BLAST

Slides adapted & edited from a set by Cheryl A. Kerfeld (UC Berkeley/JGI) & Kathleen M. Scott (U South Florida)

Kerfeld CA, Scott KM (2011) Using BLAST to Teach "E-value-tionary" Concepts. *PLoS Biology* 9(2):e1001014

Starts with a Query Sequence in FASTA Format

Amino acid sequence:

```
>ribosomal protein L7/L12 [Thiomicrospira crunogena XCL-2]
MAITKDDILEAVANMSVMEVVELVEAMEEKFGVSAAAVAVAGPAGDAGAA
GEEQTEFDVVLTGAGDNKVAAIKAVRGATGLGLKEAKSAVESAPFTLKEG
VSKEEAETLANELKEAGIEVEVK
```

Note the description line
Starts with ">", ends with carriage return
Not read as sequence data

Nucleotide sequence:

```
>gi|118139508:333094-333465 Thiomicrospira crunogena XCL-2
ATGGCAATTACAAAAGACGATATTTTAGAAGCAGTTGCTAACATGTCAGTAATGGAAG
TTGTTGAACTTGTTGAAGCAATGGAAGAGAAGTTTGGTGTTTCTGCAGCAGCAGTTGC
GGTTGCAGGTCCTGCAGGTGATGCTGGCGCTGCTGGTGAAGAACAAACAGAGTTTGAC
GTTGTCTTGACTGGTGCTGGTGACAACAAAGTTGCAGCAATCAAAGCCGTTCGTGGCG
CAACTGGTCTTGGGCTTAAAGAAGCGAAAAGTGCAGTTGAAAGTGCACCATTTACGCT
TAAAGAGGGTGTTTCTAAAGAAGAAGCAGAAACTCTTGCAAATGAGCTTAAAGAAGCA
GGTATTGAAGTCGAAGTTAAATAA
```

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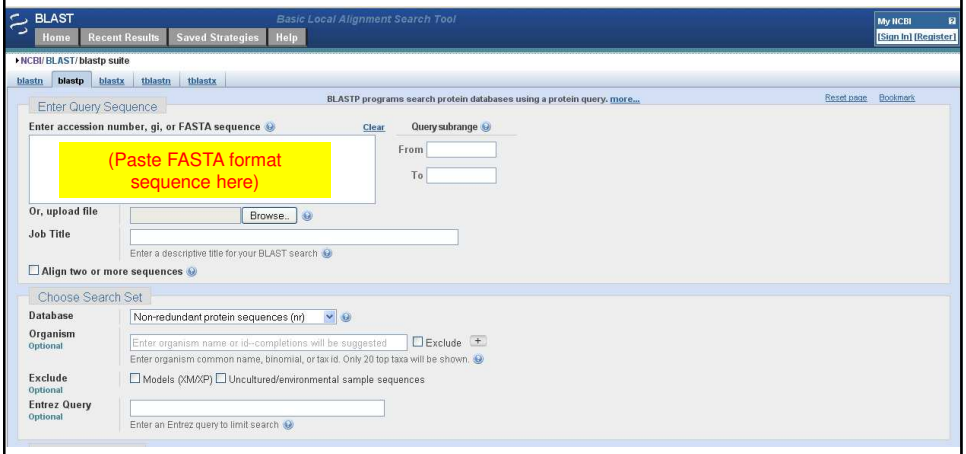
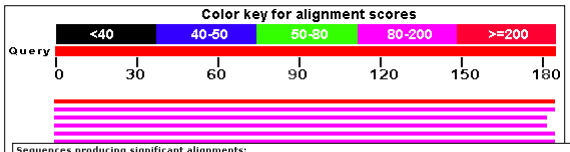
NCBI BLAST Interface
(blastp: for protein-protein alignments)



