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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

Created: May 30, 2025



Last Modified: June 16, 2025

Protocol Integer ID: 219239

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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 conversion 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
CGGTGGACAAATTGTCACNNCTGTGCAAAGGAAATTAAGGAGNNAGTGTTCA
GACATTCTTAAGCTTGTAANNATAAATTTTGGCTTTGTGTGCTGANNTATTG
GTGGAGCTAACTTAAAGCCNNTTGAATTTAGGTGAAACATTTGTCACGNNC
ACTCAAAGGGATTGTACAGNNAAGTGTGTTAAATCCAGAGAAG
```

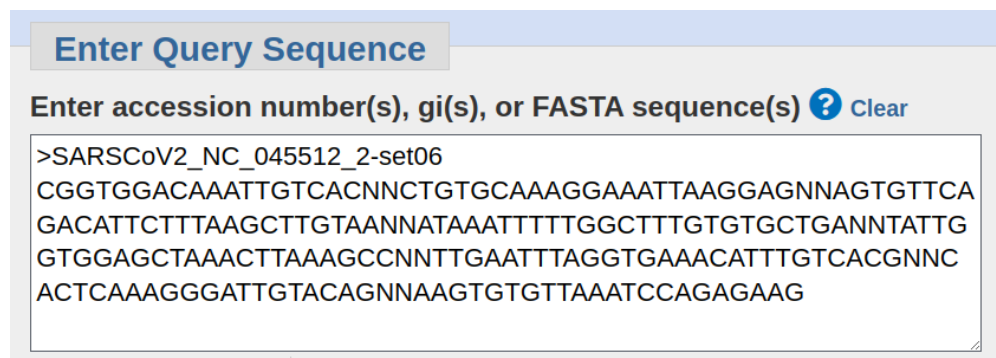
You can copy the sequence above as a test query

NCBI Blast search using lamp-blast format

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



Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) ? Clear

```
>SARSCoV2_NC_045512_2-set06
CGGTGGACAAATTGTCACNNCTGTGCAAAGGAAATTAAGGAGNNAGTGTTCA
GACATTCTTAAGCTTGTAANNATAAATTTTGGCTTTGTGTGCTGANNTATTG
GTGGAGCTAACTTAAAGCCNNTTGAATTTAGGTGAAACATTTGTCACGNNC
ACTCAAAGGGATTGTACAGNNAAGTGTGTTAAATCCAGAGAAG
```

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...

Choose Search Set

Database
☒ Standard databases (nr etc.): ☐ rRNA/ITS databases ☐ Genomic + transcript databases ☐ Betacoronavirus ☐ Experimental databases

Organism
Optional
Core nucleotide database (core_nt)
Coronavirus (taxid:694002) ☒ exclude
Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude
Optional
☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to
Optional
☐ Sequences from type material

Entrez Query
Optional
Enter an Entrez query to limit search

Optional: add filters

- 7 Select "Somewhat similar sequences (blastn)"

Program Selection

Optimize for

☐ Highly similar sequences (megablast)

☐ More dissimilar sequences (discontiguous megablast)

☒ Somewhat similar sequences (blastn)

Choose a BLAST algorithm ?

