



Universidade de São Paulo – USP
Faculdade de Medicina de Ribeirão Preto – FMRP
Ciências Biomédicas – Turma IX
RCB300 – Tópicos em Biotecnologia III (2024)

Desenvolvimento de vacina de mRNA contra Sars-CoV-2 atual

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- 2) Desenho experimental (indicar programas)
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- 5) HBA1: identificação da região 5'UTR, CDS e 3'UTR; + como fica o cDNA com essas regiões;
- 6) Otimização de códons (o que é e resultado)
- 7) Vienna RNA estrutura da spike pré e pós otimização + análise da energia livre mínima (comparar as duas)
- 8) ExPasy (tradução do cDNA) da com mutação e sem mutação; Sequência de AA com a região RBD e mutações;
- 9) Predição de epítomos de anticorpos: <http://tools.iedb.org/bcell/>. Determinar a predição de epítomos da proteína spike codificada por ambas vacinas de mRNA, antes e após a adição das mutações missense 2P. Pergunta: há diferença no número de epítomos?
- 10) Predição de epítomos de MHC classe I: <http://tools.iedb.org/mhci/>. Coloque a sequência da proteína Spike e clique em Select HLA allele reference set e em seguida clique em submit;
- 11) predição de epítomos de MHC classe II + resultados
- 12) Conclusão e recap

1. Introdução

Pandemia COVID-19

31/12/2019 - a Organização Mundial da Saúde (OMS) foi alertada sobre vários casos de pneumonia na República Popular da China. Tratava-se de uma nova cepa de coronavírus.

07/01/2020 - autoridades chinesas confirmaram que haviam identificado um novo tipo de coronavírus.

Brasil

Sem conter pandemia, Brasil perde 100 mil vidas para Covid

Vacinas de RNA

As vacinas de RNA utilizam uma molécula de RNA para fornecer instruções ao corpo sobre como produzir proteínas específicas, desencadeando uma resposta imunológica capaz de produzir anticorpos contra o antígeno, garantindo imunização contra futuras infecções do patógeno.

INOVAÇÕES NA SAÚDE

Entenda a revolução das vacinas de RNA mensageiro, que fizeram pesquisadores receber o Prêmio Nobel de Medicina 2023

Técnica utilizada na pandemia pode auxiliar o controle de outras doenças

Vacinas de RNA

Análise > Alvo de transdução de sinal.23 de março de 2022;7(1):94.

doi: 10.1038/s41392-022-00950-y.

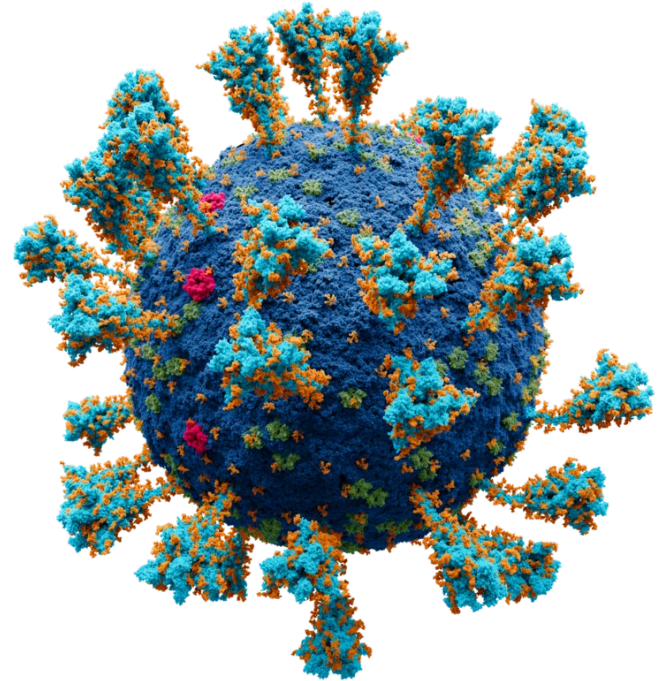
Avanços no desenvolvimento da vacina mRNA contra a COVID-19

Enyue Fang ^{# 1 2}, Xiao Hui Liu ^{# 1}, Miao Li ^{# 1}, Zelun Zhang ¹, Canção de Lifang ¹, Bai Yu Zhu ³, Xiaohong Wu ¹, Jing Jing Liu ¹, Danhua Zhao ¹, Yuhua Li ⁴

Vantagens: curto ciclo de desenvolvimento, fácil industrialização, processo de produção simples, flexibilidade para responder a novas variantes e capacidade para induzir uma melhor resposta imunológica.

Proteína Spike

- 1.200 a 1.400 resíduos de aminoácidos de comprimento;
- Glicoproteína Spike é altamente imunogênica;
- A função da glicoproteína spike é mediar a entrada viral na célula hospedeira, primeiro interagindo com moléculas na superfície externa da célula e depois fundindo as membranas viral e celular



Solodovnikov, Alexei; Arkhipova, Valeria (29 de julho de 2021)

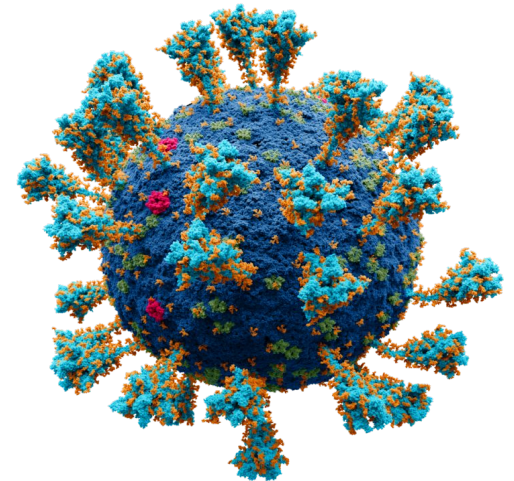
Proteína Spike

Covid: vacinas Pfizer e Moderna são as mais eficazes para reforço, indica estudo

Philippa Roxby
Repórter de saúde

3 dezembro 2021

BBC News, 2021



Solodovnikov, Alexei; Arkhipova,
Valeria (29 de julho de 2021)

2. Metodologia

Virus detail	
Virus name:	hCoV-19/Belgium/rega-47745/2023
Accession ID:	EPI_ISL_18492450
Type:	betacoronavirus
Clade:	GRA
Pango Lineage:	JN.1.1 (Pango v.4.3.1 consensus call), Omicron (BA.2-like) (Scorpio)
AA Substitutions:	Spike A27S, Spike A264D, Spike A570V, Spike D405N, Spike D614G, Spike D796Y, Spike E404K, Spike E554K, Spike F157S, Spike F406P, Spike G142D, Spike G339H, Spike G446S, Spike H69del, Spike H245N, Spike H655Y, Spike I332V, Spike ins16MPLF, Spike K356T, Spike K417N, Spike L24del, Spike L212I, Spike L216F, Spike L452W, Spike L455S, Spike N211del, Spike N440K, Spike N450D, Spike N460K, Spike N481K, Spike N501Y, Spike N679K, Spike N764K, Spike N969K, Spike P25del, Spike P26del, Spike P621S, Spike P681R, Spike P1143L, Spike Q498R, Spike Q954H, Spike R21T, Spike R158G, Spike R403K, Spike R408S, Spike S50L, Spike S371F, Spike S373P, Spike S375F, Spike S477N, Spike S939F, Spike T19I, Spike T376A, Spike T478K, Spike V70del, Spike V127F, Spike V213G, Spike V445H, Spike V483del, Spike Y144del, Spike Y505H, E T9I, M A63T, M A104V, M D3H, M Q19E, M T30A, N E31del, N G204R, N P13L, N Q229K, N R32del, N R203K, N S33del, N S413R, NS3 T223I, NS7a R25K, NS7b F19L, NSP1 S135R, NSP2 A31D, NSP2 F319L, NSP3 A1892T, NSP3 G489S, NSP3 K1155R, NSP3 N1708S, NSP3 T24I, NSP3 V238L, NSP4 L264F, NSP4 T327I, NSP4 T492I, NSP5 P132H, NSP6 F108del, NSP6 G107del, NSP6 R252K, NSP6 S106del, NSP6 V24F, NSP9 T35I, NSP12 P323L, NSP13 R392C, NSP14 I42V, NSP15 T112I
Variant:	VOI GRA (JN.1+JN.1.*) first detected in Luxembourg/Iceland
Passage details/history:	Original
Sample information	
Collection date:	2023-11-04
Location:	Europe / Belgium / Lubbeek
Host:	Human
Additional location information:	Other: Postal Code:3210
Gender:	Female
Patient age:	87
Patient status:	unknown
Specimen source:	Oropharyngeal swab
Additional host information:	
Sampling strategy:	baseline surveillance
Outbreak:	
Last vaccinated:	
Treatment:	
Sequencing technology:	Nanopore MinION
Assembly method:	Artic Network methods
Coverage:	140x (average)x
Comment:	◊ Insertion of 12 nucleotides when compared to the reference WIV04 sequence. Gap of 42 nucleotides when compared to the reference WIV04 sequence.

TGGAATGCTGATCTTTATAAGCTCATGGGACACTTCGCATGGTGGACAGCCTTTGTTACTAATGTGAATGCGTCATCATC
TGAAGCATTTTTAATTGGATGTAATTATCTTGGCAAACCACGCGAACAAATAGATGGTTATGTCATGCATGCAAATTACA
TATTTTGGAGGAATACAAATCCAATTCAGTTGTCTTCCTATTCTTTATTTGACATGAGTAAATTTCCCCTTAAATTAAGG
GGTACTGCTGTTATGTCTTTAAAAGAAGGTCAAATCAATGATATGATTTTATCTCTTCTTAGTAAAGGTAGACTTATAAT
TAGAGAAAACAACAGAGTTGTTATTTCTAGTGATGTTCTTGTTAACAACTAAACGAACAATGTTTGTTTTTCTTGTTTTA
TTGCCACTAGTCTCTAGTCAGTGTGTCATGCCGCTGTTTAATCTTATAACTACAACCTCAATCATACTAATTCTTTTAC
ACGTGGTGTATTACCCTGACAAAGTTTTTCAGATCCTCAGTTTTACATTTAACTCAGGACTTGTTCTTACCTTTCTTTT
CCAATGTTACTTGGTTCATGCTATCTCTGGGACCAATGGTACTAAGAGGTTTGATAACCCTGTCTACCATTTAATGAT
GGTGTTTATTTTGCTTCCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTTGGTACTACTTTAGATTCGAAGACCCA

Primer Show

Primer Show

Primer Show accepts a DNA sequence along with a set of primer sequences and returns a textual map showing the annealing positions of the primers. The translation of the DNA sequence can be shown in the reading frames you specify. You can also choose the number of bases per line of the map, and whether to show the DNA in its single-stranded or double-stranded form. The primer sequences you enter can contain "wild card" bases, a feature that allows Primer Show to handle degenerate primers. Use this program to produce a useful reference figure, particularly when you have designed a large number of primers for a particular template.

Paste the raw or FASTA sequence into the text area below.

```
>mRNA /gene="fem-2" (exons in uppercase)
gaacgcgaatgcctctctctcttctgatgggatgcccaattgtccacattcactcgtgtt
gcctctcttttccaacacgcaagaccagaaacgcgtcaaccaagagaaaaagacgc
cgacaacgggcagcactcgcgagagacaaaggttatcgcgttggttattatacattcgc
atccgggtcaacttttagtccttgaacatgctcttggaaaacctagttctcttaaaataa
cgttttagaagttttggtcttcagATGTCGTATTGCTAAATCATCCATCGAGTTCTACG
```

Enter the patterns in the 5' to 3' direction. An example pattern is:

/ac[gt]agcct/ (My pattern's name). The two slashes mark the boundary of the pattern and the round brackets surround the name of the pattern. The square brackets surround possible bases at a degenerate site. You can enter multiple patterns separated by commas. Primer Show automatically constructs a reverse-complement version of each primer sequence so that matches on the reverse strand can be shown. Incorrect entry of the patterns may produce errors.

```
/ATGTCGTATTGCTAAATCATCC/ (PS1),
/CTATTGTCATCTTCTTCC/ (PS2),
/AT[AC]GT[CG]ATTGGATG[CG]ATATTGG/ (PS3),
/aacagctatgaccatg/ (reverse primer),
/attaaccctcactaaag/ (T3 primer),
/cgaggtcgacggatcgc/ (KS primer),
```

SUBMIT CLEAR

- Show bases per line.
- Show the translation for

HGNC
HUGO Gene Nomenclature Committee

The resource for approved human gene nomenclature

GeneCards[®]
THE HUMAN GENE DATABASE

NIH National Library of Medicine
National Center for Biotechnology Information

Gene [Advanced](#) [Help](#) [Log in](#)

Gene

Gene integrates information from a wide range of species. A record may include nomenclature, Reference Sequences (RefSeqs), maps, pathways, variations, phenotypes, and links to genome-, phenotype-, and locus-specific resources worldwide.

