

The Pfizer Vaccine CRISPR Experiment

Introduction

The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) Technology is a genome-editing technique that has been used by Scientists to alterate the genome in a population. The synthetic alteration of a Wildtype population is called Gene Drive. A gene drive individual has an altered gene inserted into one of his chromosomes. After sexual reproduction with a Wildtype, each parent contributes one copy of each chromosome to their offspring. Thus, the offspring carries only in 50% the altered gene from the parent. The altered gene will persist in low frequency in the following generations or can even go distinct after several generations. It would take dozens of generations to recognize the characteristics of the altered gene within a substantial fraction of the population(1).

However, the new genome editing technique CRISPR CAS9 increases the odds that almost any altered gene will be inherited 100% to offspring, potentially allowing the altered gene to spread through even wild populations after only 12-15 generations for every thousand reproducing gene drive individuals(2).

The CRISPR CAS9 technology is a well-known genome editing mechanism that has been used by bacteria to defend itself from viral infections. CRISPR is a genetic sequence with short palindrome repeats interspaced with viral sequences called spacer RNA. The spacer RNA contains unique sequences of viral RNA collected from past infections. During a viral invasion, the bacterium transcribes a CRISPR RNA that contains the embedded memory of the past viral infections. If the invaded viral RNA sequence matches the transcribed CRISPR RNA with records from viral sequences, the bacterium produces an enzyme called CAS9 that cuts the viral RNA and destroys it.

This gene-editing mechanism of the bacteria has been used by researchers to insert and add altered gene sequences into the human DNA. Thus, an mRNA can be engineered that contains the genetic information for the altered gene, the information for the CAS9 enzyme and a guided RNA (gRNA) that tells the CAS9 enzyme, where to cut and paste the altered gene sequence into the human genome. After the mRNA has been injected into human cells, the information on the mRNA will be translated into a CRISPR CAS9 enzyme complex.

The CRISPR CAS 9 enzyme complex screens the human chromosomes for a specific DNA sequence that matches the sequence on the gRNA. A match activates the CAS 9 enzyme and induces a Double-Strand Break (DBS) by cutting the human DNA at a specific chromosome site. The gRNA guides the CAS9 to cut at this specific location. A short signal sequence of 2-6 nucleotides at the 3'end of the gRNA called PAM (protospacer adjacent motif), tells the CAS9 enzyme to cut the altered host RNA off the mRNA. The DNA cut stimulates the DNA repair system and inserts the free-floating altered gene sequence into the human genome(3).

In other words, the human cells copy and paste the altered gene into the Wild-type gene, when it repairs the DNA damage. Now, the gene-drive individual has two identical gene copies on each chromosome, which will be both transmitted to offspring. And the process repeats in subsequent generations causing the alteration and spread through the population(1). Because viruses are gene delivery systems, a virus is the perfect carrier for the CRISPR CAS9 technology.

Pfizer describes on his website, the Cominarty vaccine under section indication and usage as follows; COMIRNATY is a vaccine indicated for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 16 years of age and older(4).

The increasing hospitalisation for Cominarty vaccines and reports of Covid infections despite vaccination required a detailed analyzes of the Cominarty mRNA code. The aim of this study was to analyze the mRNA code for CRISPR-CAS9 gene-editing technology, which would explain the failure in the SARS CoV-2 virus immunization and the increase in fatal side effects after vaccination.

Method

1. First, the full sequenced and published mRNA code of the Pfizer BNT-162b2, also known under the product name Cominarty was base by base compared with the SARS CoV-2 Wuhan Hu-1 spike protein RNA (Genbank Association MN908947.3) (6).
2. An online Palindrome sequence finder from the DNA sequencing company, Novo Pro Lab from China, was used to find Short Palindrome Repeats and DNA spacers(6).
3. An online DNA/RNA translation tool on the Website of the Swiss Institute of Bioinformatics, called ExPasy, was used to detect Open Reading Frames(7). The results were confirmed and verified with the online NCBI ORF finder(8).
4. Two further online tools were used to search for gRNAs with cleavage sites for CAS enzymes. The gRNA search was mainly focused on the plus strand to keep the study as understandable as possible for every reader. The Giraldez Lab from Yale University offers an online CRISPR gRNA search tool. The search was customized for Human Genoms, without the occurrence of nucleotide mismatches and for cleavage sites for both CAS9 and CAS12a enzymes(9). The second online tool was the CRISPR Finder from the Sanger Institute for Genome Editing(10). The CRISPR Finder provides correspondent CRISPR ID for the gRNAs in the online Genome Database. Both CRISPR research tools gave the same results and confirmed the findings.
5. And last, the CRISPR ID of each gRNA was searched in the online BLAST Database for corresponding gene sequences in the human genome(11).