Change the blast program

- 8 Open the algorithm parameters option

BLAST

Search database core_nt using Blastn (Optimize for somewhat similar sequences)

☒ Show results in a new window

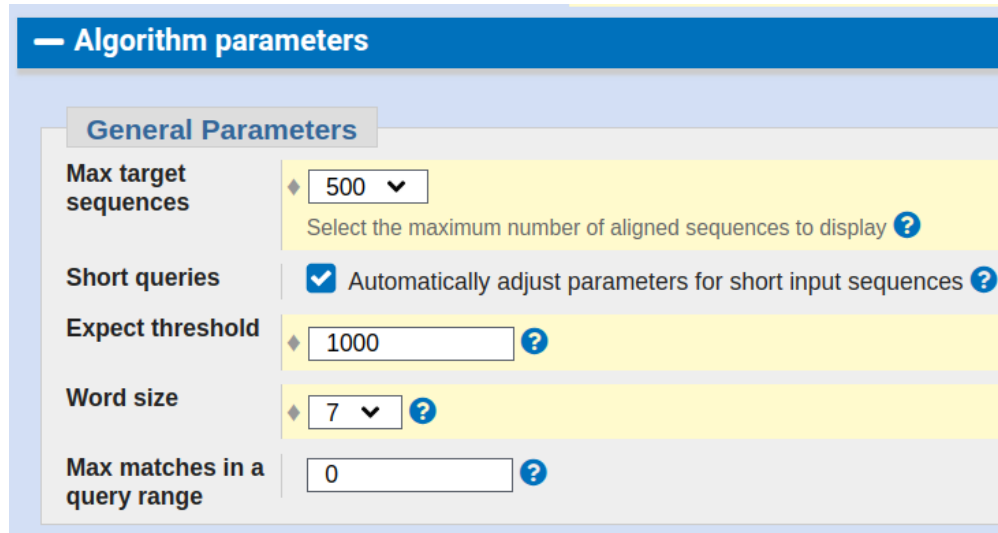
+ Algorithm parameters

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**



Algorithm parameters

General Parameters

Max target sequences 
Select the maximum number of aligned sequences to display 

Short queries ☒ Automatically adjust parameters for short input sequences 

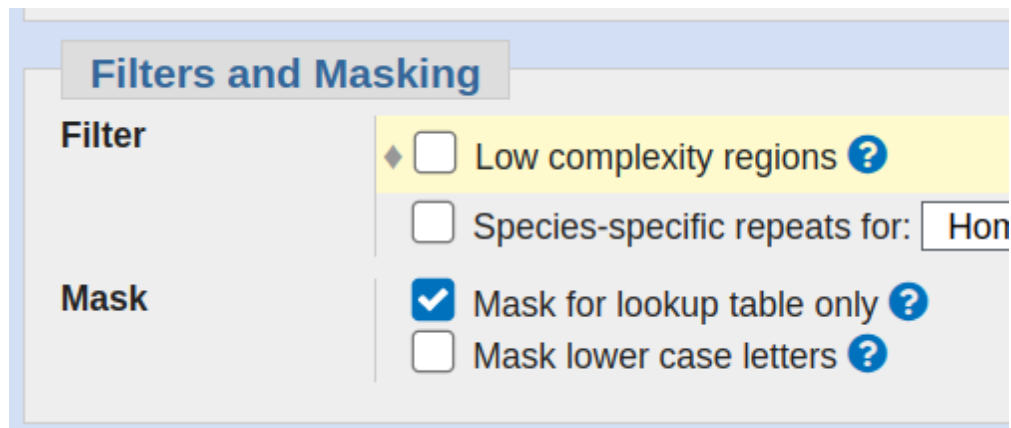
Expect threshold 

Word size 

Max matches in a query range 

Change general parameters

10 Uncheck "Low complexity regions"



Filters and Masking

Filter

☐ Low complexity regions 

☐ Species-specific repeats for:

Mask

☒ Mask for lookup table only 

☐ Mask lower case letters 

Uncheck "Low complexity regions"

11 Click the BLAST button

BLAST

Search **database core_nt** using **Blastn (Optimize for somewhat similar sequences)**

☒ Show results in a new window

Press BLAST

Lamp-blast BLAST results

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

Descriptions

Graphic Summary

Alignments

Taxonomy

Sequences producing significant alignments

DownloadSelect columnsShow500

☒ select all

500 sequences selected

GenBank

Graphics

Distance tree of results

MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒ Synthetic construct clone CDC-RG-3015799012, complete sequence	synthetic constr...	261	261	100%	3e-65	87.85%	30237	QNS71507.1