ViennaRNA Web Services
Institute for Theoretical Chemistry

■ Structure prediction ■ Folding Kinetics ■ Sequence Design ■ ncRNA Detection ■ Genome Wide Screening ■ Other

You are here: / RNA Font size:

The ViennaRNA Web Services

This server provides programs, web services, and databases, related to our work on RNA secondary structures. For general information and other offerings from our group see the main TBI homepage.

To help us providing you with even better services please take the time to rate us at [SurveyMonkey](#)

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IDT
INTEGRATED DNA TECHNOLOGIES

[Order by stock part number »](#)

PRODUCTS & SERVICES ▾ APPLICATIONS & SOLUTIONS ▾ SUPPORT & EDUCATION ▾

Codon Optimization Tool

Synthetic gene design made easy

The Codon Optimization Tool converts the DNA, or protein sequence, from one organism for expression to another. The IDT algorithm provides the best sequence option by screening and filtering sequences to lower complexity and minimize secondary structures.

OMIM[®]

An Online Catalog of Human Genes and Genetic Disorders

Updated May 10th, 2024

Translate tool

Translate is a tool which allows the translation of a nucleotide (DNA/RNA) sequence to a protein sequence.

DNA or RNA sequence

Please enter a DNA or RNA sequence - numbers and blanks are ignored

Output format

- Verbose: Met, Stop, spaces between residues
- Compact: M, -, no spaces
- Includes nucleotide sequence
- Includes nucleotide sequence, no spaces

DNA strands

- forward
- reverse

Genetic codes - [See NCBI's genetic codes](#)

Standard

reset

TRANSLATE!

Check out our new IEDB updates! (1) Learn how to [customize your database exports](#) and (2) test out the new [Next-generation Tools site](#) for all your analysis and prediction needs.

Welcome

The Immune Epitope Database (IEDB) is a freely available resource funded by NIAID. It catalogs experimental data on antibody and T cell epitopes studied in humans and other animal species in the context of infectious disease, allergy, autoimmunity and transplantation. The IEDB also hosts epitope prediction and analysis tools, and has a companion site, CEDAR (funded by NCI), which houses cancer epitopes.

[Learn More](#)

Upcoming Events & News

[Virtual User Workshop](#) Nov 1-3, 2023
 * recordings [here](#)

AACR 2024 Apr 5-10, 2024

[Festival of Biologics](#) Apr 15-17, 2024
 * [free pass registration](#)

AAI 2024 May 3-7, 2024

Summary Metrics

Peptidic Epitopes	1,613,026
Non-Peptidic Epitopes	3,188
T Cell Assays	513,389
B Cell Assays	1,403,188
MHC Ligand Assays	4,803,225
Epitope Source Organisms	4,469
Restricting MHC Alleles	1,006
References	24,735

START YOUR SEARCH HERE

Epitope ?

- Any
- Linear peptide
- Discontinuous
- Non-peptidic

Exact M



Assay ?

- T Cell
- B Cell
- MHC Ligand

Ex: neutralization

Outcome: Positive Negative



Epitope Source ?

Organism

Ex: influenza, peanut



Antigen

Ex: core, capsid, myo:

MHC Restriction ?

- Any
- Class I
- Class II
- Non-classical

Ex: HLA-A*02:01



Host ?

- Any
- Human
- Mouse
- Non-human primate

Ex: dog, camel



Disease ?

- Any
- Infectious
- Allergic
- Autoimmune

Ex: asthma



Epitope Analysis Resource

T Cell Epitope Prediction ?

Scan an antigen sequence for amino acid patterns indicative of:

MHC I Binding

MHC II Binding

MHC I Processing (Proteasome, TAP)

MHC I Immunogenicity

B Cell Epitope Prediction ?

Predict linear B cell epitopes using:

Antigen Sequence Properties

Predict discontinuous B cell epitopes using antigen structure via:

Discotope

EliiPro

Epitope Analysis Tools ?

Analyze epitope sets of:

Population Coverage

Conservation Across Antigens

Clusters with Similar Sequences

3. Resultados



Identificação da Spike no genoma

Virus detail

Virus name:	hCoV-19/Belgium/rega-47745/2023
Accession ID:	EPI_ISL_18492450
Type:	betacoronavirus
Clade:	GRA
Pango Lineage:	JN.1.1 (Pango v.4.3.1 consensus call), Omicron (BA.2-like) (Scorpio)
AA Substitutions:	Spike A27S, Spike A264D, Spike A570V, Spike D405N, Spike D614G, Spike D796Y, Spike E484K, Spike E554K, Spike F157S, Spike F486P, Spike G142D, Spike G339H, Spike G446S, Spike H69del, Spike H245N, Spike H655Y, Spike I332V, Spike ins16MPLF, Spike K356T, Spike K417N, Spike L24del, Spike L212I, Spike L216F, Spike L452W, Spike L455S, Spike N211del, Spike N440K, Spike N450D, Spike N460K, Spike N481K, Spike N501Y, Spike N679K, Spike N764K, Spike N969K, Spike P25del, Spike P26del, Spike P621S, Spike P681R, Spike P1143L, Spike Q498R, Spike Q954H, Spike R21T, Spike R158G, Spike R403K, Spike R408S, Spike S50L, Spike S371F, Spike S373P, Spike S375F, Spike S477N, Spike S939F, Spike T19I, Spike T376A, Spike T478K, Spike V70del, Spike V127F, Spike V213G, Spike V445H, Spike V483del, Spike Y144del, Spike Y505H, E T9I, M A63T, M A104V, M D3H, M Q19E, M T30A, N E31del, N G204R, N P13L, N Q229K, N R32del, N R203K, N S33del, N S413R, NS3 T223I, NS7a R25K, NS7b F19L, NSP1 S135R, NSP2 A31D, NSP2 F319L, NSP3 A1892T, NSP3 G489S, NSP3 K1155R, NSP3 N1708S, NSP3 T24I, NSP3 V238L, NSP4 L264F, NSP4 T327I, NSP4 T492I, NSP5 P132H, NSP6 F108del, NSP6 G107del, NSP6 R252K, NSP6 S106del, NSP6 V24F, NSP9 T35I, NSP12 P323L, NSP13 R392C, NSP14 I42V, NSP15 T112I
Variant:	VOI GRA (JN.1+JN.1.*) first detected in Luxembourg/Iceland
Passage details/history:	Original

S surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]

[Download Datasets](#)

Gene ID: 43740568, updated on 7-May-2024

Summary

Gene symbol	S
Gene description	surface glycoprotein
Locus tag	GU280_gp02
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Severe acute respiratory syndrome coronavirus 2 (isolate: Wuhan-Hu-1, nat-host: Homo sapiens)
Lineage	Viruses; Riboviria; Orthornavirae; Pisuviricota; Pisoniviricetes; Nidovirales; Coronidovirineae; Coronaviridae; Orthocoronavirinae; Betacoronavirus; Sarbecovirus
Also known as	spike glycoprotein
Summary	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, positive-sense, single-stranded RNA virus that causes coronavirus disease 2019 (COVID-19). Virus particles include the RNA genetic material and structural proteins needed for invasion of host cells. Once inside the cell the infecting RNA is used to encode structural proteins that make up virus particles, nonstructural proteins that direct virus assembly, transcription, replication and host control and accessory proteins whose function has not been determined.~ The structural proteins of SARS-CoV-2 include the envelope protein (E), spike or surface glycoprotein (S), membrane protein (M) and the nucleocapsid protein (N). The spike glycoprotein is found on the outside of the virus particle and gives coronavirus viruses their crown-like appearance. This glycoprotein mediates attachment of the virus particle and entry into the host cell. S protein is an important target for vaccine development, antibody therapies and diagnostic antigen-based tests.

NEW[Try the new Gene table](#)[Try the new Transcript table](#)

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

GTTAACAACATAAAGCAAAATGTTTGTCTTTCTGTTTATTGCCACTAGTCTCTAGTC 21540
-----ATGTTTGTCTTTCTGTTTATTGCCACTAGTCTCTAGTC 41

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

GTGTGTCATGCCGCTGTTTAACTTATAACTACAACCTCAATCATACTAATTCTTTCA 21600
GTGTGTTAATCTTACAACCAGA---ACTCAATTACCCCTGCATACACTAATTCTTTCA 98
***** * * * * * * * *

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

ACGTGGTGTATTACCCTGACAAAAGTTTTAGATCCTCAGTTTTACATTTAACTCAGG 21660
ACGTGGTGTATTACCCTGACAAAAGTTTTAGATCCTCAGTTTTACATTTAACTCAGG 158

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

CTTGTCTTACCTTTCTTTTCCAATGTTACTTGGTCCATGCTAT-----CTCTGGGA 21714
CTTGTCTTACCTTTCTTTTCCAATGTTACTTGGTCCATGCTATACATGCTCTCTGGGA 218

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

CAATGGTACTAAGAGGTTTGATAACCCCTGCTACCATTAAATGATGGTGTATTATTG 21774
CAATGGTACTAAGAGGTTTGATAACCCCTGCTACCATTAAATGATGGTGTATTATTG 278

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

TTCCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTGGA 21834
TTCCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTGGA 338

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

GACCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTTTATTAAAGTCTGTGAAT 21894
GACCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTTTATTAAAGTCTGTGAAT 398

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

TCAATTTTGTAAATGATCCATTTTTGGATGTTA--CCACAAAAACAACAAAAGTTGGA 21951
TCAATTTTGTAAATGATCCATTTTTGGATGTTA--CCACAAAAACAACAAAAGTTGGA 458

hCoV-19/Belgium/zegea-47745/2023
NC_045512.2:21563-25384

GGAAAAGTGAGTCAGGAGTTTATTCTAGTGCAGAAATAATTGCACCTTTTGAATATGTCTCTC 22011
GGAAAAGTGAGTTCAGGAGTTTATTCTAGTGCAGAAATAATTGCACCTTTTGAATATGTCTCTC 518

hCoV-19/Belgium/zegea-47745/2023
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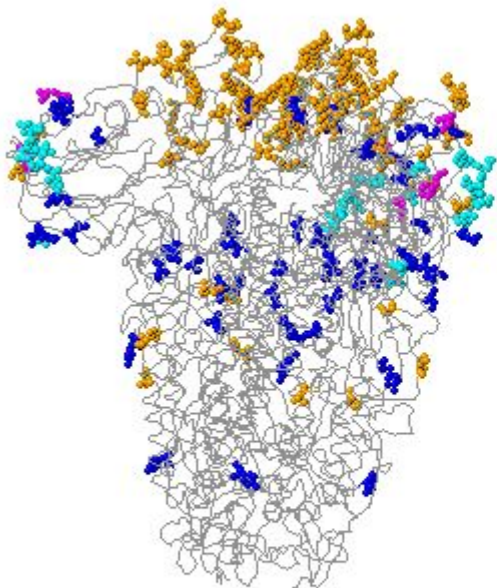
GCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAAATCTTAGGGAATTTG 22071
GCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAAATCTTAGGGAATTTG 578



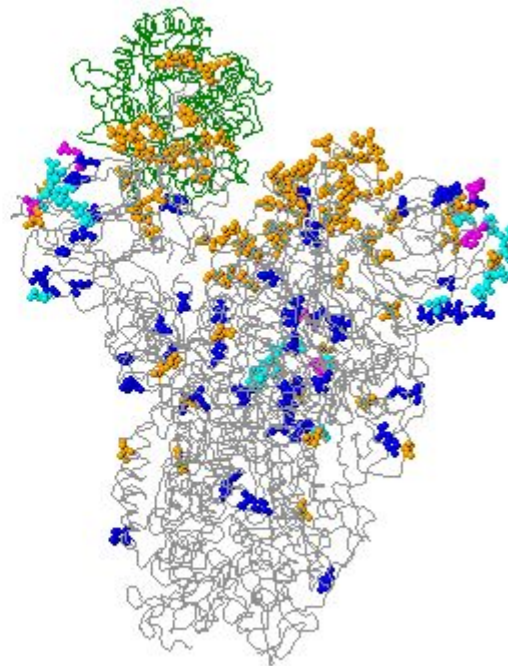
hCoV-19/Belgium/zega-47745/2023 NC_045512.2:21563-25384	TGTCCTTTGTTTCAAATGGCACACACTGGTTTGTAAACACAAAGGAATTTTATGAACCACA TGTCCTTTGTTTCAAATGGCACACACTGGTTTGTAAACACAAAGGAATTTTATGAACCACA *****	24825 3338
hCoV-19/Belgium/zega-47745/2023 NC_045512.2:21563-25384	AATCATTACTACAGACAACACATTTGTGCTCGTAACTGTGATGTTGTAATAGGAATTGT AATCATTACTACAGACAACACATTTGTGCTCGTAACTGTGATGTTGTAATAGGAATTGT *****	24885 3398
hCoV-19/Belgium/zega-47745/2023 NC_045512.2:21563-25384	CAACAACACAGTTTATGATCCTTTGCAACTTGAATTAGATTCAATCAAGGAGGAGTTAGA CAACAACACAGTTTATGATCCTTTGCAACTTGAATTAGACTCATTCAAGGAGGAGTTAGA *****	24945 3458
hCoV-19/Belgium/zega-47745/2023 NC_045512.2:21563-25384	TAAATATTTTAAAGAATCATACATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAA TAAATATTTTAAAGAATCATACATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAA *****	25005 3518
hCoV-19/Belgium/zega-47745/2023 NC_045512.2:21563-25384	TGCTTCAGTTGTAACAATTCAAAAAGAAATTGACCGCCTCAATGAGGTTGCCAAGAATTT TGCTTCAGTTGTAACAATTCAAAAAGAAATTGACCGCCTCAATGAGGTTGCCAAGAATTT *****	25065 3578
hCoV-19/Belgium/zega-47745/2023 NC_045512.2:21563-25384	AAATGAATCTCTCATCGATCTCCAAGAACTTGGAAAGTATGAGCAGTATATAAAATGGCC AAATGAATCTCTCATCGATCTCCAAGAACTTGGAAAGTATGAGCAGTATATAAAATGGCC *****	25125 3638
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hCoV-19/Belgium/zega-47745/2023 NC_045512.2:21563-25384	ATAAACGAACCTATGGATTTGTTTATGAGAATCTTCACAATTGGAACCTGTAACCTTTGAAG ATAA----- ****	25365 3822



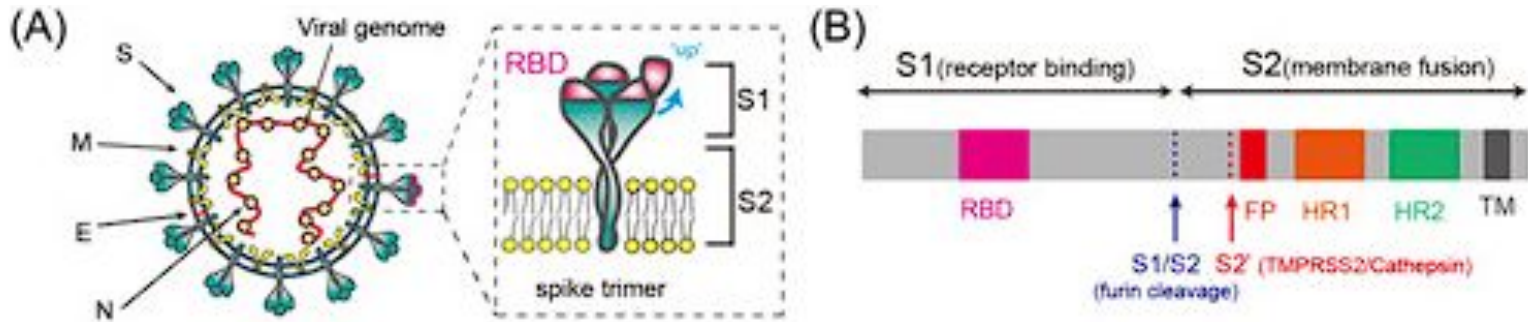
hCoV-19/Wuhan/WIV04/2019



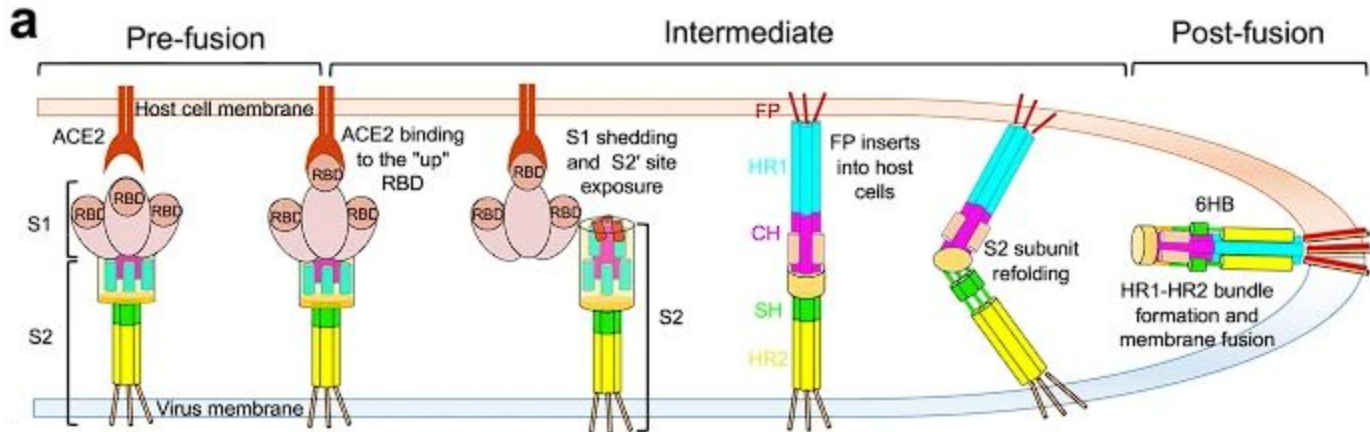
hCoV-19/Belgium/reg-47745/2023



Introdução das mutações 2P



Li X, Yuan H, Li X, Wang H. Spike protein mediated membrane fusion during SARS-CoV-2 infection. *J Med Virol.* 2023 Jan;95(1):e28212. doi: 10.1002/jmv.28212. Epub 2022 Oct 25. PMID: 36224449; PMCID: PMC9874878.



Guo L, Lin S, Chen Z, Cao Y, He B, Lu G. Targetable elements in SARS-CoV-2 S2 subunit for the design of pan-coronavirus fusion inhibitors and vaccines. *Signal Transduct Target Ther.* 2023 May 10;8(1):197. doi: 10.1038/s41392-023-01472-x. PMID: 37164987; PMCID: PMC10170451.

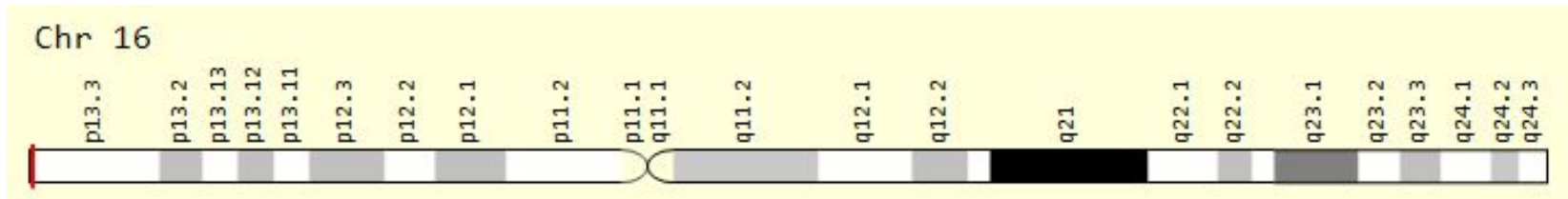
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K982P
V983P

Identificação de HBA1

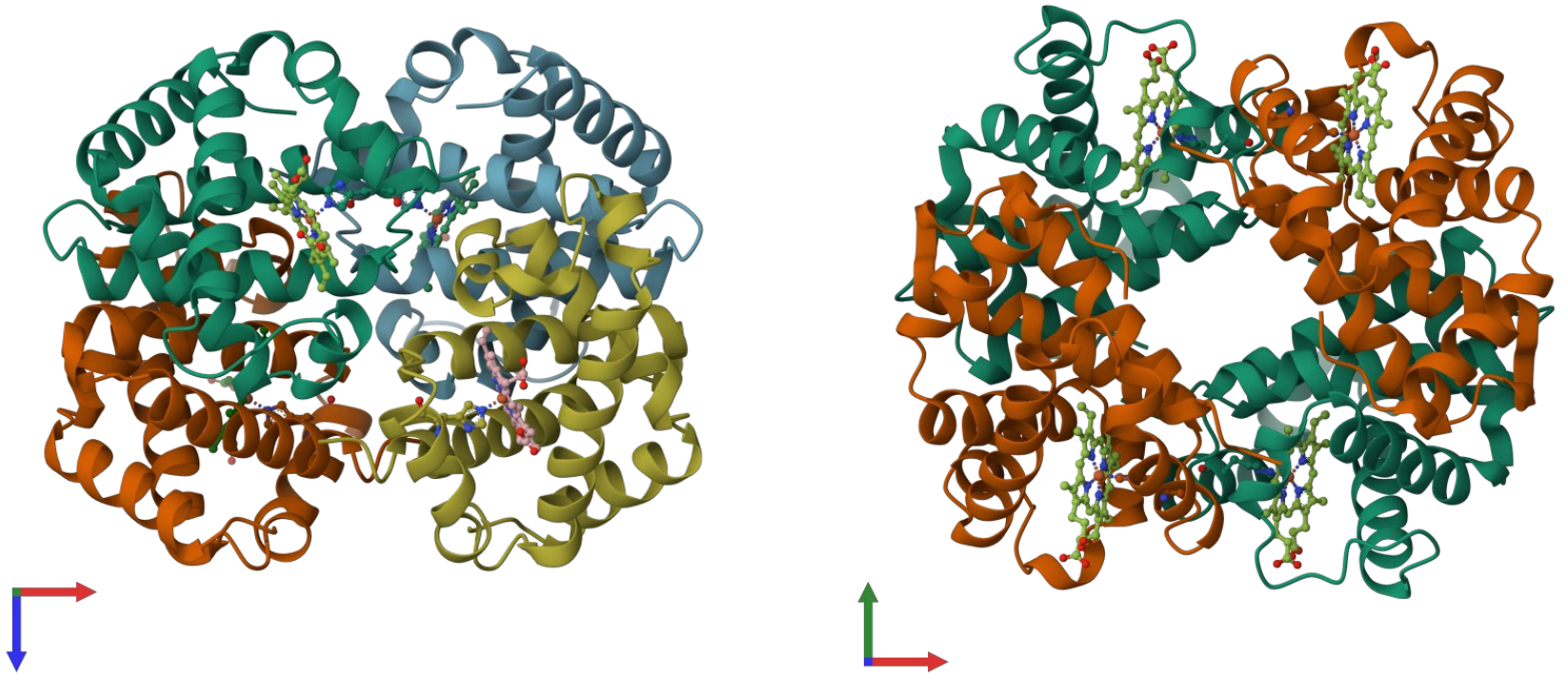
HBA1 (NM_000558.5)

- Subunidade alfa da hemoglobina;
- Região cromossômica: 16p13.3;
- 1 isoforma; transcrito maduro contendo 577pb;
- Deleções ou mutações no gene HBA1 → hemoglobinopatias.



