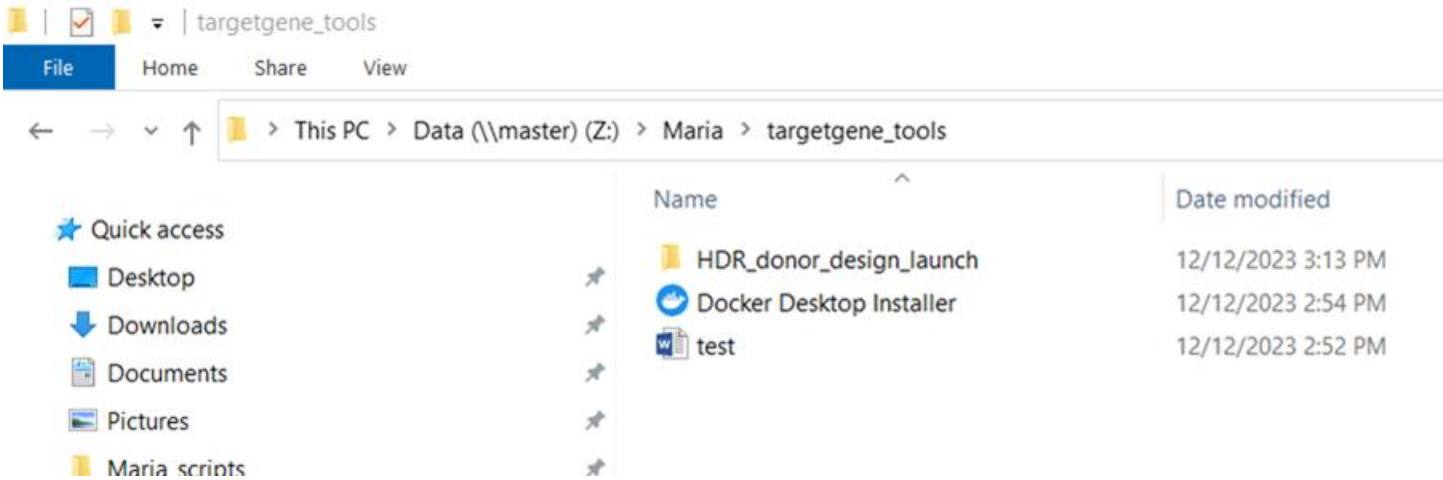
“Primer design tool” user guide

This program is designed to search for primers based on the Blast Primers and Primer3 tools. It allows one to get primer pairs near the cut site for TGEE or Cas9 guides and any other sequence. In addition, this tool collects information about off-targets and thermodynamic parameters. All of this allows you to make better decisions.

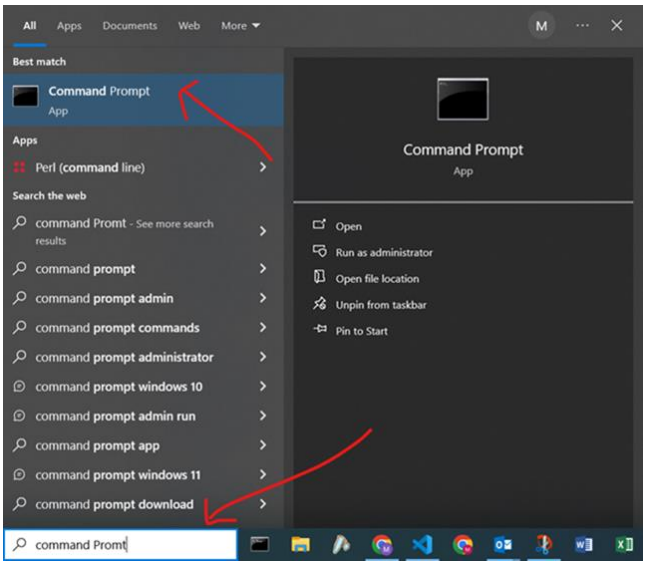
1. Tool installation.

a. Go to folder “Z:\Maria\targetgene_tools”.