☒ Synthetic construct ORF1ab_spike_ORF3_E_M_ORF6_ORF8_and N genes, complete cds	synthetic constr...	261	261	100%	3e-65	87.85%	29891	MT108784.1
☒ Synthetic construct clone CDC-RG-3015781630, complete sequence	synthetic constr...	261	261	100%	3e-65	87.85%	30238	QNS71514.1
☒ Synthetic construct clone CDC-RG-3015781631, complete sequence	synthetic constr...	261	261	100%	3e-65	87.85%	30245	QNS71504.1
☒ Synthetic construct clone icSARS-CoV-2-GFP ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1...	synthetic constr...	261	261	100%	3e-65	87.85%	30347	MT461670.1
☒ Synthetic construct clone CDC-RG-3015799005, complete sequence	synthetic constr...	261	261	100%	3e-65	87.85%	30231	QNS71506.1
☒ Synthetic construct clone CDC-RG-3015801239, complete sequence	synthetic constr...	261	261	100%	3e-65	87.85%	30227	QNS71511.1
☒ Synthetic construct clone icSARS-CoV-2-WT ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1...	synthetic constr...	261	261	100%	3e-65	87.85%	29903	MT461669.1

Reorder results

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

Filter Results

Organism *only top 20 will appear* ☐ exclude

[+ Add organism](#)

Percent Identity to

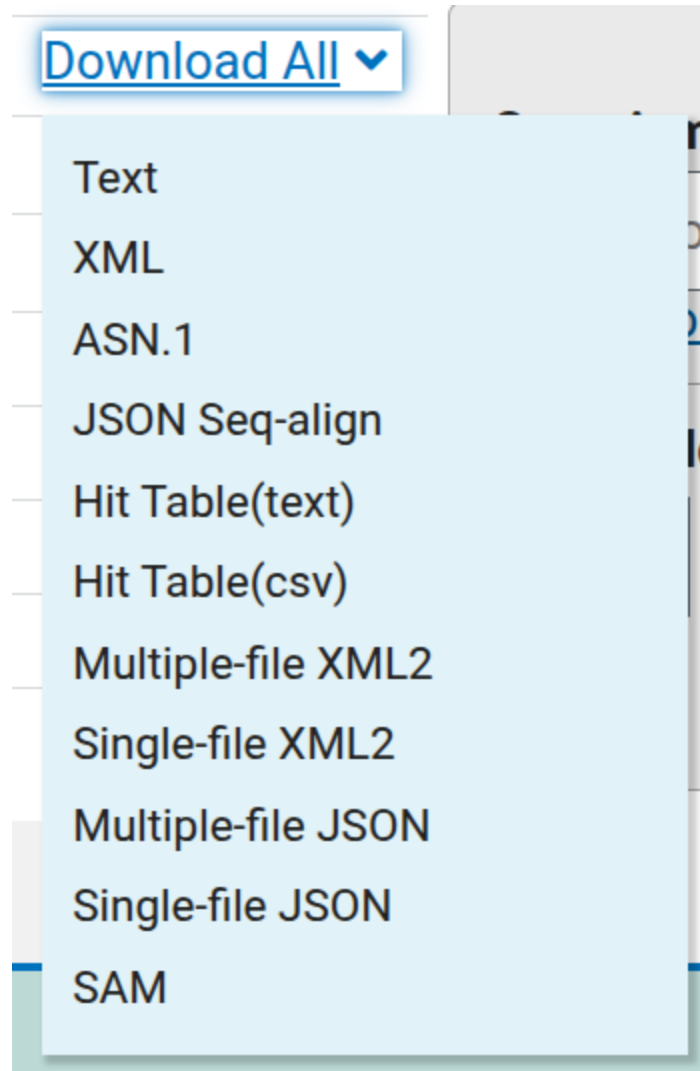
E value to

Query Coverage to

Optionally use filters in the results page

14 OPTIONAL: Download all results

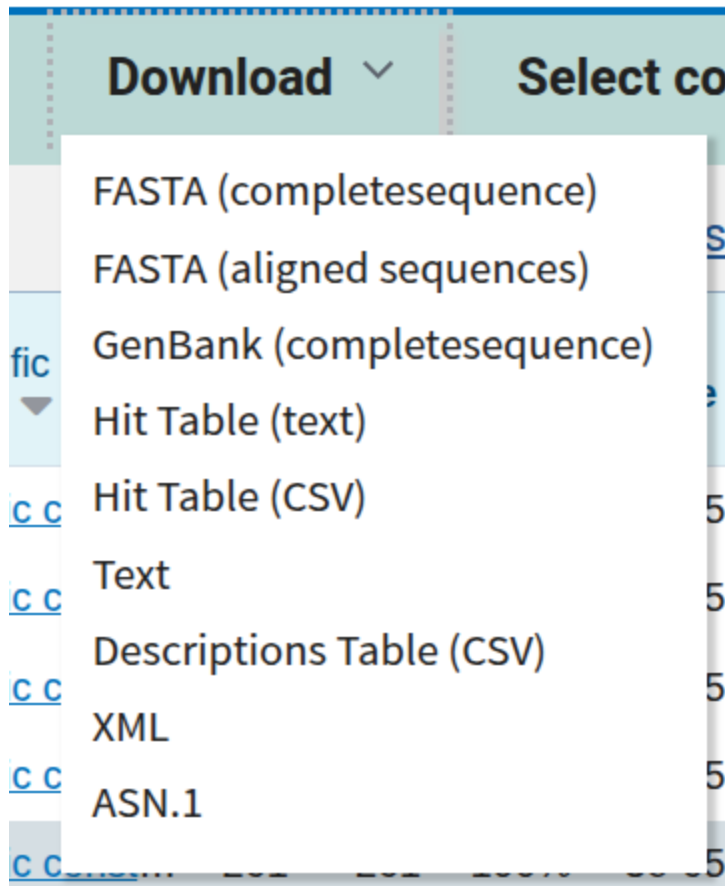
If the query is composed of more than one query, use the "Download all" link to get the results in different formats