Lorena

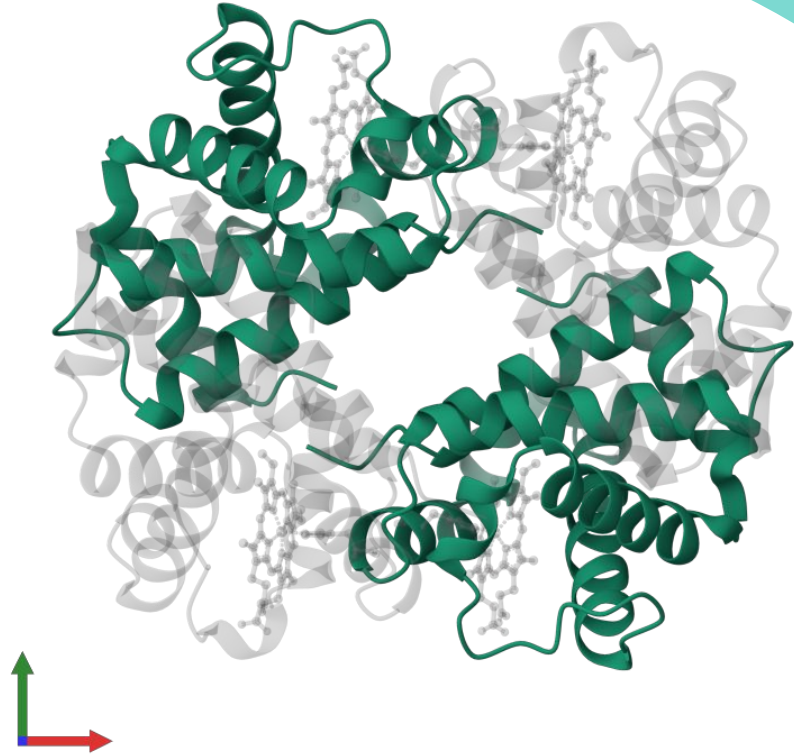
Hemoglobina



Protein Data Bank, 2023

Proteína HBA1

- Tamanho: 142 aminoácidos
- Massa molecular: 15258 Da
- Estrutura quaternária



Regiões 5'UTR e 3'UTR da HBA1

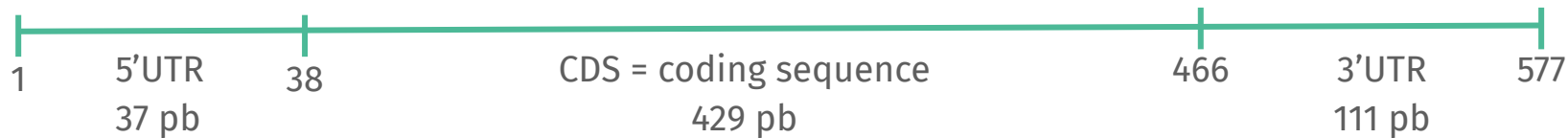
Regiões UTR

- Regiões UTR são *untranslated regions*, ou seja, regiões não traduzidas do RNA mensageiro.
- Envolvidos em mecanismos de regulação pós transcricional (Estabilidade, associação de miRNA);
- A região 5'UTR ocorre entre o CAP e o códon de iniciação;
- A região 3'UTR ocorre entre o códon de parada e a cauda Poli-A.



Regiões UTR da HBA1

- Análise do mRNA linear da subunidade alfa da hemoglobina humana (NM_000558);
- 577 bp,



Pietra Buratto De Santis

>NM_000558.5 Homo sapiens hemoglobin subunit alpha 1 (HBA1), mRNA

ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACCATGGTGCTGTCTCCTGCCGACAAGACCAACGTC

AAGGCCGCCTGGGGTAAGGTCGGCGCGCACGCTGGCGAGTATGGTGCGGAGGCCCTGGAGAGGATGTTCC

TGTCCTTCCCCACCACCAAGACCTACTTCCCGCACTTCGACCTGAGCCACGGCTCTGCCAGGTTAAGGG

CCACGGCAAGAAGGTGGCCGACGCGCTGACCAACGCCGTGGCGCACGTGGACGACATGCCCAACGCGCTG

TCCGCCCTGAGCGACCTGCACGCGCACAAGCTTCGGGTGGACCCGGTCAACTTCAAGCTCCTAAGCCACT

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GTTCTGGCTTCTGTGAGCACCGTGCTGACCTCCAAATACCGTTAAGCTGGAGCCTCGGTGGCCATGCTT

CTTGCCCCCTGGGCCTCCCCCAGCCCCCTCCTCCCCCTCCTGCACCCGTACCCCCGTGGTCTTTGAATAA

AGTCTGAGTGGGCGGCA

Pietra Buratto De Santis

Região

5' UTR

>NM_000558.5 Homo sapiens hemoglobin subunit alpha 1 (HBA1), mRNA

ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACC#GGTGCTGTCTCCTGCCGACAAGACCAACGTC
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AGTCTGAGTGGGCGGCA

Pietra Buratto De Santis

>NM_000558.5 Homo sapiens hemoglobin subunit alpha 1 (HBA1), mRNA

Região

5' UTR

ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACC#EGGTGCTGTCTCCTGCCGACAAGACCAACGTC
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GTTCTGGCTTCTGTGAGCACCGTGCTGACCTCCAAATACCGTTAACTGGAGCCTCGGTGGCCATGCTT
CTTGCCCTTGGGCCTCCCCCAGCCCTCCTCCCTTCTCGCACCCGTACCCCGTGGTCTTTGAATAA
AGTCTGAGTGGGCGCA

Região

3' UTR

ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCACCAATGTTTGTGTTTTCTTGTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTGCATGCCGCTGTTTAACTCTTATAACTACAACCTCAATCATAACA
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 CTGCACCCGTACCCCGTGGTCTTTGAATAAAGTCTGAGTGGGGGCGCA

Cassiana Mathias F. dos Santos

Otimização de códon

O que é?

- Acontece uma estruturação dos códons para leitura e tradução dos aminoácidos.

Utilizar o Codon Optimization Tool da IDT (Integrated DNA Technologies)

- A ferramenta de otimização de códons altera as trincas do DNA, de um organismo para expressão em outro, sem mudar os aminoácidos que serão traduzidos.

Otimização de códons da spike atual sem as regiões 5'UTR e 3'UTR

Resultado

```
ATG TTC GTG TTT TTG GTT TTG CTC CCC CTC GTC TCT TCA CAG TGT GTT
ATG CCT CTC TTC AAC CTG ATA ACC ACG ACC CAA TCA TAT ACC AAC TCC
TTT ACT AGG GGA GTT TAC TAC CCG GAC AAG GTG TTC CGA TCA TCA GTA
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TGG TTC CAT GCC ATA AGC GGT ACG AAC GGA ACA AAG AGA TTT GAC AAC
CCT GTA CTC CCG TTT AAT GAT GGA GTT TAT TTC GCG TCA ACG GAG AAG
TCA AAT ATC ATA AGA GGG TGG ATC TTC GGA ACA ACC CTG GAT TCC AAG
ACA CAG TCC CTT TTG ATT GTT AAC AAC GCC ACA AAT GTG TTT ATC AAG
GTC TGC GAG TTC CAA TTT TGC AAT GAT CCC TTT CTG GAC GTA TAC CAT
AAA AAT AAT AAG TCT TGG ATG GAG TCC GAA TCT GGT GTG TAC AGC TCC
GCT AAC AAT TGT ACA TTC GAA TAT GTC TCA CAG CCT TTC CTC ATG GAC
CTT GAG GGA AAG CAG GGG AAT TTC AAA AAC TTG CGA GAG TTC GTT TTT
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ATC GGC AGG GAT TTT CCG CAG GGG TTC TCA GCT CTC GAA CCG TTG GTC
GAC TTG CCC ATA GGG ATT AAC ATC ACG AGA TTC CAA ACT CTG CTT GCT
CTT AAT CGA AGC TAT CTT ACG CCT GGC GAC AGC TCC TCC GGA TGG ACC
GCC GGG GCA GCT GAT TAC TAC GTT GGT TAC TTG CAA CCA CGG ACG TTC
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ACG AGA ATT AGC AAC TGC GTA GCC GAC TAC AGT GTC CTC TAC AAT TTC
GCC CCG TTT TTT GCG TTT AAA TGT TAC GGG GTT AGT CCC ACA AAG CTG
```


Resultado

GCC CCG TTT TTT GCG TTT AAA TGT TAC GGG GTT AGT CCC ACA AAG CTG
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GAC TAT AAT TAC AAG CTC CCC GAC GAC TTT ACT GGT TGT GTG ATC GCT
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Resultado

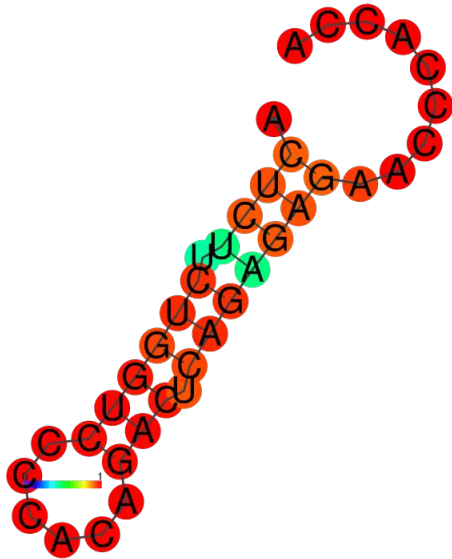
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CAA AAA TTT AAT GGG CTT ACA GTT TTG CCT CCG CTG CTC ACA GAC GAA
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GGT TGG ACC TTC GGG GCG GGA GCG GCG CTT CAA ATT CCA TTT GCT ATG
CAG ATG GCC TAC CGG TTT AAT GGG ATT GGT GTA ACT CAA AAC GTC CTC
TAC GAA AAC CAG AAG CTG ATT GCC AAT CAA TTC AAT TCC GCA ATT GGT
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AAA GGT TAC CAC TTG ATG AGC TTC CCG CAA AGT GCT CCT CAC GGG GTT
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ACC GCG CCC GCT ATC TGT CAT GAC GGA AAG GCG CAC TTT CCC AGA GAA
GGG GTT TTT GTC TCA AAT GGG ACA CAC TGG TTC GTT ACC CAG AGG AAT
TTC TAC GAG CCT CAG ATC ATC ACG ACC GAT AAT ACA TTC GTC AGT GGT
AAT TGT GAT GTG GTC ATC GGT ATT GTC AAT AAC ACT GTC TAT GAC CCG
CTG CAG CTC GAG CTT GAT TCC TTC AAG GAG GAG CTG GAT AAG TAT TTT
AAA AAC CAT ACA TCT CCC GAC GTA GAT TTG GGC GAT ATC TCA GGA ATC
AAC GCG TCC GTG GTG AAT ATC CAG AAG GAG ATA GAC CGA CTT AAT GAG
GTT GCT AAA AAC CTG AAT GAG AGT CTG ATT GAC CTG CAA GAA CTC GGG
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Estrutura Secundária e Energia Livre

Regiões 5'UTR e 3'UTR

Região 5'UTR

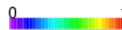
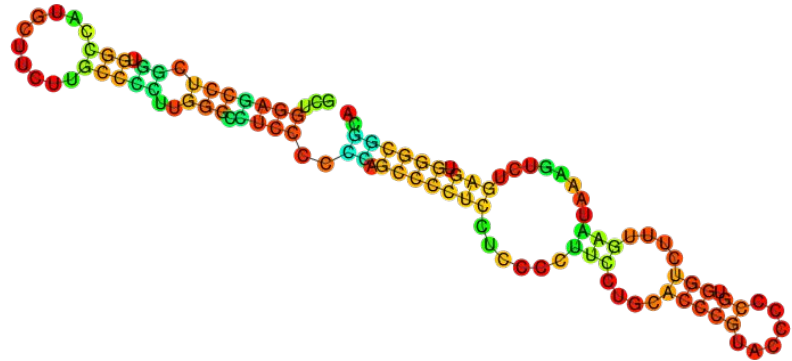
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Energia livre mínima: -10.20 kcal/mol

Região 3'UTR

GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCAG
 CCCCTCCTCCCCTTCCTGCACCCGTACCCCGTGGTCTTTGAATAAAG
 TCTGAGTGGGCGGCA



Energia livre mínima: -29.50 kcal/mol



medida de estabilidade da estrutura secundária

Vacina de mRNA (antes da otimização)

ACCTCTCGGTCGCCACAGACTCAGAGAGAACCCACCAATGTTTGTTTTCTTGTTTTATGCCACTAGTCTCTAGTCAGTGTGCATGCCGCTGTTTAATCTTATAACTACAACTCAATCATACA
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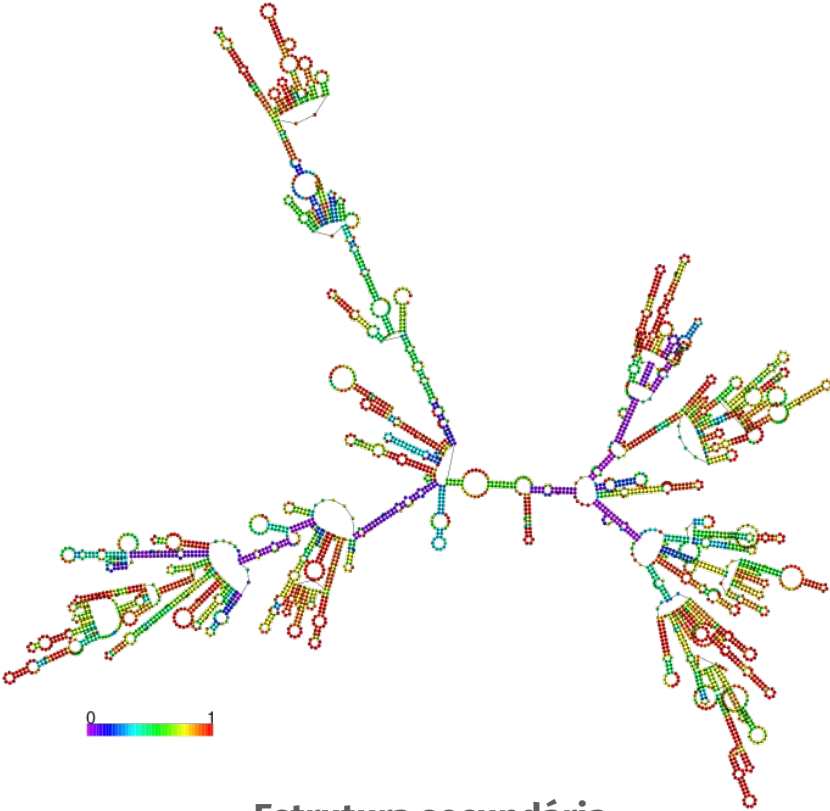


