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LAMP-Blast: Easier method to blast LAMP primersets with NCBI's Blast

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

Abstract

LAMP-blast is a method to make LAMP primers specificity check easier and faster.

Get primers

1 Get your LAMP primers or design them (many possible ways / software)
The example in this protocol uses SARS-COV2 primers from Rabe & Cepko, 2020 obtained from Lamp Bank (**https://lampprimerbank-frontend-heiderlab-53b16a.pages.imims.de/**)

Rabe BA, Cepko C. SARS-CoV-2 detection using isothermal amplification and a rapid, inexpensive protocol for sample inactivation and purification. Proc Natl Acad Sci U S A. 2020 Sep 29;117(39):24450-24458. doi: 10.1073/pnas.2011221117. Epub 2020 Sep 8. PMID: 32900935; PMCID: PMC7533677.

2 For convenience, they are expected to be in a fasta format with standardized header, like this:

[NAME] - [SET NUMBER] - [PRIMER NAME]

>name-setnumber-F3
TGCTTCAGTCAGCTGATG

>SARS_cov-set1992-F3
TGCTTCAGTCAGCTGATG
>SARS_cov-set1992-F2
TTAAATTGTCATCTTCGTCCTT
(...)

By default, individual primers follow the LAMP senseness;
F3, F2, B1, LB **are sense**; 5' - 3'
F1, B2, B3, LF are **reverse-complementary**; 3' - 5' (when compared to the F3 sequence, for instance)

Lamp-blast will automatically convert all primers to 5' - 3'

Otherwise, use lamp-blast-all_sense.sh script

If your primers are not individual nor in fasta format, please leave a comment in the github page issues

3

Convert the primers to lamp-blast format

4 Convert the primers using the lamp-blast.sh script (https://github.com/Aciole-David/lamp-blast) lamp-blast tool convertion tool
The tool expects the all primers are individual, not FIP - BIP format

Right: F3, F2, F1, B1, B2, B3, LF, LB

Wrong: F3, **FIP**, **BIP**, B3, LF, LB

The order is not important

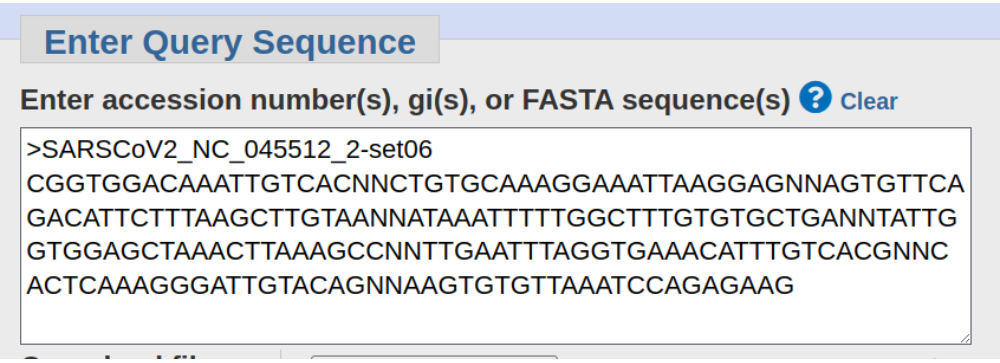
Your sequence will look like this:

>SARSCoV2_NC_045512_2-set06
CGGTGGACAAATTGTCAC**NN**CTGTGCAAAGGAAATTAAGGAG**NN**AGTGTTCAGACATTCTT
TAAGCTTGTAA**NN**ATAAATTTTTGGCTTTGTGTGCTGA**NN**TATTGGTGGAGCTAAACTTAAA
GCC**NN**TTGAATTTAGGTGAAACATTTGTCACG**NN**CACTCAAAGGGATTGTACAG**NN**AAGTG
TGTTAAATCCAGAGAAG