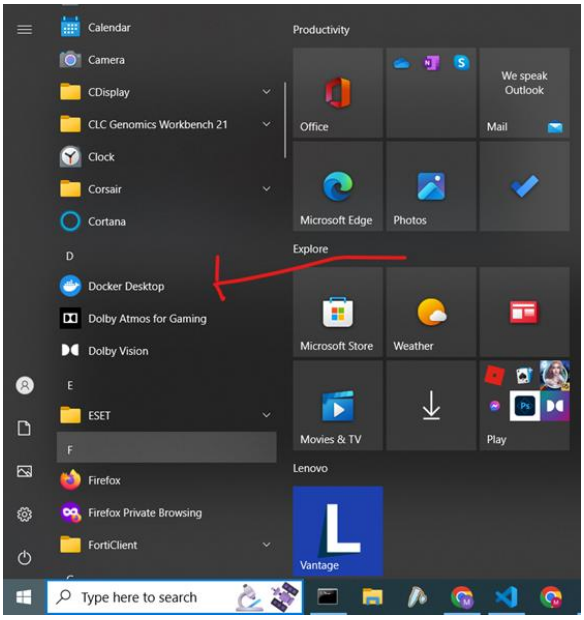
b. Launch Docker Desktop Installer with default settings.

c. Enter “Command Prompt” in the search panel and click the app.

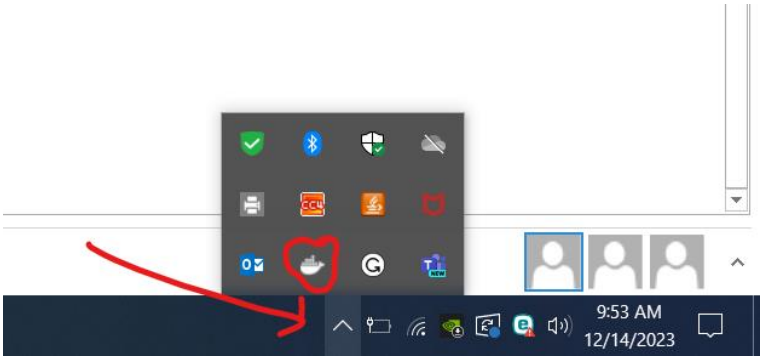


d. Enter docker pull mdyakova/primer_design_tool:v1 to opened window. Wait.

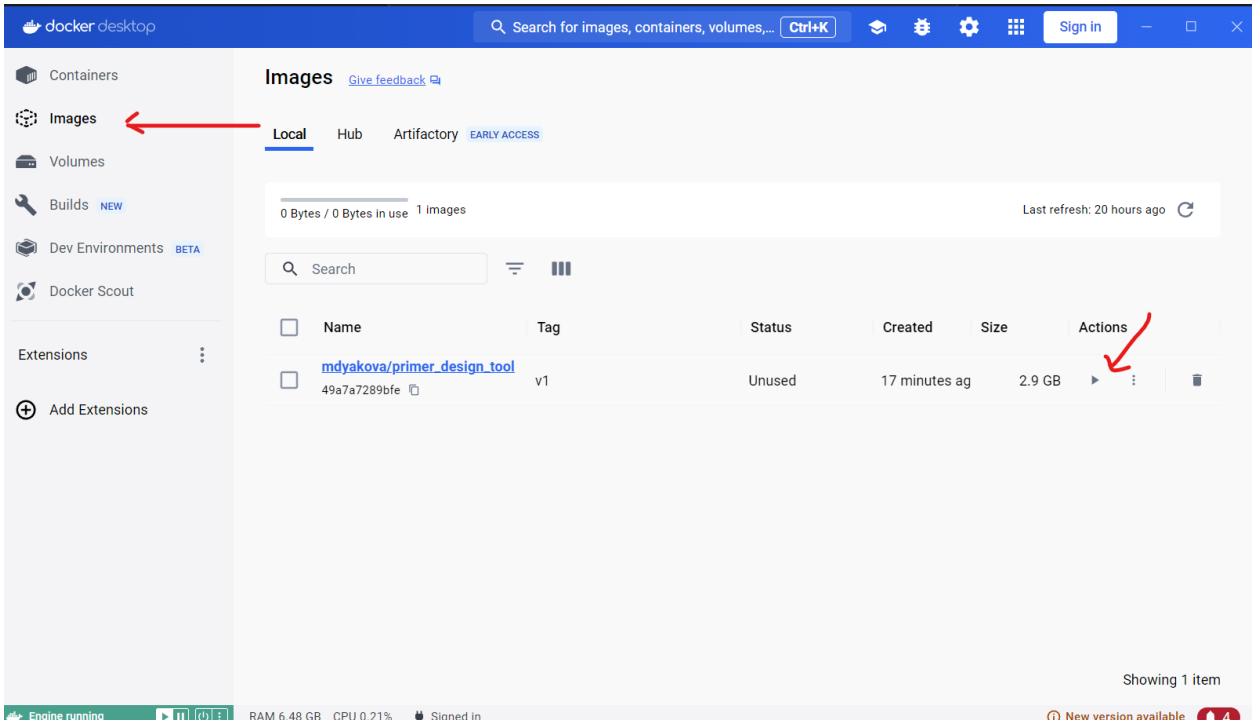
e. Open Docker Desktop. Click to icon and wait.



