats, while Gamma and Delta are avian species reservoir. Coronaviruses are responsible for wide range diseases in many animals including the human. In human they cause mild self-limiting respiratory infections. Different kinds of animals had been recorded previously historically infected with coronavirus, like Avian, Murine, Ferret, Swine, Rat, Rabbit, Whale, Camel, Bat and Human [5]. All of these types of creatures have big issues problem for us and the possibility of the virus developing inside their cells and transmission it to each other easily. For instance, in terms of SARS-

CoV strain was conveyed from civet cat to human, while the MERS-CoV strain has been transmitted from camel to human. Usually, the virus access to the human body into the respiratory system reach lung cells through attachment by S proteins that covered the outside viral surface with the human receptor and then, an inject the ssRNA inside the intact human cell and start to replicate through many process [6]. In this study, ten important proteins have been virtualized by the stated NCBI website and then tag each protein from a precise RNA site by guide RNA in order to inactivation of protein using technique called CRISPR-Cas9, that prospective will aid for stop proliferation against specified coronavirus COVID-19 . These techniques will be provided for each patient and healthy person by injectable by gene gun machine or spray aerosol to make ensure reach it the target cell. In general, CRISPR-Cas9 is very accurate technique of editing gene, which allows the scientist to manipulate the DNA for repairing and cure the gene disorder. CRISPR-Cas9 system has only two compounds, a Cas9 endonuclease and a single-strand guide RNA (sgRNA) [7, 8]. It is adopted immunity system in the bacteria to detect and match virus's DNA or RNA and stop action. CRISPR-Cas9 is having a major effect on practical genomic carried out in experimental systems [9]. The scientific term of CRISPR is derived from Clustered Regularly Interspaced Short Palindromic Repeats that tied inside the genome of the bacteria with their associated nuclease enzyme called Cas9. This specific sequencing of gene allowed the bacteria to record over the time the virus's DNA that injected to the bacteria and protect itself. CRISPR-Cas9 considered as a genetic vaccination card in the bacteria that offer an opportunity to manipulate with the gene to edit or delete any desired gene. Overall, this complex is programmable, which is meaning we can program any specific fragment of the gene within the genome and cure it [10].

2. Materials and Methods

CRIPR-Cas9 system has designed to allow inducing cleave at target 10 gene sites for essential proteins for invasion and proliferation for Coronavirus. All ten genes sequences were obtained from the NCBI database, in order to complete the coding sequence of the open reading frame (*s, e, n, m, orf3a, orf6, orf7a, orf7b, orf8, orf10*) genes. The gene code (Gene ID) for all these proteins in NCBI website were as follow: Spike protein (S) is NC_030886.1, the Envelop protein (E) is 43740570, The Nuleocapsid phosphoprotein (N) is 43740575, the Membrane glycoprotein (M) is 43740571, the ORF3a protein is 43740569, the ORF6 protein is 43740572, the ORF7a and b proteins are 43740573, the ORF8 protein is 43740577 and the ORF10 is 43740576. This study, we have designed guide RNA for only S protein which is considered the most important part of the virus to cause infection (AGGGTCCACCAAACGTAATGCGG). Then, the interference leads to synthesis dsRNA, which will be formed of the guide RNA (sgRNA), thereby the sequence of each gene to be used accurately by the CRISPR-Cas9 technique inside the infected cell. The sgRNA directs the Cas9 endonuclease to cleave both DNA strands in a specific sequencing (Table 1 and 2). DNA cleavage happens at a sequence 3 base pairs upstream of an “NGG” proto-spacer adjacent motif (PAM). The CRISPR/Cas9 requires the co-expression of a Cas9 protein with a single guide RNA vector that will be expressed by the promoter [11]. Figure 1 refers to prospective cycle treatment of COVID-19 infection by using CRISPR-Cas9 technique as a novel treatment.

Table 1. Step by step for the experiment for Crispr primers design.

Step A: Design guide-RNA criRNA.

Name	Primer Sequence
S protein_guideRNA39rvGeneArtFw	TACGACTCACTATAG**AGGGTCCACCAAACGTAATG**
S protein_guideRNA39rvGeneArtRev	TTCTAGCTCTAAAAC**CATTACGTTTGGTGGACCCT**

Step B: Lentiviral vectors: cloning with Gibson assembly.