NCBI BLAST Results Page:
Potential homologs retrieved from database



Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value
NP_440048.1	potential FMN-protein [Synechocystis sp. PCC 6803] >sp|P727	379	379	100%	1e-103
YP_001864295.1	flavin reductase domain-containing protein [Nostoc punctiform	199	199	100%	2e-49
YP_321888.1	flavin reductase-like, FMN-binding [Anabaena variabilis ATCC	198	198	98%	3e-49
NP_488484.1	flavoprotein [Nostoc sp. PCC 7120] >sp|Q8YNW7.1|DFA4_ANA	197	197	98%	6e-49
CAQ89562.1	dfa4 [Microcystis aeruginosa PCC 7806]	194	194	100%	3e-48
ZP_01630850.1	flavoprotein [Nodularia spumigena CCY9414] >gb|EAW44518.	193	193	100%	6e-48

>ref|YP_002482587.1| flavin reductase domain protein FMN-binding [Cyanothece sp. PCC 7425]
gb|ACL44226.1| flavin reductase domain protein FMN-binding [Cyanothece sp. PCC 7425]
Length=585

GENE ID: 7287783 Cyan7425_1859 | flavin reductase domain protein FMN-binding [Cyanothece sp. PCC 7425]

Score = 176 bits (446), Expect = 1e-42, Method: Compositional matrix adjust.
Identities = 95/196 (48%), Positives = 125/196 (63%), Gaps = 16/196 (8%)

```
Query  1    SGANFARQLRTHKRQRIARQATTETQADRTQQAVGRIIGSIGVVTTQTTGRH--------  52
            +G++FA+ L+  K+QR  RQ+  E Q+DRT+QAVGRIIGS+ V+T +    H
Sbjct  393  AGSDFAQVLKKAKKQRSPRQSILEVQSDRTEQAVGRIIGSLCVLTAKQQQTHPHPEVEEP  452

Query  53   -----QGILTSWVSQASFTPPGIMLAIPGEFDAYGLAGQNKAFVLNLLQEGRSVRHPFDH  107
                 +L SWVSQASF PPG+ +A+   E  A GL      AFVLN+L+EG ++RHHF
Sbjct  453  QLEVPTAMLYSWVSQASFNPPGLTLALAKE-RARGLDHSGDAFVLNVLEGDWLRHHFSK  511

Query  108  QPLPKDGDNPFSRLEHYSTQNGCLILAEALAYLECLVQSWSNIGDHVLVYATVQAGQVLQ  167
                  P  G++ F+ L      +NGC +L + LAYLEC VQS    GDH L+YATV  G+VLQ
Sbjct  512  SFAP--GEDRFAGLNIQWAENGCPVLQDCLAYLECTVQSRMECGDHVLIYATVNNGKVLQ  569

Query  168  PNGITAIRHRKSGGQY  183
            P G TA++HRKSG QY
Sbjct  570  PTGTTAVQHRKSGNQY  585
```

Overview of BLAST

1. Segment the query sequence into short “words”
2. Use the query sequence segments to scan the database for matching sequences
3. Extend the matched segments in either direction to find local alignments.
4. Create a list of hits & alignments, with best matches first

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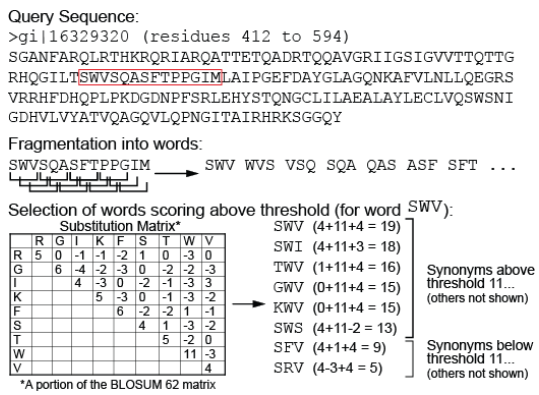
BLAST Phase 1: Segment the query sequence and identify words that could form potential alignments



- Segment the query sequence into pieces (“words”)
  - Default word length: 3 amino acids or 11 nucleic acids
- Create a list of synonyms and their scores for comparing query words to target words
  - Uses scoring matrix to calculate scores for synonyms that might be found in the database
- Save the scores (and synonyms) exceeding a given threshold T