f. Click to menu bottom right and click to icon with whale.



g. Click tab “Images”. Choose correct file and click “Run”.

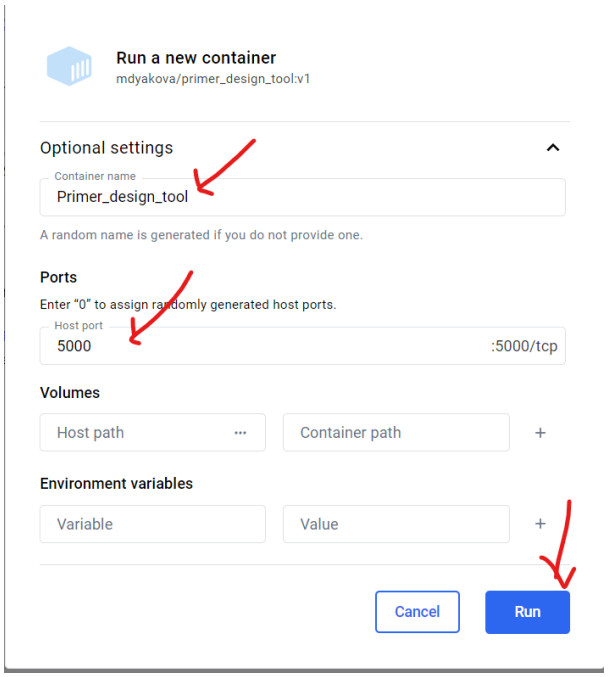


h. Click “Optional settings”.

i. Enter the container name without spaces (any good for you).

j. Enter port (**5000**).