Name	Oligonucleotide Sequence
batchOligo39rv	*GGAAAGGACGAAACACCG*AGGGTCCACCAAACGTAATG*GTTTTAGAGC TAGAAATAGCAAGTTAAAATAAGGC*

Step C: PCR to amplify the on-target site (S-protein).

Name of Primers genomic fragments	Sequences	Annealing Temperature
S_protein__OntargetGuideRna39rvLeft	GAATTGTGCGTGGATGAGGC	Tm 59.901
S_protein__OntargetGuideRna39rvRight	TGAGAGCGGTGAACCAAGAC	Tm 59.966

Table 2. Restriction map of Crispr _S protein sequence sites for Covid-19 by using Serial-cloner-V2.5, showing the green color codes regions for restriction enzymes that inactivation protein action.

TfiI
PfeI
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<58rev: Spec 100, Eff 60/48_misc_feature
>54forw: Spec 100, Eff 50/36_misc_feature
>51forw: Spec 100, Eff 45/51_misc_feature
<39rev: Spec 100, Eff 52/50_misc_feature
<38rev: Spec 100, Eff 50/31_misc_feature
<37rev: Spec 100, Eff 68/44_misc_feature >99forw: Spec 100, Eff 66/36
_misc_feature
<18rev: Spec 100, Eff 18/57_misc_feature >87forw: Spec 100, Eff 66/56_misc_feature
<17rev: Spec 100, Eff 19/42_misc_feature <81rev: Spec 100, Eff 24/37_misc_feature
<16rev: Spec 100, Eff 53/36_misc_feature >72forw: Spec 100, Eff 50/39_misc_feature
||| ||| | | || | | | | |
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M S D N G P Q N Q R N A P R I T F G G P S D S T G S N Q N G E R S G
C L I M D P K I S E M H P A L R L V D P Q I Q L A V T R M E N A V
V * * W T P K S A K C T P H Y V W W T L R F N W Q * P E W R T Q W
TACAGACTATTACCTGGGGTTTTAGTCGCTTTACGTGGGGCGTAATGCAAACCACCTGGGAGTCTAAGTTGACCGTCATTGGTCTTACCTCTTGCGTCAC
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Eco88I
Ama87I
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Sfr274I
XhoI
SciI
AvaI
StrI
<199rev: Spec 100, Eff 61/19
_misc_feature
Nli3877I
<161rev: Spec 100, Eff 65/55_misc_feature
>AccBSI >BpuAI BmeT110I
>MbiI >BpiI SlaI
<137rev: Spec 100, Eff 27/16_misc_feature PaeR7I
<136rev: Spec 100, Eff 25/37_misc_feature <189rev: Spec 100, Eff 16/43
_misc_feature
>129forw: Spec 100, Eff 63/41_misc_feature >Bbr7I BsoBI
<126rev: Spec 100, Eff 53/30_misc_feature >BbsI PspXI
<124rev: Spec 100, Eff 67/33_misc_feature >BstV2I <198rev: Spec 100, Eff 58/85