You can copy the sequence above as a test query

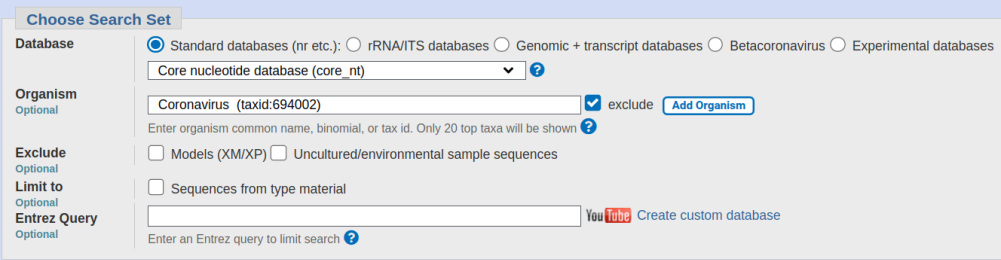
NCBI Blast search using lamp-blast format

5 Paste the converted primers sequence in the query formEnter the converted lamp primers sequence in the NCBI blast (blastn)
https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome



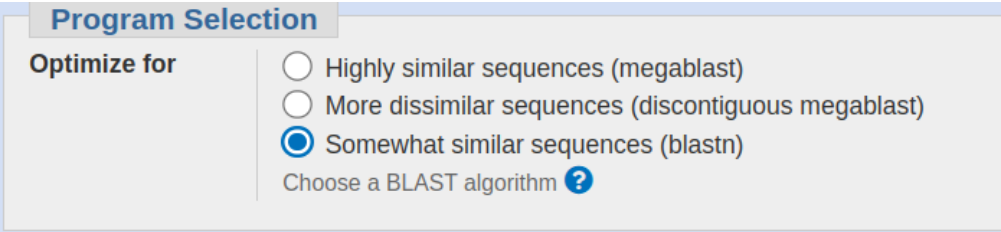
Paste the converted query sequence

6 OPTIONAL: You can add filters to refine the blast search
That will depend on what you wish to know;
Non-targets, related species, etc...



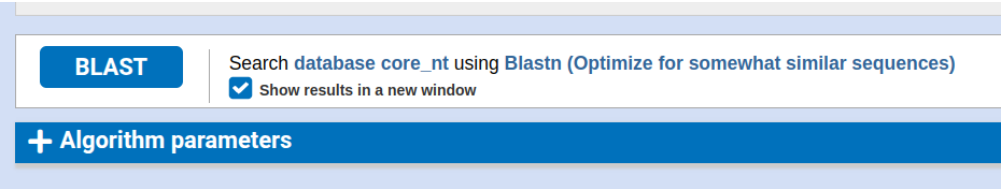
Optional: add filters

7 Select "Somewhat similar sequences (blastn)"



Change the blast program

8 Open the algorithm parameters option

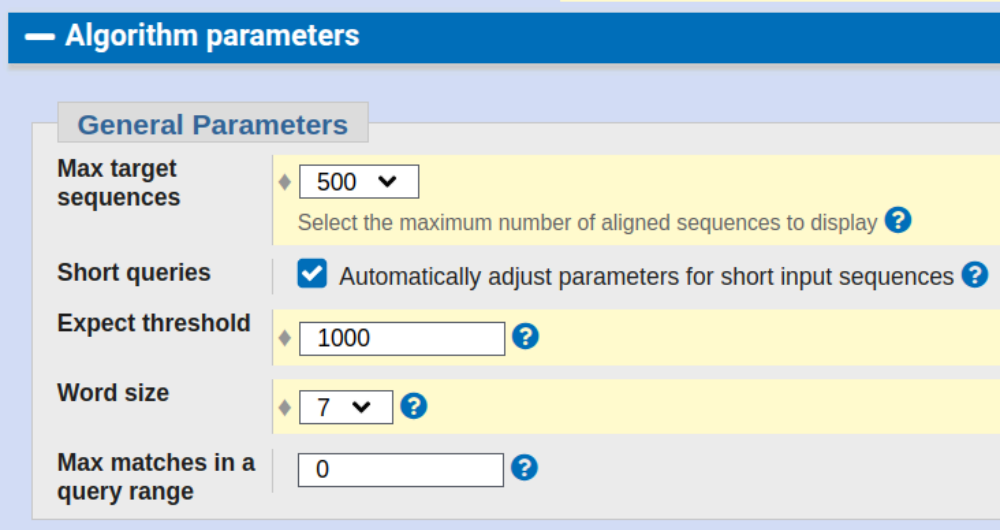


Open algorithm parameters

9 Change general parametersSet "Max target" sequences to a number you find reasonable.
Recommended not too many so we can better view the results
Otherwise, go back to **step 6** and change the filters