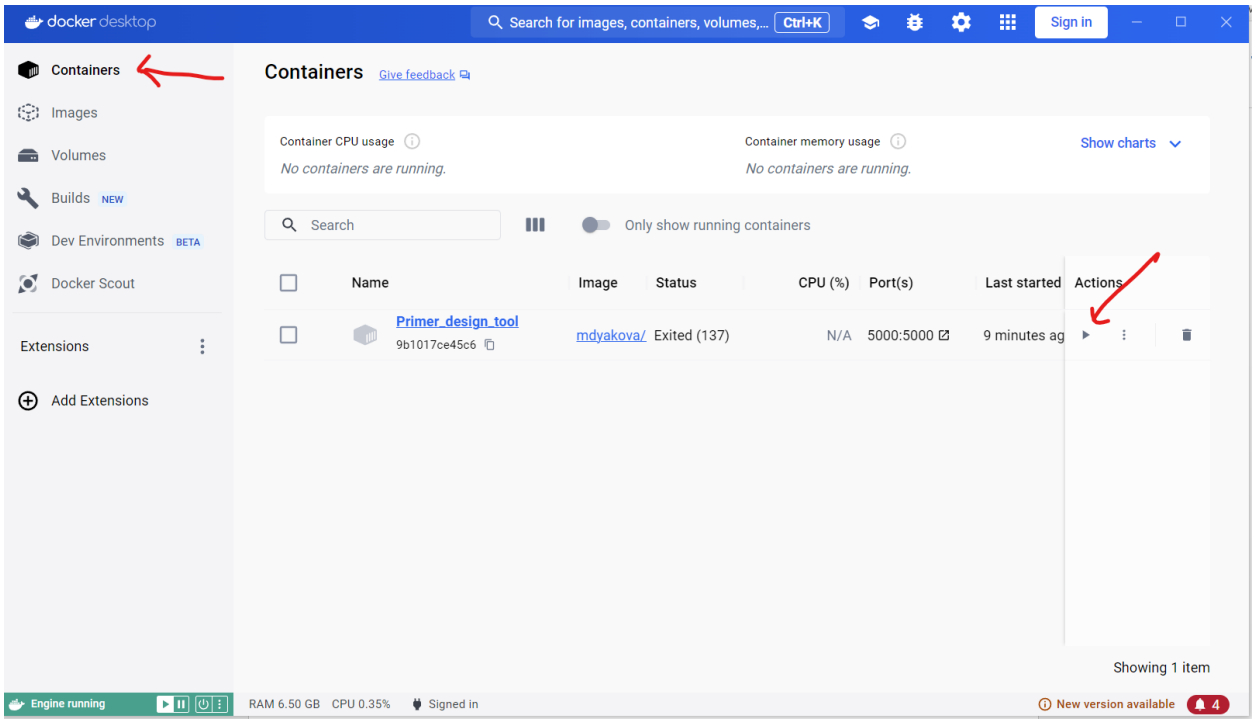
k. Click **Run**



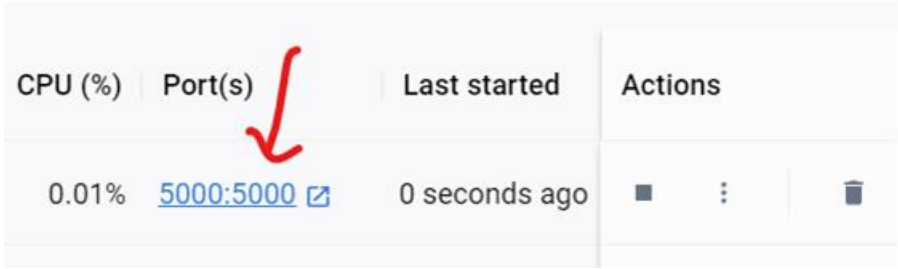
2. Tool launch

a. Click “Containers”.

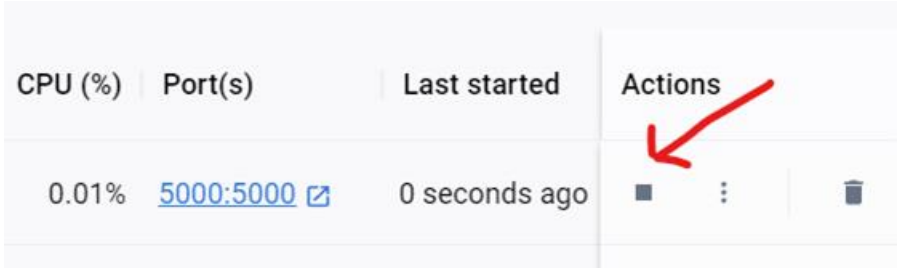
b. Click ‘Start”



c. Click link



d. To close the tool click ‘Stop”