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:

- - - Uncheck 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)

Descriptions	Graphic Summary	Alignments	Taxonomy						
Sequences producing significant alignments									
Download ▼ Select columns ▼ Show 500 ?									
☐ select all 55 sequences selected									
GenBank Graphics Distance tree of results MSA Viewer									
	Description ▼	Scientific Name ▼	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	Synthetic construct clone CDC-RG-301585163, complete sequence	synthetic constr...	267	267	100%	7e-67	89.86%	30232	ON571513.1
☒	Synthetic construct clone OTS-228_P.4-hamster_5dpi_L6499, complete sequence	synthetic constr...	267	267	100%	7e-67	89.86%	28780	PO536474.1
☒	Synthetic construct clone CDC-RG-301585261, complete sequence	synthetic constr...	267	267	100%	7e-67	89.86%	30235	ON571510.1
☒	Synthetic construct clone CDC-RG-3015782810, complete sequence	synthetic constr...	267	267	100%	7e-67	89.86%	30238	ON571508.1
☒	Synthetic construct COVI-VAC, complete sequence	synthetic constr...	267	267	100%	7e-67	89.86%	29855	MZ404503.1
☒	Synthetic construct clone CDC-RG-3015796202, complete sequence	synthetic constr...	267	267	100%	7e-67	89.86%	30235	ON571518.1
☒	Synthetic construct clone CDC-RG-3015793014, complete sequence	synthetic constr...	267	267	100%	7e-67	89.86%	30247	ON571505.1
☒	Synthetic construct clone IDCDC-RG5, complete sequence	synthetic constr...	267	267	100%	7e-67	89.86%	30189	MZ128150.1

