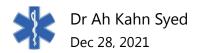
Arkmedic's blog



How to BLAST your way to the truth about the origins of COVID-19

Using BLAST is easy. I'm going to show you how easy and how to prove that SARS-Cov-2 is man-made





I've been meaning to write this blog for ever. Well, at least since Prashant Pradhan (a wonderful, honest and brave genomics scientist) raised the possibility back in February 2020 that the SARS-Cov2 virus was man made. And we have seen multiple confirmatory pieces that the virus was made in a lab, one of the better ones here on zenodo and with its own cute video for non-Bayesian peeps here. As of writing this those links are still up which at 12 months is pretty good going for any article that dares challenge the drivel propagandised by our beloved "free press [sponsored by pharma]".

Anyway, BLAST is the NCBI/NIH (aka US government) repository for genomic and proteomic sequences, amongst other things. It is where all genome scientists around the world deposit their sequences if they make a discovery. Its main function is to allow comparison of gene sequences and discovery of sequences that match one that you might have come across in your experiment. What's a gene sequence? That's easy. It's a line of code, made up of any combination of 4 letters in a sequence. Remember the film GATTACA? If you haven't watched it by now, you should - because it's yet another dystopian movie that is now too close to home

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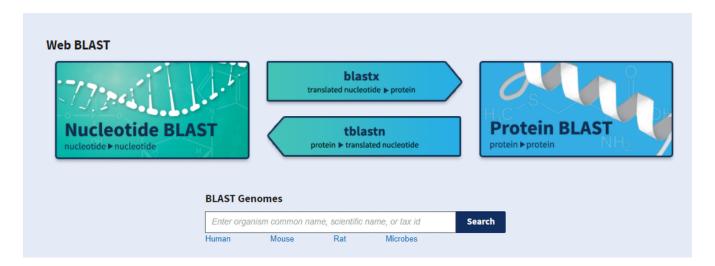


The movie's title is based on the 4 nucleotide bases (G, A, T, C) which make up the genetic code of every human's DNA. There are about 3 billion of them in each cell, making a code that is unique - resulting in you, a unique individual! The code pairs up so that G-C and A-T always combine to make the double-helix that you see in the picture, such that GATTACA would be paired with CTAATGT (the complement). The code is read in a specific direction so GATTACA on one strand would be TGTAATC on the other (the reverse complement). One of the good things about BLAST is that it doesn't care which version you give it, it will still point you to the correct gene.

One other thing to note at this point is probabilities. You did this at school with tossing a coin (where the code is H for heads or T for tails). What would be the probability of HHHH (1 in $2^4 = 1/16$). The same applies for TTTT. The same applies for THTH, or any specific sequence of coin tosses. Try it yourself if you don't believe me (predict the sequence first and then see how many times you have to run it). Genetic code is essentially a "four sided coin". So for any run of a specific sequence (e.g. GATC) the probability of getting that EXACT sequence is 1 in 4^4 , or for any number n of nucleotides (nt or bases) the chance is 1 in 4^n (this is simplified because in some situations the probability of the next base being X depends on the surrounding bases).

BLAST has two sections - nucleotide (BLASTn) and protein (BLASTp). BLASTp deals with amino acid sequences, in just the same way as nucleotide sequences. But there is a big difference because there are 20 amino acids (rather than 4 nucleotides) and therefore even short runs (e.g. QTNS = Glu-Thr-Asn-Ser) would carry a probability of somewhere

around 1 in 20⁴ (simplified), which is 1 in 160,000. The probability of a specific 5-amino acid sequence arising by random chance on the same basis jumps to 1 in 3.2million!