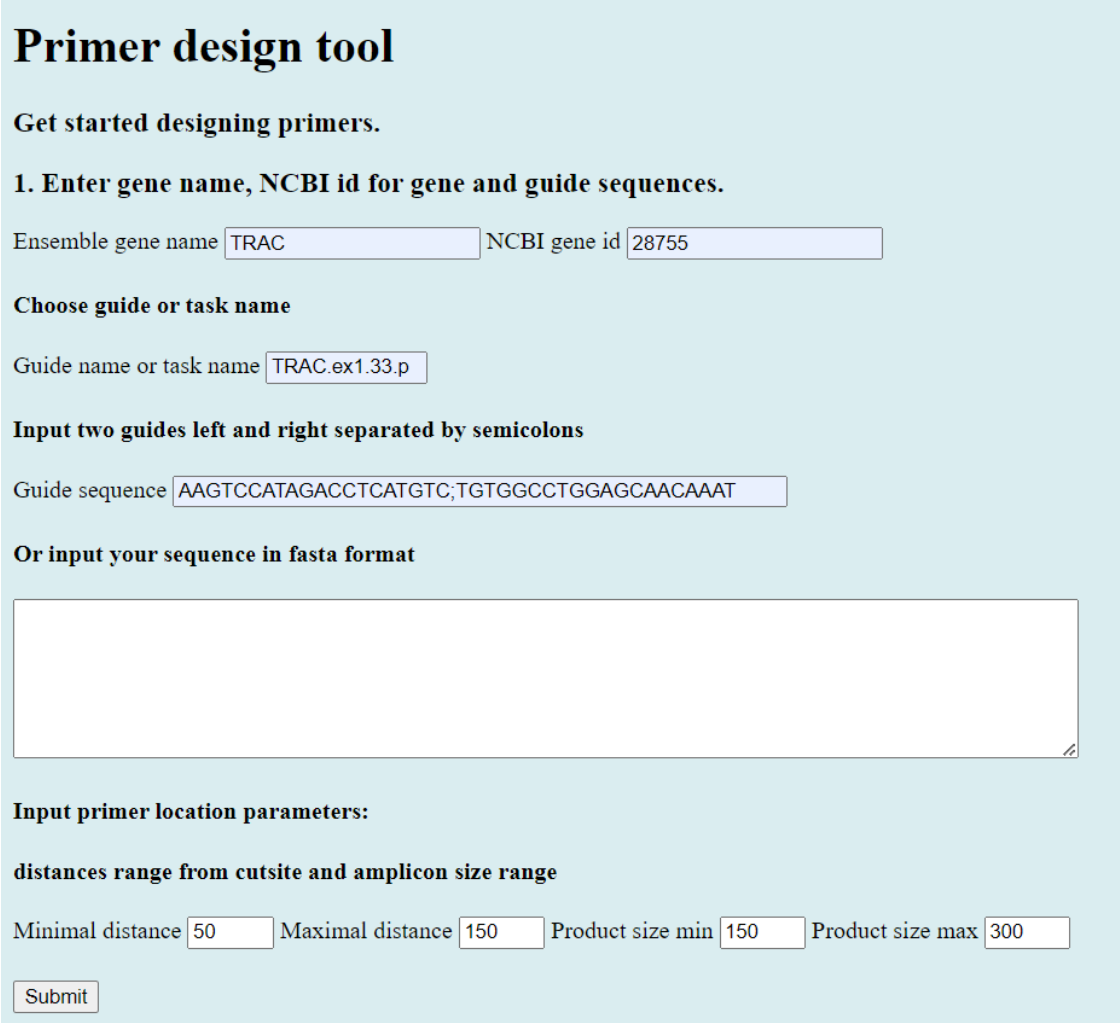
3. Search primers for guides TGEE

a. Input Ensemble gene name, NCBI gene ID, guide name, and guide sequences separated by semicolons.