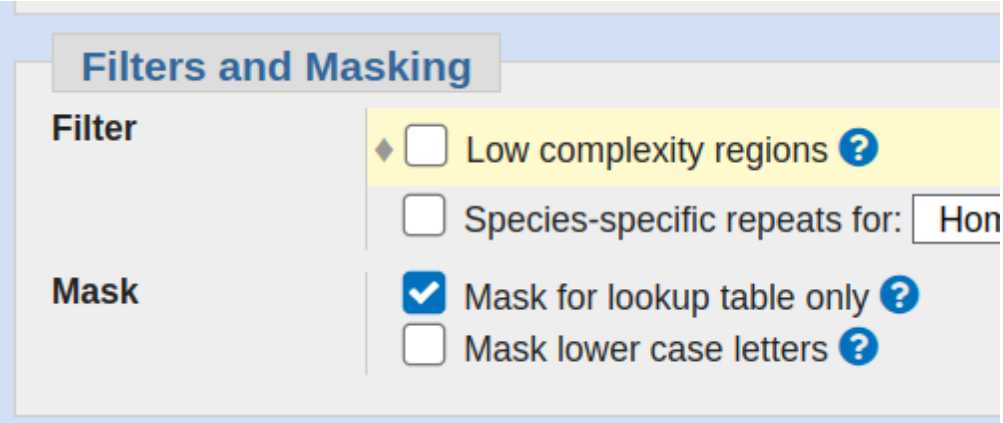
Set "Expected threshold" to **1000**

Set "Word size" to **7**



Change general parameters

10 Uncheck "Low complexity regions"



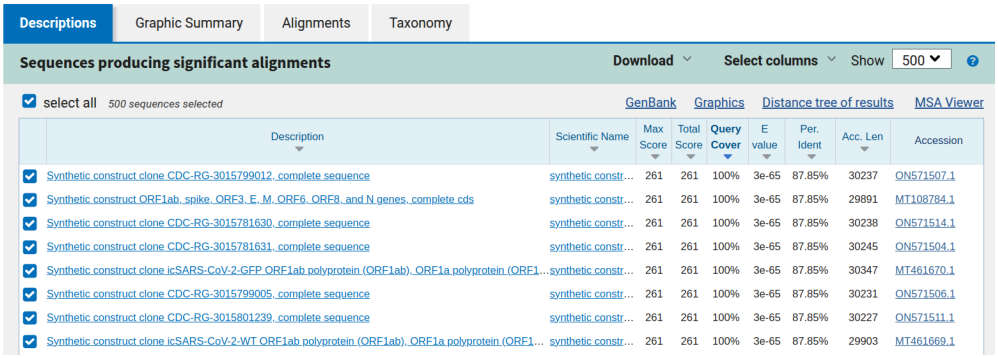
Uncheck "Low complexity regions"