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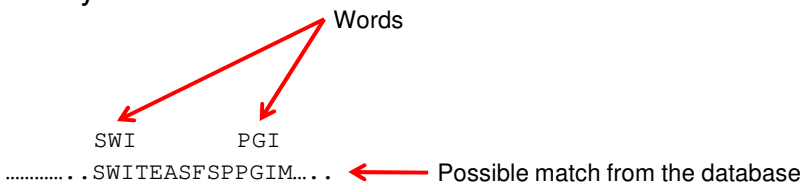
BLAST Phase 2: Using the query sequence word list, scan the database for synonyms (hits)

- Scan the database for matches to the word list with acceptable T values
- Require two matches ("hits") within the target sequence
- Set aside sequences with matches above T for further analysis



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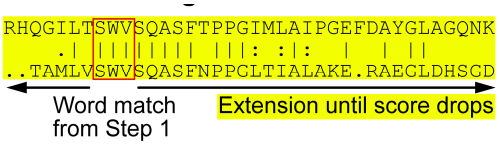
BLAST Phase 3: Extending the hits

- Search 5' and 3' of the word hit on both the query and target sequence
- Add up the score for sequence identity or similarity until value exceeds S
- Alignment is dropped from subsequent analyses if value never exceeds S



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So, to summarize:

- BLAST segments query sequence into “words” and scores potential word matches
- Scans this list for alignments that meet a threshold score T
  - uses a scoring matrix to calculate this (e.g., **BLOSUM62**)
- Uses this list of ‘synonyms’ to scan the database
- Extends the alignments to see if they meet a cutoff score S
  - uses a scoring matrix to calculate this
- Reports the alignments that exceed S

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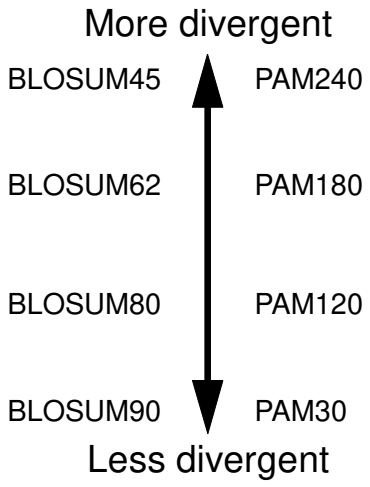
PAM and BLOSUM Matrices

- Scoring matrices are calibrated to capture different degrees of sequence similarity
- In practice, this means choosing a matrix appropriate to the suspected degree of sequence identity between the query and its hits
- PAM: empirically derived for close relatives
- BLOSUM: empirically derived for distant relatives



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Raw Scores (*S* values) from an Alignment

$$S = (\Sigma M_{ij}) - cO - dG,$$

where

M = score from a similarity matrix for a particular pair of amino acids (ij)

c = number of gaps

O = penalty for the existence of a gap

d = total length of gaps

G = per-residue penalty for extending the gap

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Limitations of Raw Scores

- S values depend on the substitution matrix, gap penalties
- Impossible to compare S values from hits retrieved from BLAST searches when different matrices and gap penalties are used

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Going from Raw Scores to Bit Scores

$$S' = [\lambda S - \ln(K)]/\ln(2)$$

(as in 0 vs 1)

where

S' = bit score

λ and K = normalizing parameters of the specific matrices and search spaces

- Larger raw scores result in larger bit scores
- Allows user to compare scores obtained by using different matrices and search spaces

Limitations of Bit Scores

- How high does a bit score have to be to suggest common ancestry?
  - Hard to evaluate hits as homologs or not, based solely on bit scores

E-value

- Number of distinct alignments with scores greater than or equal to a given value expected to occur in a search against a database of known size, based solely on chance, not homology.
  - Large E-values suggest that the query sequence and retrieved sequence similarities are due to chance
  - Small E-values suggest that the sequence similarities are due to shared ancestry (or potentially convergent evolution)

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Calculating E-values

$$E = (n \times m) / 2^{S'}$$

where

m = effective length of the query sequence

= length of query sequence – average length of alignments

(Controls for fewer alignments occurring at the ends of the query sequence)

n = effective length of the database sequence

(total number of bases)