_misc_feature
>121forw: Spec 100, Eff 76/41_misc_feature >BbvII AbsI
Hpy99I <125rev: Spec 100, Eff 39/51_misc_feature >182forw: Spec 100, Eff 54/21_misc_feature
>101forw: Spec 100, Eff 70/49_misc_feature >BsrBI >177forw: Spec 100, Eff 61/25_misc_feature
>100forw: Spec 100, Eff 61/56_misc_feature >154forw: Spec 100, Eff 54/28_misc_feature
|| | | ||| | || | || | | ||
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A R S K Q R R P Q G L P N N T A S W F T A L T Q H G K E D L K F P
G R D Q N N V G P K V Y P I I L R L G S P L S L N M A R K T L N S L
G A I K T T S A P R F T Q * Y C V L V H R S H S T W Q G R P * I P
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>204forw: Spec 100, Eff 69/33_misc_feature <261rev: Spec 100, Eff 50/45_misc_feature
<AloI <227rev: Spec 100, Eff 44/51_misc_feature >288forw: Spec 100, Eff 71/23
_misc_feature
>RpaB5I <Bsp24I <238rev: Spec 100, Eff 37/27_misc_feature >294forw: Spec 100, Eff 62/49
_misc_feature
TaqI <217rev: Spec 100, Eff 29/36_misc_feature EcoRI >285forw: Spec 100, Eff 60/36_misc_feature
EsaBC3I >210forw: Spec 100, Eff 55/28_misc_feature <BspQI <272rev: Spec 100, Eff 52/22_misc_feature
|| | | | | | | | | | | | | | | |
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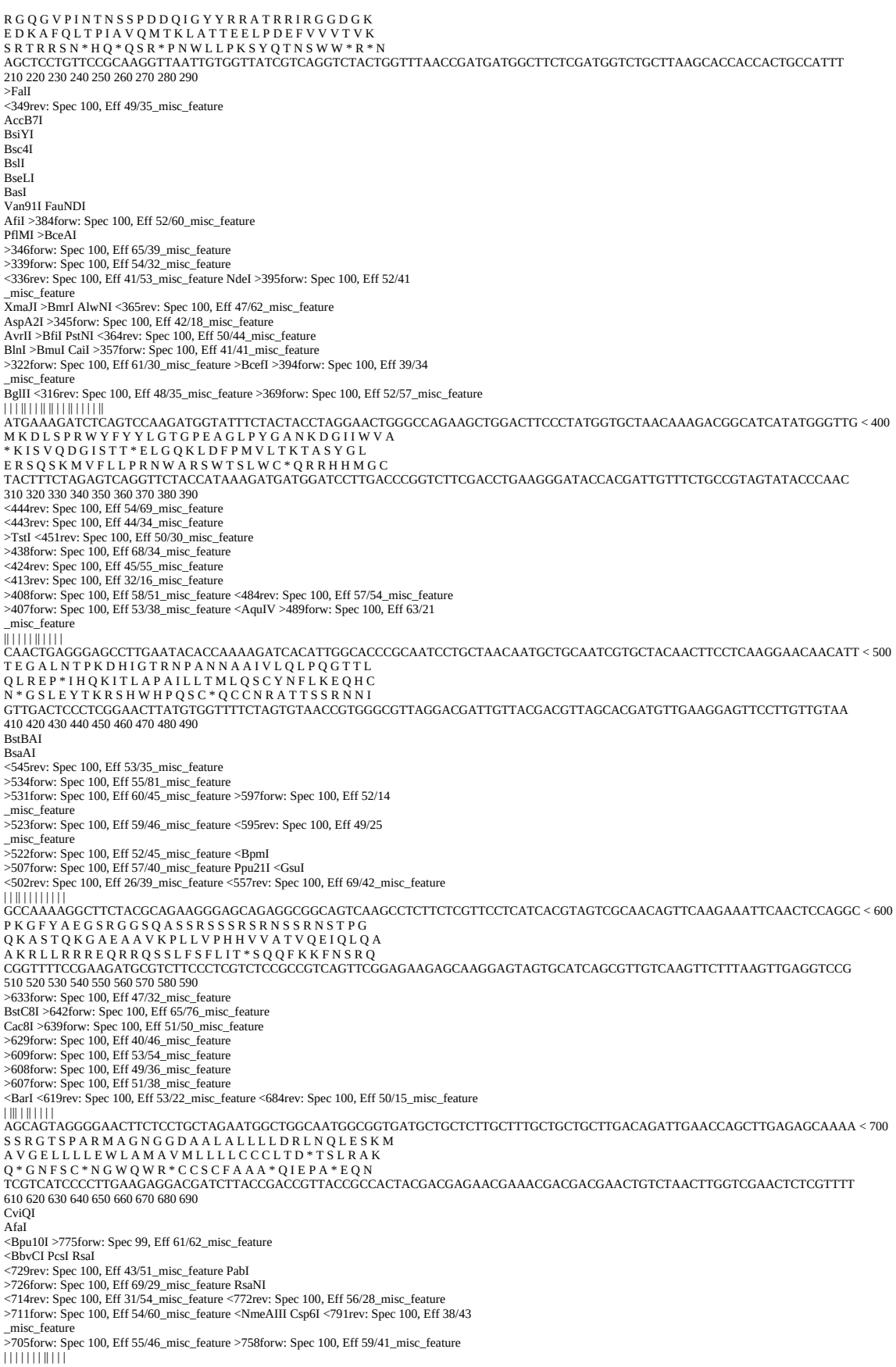
R G Q G V P I N T N S S P D D Q I G Y Y R R A T R R I R G G D G K
E D K A F Q L T P I A V Q M T K L A T T E E L P D E F V V V T V K
S R T R R S N * H Q * Q S R * P N W L L P K S Y Q T N S W W * R * N
AGCTCCTGTTCCGCAAGGTTAATTGTGGTTATCGTCAGGTCTACTGGTTTAACCGATGATGGCTTCTCGATGGTCTGCTTAAGCACCACCACTGCCATTT
210 220 230 240 250 260 270 280 290
>FalI
<349rev: Spec 100, Eff 49/35_misc_feature
AccB7I
BsiYI
Bsc4I
BslI
BseLI
BasI
Van91I FauNDI
AfiI >384forw: Spec 100, Eff 52/60_misc_feature
PflMI >BceAI
>346forw: Spec 100, Eff 65/39_misc_feature
>339forw: Spec 100, Eff 54/32_misc_feature
<336rev: Spec 100, Eff 41/53_misc_feature NdeI >395forw: Spec 100, Eff 52/41
_misc_feature
XmaJI >BmrI AlwNI <365rev: Spec 100, Eff 47/62_misc_feature
AspA2I >345forw: Spec 100, Eff 42/18_misc_feature
AvrII >BfiI PstNI <364rev: Spec 100, Eff 50/44_misc_feature