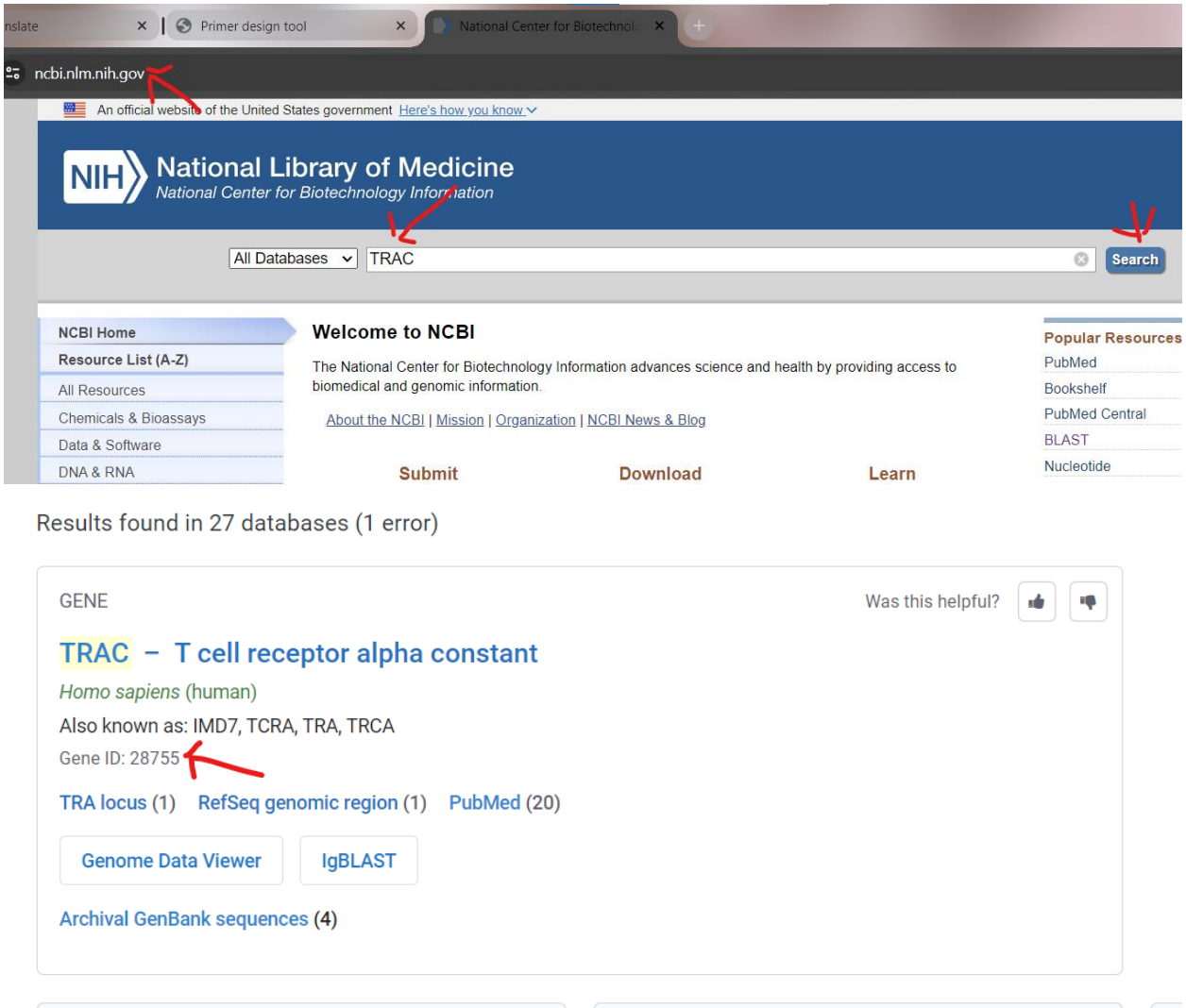
Choose primer location parameters:

- If you want to find primers for NGS and similar tasks, you can keep default parameters.
- If you want to find primers for PCR1 (amplicon with whole homology arms), change the minimal distance to equal homology arm size + 20..25. The maximum distance should be more than the minimal distance. Also, increase product size (minimal size should be more than two homology arm sizes).
- For any other case, choose the correct parameters for your tasks.