The value of E decreases exponentially with increasing S

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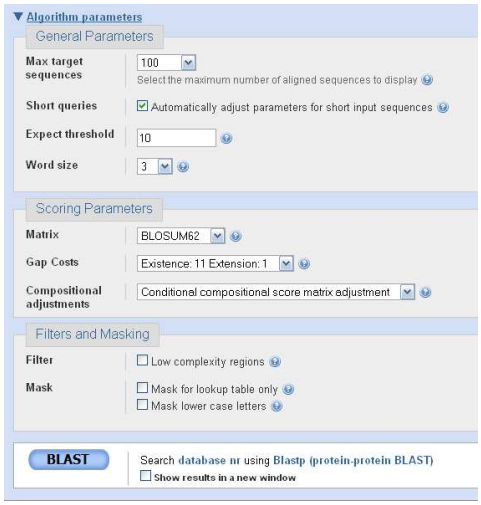
BLAST Parameters

- Expect
- Word size
- Matrix
- Gap costs
- Filter
- Mask



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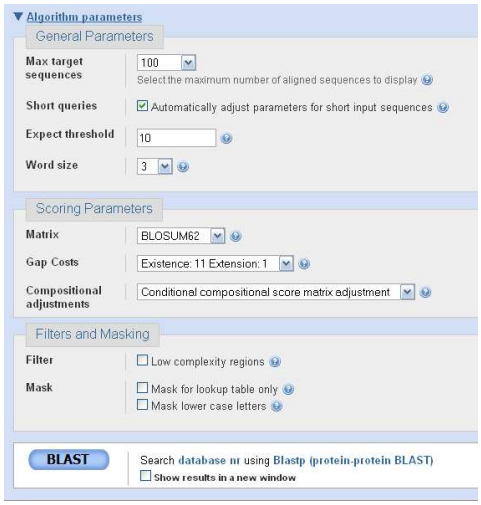
E value Threshold

- Alignments will be reported with E-values less than or equal to the expect values threshold
  - Setting a larger E threshold will result in more reported hits
  - Setting a smaller E threshold will result in fewer reported hits



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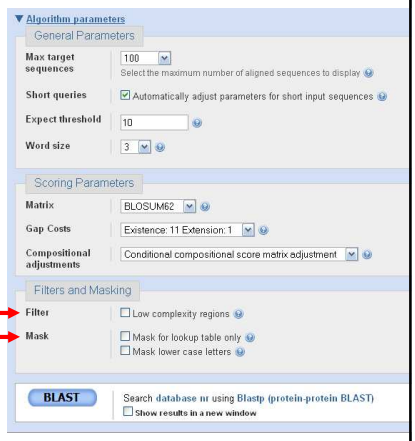
Filter and Mask

- Filter: Low complexity
  - Replaces the following with N (nucleotides) or X (amino acids)
    - Dinucleotide repeats
    - Amino acid repeats
    - Leader sequences
    - Stretches of hydrophobic residues
- Mask: Lower case
  - Replaces lowercase letters in sequence with N or X
    - Lowercase letters typically indicate base or amino acid not known with certainty



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Parameter Summary is Found at the Bottom of the Output…..

Search Parameters	
Program	blastp
Word size	3
Expect value	10
Hitlist size	100
Gapcosts	11,1
Matrix	BLOSUM62
Filter string	F
Genetic Code	1
Window Size	40
Threshold	11
Composition-based stats	2

Database	
Posted date	Sep 6, 2010 4:42 AM
Number of letters	4,014,994,744
Number of sequences	11,756,863
Entrez query	none

Karlin-Altschul statistics		
Lambda	0.319424	0.267
K	0.13352	0.041
H	0.397413	0.14

Results Statistics	
Length adjustment	129
Effective length of query	54
Effective length of database	2498359417
Effective search space	134911408518
Effective search space used	134911408518

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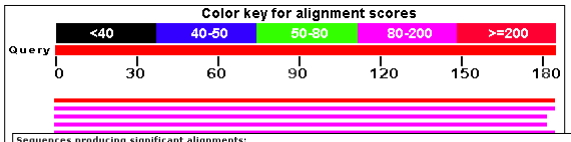
Evaluating BLAST Results