So let's hit the Protein BLAST button and away we go... and this is the screen you will get to, that I'm going to walk you through

BLAST®,	» blastp suite	Home				
blastn bl a	Standard Protein BLAST blastx tblastx tblastx					
Enter Query S	BLASTP programs search protein databases using a protein query. more					
	umber(s), gi(s), or FASTA sequence(s) Clear Query subrange					
>unnamed protein pro TNGTKR	11211					
Or, upload file	Choose file No file chosen					
Job Title	Protein Sequence					
	Enter a descriptive title for your BLAST search					
Align two or mor	re sequences					
Choose Searc	h Set					
Database	Non-redundant protein sequences (nr)					
Organism	Viridae (tavid:10220)					
Optional	Optional SARS-CoV-2 (taxid:2697049) SARS-CoV-2 (taxid:2697049)					
	Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.					
Exclude						
Program Selec	ction					
Algorithm	Quick BLASTP (Accelerated protein-protein BLAST)					
	blastp (protein-protein BLAST) PSI-BLAST (Position-Specific Iterated BLAST)					
	O PHI-BLAST (Pattern Hit Initiated BLAST)					
	DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST) Choose a BLAST algorithm					
BLAST	Search database nr using Blastp (protein-protein BLAST)					
	Show results in a new window					

In [1] you need to enter your amino acid sequence of interest (BLAST adds ">unnamed

protein product" automatically). Fortunately you don't need to look hard for this because we are going to concentrate on only 4 sequences within the SARS-CoV-2 viral genome/proteome and these are laid out for us in Prashant Pradhan's wonderful paper "Uncanny similarity of unique inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag" published 31st Jan 2020 a few days after the genome sequence was released.

The bit you need is in table 1 which I am reposting here and you will see that I have posted the 6-amino acid sequence TNGTKR in the box [1] marked in red on the BLASTp screen.

Motifs	Virus Glycoprotein	Motif Alignment	HIV protein and Variable region	HIV Genome Source Country/ subtype	Number of Polar Residues	Total Char ge	pI Valu e
Insert 1	2019- nCoV (GP) HIV1(GP120)	71 76 TNGTKR TNGTKR 404 409	gp120- V4	Thailand */ CRF01_ AE	5 5	2 2	11 11
Insert 2	2019- nCoV (GP) HIV1(GP120)	145 150 HKNNKS HKNNKS 462 467	gp120- V5	Kenya*/ G	6 6	2 2	10 10
Insert 3	2019- nCoV (GP) HIV1(GP120)	245 256 RSYLTPGDSSSG RTYLFNETRGNSSSG 136 150	gp120- V1	India*/C	8 10	2	10.84 8.75
Insert 4	2019- nCoV (Poly P) HIV1(gag)	676 684 QTNSPRRA QTNSSILMQRSNFKG PRRA 366 384	Gag	India*/C	6 12	2 4	12.00 12.30

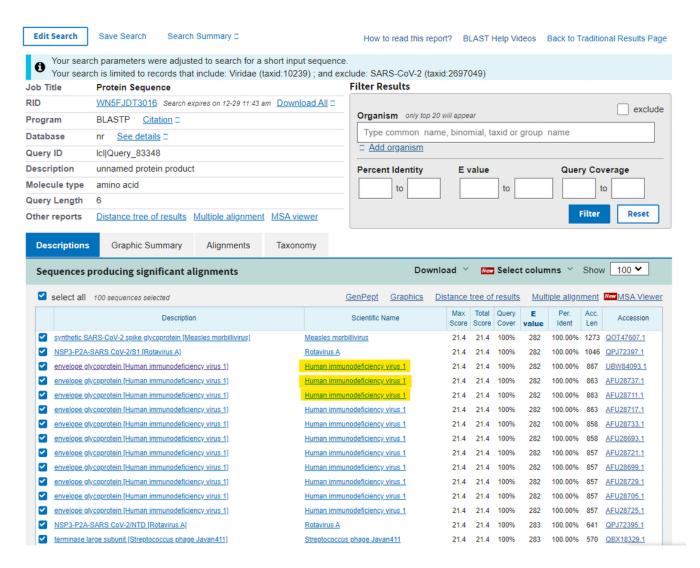
Table 1: Aligned sequences of 2019-nCoV and gp120 protein of HIV-1 with their positions in primary sequence of protein. All the inserts have a high density of positively charged residues. The deleted fragments in insert 3 and 4 increase the positive charge to surface area ratio. *please see Supp. Table 1 for accession numbers