BlnI >BmuI CaiI >357forw: Spec 100, Eff 41/41_misc_feature
>322forw: Spec 100, Eff 61/30_misc_feature >BcefI >394forw: Spec 100, Eff 39/34
_misc_feature
BglII <316rev: Spec 100, Eff 48/35_misc_feature >369forw: Spec 100, Eff 52/57_misc_feature
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M K D L S P R W Y F Y Y L G T G P E A G L P Y G A N K D G I I W V A
* K I S V Q D G I S T T * E L G Q K L D F P M V L T K T A S Y G L
E R S Q S K M V F L L P R N W A R S W T S L W C * Q R R H H M G C
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<443rev: Spec 100, Eff 44/34_misc_feature
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<413rev: Spec 100, Eff 32/16_misc_feature
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>407forw: Spec 100, Eff 53/38_misc_feature <AquIV >489forw: Spec 100, Eff 63/21
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T E G A L N T P K D H I G T R N P A N N A A I V L Q L P Q G T T L
Q L R E P * I H Q K I T L A P A I L L T M L Q S C Y N F L K E Q H C
N * G S L E Y T K R S H W H P Q S C * Q C C N R A T T S S R N N I
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BsaAI
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Q K A S T Q K G A E A A V K P L L V P H H V V A T V Q E I Q L Q A
A K R L L R R R E Q R R Q S S L F S F L I T * S Q Q F K K F N S R Q
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A V G E L L L L E W L A M A V M L L L L C C C L T D * T S L R A K
Q * G N F S C * N G W Q W R * C C S C F A A A * Q I E P A * E Q N
TCGTCATCCCCTTGAAGAGGACGATCTTACCGACCGTTACCGCCACTACGACGAGAACGAAACGACGACGAACTGTCTAACTTGGTCGAACTCTCGTTTT
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AfaI
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_misc_feature
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C L V K A N N N K A K L S L R N L L L R L L R S L G K N V L P L K H
V W * R P T T T R P N C H * E I C C * G F * E A S A K T Y C H * S
ACAGACCATTTCCGGTTGTTGTTGTTCCGGTTTGACAGTGATTCTTTAGACGACGACTCCGAAGATTCTTCGGAGCCGTTTTTGCATGACGGTGATTTCG
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AspI XcmI >858forw: Spec 100, Eff 22/19_misc_feature
PflFI <845rev: Spec 100, Eff 39/46_misc_feature