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            +G++FA+ L+  K+QR  RQ+  E Q+DRT+QAVGRIIGS+ V+T +     H
Sbjct  393  AGSDFAQVLKKAKKQRSPRQSILEVQSDRTEQAVGRIIGSLCVLTAKQQQTHPHPEVEEP  452

Query  53   -----QGILTSWVSQASFTPPGIMLAIPGEFDAYGLAGQNKAFVLNLLQEGRSVRRHFDH  107
                     +L SWVSQASF PPG+ +A+  E  A GL     AFVLN+L+EG ++RRHF
Sbjct  453  QLEVPTAMLVSWVSQASFNPPGLTIALAKE-RAEGLDHSGDAFVLNVLKEGMNLRRHFSK  511

Query  108  QPLPKDGDNPFSRLEHYSTQNGCLILAEALAYLECLVQSWSNIGDHVLVYATVQAGQVLQ  167
             P  G++ F+ L      +NGC +L + LAYLEC VQS    GDH L+YATV  G+VLQ
Sbjct  512  SFAP--GEDRFAGLNIQWAENGCPVLQDCLAYLECTVQSRMECGDHWLIYATVNNGKVLQ  569

Query  168  PNGITAIRHRKSGGQY  183
            P G TA++HRKSG QY
Sbjct  570  PTGTTAVQHRKSGNQY  585
```


Examine the BLAST Alignment

>ref|YP_002482587.1| flavin reductase domain protein FMN-binding [Cyanothece sp. PCC 7425]
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Length=585

Does it cover the whole length of both the query and subject sequences?

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            +G++FA+ L+  K+QR  RQ+  E Q+DRT+QAVGRIIGS+ V+T +     H
Sbjct  393  AGSDFAQVLKKAKKQRSPRQSILEVQSDRTEQAVGRIIGSLCVLTAKQQQTHPHPEVEEP  452

Query  53   -----QGILTSWVSQASFTPPGIMLAIPGEFDAYGLAGQNKAFVLNLLQEGRSVRRHFDH  107
                     +L SWVSQASF PPG+ +A+  E  A GL     AFVLN+L+EG ++RRHF
Sbjct  453  QLEVPTAMLVSWVSQASFNPPGLTIALAKE-RAEGLDHSGDAFVLNVLKEGMNLRRHFSK  511

Query  108  QPLPKDGDNPFSRLEHYSTQNGCLILAEALAYLECLVQSWSNIGDHVLVYATVQAGQVLQ  167
             P  G++ F+ L      +NGC +L + LAYLEC VQS    GDH L+YATV  G+VLQ
Sbjct  512  SFAP--GEDRFAGLNIQWAENGCPVLQDCLAYLECTVQSRMECGDHWLIYATVNNGKVLQ  569

Query  168  PNGITAIRHRKSGGQY  183
            P G TA++HRKSG QY
Sbjct  570  PTGTTAVQHRKSGNQY  585
```

High E-value: Discovery of a Distant Homolog or Garbage?

- Take another look at the target (subject) sequence(s) that have high E-values
  - Similar length?
  - Recurring motifs?
  - Similar biological functions?
- Use target sequences as query sequences for another BLAST search
  - Does the original query sequence come up in report?

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Or to take a more topical BLAST search, a high-profile, now retracted, *bioRxiv* preprint:

Uncanny similarity of unique inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag This article has been withdrawn. Click here for details