For step [2] in the BLASTp entry screen you need to add some filters. The first filter is to restrict the search to "viridae (viruses)" (or you can just enter 10239 which is the taxonomy ID). The reason for this is that there are gazillions of species on the planet and BLASTp will search for all of them, but you really only want to know which virus this motif came from. You're not really interested if the motif is found in a squid, although it is possible that a squid also got in on the action with the famous bat-and-pangolin tetea-tete touted by the likes of Peter Daszak and Dominic Dwyer, making it a zoonotic menagerie-a-trois, but let's stick to reality.

The second condition is to exclude all the references to SARS-CoV-2 that have now

accumulated in the database, because those will all pop up (thousands of them) and we're not interested.

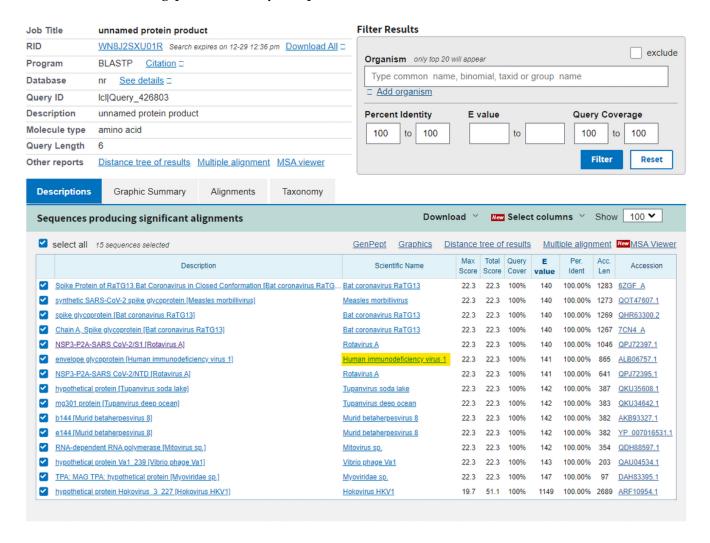
Once you've entered those hit the BLAST button and what do you get? You will get a list of candidates that have close homology to that sequence. Because it is a very short sequence the homology (likeness) should be 100%. The top of the page will be a summary of what you have requested and the rest of the page is a list of the matching sequences. What you will see immediately is near the top of the list are two synthetic viruses which are a chimaera of SARS-Cov-2 and another virus, which have appeared in the last 2 years by labs making more viruses (because we don't have enough). The next in the list is a whole bunch of references to HIV-1.



You can click on any of these and you will be taken to the alignment screen where the alignments between the subject (your TNGTKR) and query (all viruses) are shown, and you will see as you go down the page that the alignments only hold for HIV-1 until you start getting synthetic and hypothetical proteins, until the next real virus in the list which is HIV-2...

OK but one hit like this could be coincidental. In the list you will see the "E-value" which is an indicator of the probability of finding matches like this and should be as close to zero as possible. Here it's 282 which really just reflects the likelihood of finding matches on a short sequence.