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>WviI >822forw: Spec 100, Eff 43/40_misc_feature >882forw: Spec 100, Eff 68/20_misc_feature
| | | | | | | | | | | | | |
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Y N V T Q A F G R R G P E Q T Q G N F G D Q E L I R Q G T D Y K H
T M * H K L S A D V V Q N K P K E I L G T R N * S D K E L I T N I
I Q C N T S F R Q T W S R T N P R K F W G P G T N Q T R N * L Q T L
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Aor51HI
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EaeI <924rev: Spec 100, Eff 59/47_misc_feature >980forw: Spec 100, Eff 49/74_misc_feature
CfrI >BseYI SmiMI >965forw: Spec 100, Eff 53/44_misc_feature
AcoI BstAPI >GsaI <Eco57I >945forw: Spec 100, Eff 40/47_misc_feature
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|| | | |||||| | | | | | | | | || | |
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W P Q I A Q F A P S A S A F F G M S R I G M E V T P S G T W L T Y T
G R K L H N L P P A L Q R S S E C R A L A W K S H L R E R G * P T
A A N C T I C P Q R F S V L R N V A H W H G S H T F G N V V D L H
ACCGGCGTTTAACGTGTTAAACGGGGGTCGCGAAGTCGCAAGAAGCCTTACAGCGCGTAACCGTACCTTCAGTGTGGAAGCCCTTGCACCAACTGGATGT
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| || | | | | | | |
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G A I K L D D K D P N F K D Q V I L L N K H I D A Y K T F P P T E
Q V P S N W M T K I Q I S K I K S F C * I S I L T H T K H S H Q Q S
R C H Q I G * Q R S K F Q R S S H F A E * A Y * R I Q N I P T N R
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24/45_misc_feature
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| | | | | | | | | | |
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P K K D K K K K A D E T Q A L P Q R Q K K Q Q T V T L L P A A D L
L K R T K R R R L M K L K P Y R R D R R N S K L * L F F L L Q I W
A * K G Q K E E G * * N S S L T A E T E E T A N C D S S S C C R F G
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>1253forw: Spec 100, Eff 55/54_misc_feature
PceI
Eco147I
StuI
MunI SseBI
MfeI <1229rev: Spec 100, Eff 54/23_misc_feature
>Hin4I <1211rev: Spec 100, Eff 35/45_misc_feature
| | | | ||