Results

1. Cominarty analogy with SARS CoV-2 Wuhan Hu-1 Spike protein

(Genbank Association MN908947.3)

The Cominarty mRNA matches in 24% with the SARS CoV-2 Wuhan Hu-1 Spike Protein. But 76% of the Cominarty genetic code is not related to the SARS CoV-2 spike protein. The inserted spike protein sequences are located mainly in the 3' end of the mRNA. Figure 1 shows highlighted in different colours gene sequences that match the SARS CoV-2 spike protein. As we can see from figure 1, fragmented spike protein sequences are added to new DNA sequences (not highlighted).

2. Short Palindrome Repeats

The palindrome sequencer found 12 short palindrome repeats in the mRNA. These are CAGCTG, CTGCAG, GCCGGC, CACGTG, TGTACA, AGATCT, GCATGC, GATATC, TGATCA, TGGCCA, GGCGCC, GCTAGC. The palindrome repeat CAGCTG appeared seven times and its reverse version CTGCAG occurred six times in the mRNA. The Short Palindrome repeats are highlighted in figure 2 in bright blue.

Between the short palindrome repeats are interspaced fragmented Spike protein sequences. The same in colour highlighted spike protein sequences from figure 1 are highlighted in figure 2 in gray. We can see in figure 2 that the mRNA exists of fragmented spike protein pieces. Not the full-length spike protein appears in the Cominarty mRNA as mentioned in the WHO Program paper from September 2020 but fragments of spike protein sequences that have been added to new gene sequences (12). Gene sequences from the spike protein were even used to create some of the Short Palindrome Repeats, which are presented in figure 3 as highlighted gray nucleotides within bright blue fields. Figure 3 shows an example of a DNA spacer. Fragmented virus spike protein sequences occur between short palindrome repeats.

3. Open Reading Frames in the mRNA

Both the WHO Program Paper for the Cominarty vaccine and the published Pfizer mRNA sequence show one large Open Reading Frame of over 3800 bp (12,5). This one open reading frame cannot possibly be translated into a full-length spike protein due to two features in the mRNA. First, the spike protein sequences express just fragmented amino acids and not complete proteins. Second, the mRNA has stop and start codons in the middle of the open reading frame, which triggers nonsense-mediated decay and degradation of the mRNA (13). Noticeable is that the Stop codon UGA was changed into UGAU.

But with the help of both, the expasy DNA/RNA translation tool and NCBI's ORF finder, 13 hidden open reading frames were found within the long open reading frame, which is translated into different fully functional proteins. Figure 2 shows highlighted in dark and bright pink several open reading frames.

Interestingly, the first protein nt 275-410 does not contain a single fragmented sequence from the spike protein (figure 4). The BLAST Protein search for this protein results in the human Coronavirus HKU1 isolate N5 (figure 5). Another hidden open reading frame expresses into a hypothetical protein. A hypothetical protein is a computer-predicted protein that does not exist in nature. This hypothetical protein expresses from nt 2795-2822 including the start and stop codon (figure 6). The modified stop codon UGAU for the hypothetical protein matches in position with the spike protein sequence. Figure 7 shows the BLAST result.

The Cominarty mRNA expresses also two short proteins, both of which play a role in the human cell signalling pathway. The sequence CCCAGGCAC expresses the PRH protein, which has a role in cancer suppression. And the CCAGCGTCG expresses the PAS protein, which plays a role in cell signalling.

And we also find fully synthetic spike proteins that resemble either 96% or in another case 98% a measles mutant virus protein (figure 8 and 9). Right at the 3' end of the mRNA at nt 2254-3367 is a synthetic protein that shows in a FASTA search only analogy with the Human Homeobox Hox A-4. Interestingly, this synthetic protein is the only protein that contains mainly spike protein sequences (figure 3,10).

4. CRISPR gRNA findings

Three gRNAs including the PAM sequence were found for the plus strand by Yale's University CRISPR research tool and confirmed with the CRISPR sequence finder by the Sanger Institute. Each gRNA sequence ends with the PAM sequence GGG.

4.1. The first gRNA has the CRISPR ID1161208236. The 21 bp long sequence is AGCTGCCCTTCCCGTCCT GGG (figure 11).

4.2. The second gRNA has the CRISPR ID 992837047. The 21 bp long sequence is TTAGCCTAGCCACACCCAC GGG (figure 12).

4.3. The third gRNA has the CRISPR ID 1202628979. The 23 bp long sequence is AACTAAGCTATACTAACCCCA GGG (figure 13).

5. Corresponding gRNAs on human reference genome

All three gRNAs were searched in the BLAST Database for the human genome. The results show that all gRNA sequences correspond to the Human Reference Genome GRCh38.

The gRNA with CRISPR ID 1161208236 originates from Chromosome 5 (figure 14) of GRCh38. The gRNA with CRISPR ID 992837047 also originates from Chromosome 5 (figure 15). And gRNA with CRISPR ID 1202628979 originates from Chromosome 19 of the GRCh38 Human Reference Genome (figure 16).