11 Click the BLAST button

Press BLAST

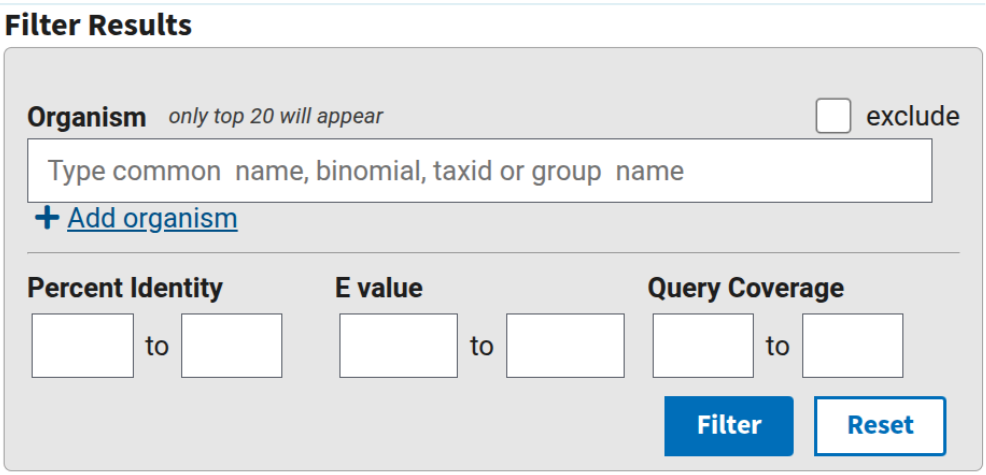
Lamp-blast BLAST results

12 On results page, click "Query Cover" to reorder results by coverage on query sequence



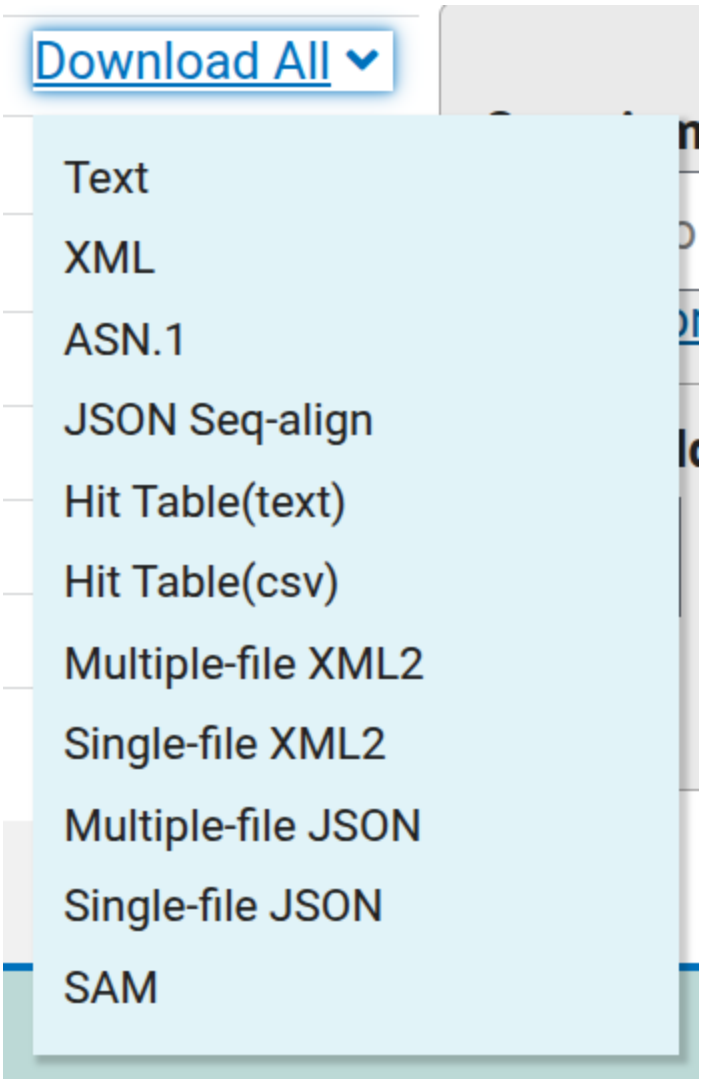
Reorder results

13 OPTIONAL: Filter results
Filters are available if one wish to include or exclude some of the resultsOptionally add filters to the results pageusein