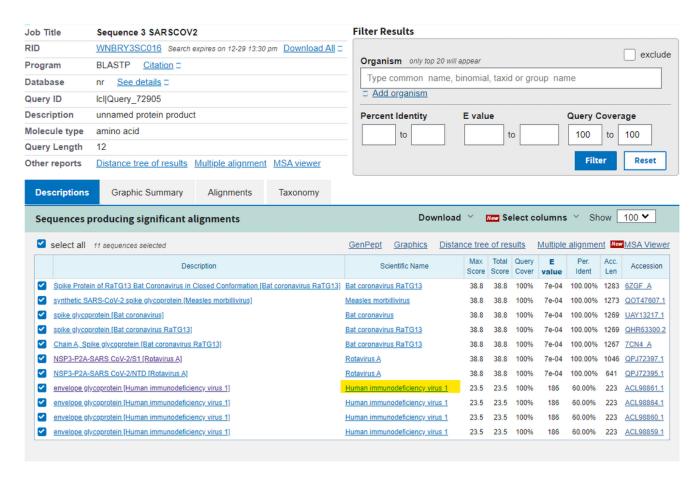
So, here's the rub. Either these sequences are by chance going to match up with a whole bunch of other viruses (because the E-value is high and therefore we should expect a lot of matches) or they are really unusual sequences that have specifically, preferentially or uniquely match with HIV-1. How shall we address this? Well let's go to the next sequence - HKNNKS, which is another short sequence. Just to tidy up the screen we can set a filter for short sequences to ensure that 100% of the sequence matches (top right). Now remember that if the match to HIV-1 was random, we shouldn't really see a match on this list, because it should be bumped off by all the other hundreds of viruses that should be matching preferentially. Oops...



Note the other matches - Bat RaTG13 which didn't appear in this database until after people started questioning the origin of the coronavirus, and is likely to be a synthetic sequence, and the same synthetic viruses that came after outbreak. So, HIV-1 is the

ONLY match for both of these sequences.

Let's go to sequence 3. This is a longer sequence. RSYLTPGDSSG. I wonder what virus (or set of random viruses, because it's a random sequence, remember) this will match to.... Oh look...

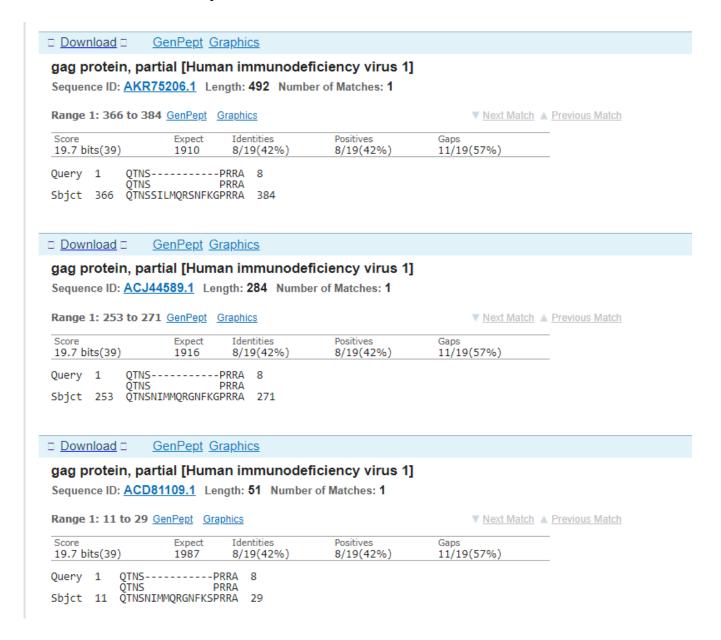


So in this search I have restricted the query coverage to 100% to get rid of the noise and the hypothetical proteins. All we are left with are the synthetic viruses from post-covid and RaTG13 (also post-covid). The only remaining virus in this list is, you guessed it, HIV-1. What are the odds that HIV-1 would pop up in all 3 searches?

And just to complete the quad-trick we need the last insert sequence identified in Pradhan's paper which is QTNS——PRRA. This is a really interesting sequence which we will come to later, because is the **furin cleavage site**. It's interesting because beta coronaviruses likes this don't have a furin cleavage site, this is the only one. Surely this site couldn't have come from HIV-1? Well, it's not from the GP120 protein like the other three sequences, it's completely different and on a different location of the virus which I'll show you soon but for now let's run the BLASTp.

This time it's a bit messier because there have been a bunch of hypothetical and synthetic proteins added since SARS-CoV-2 was released (I should have written this piece last year). But HIV-1 makes its appearance on the list again and this time I'll just

show the alignments between the gag protein and the coronavirus - in this case there is a deletion from the HIV-1 protein.