6. In addition, this study found a protein sequence at nt 3879-4050 that translated into a synthetic novel gp130 protein. The novel gp130 protein was already engineered in 1998 by Y. Liu. The publication from 1998 has been completely removed from the web. The function of this protein, therefore, remains unclear.

Conclusion

The analysis clearly shows that the Cominarty vaccine cannot induce immunization because it translates not into the full Covid spike protein pathogen. Although all genome database searches result in fully synthetic spike protein production for the Cominarty mRNA, the complete spike protein, however, cannot possibly be translated due to the fragmented RNA sequences and induced translation discontinuation, which would lead to mRNA degradation. Thus, the inserted spike protein sequences mask the real intention behind the Cominarty vaccine. The manipulation of the human genome at Chromosome 5 and Chromosome 19 as part of a human gene drive experiment.

This human gene drive experiment consists of three parts. The first part hosts CRISPR RNA with typical features such as Short Palindrome Repeats interspaced with viral RNA spacer.

The second part has hidden open reading frames that are translated into 13 several synthetic proteins such as hypothetical protein, a measles virus mutant protein or even human coronavirus protein. The different fully synthetic proteins have nothing to do with the SARS Cov-2 virus pathogen and are designed to cause both, the collapse and breakdown of the immune system.

A vaccine designed to support the immune system would never contain a hypothetical protein, whose post-translational function in human cells remains unknown. Why would a vaccine manufacturer risk the overload of the immune system with such a protein? Also questionable is why the mRNA translates into a synthetic protein that is analogue to the human Coronavirus? The human Coronavirus is a completely different type of virus than the SARS CoV-2 (14). And finally, why should the vaccine manufacturer go through so much trouble hiding 13 protein sequences between Spacer DNAs instead of creating a simple mRNA that will be translated into 13 full functional spike proteins?

The third part of Pfizer's CRISPR experiment contains three gRNAs including the PAM sequence that cleavages for the CAS 9 and CAS 12a enzyme. The analyzed gRNAs relate only to the plus strand. Additional gRNAs emerged in the analysis for the reverse strand but hasn't been discussed in this study. A pharmaceutical company cannot market a new mRNA technology under the disguise of vaccines that actually contains a technology to manipulate the human genome, where the public has not been informed about.

The only individual, who could create such gRNAs in a stable mRNA environment is Feng Zhang from the MIT Zhang lab. And the research reveals that Pfizer announced back in 2016, a collaboration with MIT's Zhang lab for cloud-based storage and analysis of human gene regulation datasets(15). And in return, Pfizer, along with former Pfizer executive Devyn Smith, raised \$215 million in funds for Arbor Technology, Feng Zhang's newly founded CRISPR company(16).

It comes as no surprise that MIT is the number one institution involved in the human genome project. The complete mapping of the human genome led to a database of stored reference genomes. The digital genome sequences of 13 volunteers were stored under the human reference genome GRCh38(17). Since the beginning of the Human Genome Project, the GRCh38 genome has been modified in the database (18). The aim is to create the desired human race using digital calculations and computer predictions. Such computer-predicted perfect gene sequences have now emerged in the Cominarty mRNA vaccine.

The individual, who was injected with a reference human genome from 13 different volunteers and the CRISPR CAS9 gene-editing tool was not informed before vaccination and did not give consent for his genome to be manipulated. Thus, this study also serves for criminal charges and legal disputes against the Pfizer company for conducting genome experiments on humans.

Reference

1. Youtube. Gene Drives from Harvard Wyss Institute. July 2014. <https://wyss.harvard.edu/media-post/crispr-cas9-gene-drives/> [Accessed 09/02/2022]
2. Wikipedia. Gene Drive. February 2022. https://en.wikipedia.org/wiki/Gene_drive [Accessed 09/02/2022]
3. Matthew Behra, Jing Zhou, Bing Xu, Hongwei Zhang. In vivo delivery of CRISPR-Cas9 therapeutics: Progress and challenges. *Acta Pharmaceutica Sinica B*. 2021;11(8):2150e2171.
4. Cominarty U.S. Physician Prescribing Information (Requires Dilution). <https://labeling.pfizer.com/ShowLabeling.aspx?id=15623> [Accessed 09/02/2022].
5. Dae-Eun Jeong, Matthew McCoy, Karen Artiles, Orkan Ilbay, Andrew Fire, Kari Nadeau, Helen Park, Brooke Betts, Scott Boyd, Ramona Hoh, and Massa Shoura. Assemblies of putative SARS-CoV2-spike-encoding mRNA sequences for vaccines BNT-162b2 and mRNA-1273. *Virological.org*. 2021. <https://virological.org/t/assemblies-of-putative-sarscov2-spike-encoding-mrna-sequences-for-vaccines-bnt-162b2-and-mrna-1273/663> [Accessed 6.1.2022].
6. Novo Pro Labs. Tools. DNA Palindrome. <https://www.novoprolabs.com/tools/dna-palindrome> [Accessed 06/02/2022]
7. ExPASy.org. Translate. <https://web.expasy.org/translate/> [Accessed 06/02/2022].
8. NCBI. ORF Finder. <https://www.ncbi.nlm.nih.gov/orffinder/> [Accessed 06/02/2022]
9. CRISPR SCAN.org. Sequence. <https://www.crisprscan.org/sequence/> [Accessed 06/02/2022]
10. WGE, Stem Cell, Sanger.ac.uk. Search by Sequence. https://wge.stemcell.sanger.ac.uk/search_by_seq [Accessed 06/02/2022]