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Sequência da vacina de mRNA antes da otimização de códons

Vacina de mRNA (antes da otimização)



Estrutura secundária

Energia livre mínima: -1110.70 kcal/mol



Vacina de mRNA (após a otimização)

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 ACC CAA TCA TAT ACC AAC TCC TTT ACT AGG GGA GTT TAC TAC CCG SAC AAG GTG TFC CGA TCA TCA GTA CTC CAC CTC ACA CAG GAC CTT TTC CTG CCG TTT TT
 T TCA AAT GTC ACC TGG TTC CAT GCC ATA AGC GGT ACG AAC GGA ACA AAG AGA TTT GAC AAC CCT GTA CTC CCG TTT AAT GAT GGA GTT TAT TTC GCG TCA ACG
 GAG AAG TCA AAT ATC ATA AGA GGG TGG ATC TTC GGA ACA ACC CTG GAT TCC AAG ACA CAG TCC CTT TTG ATT GTT AAC AAC GCC ACA AAT GTG TTT ATC AAG GT
 C TGC GAG TTC CAA TTT TGC AAT GAT CCC TTT CTG GAC GTA TAC CAT AAA AAT AAT AAG TCT TGG ATG GAG TCC GAA TCT GGT GTG TAC AGC TCC GOT AAC AAT
 TGT ACA TTT GAA TAT GTC TCA CAG CCT TTC CTC ATG GAC CTT GAA AAG CAG GGG AAT TTC AAA AAC TTG CGA GAG TTC GTT TTT AAG AAC ATA GAC GGC TA
 T TTT AAG ATC TAT AGC AAA CAC ACC CCC ATA ATC GGC AGT GAT TTT CCG GAC GGG TTC TCA GCT CTC GAA CCG TTG GTC GAC TTG CCC ATA GGG ATT AAC ATC
 ACG AGA TTC CAA ACT CTG CTT GCT CTT AAT CGA AGC TAT CTT ACG CCT GGC GAC AGC TCC TCC GGA TGG ACC GCC GGG SCA GCT GAT TAC TAC GTT GGT TAC TT
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 T GAG GTA TTC AAC GCC ACA CGC TTC GCC AGT GTA TAC GCC TGG AAT AGG ACG AGA AAT AGC AAC TGC GTA CCG GAC TAC AGT GTC CTC TAC AAT TTC GCC CCG
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 A CAG ATT GCC CCC GGT CAA ACA GGG AAT ATC GCG GAC TAT AAT TAC AAG CTC CCC GAC TTT ACT GGT TGT GTG ATC GCT TGG AAT AGT AAT AAG CTT GAC
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 CTC AGC TTC GAG CTC CTT CAT GCC CCA GCC ACT GTG TGC GGG CCG AAA AAA TCC ACA AAC TTC GTG AAG AAT AAA TGC GTC AAC TTC AAC TTT AAT GGG CTT AC
 A GGT ACG GGA GTA CTG ACA AAA TCT AAC AAA AAG TTT CTT CCG TTT CAG CAG TTT GGT AGG GAC ATC GTC GAC ACG ACT GAT GCG GTT AGA GAT CCA CAG ACA
 CTT GAA ATC CTC GAC ATA ACT CCA TGT TCC TTC GGG GSA GTT TCC GTG ATA ACC CCA GGG ACC AAT ACA AGC AAC CAA GTA CCG GTA CTG TAT CAA GGA GTT AA
 T TGC ACG GAA GTG AGC GTT GCA ATC CAC GCG GAC CAG CTT ACT CCT ACC TGG CGA GTA TAC AGT ACC GGC TCC AAT GTA TTT CAA ACT CCG GCG GCG TGT TTG
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 TAC AAA ACA CCG CCT ATC AAA TAC TTC GGC GGT TTT AAC TTC TCT CAA ATC CTG CCG GAT CCC TOT AAG CCC TCT AAG CGA AGC TTC ATA GAA GAC TTG CTT TT
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 T GAA TGT GTC CTC GGG CAG AGC AAG GGT TAT TTT GTC GGC AAA GGT TAC CAC TTG ATG AGC TTC CCG CAA AGT GGT CCT CAC GGS GTT GTT TTT CTT CAC
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 G ACA CAC TGG TTC GTT ACC CAG AGG AAT TTC TAC GAG CCT CAG ATC ATC AGC ACC GAT AAT ACA TTC CTC AGT AAT TGT GAT GTG CTC ATC GGT ATT GTC
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 T ATC TCA GGA ATC AAC GOG TCC GTG GTG AAT ATC CAG AAG GAG ATA GAC CGA CTT AAT GAG GTT GGT AAA AAC CTG AAT GAG AGT CTG ATT GAC CTG CAA GAA
 CTC GGG AAA TAC GAA CAA TAT ATA AAA TGG CCC TGC TAC ATC TGG CTG GGT TTT ATA CCG GGT CTT ATA GCC ATA GTT ATG GTC ACC ATT ATG CTT TGT TGT AT
 G ACC TCC TGT TGC AGT TGC CTT AAG GGA TGT TGT TCA TGC GGT AGC TGC TGC AAG TTT GAT GAA GAT TAC GGC CCA GTC CTT AAG GGC GTC AAA CTC CAC
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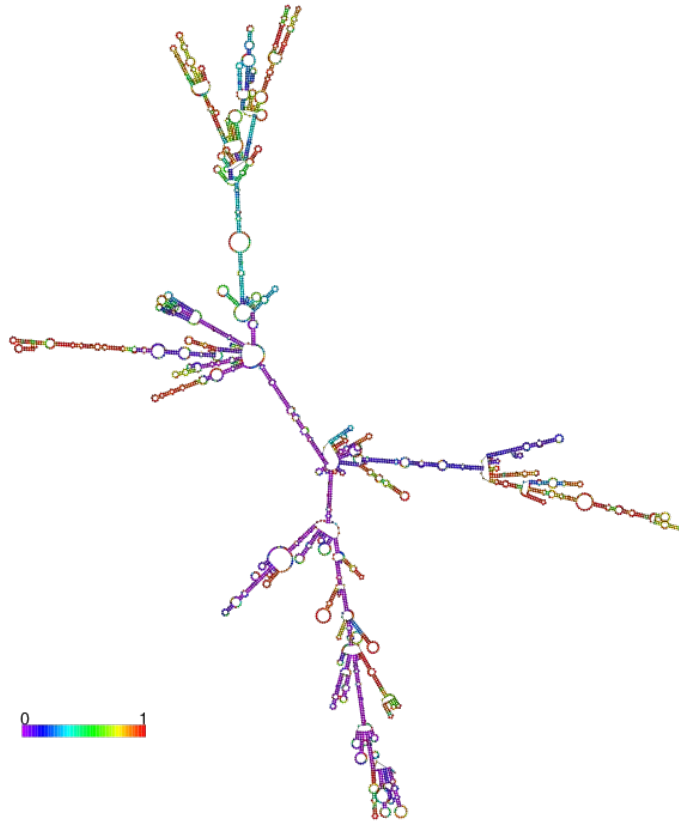


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Sequência da vacina de mRNA após a otimização de códons

Vacina de mRNA (após a otimização)

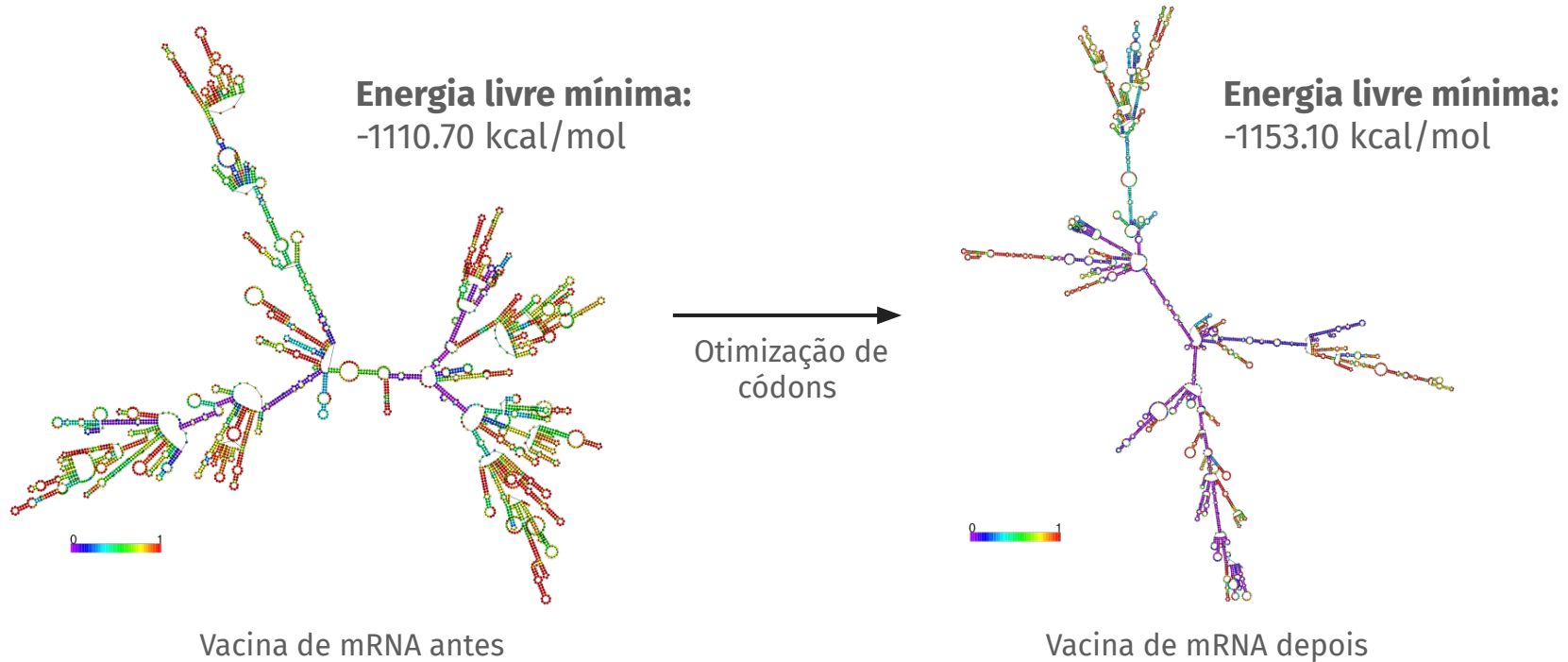


Energia livre mínima: -1153.10 kcal/mol



Estrutura secundária

Estruturas Secundárias e Energia Livre



Otimização de códons → Aumento da estabilidade da estrutura secundária

Helena

Sequências da Spike da linhagem JN.1 antes e após as mutações

Sequência cDNA Spike JN.1: sem mutação

ATGTTTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTGCATGCCGCTGTTTAATCTTATAACTACAACCTCAATCATACTAATCTTTCCACACGT
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 CTCAAAGGAGTCAAATTTACATTACACATA

Sequência traduzida Spike JN.1: sem mutação

5'3' Frame 1

MFVFLVLLPLVSSQCVMLPLFNLITTTQSYTNSFTRGVYYPDKVFRSSVLHLTQDLFLPFFSNVTFWFAISGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNA
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 IAIVMVTIMLCCMTSCCSCLGCCSCGSCCKFDEDDSEPVLLKGVKLYHT-

1269 resíduos

Domínio RBD Spike JN.1: sem mutação

MFVFLVLLPLVSSQCVMLFNLITTTQSYTNSFTRGVYYPDKVFRSSVLHLTQDLFLPFFSNVTWFHAI SGTNGTKRFDNPFVLPFNDGVYFASTEKS
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ARSVASQSI IAYTMSLGAENSVAYSNN SIAIPTNFTI SVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLKRALTGIAVEQDKNTQE
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LAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFN SAI GKIQDSL FSTASALGKLQDVVNHNAQALNTLVKQLSSKFGAIS
SVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHV TYVPAQ
EKNFTTAPAICH DGKAHF PREGVFVSN GTHWFVTQRNFYEPQIITTDNTFVSGNCDV VIGIVNNTVYDPLQLELDSFKEELDKYFKNHTSPD VDLGD
ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSC LKGCCSCGSCCKFDEDDSE PVL
KGVKLYHT

Segundo o roteiro disponibilizado (316-533) = 217 resíduos*

Domínio RBD Spike original: sem mutação

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SPRRARSVASQSI IAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKT SVDCTMYICGDSTEC SNLLLQYGSFCTQLNRALTGIAVEQDK
NTQEVAQVKQIYKT **KV**IKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQY
TSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNF
GAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQ LIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTY
VPAQEKNTTAPAICHGDKAHFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVI GIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDV
DLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDSDS
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Segundo o roteiro disponibilizado (319-537) = 218 resíduos



Sequence, in FASTA format

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>spike atual sem mutação
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VEKGIYQTSNFRVQPTESI VRFPNVTNLCPFHEVFVNATRFASVYAWNRT RISNCVADYSVLYNFAPFFAFKCYGVSPTKLN DLCFTNVYADSFVIK GNEVSIAPGQTGN IADYNYKLPDDFTGCVI AWNSNKLDSKHSGNYDYWYRSFRKS  
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YENQKLIANQFNSAIGKIQDSLFS TASALGKLQDVVNHNAQALNTLVKQLS SKFGAISV LNDILSRLDKVEAEVQIDRLITGR LQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYV  
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Valid Sequence.