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D D F S K Q L Q Q S M S S A D S T Q A *
M I S P N N C N N P * A V L T Q L R P X
* F L Q T I A T I H E Q C * L N S G L X
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1210 1220 1230 1240 1250

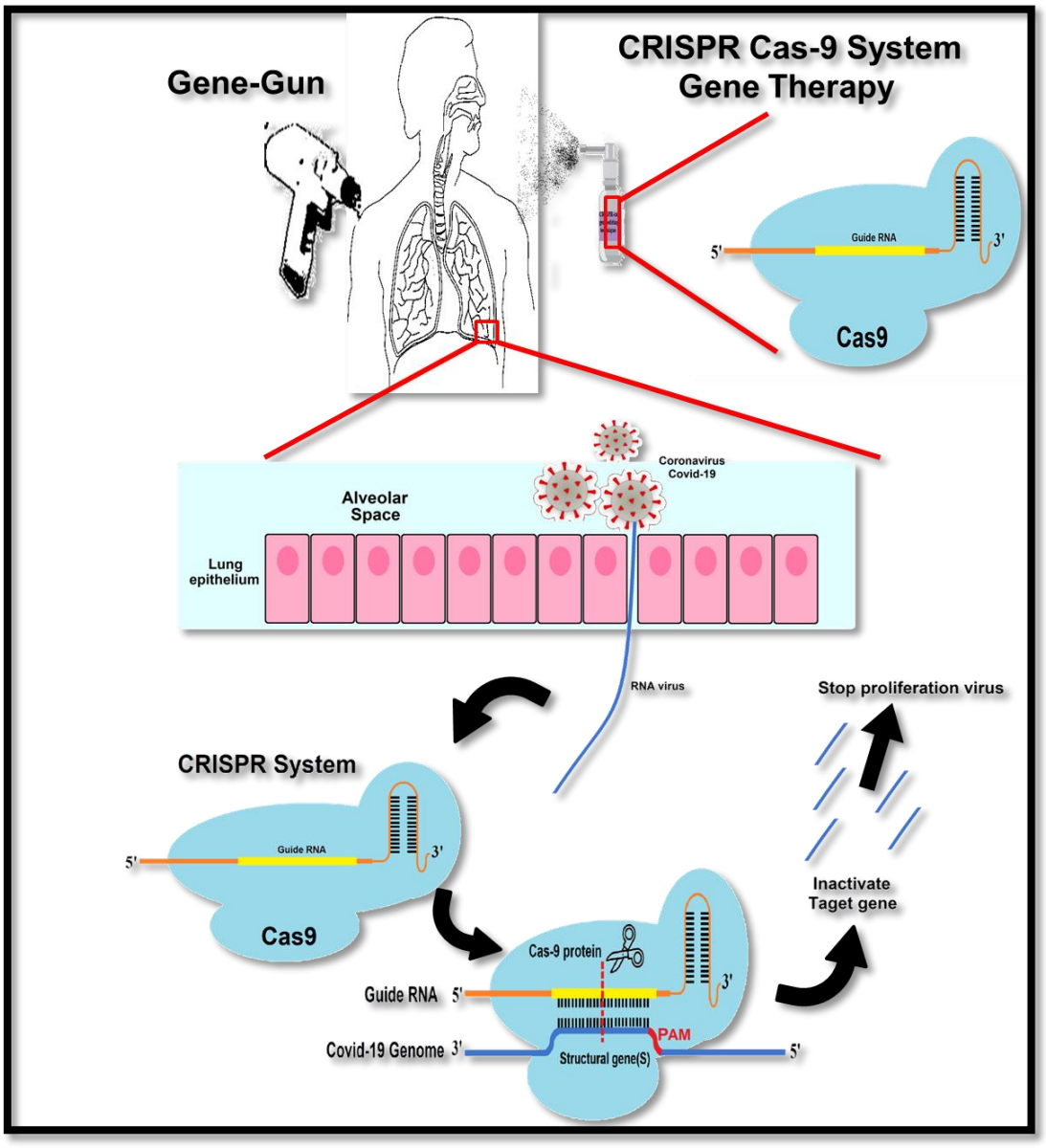

Figure 1. Overview of the stages for how to delivering gene therapy via CRISPR/Cas9 system with a specific guide-RNA for fighting Coronavirus Covid-19 by using a Gun-gene machine or spray aerosol. Also, how the CRISPR/Cas9 binds to the target gene site within sgRNA virus.

2. Results and Discussion

This Even though most of the researchers are considering finding a safe vaccine against Covid-19. However, in this study has suggested that CRISPR/Cas9 systems can accurately link certain gene with specific single guide-RNA in many essential sites into the Coronavirus genome (*s*, *e*, *n*, *m*, *orf3a*, *orf6*, *orf7a*, *orf7b*, *orf8*, *orf10*). Our study proposes to complete inactivation for each protein target, which has ability caused the infection by using bioinformatics analysis to do cleavage each gene through

binding with guide-RNA and stop expression. In addition, it has believed that the using design guidelines will induce decrease potential effects of proliferation coronavirus inside human cells and gives our body at least an initiate immunity. In this study, we just focus an example of one important protein (Spike -protein) out of ten proteins persist into the virus, which have capability inhibited for invasion and proliferation action inside the host cell. The idea has been developed throughout designed guide-RNA for S-gene sequences, in order to inactivate and impeding gene expression into the S-gene and ceases their action. Conservatively determining target gene sequences in order to avoid interfering with human genes contents, thereby make ensure to destroy the only target virus away from others. The limitation of facility and to save the time, the overall aim of our research is to bring together a serious detecting of genes and proteins sequencing of the essential proteins of COVID-19 coronavirus to make them as a target to inactivate the infection of virus by using CRISPR-Cas9 technique. The editing gene- CRISPR/Cas9- permits for precise genome destruction and replacement in a flexible way resulting in high activity and low cellular toxicity [10]. This will be a building block towards these potential proteins in the future studies that could then explore and exploit very applied method clinically to destroy this disease genetically and radically.

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