11. BLAST. NCBI. <https://blast.ncbi.nlm.nih.gov/Blast.cgi> [Accessed 07/02/2022]
12. WHO International Nonproprietary Names Programme. Berthub.EU. <https://berthub.eu/articles/11889.doc> [Accessed 30/12/2021].
13. Youtube. Nonsense mediated and non stop mediated mRNA decay: mRNA surveillance. <https://www.youtube.com/watch?v=UTxiohosPro> [Assecced 02/02/2022].
14. Zigui Chen, Siaw S Boon, Maggie H Wang, Renee W Y Chan, Paul K S Chan, Genomic and evolutionary comparison between SARS-CoV-2 and other human coronavirus nCoV-229E. J Virol Methods. 2021;289:114032.
15. Broadinstitute.News. <https://www.broadinstitute.org/news/8064>. [Assecced 10/02/2022].
16. Endpoints.News.<https://endpts.com/feng-zhangs-quiet-spinout-snares-215m-in-a-race-for-the-next-big-crispr-company/>. [Accessed 10/02/2022].
17. E pluribus unum.Nat Methods.2010; 7:331. <https://doi.org/10.1038/nmeth0510-331>
18. Human Genome Project Information Archive. 2019. https://web.ornl.gov/sci/techresources/Human_Genome/project/index.shtml [Accessed 10/02/2022]

Figures

Figure 1
Cominary mRNA. Highlighted in colour are SARS CoV-2 spike protein sequences.



Figure 2
Highlighted in gray are fragmented spike protein interspaced with short palindromic repeats, which are highlighted in bright blue. Between the short palindromic repeats are open reading frames, which express full functional proteins, here high-lighted in dark and bright pink. The open reading frames have in some cases

modified stop codons TGAT (UGAU), which are highlighted in bright green. Also visible in figure 2 are start codons in dark blue and stop codons in dark green that occur in the middle of the open reading frame, which cause nonsense decay and mRNA degradation. Gray highlighted nucleotides within open reading frames in pink or within short palindrome repeats in bright blue matches in position with the spike protein sequence.