[Choose file](#)[Example protein sequence](#)

▸ Advanced options

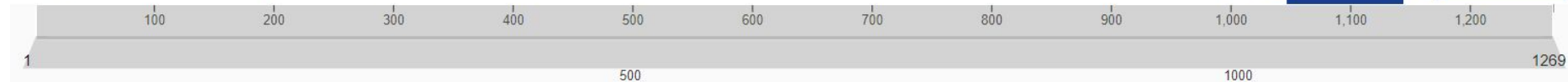
[Search](#)[Clear](#)

Entry matches to this proteinⁱ

Options ▾



Export ▾



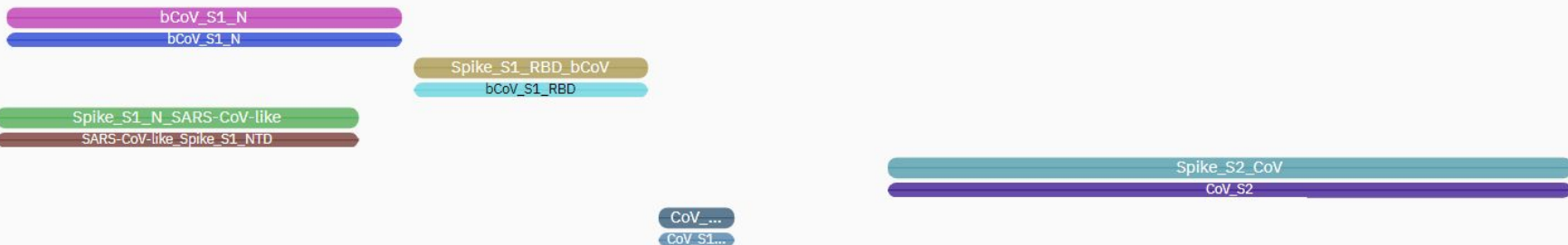
▼ Representative Domains



▼ Family



▼ Domain



▼ Homologous Superfamily



Domínio RBD Spike JN.1: sem mutação

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 LKGVKLHYT

Segundo o Interpro (345-522) = 177 resíduos



Sequência cDNA Spike JN.1: com mutação

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CCU	} Pro
CCC	
CCA	
CCG	

Sequência traduzida Spike JN.1: com mutação

5'3' Frame 1

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 LSRLDPEAEVQIDRLITGR LQSLQTYVTQQLIRAAEIRASANLAATKMSECVL GQSKRVDFCGKGYHLSFQPQSAPHGVVFLHVTVYVPAQEKNFTTAPAI CHDGAHFPREGV FVVSNGTHW
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1269 resíduos

Domínio RBD Spike JN.1: com mutação

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Segundo o roteiro disponibilizado (316-533) = 217 resíduos



Sequence, in FASTA format

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>Spike atual com mutação
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Valid Sequence.

Choose file

Example protein sequence

▸ Advanced options

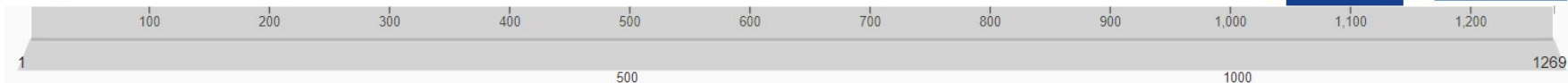
Search

Clear

Entry matches to this proteinⁱ

Options ▾

Export ▾



▼ Representative Domains

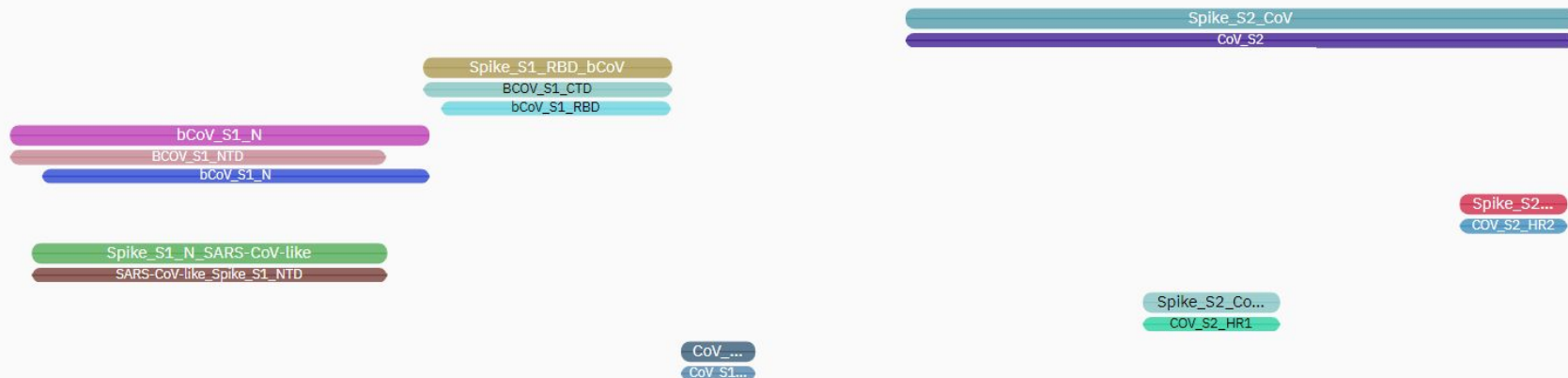


▼ Family

BETA_CORONA_SPIKE

BETA_CORONA_SPIKE

▼ Domain



▼ Homologous Superfamily



Domínio RBD Spike JN.1: com mutação

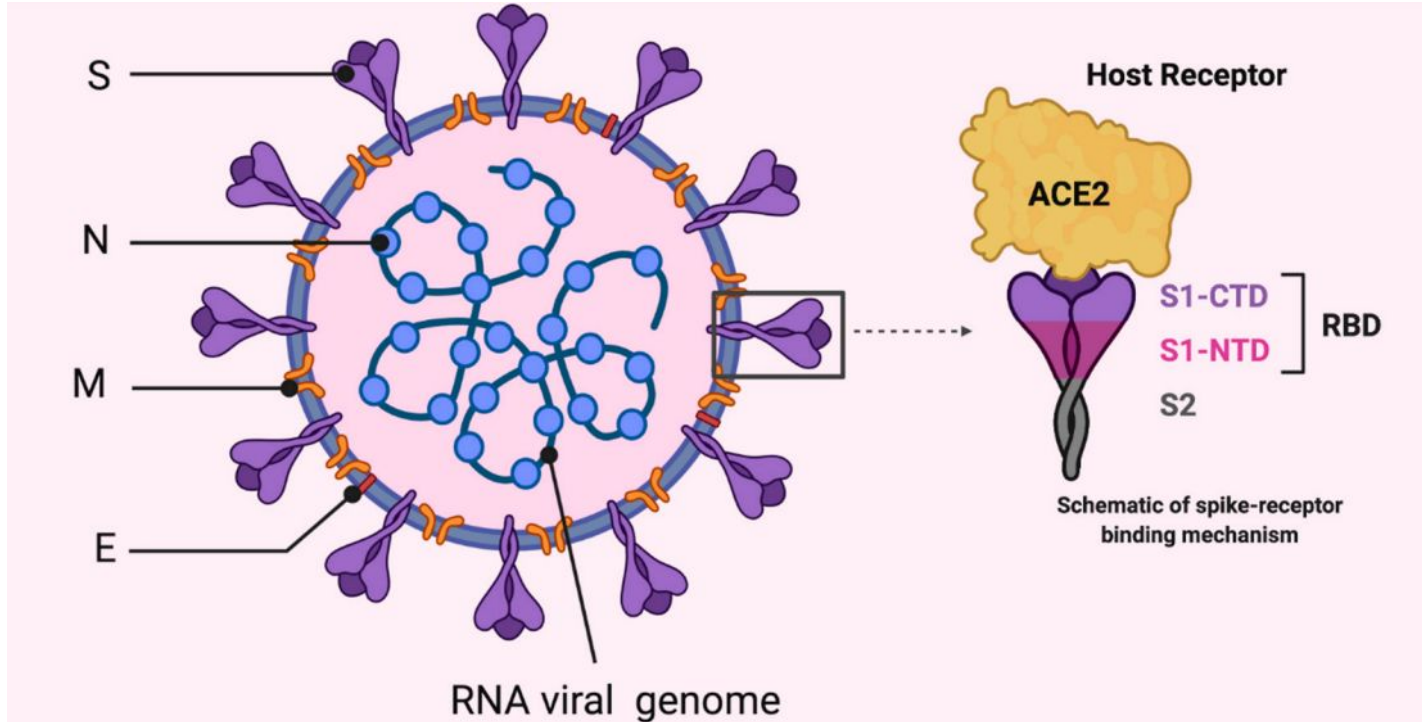
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 LKGVKLHYT

Segundo o Interpro (345-522) = 177 resíduos



Domínio RBD

Receptor Binding Domain



4. Perfil Imunológico

Predição de epítomos (pré-mutação)

Predicted peptides:

No. ↕	Start ↕	End ↕	Peptide ↕	Length ↕
1	22	32	LITTTQSYTNS	11
2	56	77	FLPFFSNVTWFHAISGTNGTKR	22
3	137	152	DPFLDVYHKNNKSWME	16
4	173	187	FLMDLEGKQGNFKNL	15
5	206	217	TPIIGRDFPQGF	12
6	246	258	LTPGDSSSGWTAG	13
7	291	291	D	1
8	293	293	L	1
9	303	321	FTVEKGIYQTSNFRVQPTTE	19
10	326	362	FPNVTNLCPFHEVFNATRFASVYAWNRRTRISNCVADY	37
11	370	392	PPFAFKCYGVSPTKLNDLCFTNV	23
12	398	423	VIKNEVSQIAPGQTGNIADYNYKLP	26
13	436	494	NKLDSKHSGNYDYWYRSFRKSKLKPFERDISTEIQAGNKPKGKGPNCYFPLQSYGFR	59
14	513	530	LLHAPATVCGPKKSTNLV	18
15	553	557	KKFLP	5
16	576	578	QTL	3

Obs. RBD 316-533

Predição de epítomos (pré-mutação)

17	598	602	TNTSN	5
18	612	616	NCTEV	5
19	620	628	IHADQLTPT	9
20	633	643	STGSNVFQTRA	11
21	651	661	YVNSYECDIP	11
22	669	686	SYQTQTKSRRRARSVASQ	18
23	692	705	TMSLGAENSVAYSN	14
24	744	744	E	1
25	769	775	EQDKNTQ	7
26	782	810	KQIYKTPPIKYFGGFNFSQILPDPSKPSK	29
27	824	839	LADAGFIKQYGDCLGD	16
28	984	988	EAEVQ	5
29	1031	1039	GQSKRVDFC	9
30	1104	1114	NFYEPQIITTD	11
31	1129	1170	VNNTVYDPLQLELDSFKEELDKYFKNHTSPDVLGDISGINA	42
32	1199	1203	LGKYE	5
33	1249	1263	CCKFDEDDSEPVKKG	15
34	1265	1265	K	1

Total: 34 epítomos

Predição de epítomos (pós-mutação)

Predicted peptides:

No.	Start	End	Peptide	Length
1	23	32	ITTTQSYTNS	10
2	56	77	FLPFFSNVTWFHAISGTNGTKR	22
3	137	152	DPFLDVYHKMKNSWME	16
4	173	187	FLMDLEGKQGNFKNL	15
5	206	217	TPIIGRDFPQGF	12
6	246	258	LTPGDSSSGWTAG	13
7	291	291	D	1
8	293	293	L	1
9	303	320	FTVEKGIYQTSNFRVQPT	18
10	327	351	PNVTNLCPFHEVFNATRFASVYAWN	25
11	353	353	T	1
12	355	362	ISNCVADY	8
13	368	392	FAPFFAFKCYGVSPKLNLCFTMV	25
14	399	418	IKGNEVSQIAPGQTGNIADY	20
15	420	423	YKLP	4
16	437	495	KLDSKHSNGYDYWYRSFRKSKLKPFERDISTEIQAGNKPKGKGPNCYFPLQSYGFRP	59
17	513	530	LLHAPATVCGPKKSTNLV	18
18	553	557	KKFLP	5
19	576	578	QTL	3
20	598	602	TNTSN	5
21	612	616	NCTEV	5

Obs. RBD 316-533

Predição de epítomos (pós-mutação)

22	620	628	IHADQLTPT	9
23	633	643	STGSINVFQTRA	11
24	651	661	YVNNSYECDIP	11
25	669	686	SYQTQTKSRRRARSVASQ	18
26	692	705	TMSLGAENSVAYSN	14
27	744	744	E	1
28	769	775	EQDKNTQ	7
29	782	810	KQIYKTPPIKYFGGFNFSQILPDPSKPSK	29
30	824	839	LADAGFIKQYGDCLGD	16
31	981	989	DPPEAEVQI	9
32	1031	1039	GQSKRVDFC	9
33	1103	1114	RNFYEPQIITTD	12
34	1129	1170	VNNTVYDPLQLELDSFKEELDKYFKNHTSPDVDLGDISGINA	42
35	1198	1203	ELGKYE	6
36	1249	1263	CCKFDEDDSEPVLKG	15
37	1265	1265	K	1

Total: 37 epítomos



Epítomos de MHC I e II

MHC-I

	Epítopo	Start	End	Tamanho	Aminoácidos	Score	Afinidade
Sem mutação	HLA-A*11:01	446	454	9	PREGVFVSN	0.0	100%
Com mutação	HLA-A*11:01	413	422	10	PHGVVFLHVT	0.0	100%

Fonte: autoral, 2024

- Tamanho (início e fim);
- Sequência de aminoácidos;
- *Score*;
- Afinidade do epítopo ao MHC I.

MHC-II

Fonte: Autorial, 2024

	Epítopo	Start	End	Tamanho	Aminoácidos	Score	Afinidade
Sem mutação	HLA-DRB4*01:01	472	486	15	CKGKGPNKY	0.0003	100.0
Mutação 2P	HLA-DRB4*01:01	472	486	15	CKGKGPNKY	0.0003	100.0

Quantidade de epítomos:

MHC-II original

sem mutação: 27.945 epítomos

com mutação: 27.945 epítomos

MHC-II atual

sem mutação: 27.945 epítomos

com mutação: 33.885 epítomos

Sem mutação

Method used: netmhciiipan_el

allele	seq_num	start	end	length	core_peptide	peptide	score	rank		
HLA-DRB5*01:01	1	341	354	14	FASVYAMNR	ATRFASVYAMNRT	0.8510	0.06		
HLA-DRB3*01:01	1	459	472	14	FERDISTEI	KPFERDISTEIQQA	0.9192	0.06		
HLA-DRB3*01:01	1	458	471	14	FERDISTEI	LKPFERDISTEIQ	0.9261	0.06		
HLA-DRB5*01:01	1	932	946	15	FSTASALGK	DSLSTASALGKLQD	0.8472	0.08		
HLA-DRB3*01:01	1	457	472	16	FERDISTEI	KLKPFERDISTEIQQA	0.9121	0.08		
HLA-DRB3*01:01	1	458	472	15	FERDISTEI	LKPFERDISTEIQQA	0.9213	0.08		
HLA-DRB5*01:01	1	340	354	15	FASVYAMNR	NATRFASVYAMNRT	0.8232	0.1		
HLA-DRB3*01:01	1	457	470	14	FERDISTEI	KLKPFERDISTEIQ	0.8715	0.1		
HLA-DRB3*01:01	1	457	471	15	FERDISTEI	KLKPFERDISTEIQ	0.9054	0.1		
HLA-DRB5*01:01	1	932	945	14	FSTASALGK	DSLSTASALGKLQ	0.7789	0.11		
HLA-DRB5*01:01	1	341	355	15	FASVYAMNR	ATRFASVYAMNRT	0.8078	0.12		
HLA-DRB5*01:01	1	931	946	16	FSTASALGK	QDSLSTASALGKLQD	0.8292	0.12		
HLA-DRB3*01:01	1	458	473	16	FERDISTEI	LKPFERDISTEIQAG	0.8696	0.12		
HLA-DRB3*01:01	1	456	471	16	FERDISTEI	SKLKPFERDISTEIQ	0.8698	0.12		
HLA-DRB3*01:01	1	459	473	15	FERDISTEI	KPFERDISTEIQAG	0.8415	0.14		
HLA-DRB3*01:01	1	456	470	15	FERDISTEI	SKLKPFERDISTEIQ	0.8108	0.16		
HLA-DRB5*01:01	1	932	947	16	FSTASALGK	DSLSTASALGKLQDV	0.7744	0.18		
HLA-DRB5*01:01	1	340	355	16	FASVYAMNR	NATRFASVYAMNRT	0.7884	0.18		
HLA-DRB5*01:01	1	339	354	16	FASVYAMNR	FNATRFASVYAMNRT	0.7888	0.18		
HLA-DRB5*01:01	1	340	353	14	FASVYAMNR	NATRFASVYAMNRT	0.7335	0.19		
HLA-DRB5*01:01	1	775	789	15	FAQVKQIYK	QEVFAQVKQIYKTPP	0.7751	0.2		
HLA-DRB5*01:01	1	342	355	14	FASVYAMNR	TRFASVYAMNRT	0.7187	0.21		
HLA-DRB5*01:01	1	775	788	14	FAQVKQIYK	QEVFAQVKQIYKTP	0.7189	0.21		
HLA-DRB5*01:01	1	931	945	15	FSTASALGK	QDSLSTASALGKLQ	0.7483	0.22		
HLA-DRB5*01:01	1	774	789	16	FAQVKQIYK	TQEVFAQVKQIYKTPP	0.7520	0.22		
HLA-DRB5*01:01	1	341	356	16	FASVYAMNR	ATRFASVYAMNRT	0.7394	0.24		
HLA-DRB1*01:01	1	535	550	16	FNGLTGTGV	VNFNGLTGTGVLTK	0.9358	0.25		
HLA-DRB1*01:01	1	536	550	15	FNGLTGTGV	NFNFNGLTGTGVLTK	0.9435	0.26		
HLA-DRB5*01:01	1	933	946	14	FSTASALGK	SLSTASALGKLQD	0.6997	0.27		
HLA-DRB5*01:01	1	232	245	14	FQTLALNRSY	ITRFQTLALNRSY	0.6855	0.28		
HLA-DRB3*01:01	1	455	470	16	FERDISTEI	KSKLKPFERDISTEIQ	0.7190	0.28		
HLA-DRB3*01:01	1	459	474	16	FERDISTEI	KPFERDISTEIQAGN	0.7252	0.28		
HLA-DRB5*01:01	1	775	790	16	FAQVKQIYK	QEVFAQVKQIYKTPPI	0.7016	0.29		
HLA-DRB1*01:01	1	312	325	14	FRVQPTESI	TSNFRVQPTESI	0.9072	0.34		
HLA-DRB1*01:01	1	63	77	15	FHAISGTNG	VTVFHAISGTNGTKR	0.9222	0.34		
HLA-DRB5*01:01	1	231	244	14	FQTLALNRS	NITRFQTLALNRS	0.6566	0.35		
HLA-DRB5*01:01	1	930	945	16	FSTASALGK	IQDSLSTASALGKLQ	0.6749	0.36		
HLA-DRB3*01:01	1	1250	1263	14	FDEDDSEPV	CKFDEDDSEPVKLG	0.6972	0.36		
HLA-DRB5*01:01	1	339	353	15	FASVYAMNR	FNATRFASVYAMNRT	0.6690	0.37		
HLA-DRB5*01:01	1	774	788	15	FAQVKQIYK	TQEVFAQVKQIYKTP	0.6734	0.37		
HLA-DRB1*01:01	1	536	551	16	FNGLTGTGV	NFNFNGLTGTGVLTKS	0.9067	0.4		
HLA-DRB5*01:01	1	775	789	14	FAQVKQIYK	QEVFAQVKQIYKTPP	0.6393	0.41		