So, there we have 4 matches to HIV sequences with **no other viruses*** **appearing in all 4** match lists (*barring synthetic ones created after the event). What are the odds of that close to zero.

But look at this. These were not just random sequences from HIV. In his paper, Pradhan went further and recreated the structure of the virus with location of the four inserts. Lo and behold, these "random" inserts - all from HIV - are all at binding sites of the coronavirus. What are the odds?

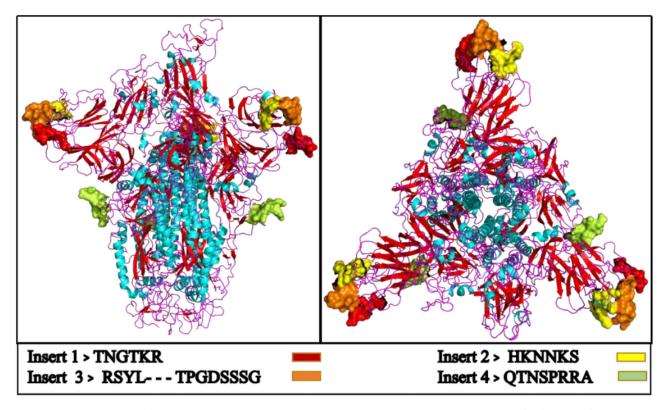


Figure 3. Modelled homo-trimer spike glycoprotein of 2019-nCoV virus. The inserts from HIV envelop protein are shown with colored beads, present at the binding site of the protein.

Now, it's possible that you aren't convinced. Despite the fact that the only virus to appear in match lists for all four inserts, from the hundreds of thousands of viruses around, happens to be HIV-1. And HIV-1 should have no real chance of forming recombinant viruses with bat coronaviruses in nature, and no real chance of forming 4 different recombinations that just happen to be at binding sites for the virus. But if that doesn't convince you there is one special feature of insert 4 we need to look at.

Now we are going back to nucleotides, the G-A-C-T's that make up the sequence that code for the amino acids that we have so far been talking about. The original reference genome sequence for the coronavirus has a genbank ID of NC_045512.1 and can be seen here: https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.1

You can play around with the genome sequence using BLASTn (for nucleotide) by going to that page and selecting "Run BLAST" from the right hand column which will take you to a similar BLAST page as had with the proteins above.

blastn blas	tp blastx tblastn tblastx	Standard Nucleotide BLAST
Enter Query Se		TN programs search nucleotide databases using a nucleotide query. <u>n</u>
	mber(s), gi(s), or FASTA sequence(s) Clear	Query subrange
ref NC_045512.1	1	From
0		То
Or, upload file	Choose file No file chosen	
Job Title	ref NC_045512.1 Enter a descriptive title for your BLAST search	
Align two or more		
Choose Search	ı Set	
Database	Standard databases (nr etc.): rRNA/ITS databases (nr etc.):	abases O Genomic + transcript databases O Betacoronaviru
	Nucleotide collection (nr/nt)	•
Organism Optional	Viridae (taxid:10239)	exclude Add organism
Exclude	Enter organism common name, binomial, or tax id. Only a Models (XM/XP) Uncultured/environmental	
Optional		sample sequences
Continuation Conti	Sequences from type material	You Tube Create custom database
Entrez Query Optional	Enter an Entrez query to limit search	Tourist Create Custom database
Program Selec	tion	
Optimize for	Highly similar sequences (megablast) More dissimilar sequences (discontiguous megablast) Somewhat similar sequences (blastn) Choose a BLAST algorithm	ablast) 3
BLAST	Search database Nucleotide collection (nr/nt) us Show results in a new window	sing Megablast (Optimize for highly similar sequences)