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GAGAATAAACTAGTATTCTTCTGGTCCCCACAGACTCAGAGAGAACC
CGCCACC
ATGTTCTGTTTCTGGTGTCTGCTCTGGTTCAGCCAGTGTGT
GAACCTGACCACCAAGAACACGCTGCCTCCAGCTACACCAACAGC
TTTACAGAGGCGTGTACTACCCGGACAGGTTTCAGATCCAGCGT
GCTGCACTTACCAGGACTGTCTGCTTCTTTCAGCAACGTGA
CCTGGTTCACCGCTCTGAGTCTCCGGACCATTGGCCAGAG
ATTGGACACCCGGTGGCTGCTTCAAGGAGGGGGTACTTTGGCA
GCACCGAGAAGTCCAAATATCAGAGGCTGGATCTTGGCCAC
ACTGGACAGCAAGCCAGAGCTGTGATCTGAAACAACGCCAC
CAACGTGGTCAATAAAGTGGCGAGTTCAGTTCGCAAGCACCCC
TTCTTGGGGTCTACTACCACAGAAACAACAGAGCTGTCTGAAA
CTCAGTTCACCGCTTGTAGCAACCCCAAAATCTCCAGTTCAGTA
CGTGTCCAGCCCTTCTGAGCGACCTGGAAGCAAGAGGGCAAC
TTCAGAACCTGGCGAGTCTGTTAAGAAATCTGACGGTACTT
CAAGATCTACAGCAAGCAACCCCTAACCTCCTGTCGGGATCTGC
CTCAGGGTTCTCTGCTCTGGAACCCCTGGTGGATCTGCCATCGGC
ATCAATACACCCGGTTCTGCACTGGCTGGCCCTGCAAGAACTA
CTTGACCCTGGCGTAGCAGCACCGGATGGCACTGTGGTGGCC
GCTTACATGTGGGCTACTCGAGCTGAGAACCCTCTCTGAGTA
CAACGAGAACGGCACCATCACCGAGCCGTGGATGTCTTGGAT
CCTCTGAGGGAGACAAAGTCACCTGAACTCTACCCGTGGAAA
AGGGCATCTACAGACCAGCAACTTCCGGGTGACGCCACCGAATC
CATGTGGCTTCCCAATACCAACTGTGGCCCTTGGCGAGG
TGTTCAATCTCTGAGATTTGGCTCTGTCTTGGAAAGGAA
CGGGATCAGCCTTGGGTGGCGAGTACTCTGGTGTGACACTCCG
CCAGCTTACGACCTTCAAGTGCTAAGGTGCCCTAAGCTTCCCT
GACGACCTGTGGCTTCAAAAGGTGACCGCGACAGCTCTGTATCC
GGGGAGTTGAAAGTGGCGAGATGGCCCTGTCTGACAGGCAAG
TTGGCGATACAGTACAGTAAAGCTGCGCGAGCACTTGGCTGGT
GATTGGCTGGAACGCAAACTGGATCTCCAAAAGTCGGCGCAAC
TACAATTACTGTACCGGCTTCGGGAAGTCAAATGAAGCCCTT
CGAGCGGACATCTCCACGAGATCTATCAGCGCGGAGCCCTT
GTAACGGCGTGGAAGGCTTCAACTGTACTTCCACTGCAGTCTTAC
GGCTTCTAGCCCAAAATCTGGTGGGATCAGCCCTACAGAGTGT
GGCTCTTCACTTGGATGCTGATGCTGGCGCACTTGGGGAG
CTTAAGAAGCACCTCTCTGGAAGAACAAATGGGTAACTTCA
ACTTCAACGGCTTAGCCGGACCCGGTGTTAGCAGAGAGCAACA
AGAAAGTCTCCATTCCAGCAGTTTGGCCGGGATATCGCGGATACC
ACAGACCGCGTTTAGATCCCCAGACACTGGAATCTGGACATCA
CTGGTTCAGCTTGGCGGAGTGTCTGTGATCACTTGGCACTA
BACACGAACTCAGTGTGATACAGACGAGCTGACTGATC
GAAGTCCCGTTGGCATTACCGCGATAGCTGACACCTCATGGCG
GGTGTACTCCACCGGCAGACTGTTTTCAGACAGAGCCGGCTG
TCGGAGCCGAGCACGTGACAAATAGCTACGAGTGGGACATCC
CGGGTTGAAATCTGCCCGAGTTACAGACACAGAAACAGCCCT
CGAGATCGAGATCTGGCAGCCAGAGCTTGTCTTCTAGCA
TTCTCTGAGCTCGGACAGCACTGCTCTCACAAACTAT
CGTATCCCAAACTTACCACTAGCTGACAGAGATCTCTG
CTGTCTATGACCAAGACCGAGCTGGATGCACATGTACTG
GGGATCCACCGAGTCTCACAACTGTGCTGAGTACGGCAGCT
TCTGCACTCAGCTAATAGAGCCCTGACGGGATCGCTGTGAACA

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GTACAAGAAACCCAAGAGTGTGCCCAAGTGAAGCAGATCTA
CAAGACCCCTCTATAAAGACTTCCGGCTTCAATTTCAG
CCAGATTTGCCCGATCTAGCAAGCCAGCAAGCGGAGTTCATCG
AGGACCTGTGTTCAAAAACACTGGCCGACCGGGTTCAT
CAAGCAGTATGGCGATTGCTGGCGACTTTGCCGAGGATCTG
TTTGGCCCAAGAGTTTAAGGACGCACTTGTCTCTCTTGGC
ACCGATGAGTGACCACAGAGATTTGCCCTCTGCTGGCA
CAATCAAAAGCGCTGACATTTGGAGAGGCCGCTCTGAGAT
CCCTTTGCTATCCAGATGCCTACCGGTTCAACGGATCGGAGTGA
CCGAATGTGCTGTAGGAGAAACAGCAAGCCTCGCACAACAGTT
CAACAGCGCCATCGCAAGATCCAGGACAGCTGAGCAGCACAGCA
AGCCCCCTGGGAAAGTTGAGGACGTGTTAACAATGGGCA
TCAGCTCTGTGTGACGATATCTGAGCAGCTGGACCTCTGAG
GCCAGGTGCAGATCGACAGATGATCAAGGCAAGCTGAGAGCC
TCCAGACATAGTGACCAGCAGCTGATCGCCGGCGAGATAG
AGCCCTGCAATCTGCCGCACAGATCTGAGTGTGGTGTG
GGCCAGAGTAAGAGATGGACTTTCCGCAAGGGTTACCACTG
TGACCTTCCCTAGTTTGGCTACCGCGGTGGTTCTGAGGTG
ACATAGTCTTGTCTAAGAGATTTCACCACCTTCAAGCTT
ATCGCCAGGCCAAGCTTCGTTGAGAGAGGGCGGTG
GTTTCAACGGCACTTGGTCGTTGACAGGGGAACGTCTCTAC
GAGCCAGATCACTCACCGCAACACTTTCTGTCTGTGCACT
CGACCTGTGATCTGGATCTGAAACCTTTGCACTCTT
CAGCCGAGCTGACAGTTGAAAGAGAAATTTGACAAGTAA
AGAACCACAAGCCCGAGTGGACCTGGCGATATAGGGAAAT
CAATGCCAGCGCTGACATCAAGAAAGATGCACGCGCTAC
GAGGTGCCAGAATTAAGAGAGCTGATCACTCAAGAA
TTGGGAAGTACAGAGTCACTAAGTTGGCTGGTACATCTGG
CGKTTATCCGGACTATGGCTGATGGTCAATATGT
TTGTTGTACCTCTGAGCTGGTTGAGGGCTGTTTAG
TTGGCACTCTGCAAGTCAAGGGAGCTGAGCCG
TGAAGGGCTGAACTGCAATGATGATCTGAGCTGGACT
GCATGCACGCAGTGCCCTTCCCGCTCTGGCTTACCCCGA
GTCTCCCGACTCGGGTCCAGATGCTCCCACTCCACTGGC
CCACTCACTCTGTAGTTCAGCACTCCCAAGCACGG
AATGCACTCAAACGTTGGCTAGCGAGCCCACTGGAAACAG
CAGTATAACTTTACAATAAAGAAAGTTAACAAGCTATACT
AACCCAGGGTTGTGCAATTCTGGCACGCACTGGAGCTAG
CA