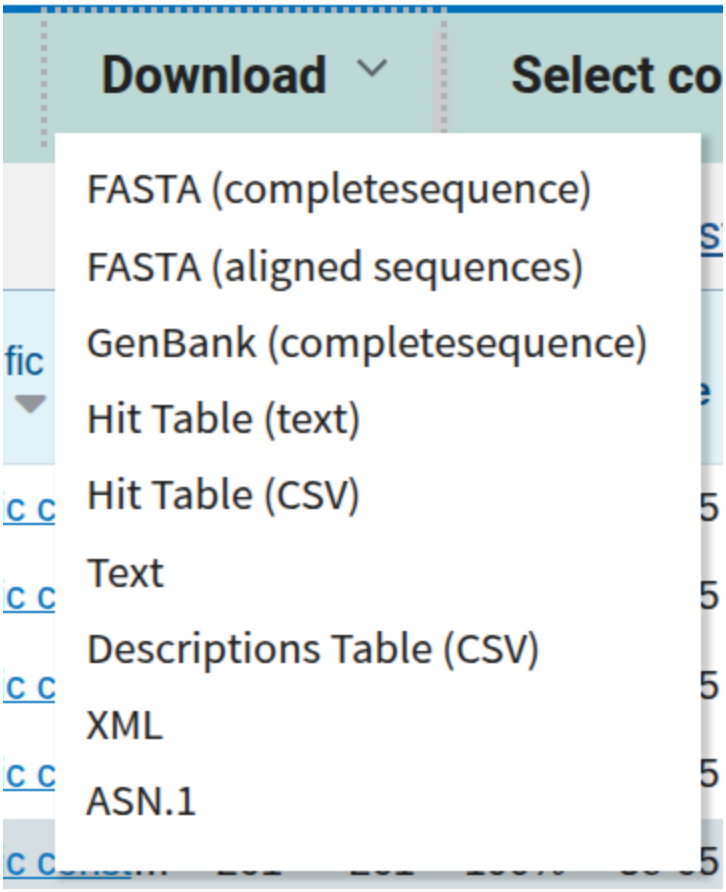
Optionally use filters in the results page

14 OPTIONAL: Download all results
If the query is composed of more than one query, use the "Download all" link to get the results in different formats



Optionally "Download all" results

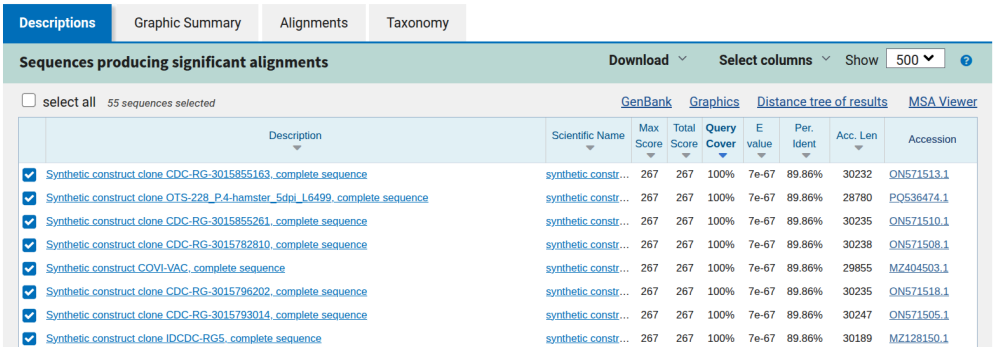
15 Optional: Download results
Use the "Download" button in the section "Sequences producing significant alignments" to get the current results in different formats



Download current results

16 Optional: Select a portion of the hits before visualizing
One can uncheck the "select all"
Then, select certain hits
As a shortcut:
- - - Unchek the "select all"
- - - Check the first result
- - - Hold the **SHIFT** key
- - - Scroll down until you find a certain hit with a low e-value / cov / etc;
this will limit the number of results in the view step to be between the first (top) result until the last selected result
- - - check the last hit you wish to see in the viewer

The image below, for example, shows we'll (arbitrarily) limit to see only:
the best results (100% query cover / 7e-67 e-value / 89.86% identity)
down to
not good results (21% query cover / 587 e-value / 80.49% identity)