27.945 epitopos

Fonte: Auroral, 2024

Com mutação 2P

Method used: netmhciiipan_e1

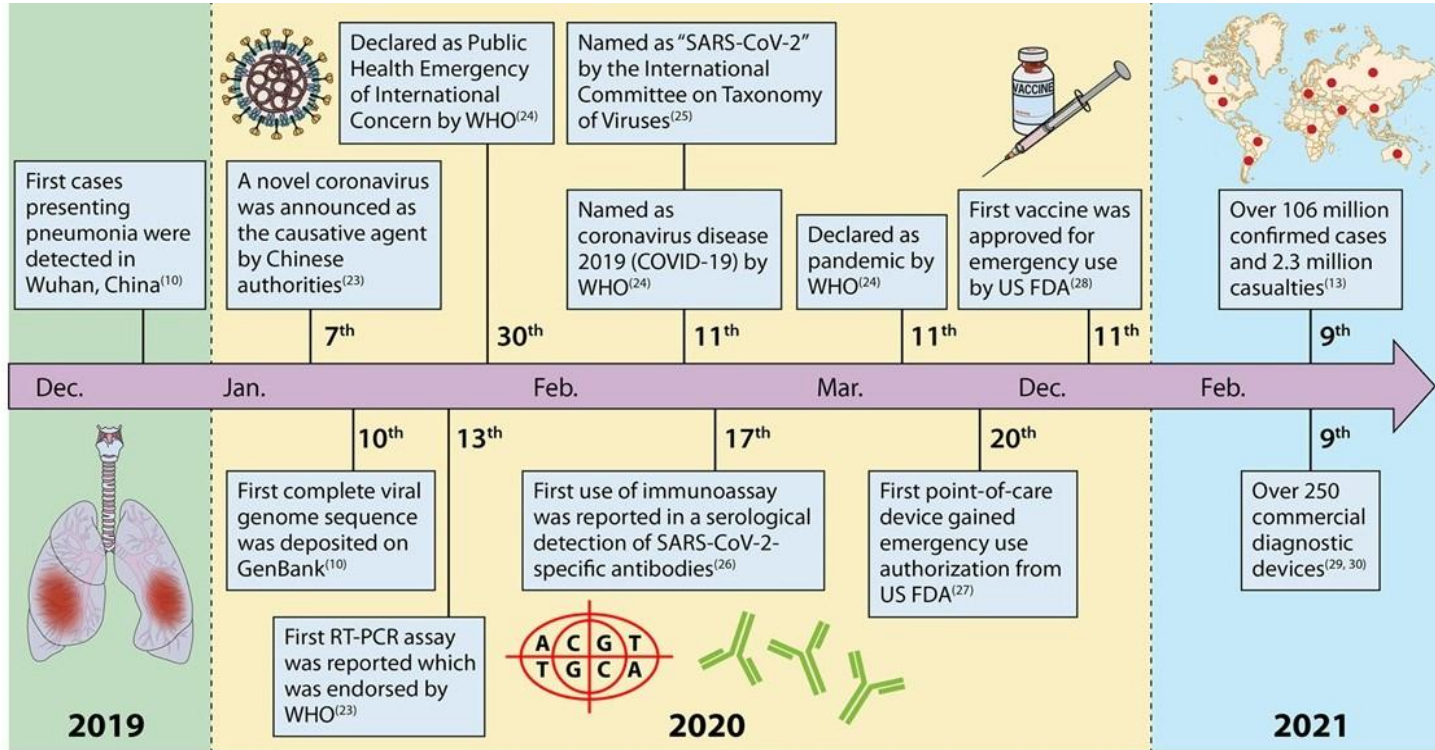
allele	seq_num	start	end	length	core_peptide	peptide	score	rank
HLA-DRB5*01:01	1	341	354	14	FASVYAMNR	ATRFASVYAMNRTR	0.8510	0.06
HLA-DRB3*01:01	1	459	472	14	FERDISTEI	KPFERDISTEIYQA	0.9192	0.06
HLA-DRB3*01:01	1	458	471	14	FERDISTEI	LKPFERDISTEIYQ	0.9261	0.06
HLA-DRB5*01:01	1	932	946	15	FSTASALGK	DSLFFSTASALGKLQD	0.8472	0.08
HLA-DRB3*01:01	1	457	472	16	FERDISTEI	KLKPFERDISTEIYQA	0.9121	0.08
HLA-DRB3*01:01	1	458	472	15	FERDISTEI	LKPFERDISTEIYQA	0.9213	0.08
HLA-DRB5*01:01	1	340	354	15	FASVYAMNR	NATRFASVYAMNRTR	0.8232	0.1
HLA-DRB3*01:01	1	457	470	14	FERDISTEI	KLKPFERDISTEIY	0.8715	0.1
HLA-DRB3*01:01	1	457	471	15	FERDISTEI	KLKPFERDISTEIYQ	0.9054	0.1
HLA-DRB5*01:01	1	932	945	14	FSTASALGK	DSLFFSTASALGKLQ	0.7789	0.11
HLA-DRB5*01:01	1	341	355	15	FASVYAMNR	ATRFASVYAMNRTRI	0.8078	0.12
HLA-DRB5*01:01	1	931	946	16	FSTASALGK	QDSLFFSTASALGKLQD	0.8292	0.12
HLA-DRB3*01:01	1	458	473	16	FERDISTEI	LKPFERDISTEIYQAG	0.8696	0.12
HLA-DRB3*01:01	1	456	471	16	FERDISTEI	SKLKPFERDISTEIYQ	0.8698	0.12
HLA-DRB3*01:01	1	459	473	15	FERDISTEI	KPFERDISTEIYQAG	0.8415	0.14
HLA-DRB3*01:01	1	456	470	15	FERDISTEI	SKLKPFERDISTEIY	0.8108	0.16
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HLA-DRB5*01:01	1	340	355	16	FASVYAMNR	NATRFASVYAMNRTRI	0.7884	0.18
HLA-DRB5*01:01	1	339	354	16	FASVYAMNR	FNATRFASVYAMNRTR	0.7888	0.18
HLA-DRB5*01:01	1	340	353	14	FASVYAMNR	NATRFASVYAMNRT	0.7335	0.19
HLA-DRB5*01:01	1	775	789	15	FAQVKQIYK	QEVFAQVKQIYKTPP	0.7751	0.2
HLA-DRB5*01:01	1	342	355	14	FASVYAMNR	TRFASVYAMNRTRI	0.7187	0.21
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HLA-DRB5*01:01	1	774	789	16	FAQVKQIYK	TQEVFAQVKQIYKTPP	0.7520	0.22
HLA-DRB5*01:01	1	341	356	16	FASVYAMNR	ATRFASVYAMNRTRIS	0.7394	0.24
HLA-DRB1*01:01	1	535	550	16	FNGLTGTGV	VNFNFNGLTGTGVLTG	0.9358	0.25
HLA-DRB1*01:01	1	536	550	15	FNGLTGTGV	NFNFNGLTGTGVLTG	0.9435	0.26
HLA-DRB5*01:01	1	933	946	14	FSTASALGK	SLFFSTASALGKLQD	0.6997	0.27
HLA-DRB5*01:01	1	232	245	14	FQTLALNLR	ITRFQTLALNRSY	0.6855	0.28
HLA-DRB3*01:01	1	455	470	16	FERDISTEI	KSKLKPFERDISTEIY	0.7190	0.28
HLA-DRB3*01:01	1	459	474	16	FERDISTEI	KPFERDISTEIYQAGN	0.7252	0.28
HLA-DRB5*01:01	1	775	790	16	FAQVKQIYK	QEVFAQVKQIYKTPPI	0.7016	0.29
HLA-DRB1*01:01	1	312	325	14	FRVQPTESI	TSNFRVQPTESIVR	0.9072	0.34
HLA-DRB1*01:01	1	63	77	15	FHAISGTNG	VTWFHAIISGTNGTKR	0.9222	0.34
HLA-DRB5*01:01	1	231	244	14	FQTLALNLR	NITRFQTLALNRS	0.6566	0.35
HLA-DRB5*01:01	1	930	945	16	FSTASALGK	IQDSLFFSTASALGKLQ	0.6749	0.36
HLA-DRB3*01:01	1	1250	1263	14	FDEDDSEPV	CKFDEDDSEPVLLKG	0.6972	0.36
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HLA-DRB5*01:01	1	774	788	15	FAQVKQIYK	TQEVFAQVKQIYKTP	0.6734	0.37
HLA-DRB1*01:01	1	536	551	16	FNGLTGTGV	NFNFNGLTGTGVLTGS	0.9067	0.4
HLA-DRB5*01:01	1	776	789	14	FASVYAMNR	QEVFAQVKQIYKTPP	0.6383	0.42

33.885 epítipos

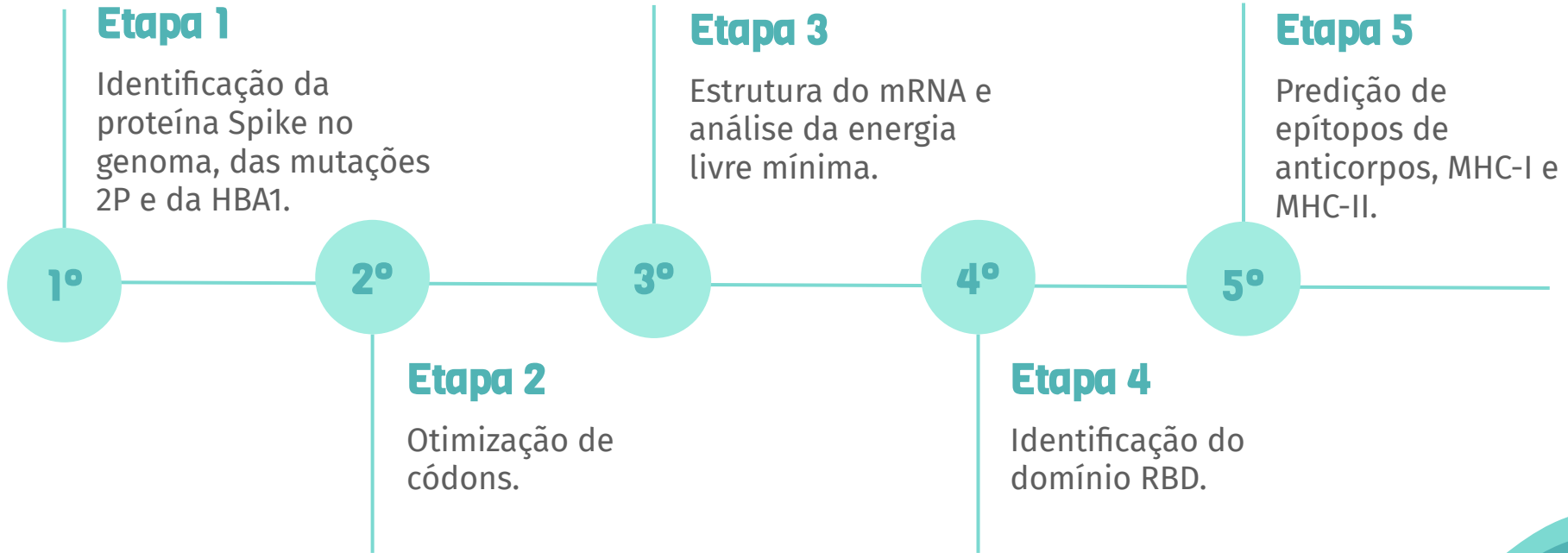
5. Pontos-chave e conclusão

Panorama geral

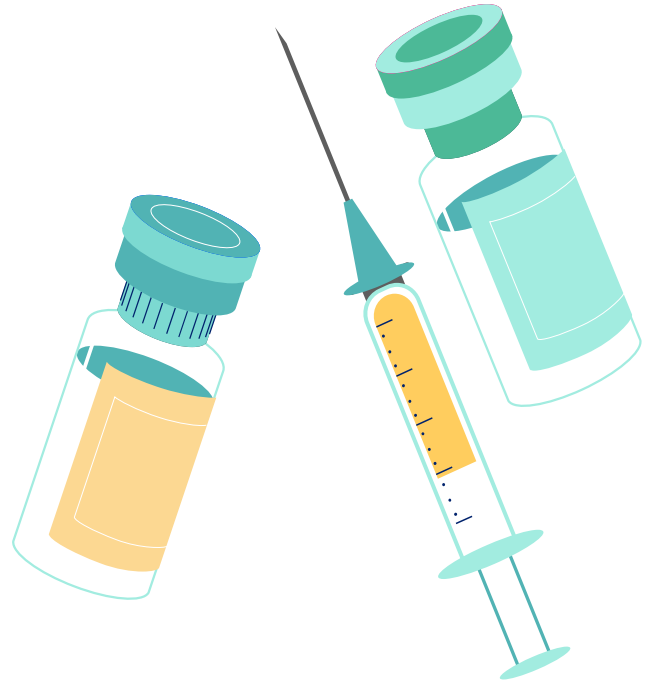
Thuany



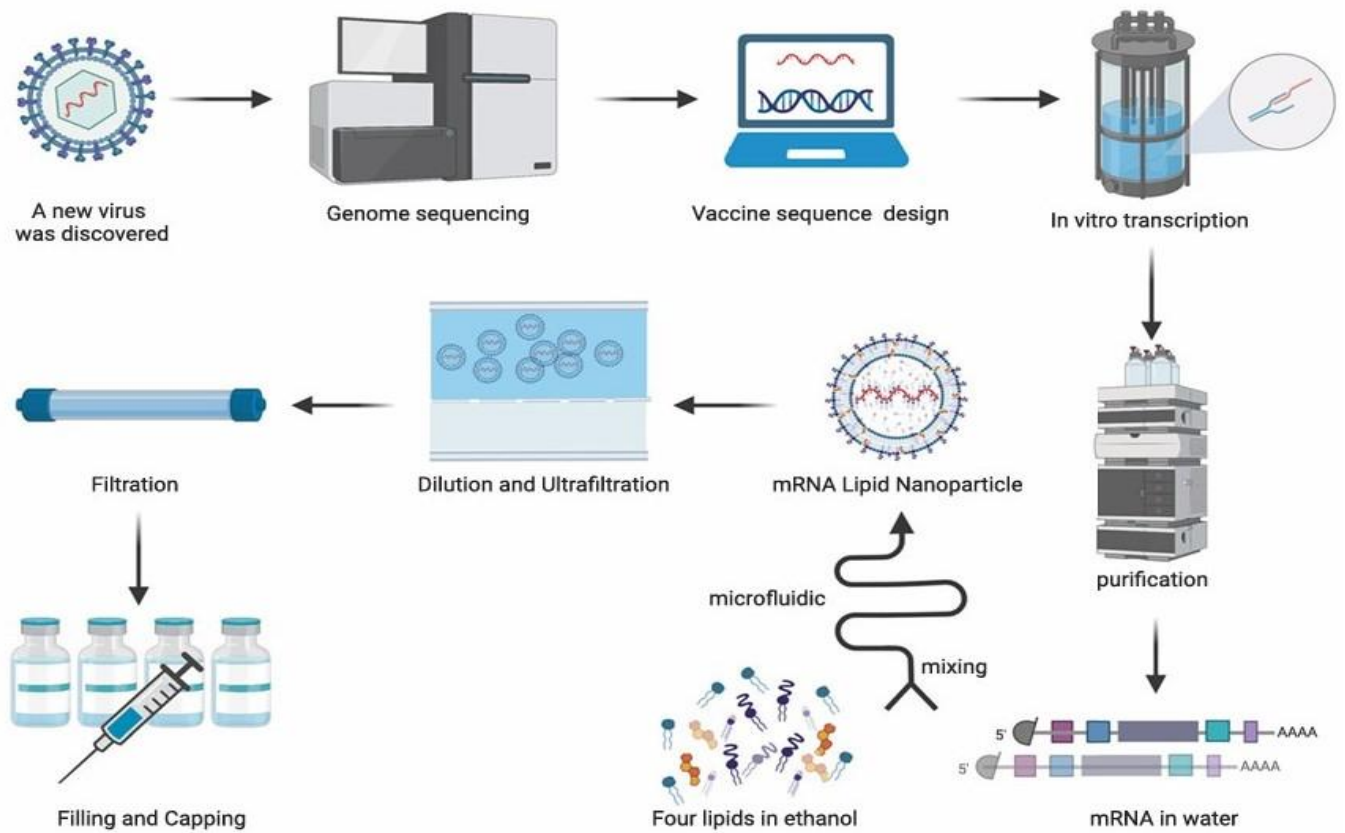
Recapitulação



**A construção de uma
vacina de mRNA
contra a linhagem
atual de SARS-CoV-2
apresentou
desenvolvimento
satisfatório.**



Perspectivas



Fonte: Fang et al., 2022

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Muito
obrigado!