Best results

☒ Meleagris gallopavo genome assembly, chromosome: 10	Meleagris gallo...	38.3	38.3	22%	587	79.55%	29439779	HG999689.4
☒ PREDICTED: Pecten maximus protein capicua homolog (LOC117341011), mRNA	Pecten maximus	37.4	37.4	22%	587	77.27%	8233	XM_033902806.1
☒ Alectoris rufa genome assembly, chromosome: 40	Alectoris rufa	38.3	38.3	22%	587	79.55%	77739472	OZ238805.1
☒ Scirpus scirpus genome assembly, chromosome: 11	Scirpus scirpus	41.0	41.0	21%	48	81.40%	22691464	OZ228728.1
☒ Bernardetia sp. ABR2-2B chromosome, complete genome	Bernardetia sp...	38.3	38.3	21%	587	78.57%	5299216	CP147020.1
☒ Haplosporidia sp. GHCO000330 genome assembly, chromosome: 1	Unknown	41.0	41.0	21%	48	80.49%	29858730	OZ242225.1
☒ PREDICTED: Ceratitis capitata protein expanded (LOC101462585), transcript variant X3, mRNA	Ceratitis capitata	37.4	37.4	21%	587	80.49%	7085	XM_004530257.3
☒ PREDICTED: Ceratitis capitata protein expanded (LOC101462585), transcript variant X1, mRNA	Ceratitis capitata	37.4	37.4	21%	587	80.49%	7183	XM_004530256.3
☒ PREDICTED: Ceratitis capitata protein expanded (LOC101462585), transcript variant X2, mRNA	Ceratitis capitata	37.4	37.4	21%	587	80.49%	7184	XM_004530258.3
☐ Dipodops deserti UQA04_Rod_734, complete genome	Dipodops deserti	38.3	38.3	20%	587	80.49%	5515	OQ686903.1
☐ Mus musculus BAC clone RP24-530L11 from 3, complete sequence	Mus musculus	38.3	38.3	20%	587	80.49%	160259	AC142502.3