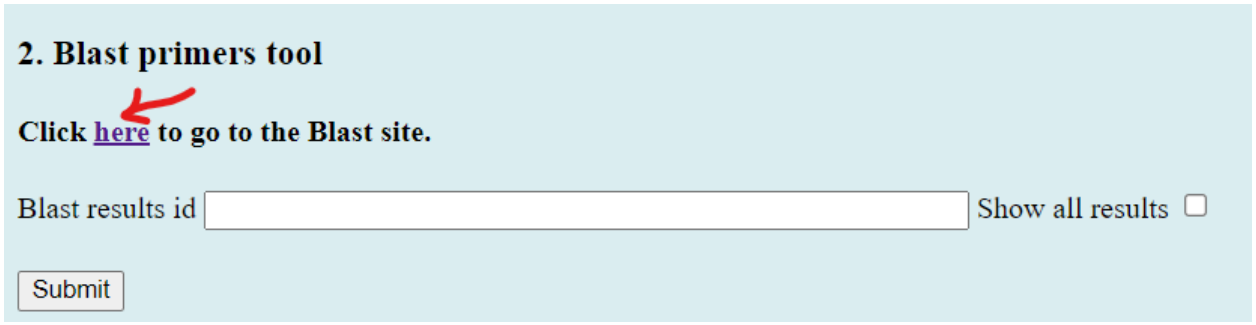
Click Submit. Wait.



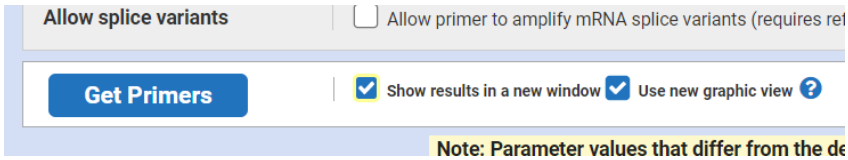
NCBI gene ID:



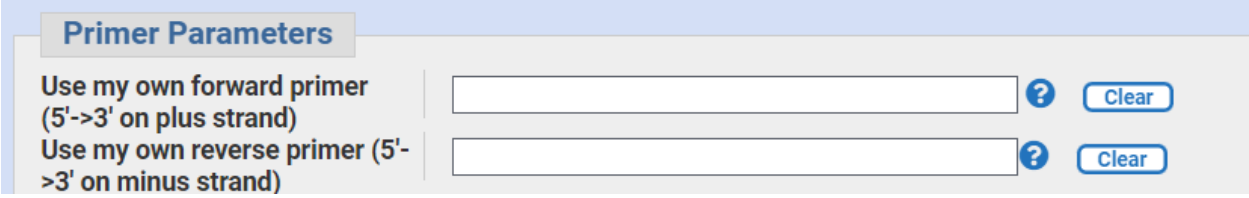
b. Click the link. You go to the Blast Primers page with all necessary parameters.



c. Click ‘Get primers”.

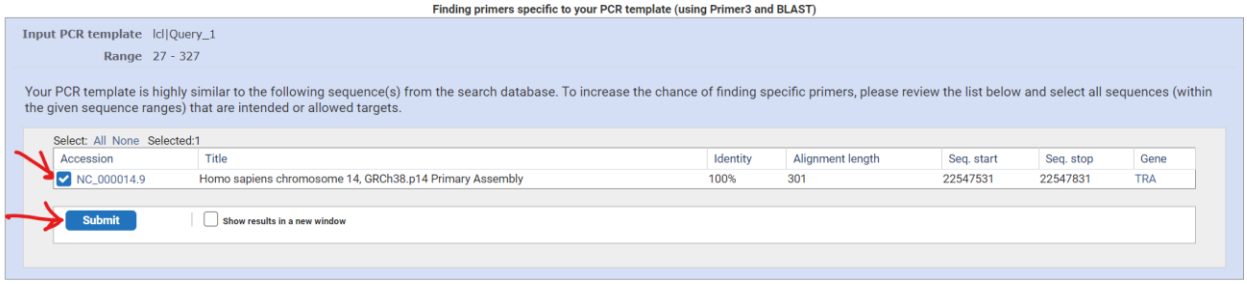


d. If you already have left or right primers and need to find only second one, input it to these forms.

