

COVID-19 Tutorial



This tutorial will guide you through using [Primer-BLAST](#) and making sense of the outputs.

1. Open [Primer-BLAST](#). This is a tool developed for scientists to design and optimize primer sequences. One of the things that it does really well is to search the database of genome sequences looking for places where given primer sequences would bind.
2. Enter the primers into the appropriate forward and reverse primer sequence boxes. There are several different COVID-19 test sequences and you can try any here, just make sure that you use a forward and a reverse sequence from the same test as they have to work as a pair.

3. You will NOT enter a PCR Template (because you want to see *all* of the matches that the database can show you) and you will leave all of the standard parameters as-is.
4. Finally, scroll down toward the bottom of the form. For the Database, type “nr” to search all sequences. Make sure the Organism field is also empty to search broadly.



5. Click Get Primers and wait for your results 🕒🕒🕒

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6. If everything runs smoothly, your results should appear as a list.
 7. The area of interest is the list of “Products on target templates” which tells you all of the sequences in the database that would produce a “hit” or positive signal in the PCR assay due to these primers binding and amplifying the identified sequence of DNA. Let’s break down the information that you’re given:

The hyperlinked number is the unique code that refers to this sequence in the genome database and the rest of that line is the name given to that sequence. You’ll notice that the naming isn’t always consistent but gives you a pretty good idea of the organism and relevant information about its collection. **What organism(s) are your sequences from and what other information can you tell about them? Even if the naming seems very different, are they all from SARS-CoV2? What factors explain the differences in naming?**

Product length tells how long the piece of DNA is between the two primers. **How long is this PCR product?**

Then it shows the forward primer and below it the numbers of the positions of the corresponding bases in the identified sequence. The dots tell you that the sequence forms a perfect match, while any letters in that space would identify the specific mismatches. Then, same thing for the reverse primer. **Do these primers make a perfect match?**

8. Now you can scroll down to see the range of matches or use Ctrl+F to do a search for relevant sequence or organism names, or to give you a count of the number of sequences found. **How many matches did you get with these primers? What factors determine how many matches you see?**

Were there any matches NOT to SARS-CoV2? Why would that be? Would that be a problem for administering this test to manage the pandemic? Why or why not?