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STOP Codon TGA Modified to **TGAT**

gRNA **GCTAGCTGGCCCTTTCCCTGCTGGG** CAS9 21bp ID 1161208236

gRNA **GCAATAAACCGAAAGTTAACTAA** CAS12a 23bp

gRNA **AACTAAGCTATACTAACCCAGGG** CAS12a 23bp ID 1202628979

gRNA **AGCTATACTAACCCAGGGT** CAS9

gRNA **TTGGCTAGCCCTGCTGG** CAS9 21bp ID 992837047

PAM **GGG**

Repeating Palindromes **CTGCAG**

Reading frame 2

Reading frame 3

Figure 3. Between two palindrome repeats in bright blue appear interspaced spike protein fragments as an example for a DNA spacer. Gray highlighted nucleotides within the palindromes in bright blue matches in position the spike protein sequence. The DNA spacer is followed by an open reading frame for a protein that has 58% analogy with the HoxA4 protein, here highlighted in pink. This fully synthetic protein contains mainly of spike protein amino acids.

TCCAGACATACG TGA CCCAG CAGC TGATCA GAGCCGCCGAGAT TAG
 AGCCTCTGCCAATCTGGCCGCCACCAAG ATGTC T GAGTGTGTGCTG
 GGCCAGAGCAAGAGAGTGGACTTTTGGGCAAGGGCTACCACCTGA
 TGAGCTTCCCTCAGTCTGCCCTCACGGCGTGGTGTTC TG CACGTG
 ACATATGTGCCCGCTCAAGAGAAGAATTCACCACCGCTCCAGCC
 ATCTGCCACGACGGCAAAGCCCACTTTCCTAGAGAAGGCGTGTTC
 GTGTCCAACGGCACCCAT TGGTTCG TGA CACAGCGGAATTCTAC

Figure 4
 Human Coronavirus protein
 without any analogy to the spike
 protein. The stop codon UGAU is
 modified.

CCTGGTCCACGCCATC CACGTG TCCGGCACCA ATGGCACCAAGAG
 ATTCGACAACCCCGTGTGCCCTTCAACGACGGGGTGTACTTTGCCA
 GCACCGAGAAGTCCAACATCATCAGAGGCTGGATCTTCGGCACCCAC
 ACTGGACAGCAAGACCCAGAGCCTGCTGATCGTGAACAACGCCAC

Figure 5
 BLAST result for Human
 Coronavirus HKU1 isolate N5

RecName: Full=Replicase polyprotein 1a; Short=pp1a; AltName: Full=ORF1a polyprotein; Contains: RecName: Full=Non-structural protein 1; Short=nsP1; AltName: Full=p28; Contains: RecName: Full=Non-structural protein 2; Short=nsP2; AltName: Full=p65; Contains: RecName: Full=Non-structural protein 3; Short=nsP3; AltName: Full=PL1-PRO/PL2-PRO; AltName: Full=PL1/PL2; AltName: Full=Papain-like proteinases 1/2; AltName: Full=p210; Contains: RecName: Full=Non-structural protein 4; Short=nsP4; AltName: Full=Peptide HD2; AltName: Full=p44; Contains: RecName: Full=3C-like proteinase; Short=3CL-PRO; Short=3CLp; AltName: Full=M-PRO; AltName: Full=nsP5; AltName: Full=p27; Contains: RecName: Full=Non-structural protein 6; Short=nsP6; Contains: RecName: Full=Non-structural protein 7; Short=nsP7; AltName: Full=p10; Contains: RecName: Full=Non-structural protein 8; Short=nsP8; AltName: Full=p22; Contains: RecName: Full=Non-structural protein 9; Short=nsP9; AltName: Full=p12; Contains: RecName: Full=Non-structural protein 10; Short=nsP10; AltName: Full=Growth factor-like peptide; Short=GFL; AltName: Full=p15; Contains: RecName: Full=Non-structural protein 11; Short=nsP11 [Human coronavirus HKU1 (isolate N5)]
 Sequence ID: POC6U5.1 Length: 4421 Number of Matches: 4
 Range 1: 2781 to 2795