In a similar way you would enter NC_045512.1 (or the updated NC_045512.2) into the first box, choose the options shown in the part marked [2], select megablast in part 3 and click go, and you will get a list of SAR-CoV-2 genomes that match (obviously). I won't show that screen because it's not important here but this is the screen you get if you were to look at two closely matched sequences and you can click "CDS feature" to superimpose the amino acid sequence. You will end up with pages of something that looks like this:

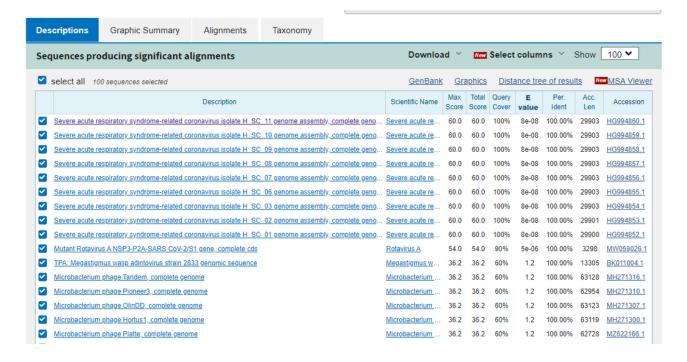
CDS:surface glycopro Query	657 23548	N N S Y E C D I P I G A G I C A S Y Q T ACAACTCATATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTC	23607
Sbjct	23532	ACAACTCATATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTC	23591
CDS:surface glycopro	657	N N S Y E C D I P I G A G I C A S Y Q T	
CDS:surface glycopro	677	Q T N S P R A R S V A S Q S I I A Y T	23667
Query	23608	AGACTAATTCTCCTCGGCGGGCACGTAGTGTAGCTAGTCAATCCATCATTGCCTACACTA	
Sbjct	23592	AGACTAATTCTCCTCGGCGGGCACGTAGTGTAGCTAGTCAATCCATCATTGCCTACACTA	23651
CDS:surface glycopro	677	Q T N S P R R A R S V A S Q S I I A Y T	
CDS:surface glycopro Query	697 23668	M S L G A E N S V A Y S N N S I A I P T TGTCACTTGGTGCAGAAAATTCAGTTGCTTACTCTAATAACTCTATTGCCATACCCACAA	23727
Sbjct	23652	TGTCACTTGGTGCAGAAAATTCAGTTGCTTACTCTAATAACTCTATTGCCATACCCACAA	23711
CDS:surface glycopro	697	M S L G A E N S V A Y S N N S I A I P T	
CDS:surface glycopro Query	717 23728	N F T I S V T T E I L P V S M T K T S V ATTTTACTATTAGTGTTACCACAGAAATTCTACCAGTGTCTATGACCAAGACATCAGTAG	23787
Sbjct	23712	ATTTTACTATTAGTGTTACCACAGAAATTCTACCAGTGTCTATGACCAAGACATCAGTAG	23771
CDS:surface glycopro	717	N F T I S V T T E I L P V S M T K T S V	

In this particular section you can see it's a sequence of the "spike protein" (surface glycoprotein) and the nucleotides are labelled 23548...23771 (of about 30,000 nucleotides or bases i.e. G-C-A-T). [NB: in actual fact this is RNA so should have a U in place of every T, but BLAST compensates for this automatically for simplicity]. The smaller number is the number of amino acid in the protein sequence so for each 3 nucleotides, the number goes up by 1 amino acid. The highlight is amino acid 677 (Q) to 686 (S), giving $677\rightarrow686 = QTNSPRRARS$.