Best results

Not so good results

MSA viewer of results

17 Open MSA viewer
Use the Multiple Sequence Alignment (MSA) viewer

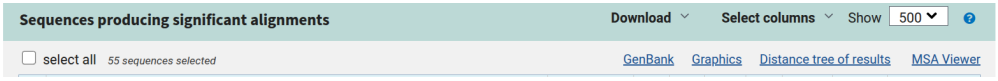
17.1 MSA on all hits

To see a MSA for all the hits, without any filter, click the **MSA viewer** button next to "Other reports Distance tree of results"

MSA viewer button of all results

17.2 MSA on selected hits

To see a MSA for the selected hits, click the **MSA viewer** button in the rightmost of "Sequences producing significant alignments" area
The image below shows the example from step 16



MSA viewer button of selected results

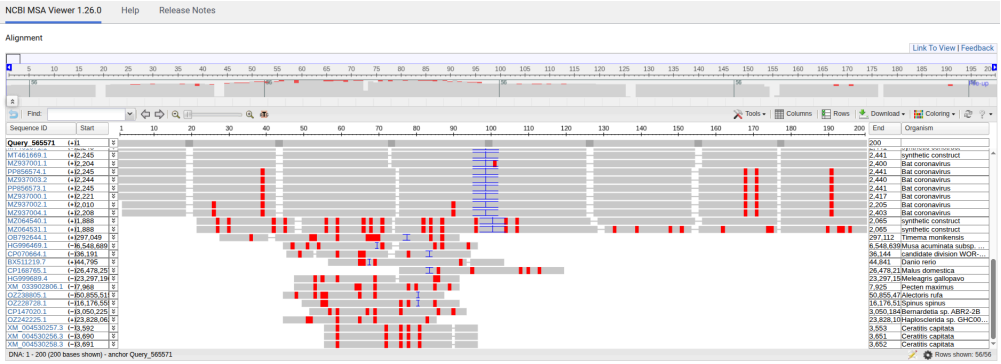
18 The MSA viewer page

MSA viewer shows a query-anchored alignment of hits, so the first sequence is the query

The query is the joined, converted primers, so you'll see all primers if they have a good overall match
Dark Intervals in the query are the 'NN'
The 'best' results are lightgrey

top 'best results'

Mismatches are highlighted red by default



Bottom 'not so good' results

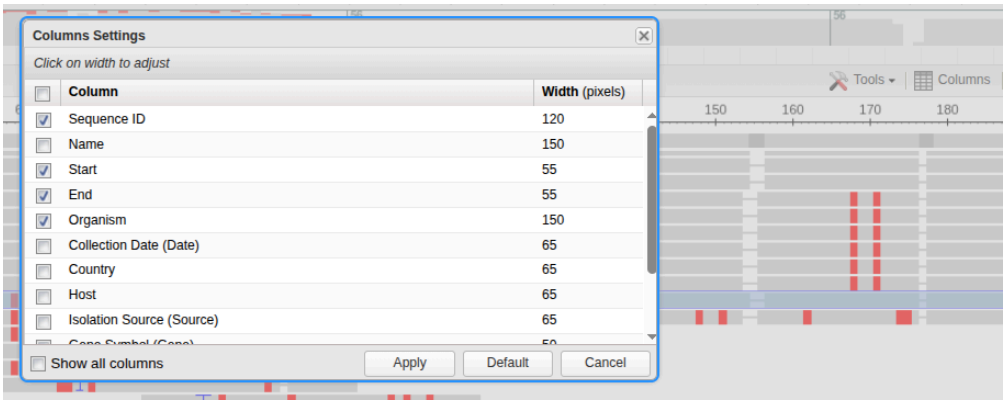
The example shows that
'**MZ937001.1** ', 'bat coronavirus' has only one mismatch around the position 100, while '**MZ064531.1**', 'synthetic construct' has many mismatches and no F3 aligned

One would expect, therefore, that '**MZ937001.1**' is more likely to potentially amplify than '**MZ064531.1**' and all the results below the later one

'Potentially' because we know that other reasons (reagents, pipetting, dna input, etc) may reflect a good amplification
Nonetheless, we are able to see here which sequences are clearly expected to amplify or not amplify if both have the same reagents, preparation, etc