Score	Expect	Identities	Positives	Gaps	Frame
26.9 bits(56)	9.5()	11/15(73%)	11/15(73%)	1/15(6%)	
Query 12	TP-SLKGSTGLSNLL 25				
Sbjct 2781	TP-SLKG LSNLL 2795				

Figure 6
 Open Reading Frame that
 translates into a Hypothetical
 Protein. Stop codon has been
 modified and matches in
 nucleotide position with the spike
 protein.

.ATGTGCTGTACGAGAACCAGAAGC TGA

Figure 7
BLAST result for the Hypothetical Protein

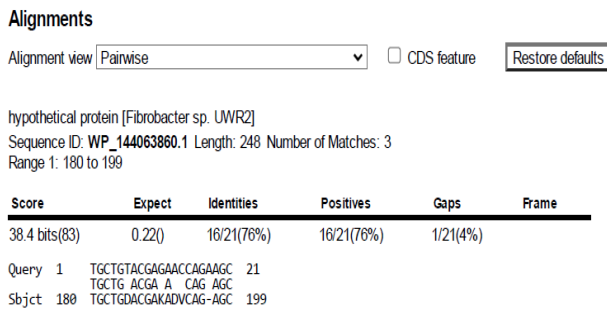


Figure 8
BLAST result for fully synthetic protein with 96% analogy to a mutant measles virus strain.

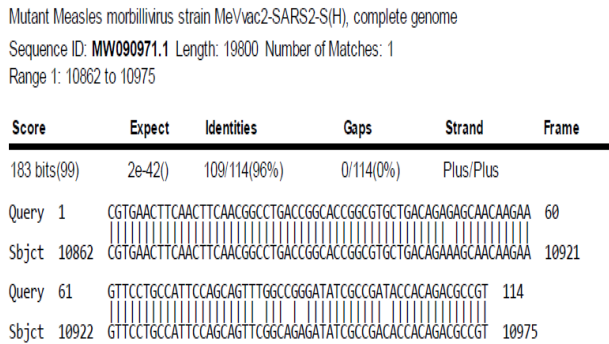


Figure 9
BLAST result for another fully synthetic spike protein with 98% analogy to a mutant measles virus strain.

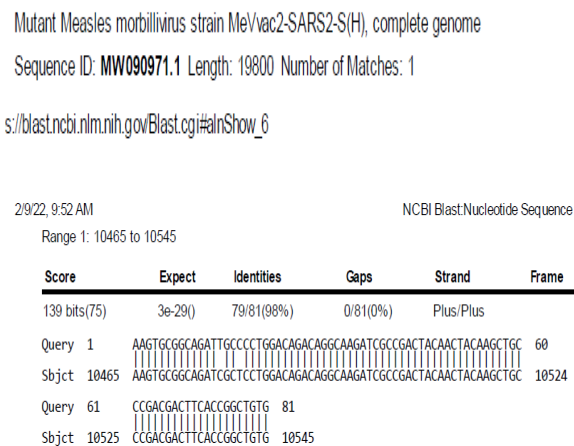


Figure 10 FASTA result for Hox A-4 protein

Statistics: Expectation_n fit: $\rho(\ln(x)) = 3.9134 \pm 0.000885$; $\mu = 10.9802 \pm 0.050$
 mean var=42.5317 +/- 8.173, 0's: 3 Z-trim(108.2): 8 B-trim: 134 in 1/60
 Lambda= 0.196661
 statistics sampled from 6390 (6391) to 6390 sequences
 Algorithm: FASTA (3.8 Nov 2011) [optimized]
 Parameters: BL50 matrix (15:-5), open/ext: -10/-2
 ktup: 2, E-join: 1 (0.873), E-opt: 0.2 (0.486), width: 16
 Scan time: 0.160