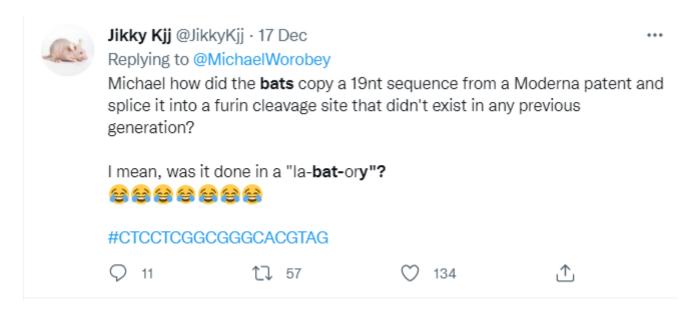
Now, this is *really* interesting because not only have we seen that the QTNS section is derived from HIV but there is something very special about the adjacent PRRAR because that is a **furin cleavage** site and as we have seen, these don't exist in this type of SARS-like virus. It's an insertion to the viral genome, but nobody really knows how it got there (just like the HIV sequences). In order to see where it came from we need to look outside the amino acid sequence and back to the genome sequence.

The genome sequence that you can see for this amino acid sequence is:

CAGACTAATTCTCCTCGGCGGGCACGTAGT which is 30 nucleotides coding for 10 amino acids. For this sequence to arise by chance would be an infinitesimally small number, so it has to have arisen somewhere (i.e. from another virus) or else some of it must be synthetic. So let's BLAST(n) it, and this time we exclude "synthetic constructs" from our search (because we are looking for real viruses, not synthetic ones). What do we get?



So, now you are getting used to these displays we can see that the only viral sequences in here are synthetic, and if you were to click on each of these you would find their registration date after Feb 2020. In other words, no virus in existence has this genetic sequence. Well this is strange, because in order for a virus to acquire a large sequence like this it has to get it from another organism. It has no lab to manipulate gene sequences, neither do the bats (hence Jikky the lab mouse's little joke)...

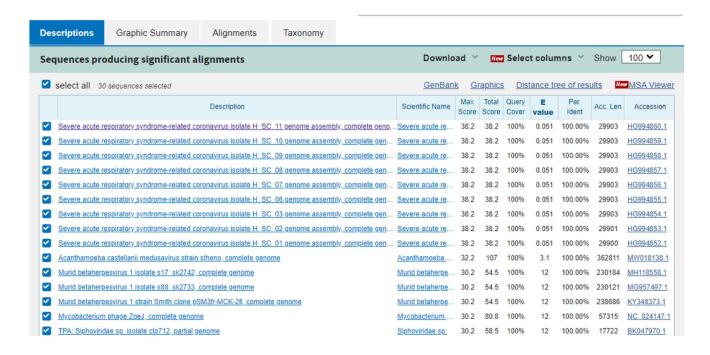


It's easy enough to change a single nucleotide (a single point mutation or SNP) or even insert or delete nucleotides (less common) but to insert 20 or 30 nucleotides with a code that works? Nope, that has to come from another virus or else it's been done in a lab.

So, where did this code come from? Well it turns out that BLAST can tell us - with some degree of certainty - where some of this code, particularly the bit that codes for the

PRRAR section (the furin cleavage site which is so unique), came from.

The bit that we are interested in is in the clue from Jikky. CTCCTCGGCGGCACGTAG. Let's BLAST it

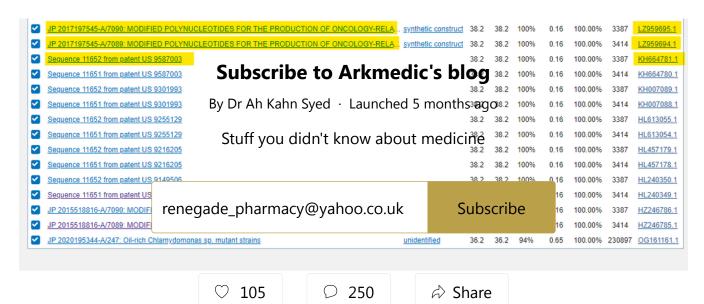


What you see is the same (misclassified SARS-CoV-2) sequences in the first 9 hits, and then none of the remaining hits have 19/19 matches. What this means is that there is no virus known to man that has this particular sequence in its genome prior to the discovery of SARS-Cov-2. So where on earth has it come from? For this you need to select a different database. Let's go back to the BLASTn query screen and change the database option to "Patent sequences (pat)". Remove all the exclusions and run the BLAST.