19 Optional: Tidy up the MSA page

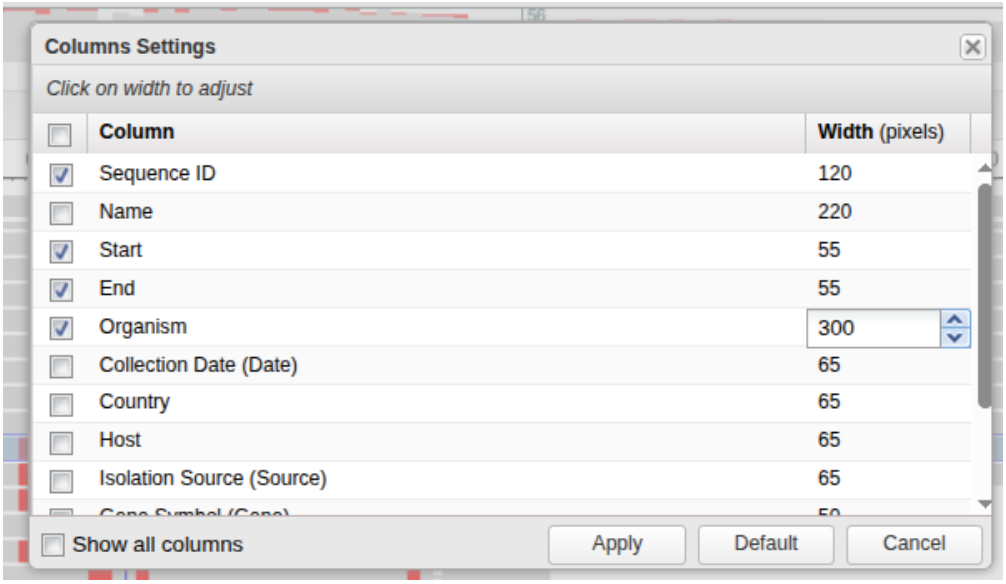
19.1 Columns
Click the 'columns' button to select columns shown
This is different depending on which databases / hits you get



Optional: Select columns

19.2 Column width

Optional: Change the width of a column

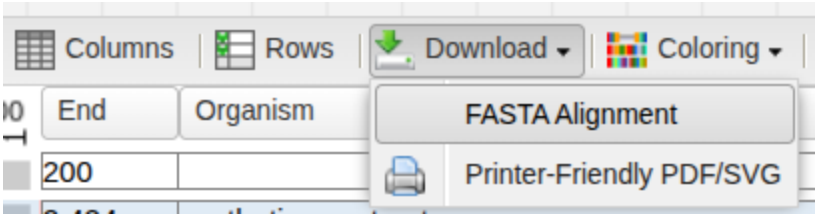


Optional: Column width

19.3 Zoom in or out, in order to see many or few results

20 Download MSA results

One can download fasta or PDF of the results



More refined specificity check

21 If you want more control and be able to visualize much more results in a single specificity check,

consider using another tool of ours, LampAID: https://github.com/Aciole-David/lampAID

LampAID is easier to run, interpret and share results of lamp primer specificity
The tool can parallel direct searches of multiple primersets against thousands of sequences in minutes

LampAID results in a web browser

Paper is coming soon!

Protocol references

Aciole Barbosa, David (2025) *Lamp-blast: Easier method to blast lamp primersets with NCBI's blast*, *GitHub*. Available at: https://github.com/Aciole-David/lamp-blast (Accessed: 16 June 2025).

ARABI-JESHVAGHANI, Fatemeh et al. LAMPPrimerBank, a manually curated database of experimentally validated loop-mediated isothermal amplification primers for detection of respiratory pathogens. **Infection**, v. 51, n. 6, p. 1809-1818, 2023.

Rabe BA, Cepko C. SARS-CoV-2 detection using isothermal amplification and a rapid, inexpensive protocol for sample inactivation and purification. Proc Natl Acad Sci U S A. 2020 Sep 29;117(39):24450-24458. doi: 10.1073/pnas.2011221117. Epub 2020 Sep 8. PMID: 32900935; PMCID: PMC7533677.