☐ PREDICTED: Gambusia affinis cytochrome c oxidase subunit 7A-related protein, mitochondrial (LOC122...	Gambusia affinis	37.4	37.4	20%	587	82.05%	860	XM_044103841.1
☐ Meleagris gallopavo genome assembly, chromosome: 5	Meleagris gallo...	37.4	37.4	20%	587	79.40%	29439779	HG999689.4

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

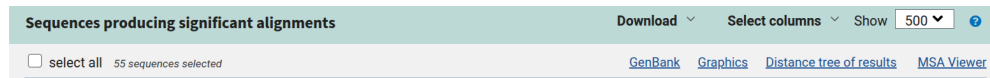
To see a MSA for all the hits, without any filter, click the **MSA viewer** button next to "Other reports Distance tree of results"

Other reports Distance tree of results MSA viewer ?

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

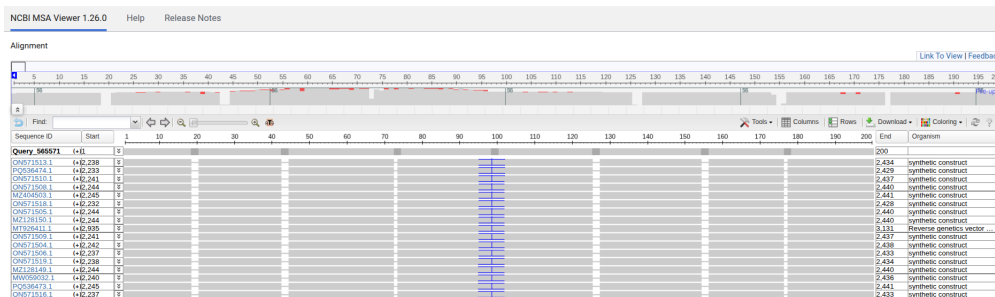
18 The MSA viewer page

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

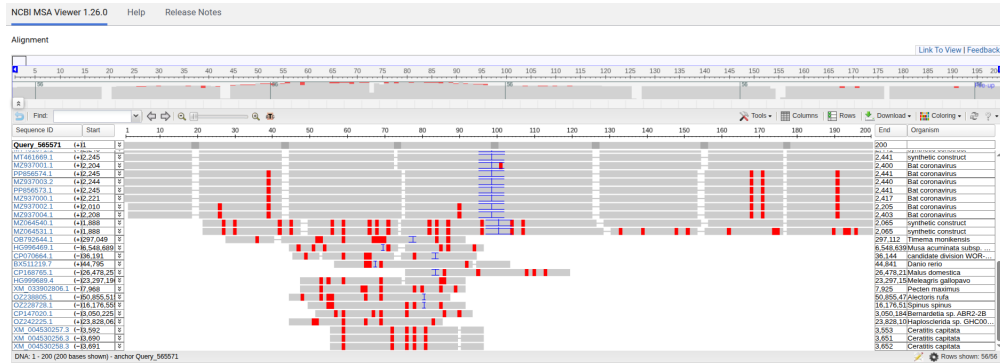
Dark Intervals in the query are the 'NN'

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

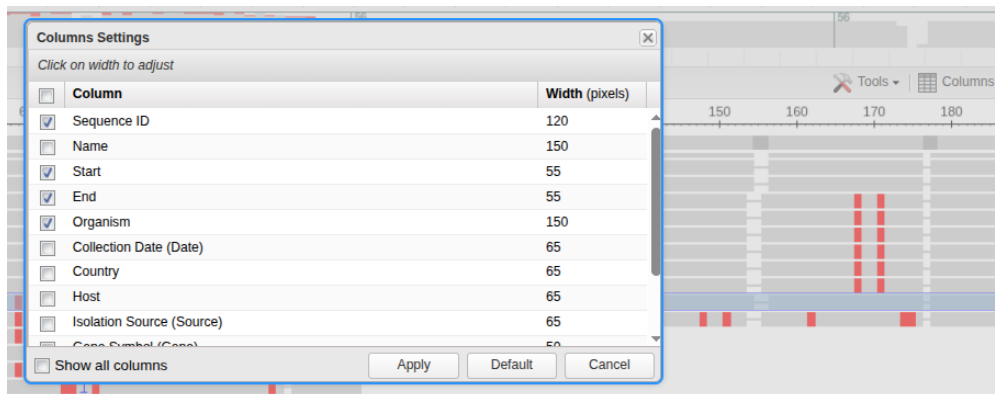
Nonetheless, we are able to see here which sequences are clearly expected to amplify or not amplify if both have the same reagents, preparation, etc

19 Optional: Tidy up the MSA page

19.1 Columns

Click the 'columns' button to select columns shown

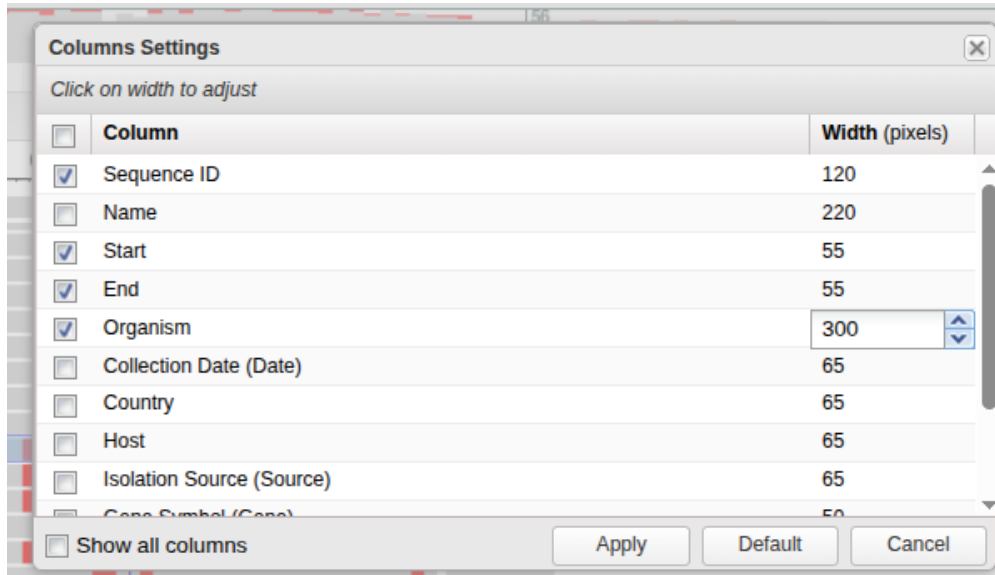
This is different depending on which databases / hits you get



Optional: Select columns

19.2 Column width

Optional: Change the width of a column

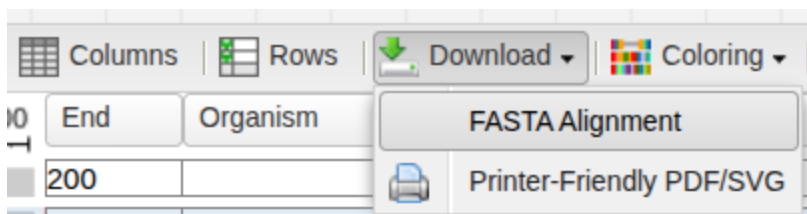


Optional: Column width

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.