Standard Nucleotide BLAST

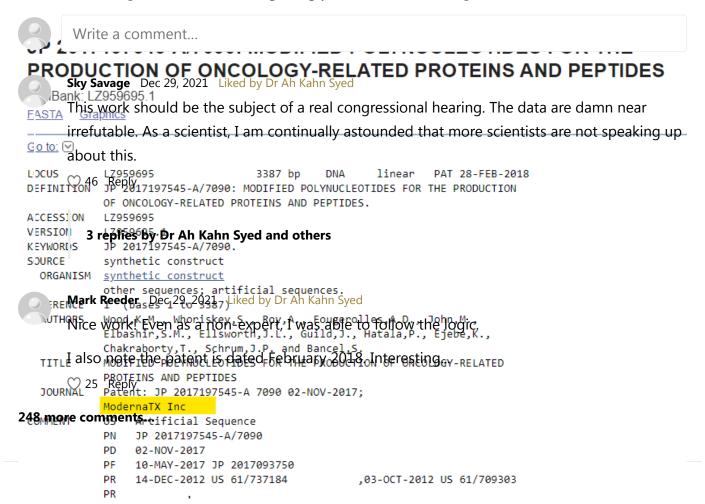
blastn	blastp	blastx	tblastn	tblastx		
Enter O	BLASTN programs search nucleotide databases using a nucleotide query. more					
	Enter Query Sequence Enter accession number(s), gi(s), or FASTA sequence(s) Clear Query subrange					
	CTCCTCGGCGGGCACGTAG From					
					То	
Or, upload	Or, upload file Choose file No file chosen					
Job Title	SA	SARS2 FCS genome patent BLAST				
Alian tu	Enter a descriptive title for your BLAST search Align two or more sequences					
		ences				
Choose	Search Set	tandard databa	ses (nr etc.):	rRNA/ITS data	hases Genomic + transcript databases Betacoronavirus	
	Database Standard databases (nr etc.): ○ rRNA/ITS databases ○ Genomic + transcript databases ○ Betacoronavirus Patent sequences(pat)					
Organism	Enter organism name as id completions will be auggested avaluate Add associated					
Optional		Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown				
Exclude Optional	_ 1	☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences				
Limit to Optional		Sequences from type material				
Entrez Que	,	You to Create custom database Enter an Entrez query to limit search				
Program Selection						
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More dissimilar sequences (discontiguous megablast) Somewhat similar sequences (blastn)				blast)		
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BLA		rch database p Show results in a	_	(Optimize for s	somewhat similar sequences)	

The results here need a little bit of sifting through because the top of the list include results from patents from this year. They are prefixed WO2021 and WO2020 so can be ignored. Just below those are the ones that we are interested in. I have just highlighted the top three but the whole list of many are patents owned by the same company, you just have to click on the accession number on the right.



So let's do it and see which company, that we all know (now), that is a pharma company

that has never produced a working drug yet has a market cap of over \$80bn...



Yes, that's right. Every single one of these patents that contains that 19nt sequence (for which the probability of occurring by random chance is less than 1 in a billion) is from Moderna. [Note the sequence is **Readly**htorwall plement sequence but this is likely a direct result of the cell lines that it occurred in - MSH3_mutated cell lines designed for developing renegade_pharmacy@ya derr was actually for a mutated MSH3 gene for this purpose]

In order for that sequence to have arisen in that virus, the virus which was manufactured with its HIV inserts, had to have had been infected into patented cell lines supplied by Moderna that had that unique sequence not seen in any other virus.

In theory nothing is impossible in science, medicine or genomics. A SARS virus emerging naturally with 3 HIV inserts at its binding sites and also containing a furin cleavage site that doesn't exist in nature but does exist in a Moderna patent... that's seriously crazy talk. It doesn't exist. A flying pink elephant would be a million times more likely.