The best scores are:

		opt bits	E(13144)	%_id	%_sim	alen		
sp Q00056 HXA4_HUMAN	Homeobox protein Hox-A4; Homeobox (320)	78	28.0	0.57	0.412	0.588	34	align
sp P08050 CXA1_RAT	Gap junction alpha-1 protein; Connex (382)	73	26.6	1.8	0.423	0.692	26	align
sp P23242 CXA1_MOUSE	Gap junction alpha-1 protein; Conn (382)	73	26.6	1.8	0.423	0.692	26	align
sp P18246 CXA1_BOVIN	Gap junction alpha-1 protein; Conn (383)	73	26.6	1.8	0.423	0.692	26	align
sp P06485 US02_HMV11	Protein US2 (291)	70	25.7	2.6	0.308	0.577	26	align
sp P01425 WNS1_HEMHA	Short neurotoxin 1; Toxin II (61)	62	22.9	3.7	0.412	0.824	17	align
sp P01426 WNS1_NA3PA	Short neurotoxin 1; Neurotoxin alp (61)	62	22.9	3.7	0.412	0.824	17	align
sp P60775 WNS1_LATSE	Eradutoxin a; Short neurotoxin 1a; (83)	62	23.0	4.7	0.412	0.824	17	align

Figure 11 CRISPR gRNA result for ID1161208236

ID: 1161208236

[View in Genome Browser \(https://wge.stemcell.sanger.ac.uk/genoverse_browse?view_single=1&crispr_id=1161208236\)](https://wge.stemcell.sanger.ac.uk/genoverse_browse?view_single=1&crispr_id=1161208236)

Species Human (GRCh38)
 Location 19:3053386-3053408 (https://www.ensembl.org/Homo_Sapiens/Location/View?r=19:3053386-3053408)
 Sequence AGCTGCCCTTTCCCGTCCT GGG (reverse)
 Strand -
 Crispr in exon? Yes
 Crispr in intron? No

Figure 12 CRISPR gRNA result for ID 992837047

ID: 992837047

[View in Genome Browser \(https://wge.stemcell.sanger.ac.uk/genoverse_browse?crispr_id=992837047&view_single=1\)](https://wge.stemcell.sanger.ac.uk/genoverse_browse?crispr_id=992837047&view_single=1)

Species Human (GRCh38)
 Location 5:80651907-80651929 (https://www.ensembl.org/Homo_Sapiens/Location/View?r=5:80651907-80651929)
 Sequence TTAGCCTAGCCACACCCCA CGG (reverse)
 Strand -
 Crispr in exon? No
 Crispr in intron? Yes

Figure 13 CRISPR gRNA result for ID 1202628979

ID:
1202628979

[View in Genome Browser \(https://wge.stemcell.sanger.ac.uk/genoverse_browse?view_single=1&crispr_id=1202628979\)](https://wge.stemcell.sanger.ac.uk/genoverse_browse?view_single=1&crispr_id=1202628979)

Species Human (GRCh38)
 Location M:851-873 (https://www.ensembl.org/Homo_Sapiens/Location/View?r=M:851-873)
 Sequence AACTAAGCTATACTAACCC AGG
 Strand +
 Crispr in exon? No
 Crispr in intron? No

Figure 14
Corresponding BLAST result for
CRISPR ID 1161208236 on
Chromosome 19 of GRCH38

Homo sapiens chromosome 19, GRCh38.p13 Primary Assembly

Sequence ID: **NC_000019.10** Length: 58617616 Number of Matches: 135

Range 1: 3053386 to 3053408

Score	Expect	Identities	Gaps	Strand	Frame
46.1 bits(23)	3e-04()	23/23(100%)	0/23(0%)	Plus/Minus	

Features:
24482 bp at 5' side: transducin-like enhancer protein 2 isoform x2411 bp at 3' side: tle family member 5 isoform x1

```
Query 1      AGCTGCCCCCTTCCCGTCCTGGG 23
            |||
Sbjct 3053408 AGCTGCCCCCTTCCCGTCCTGGG 3053386
```

Figure 15
Corresponding BLAST result for
CRISPR ID 992837047 on
Chromosome 5 of GRCH38

Homo sapiens chromosome 5, GRCh38.p13 Primary Assembly

Sequence ID: **NC_000005.10** Length: 181538259 Number of Matches: 223

Range 1: 80651907 to 80651929

Score	Expect	Identities	Gaps	Strand	Frame
46.1 bits(23)	3e-04()	23/23(100%)	0/23(0%)	Plus/Minus	

Features:
dihydrofolate reductase isoform 1dihydrofolate reductase isoform 3

```
Query 1      TTAGCCTAGCCACACCCACGCG 23
            |||
Sbjct 80651929 TTAGCCTAGCCACACCCACGCG 80651907
```

Figure 16
Corresponding BLAST result for
CRISPR ID 1202628979 on
Chromosome 5 of GRCH38

Homo sapiens chromosome 5, GRCh38.p13 Primary Assembly

Sequence ID: **NC_000005.10** Length: 181538259 Number of Matches: 133

Range 1: 80651848 to 80651866

Score	Expect	Identities	Gaps	Strand	Frame
38.2 bits(19)	0.061()	19/19(100%)	0/19(0%)	Plus/Minus	

Features:
dihydrofolate reductase isoform 1dihydrofolate reductase isoform 3

```
Query 1      AACTAAGCTATACTAACCC 19
            |||
Sbjct 80651866 AACTAAGCTATACTAACCC 80651848
```