# PCR LAB Experience Tutorial

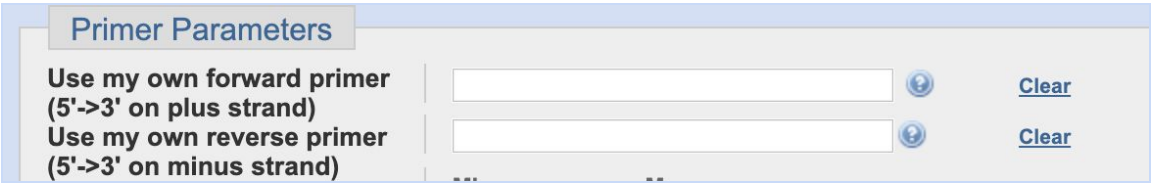
RockEDU SCIENCE OUTREACH

This step-by-step tutorial will guide you through using Primer-BLAST and making sense of the outputs.

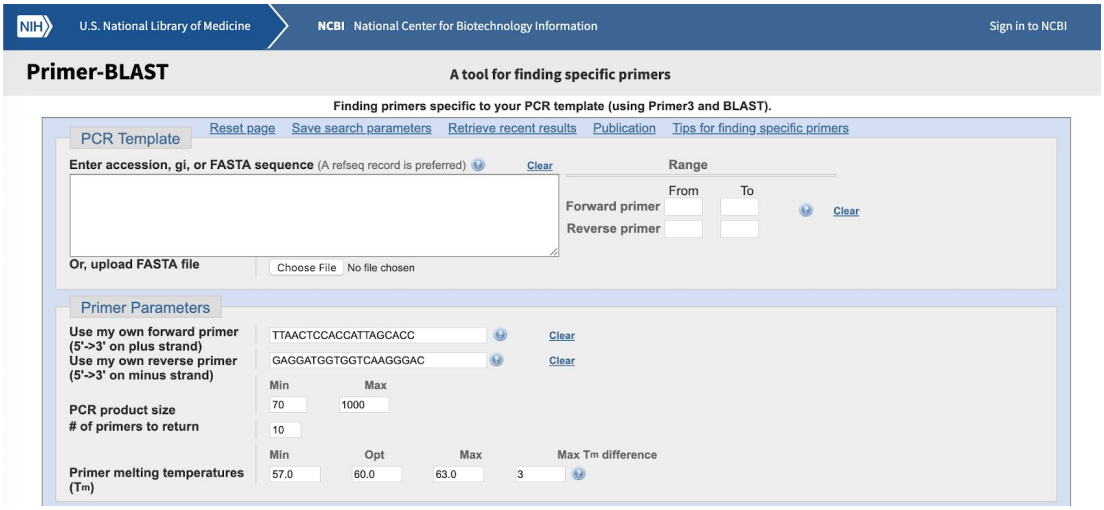
1. Open Primer-BLAST. This a tool developed for scientists to design and optimize primer sequences. One of the things that it does really well is to search the database of genome sequences looking for places where given primer sequences would bind.

We will use a set of primers that RockEDU frequently uses with students in our research laboratory at The Rockefeller University, where we have students scrape off some cheek cells with a toothpick (no blood, it doesn't even hurt) and then mix them with the PCR reagents including the set of primers below:

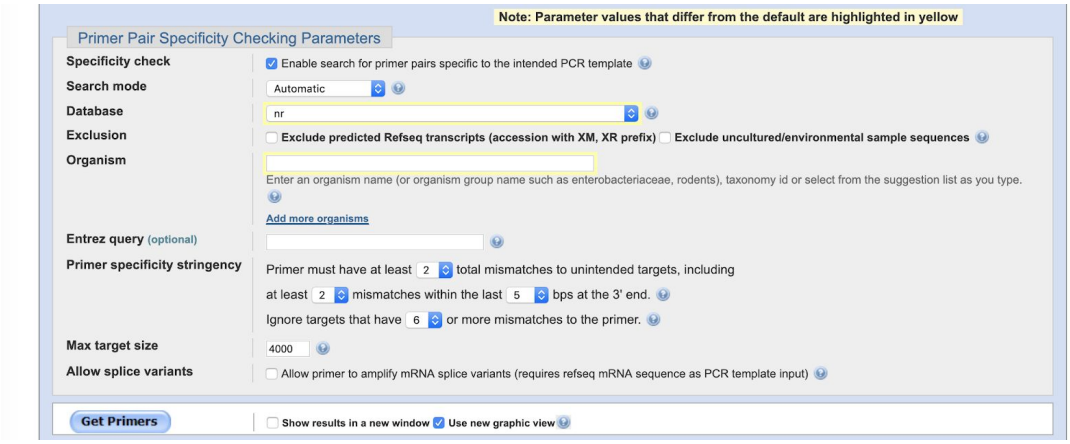
2. Enter the primers into the appropriate forward and reverse primer sequence boxes:
   FORWARD: TTAACTCCACCATTAGCACC
   REVERSE: GAGGATGGTGGTCAAGGGAC




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. Thus, the top of the form should now look like this:



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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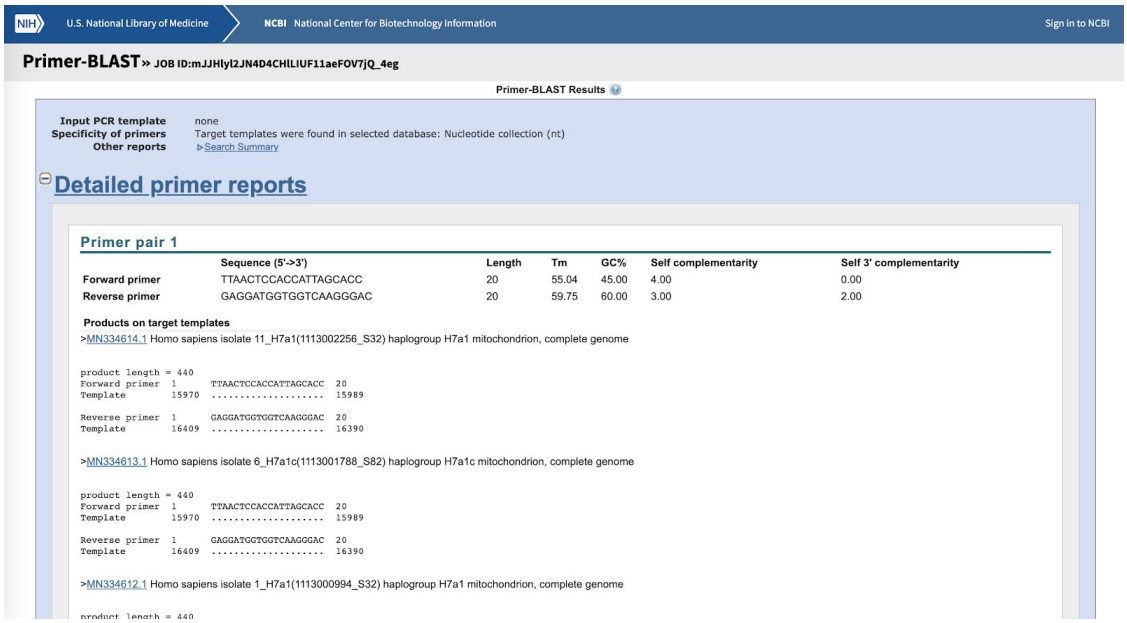
6. If everything runs smoothly, your results should appear as a list, starting like this...




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:

>MN334614.1 Homo sapiens isolate 11_H7a1(1113002256_S32) haplogroup H7a1 mitochondrion, complete genome

```
product length = 440
Forward primer  1      TTAACTCCACCATTAGCACC  20
Template        15970  ....................  15989

Reverse primer  1      GAGGATGGTGGTCAAGGGAC  20
Template        16409  ....................  16390
```

MN334614.1 is the unique code that refers to this sequence in the genome database. Tand the remainderst 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. **In this sample name, where does it tell you this is from human mitochondrial DNA?**

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 sequences or organism names, or to give you a count of the number of sequences found. **How many human sequences matched with these primers?**