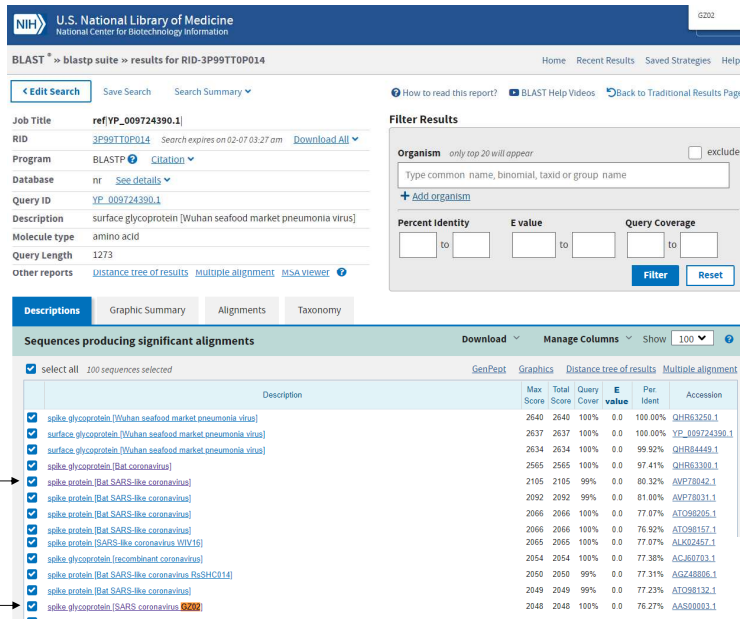
Prashant Pradhan, Ashutosh Kumar Pandey, Akhilesh Mishra, Parul Gupta, Praveen Kumar Tripathi, Manoj Balakrishnan Menon, James Gomes, Perumal Vivekanandan, Bishwajit Kundu

"We … compared the spike glycoprotein sequences of the 2019-nCoV to SARS …we found that the 2019- nCoV spike glycoprotein contains 4 insertions"

"To further investigate if these inserts are present in any other corona virus, **we performed a multiple sequence alignment of spike glycoprotein sequences of all available coronaviruses** in NCBI refseq. We found that **these 4 insertions are unique to 2019-nCoV and are not present in other coronaviruses analyzed**."

"To our surprise, **all the 4 inserts in the 2019-nCoV mapped to short segments of amino acids in the HIV-1 gp120 and Gag among all annotated virus proteins in the NCBI database.** This uncanny similarity of novel inserts in the 2019- nCoV spike protein to HIV-1 gp120 and Gag is unlikely to be fortuitous."

Let's repeat their BLAST analysis: Wuhan coronavirus spike protein x nr database

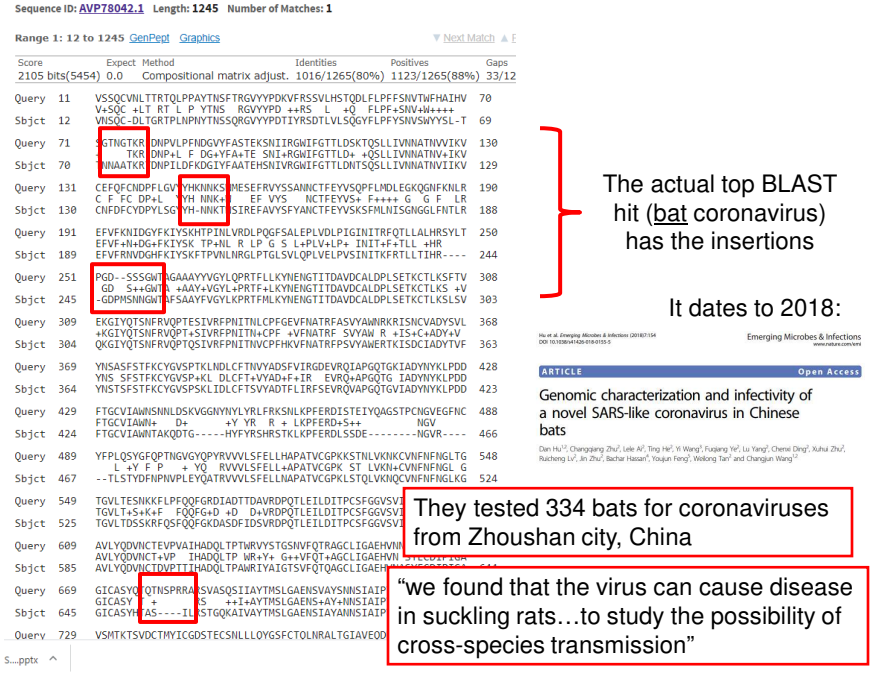


A better top hit

Their top hit

Score %ID
2105 80%
vs
2048 76%

25



The actual top BLAST hit (bat coronavirus) has the insertions

It dates to 2018:

They tested 334 bats for coronaviruses from Zhoushan city, China

"we found that the virus can cause disease in suckling rats…to study the possibility of cross-species transmission"

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