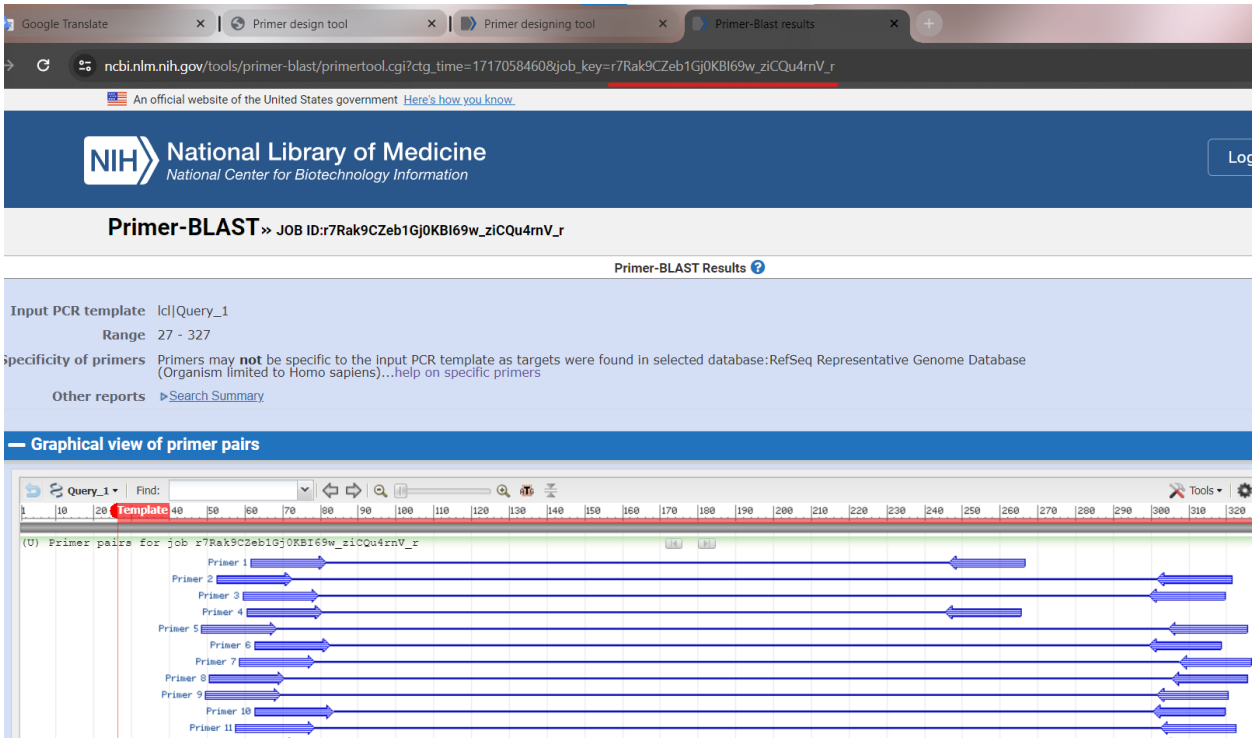


e. A new page will open. Please wait.

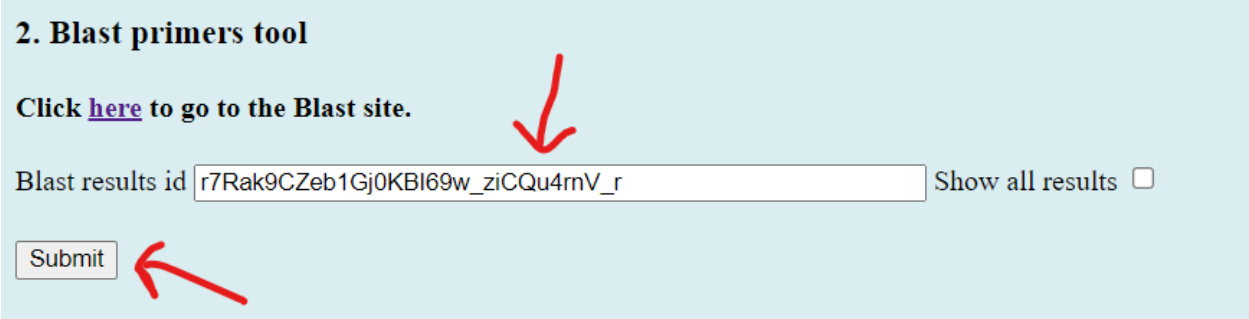
f. Choose template id if you want (recommended). Click Submit. Wait. It's not faster.



g. When you get a page with results, copy job_key (all after "job_key=").



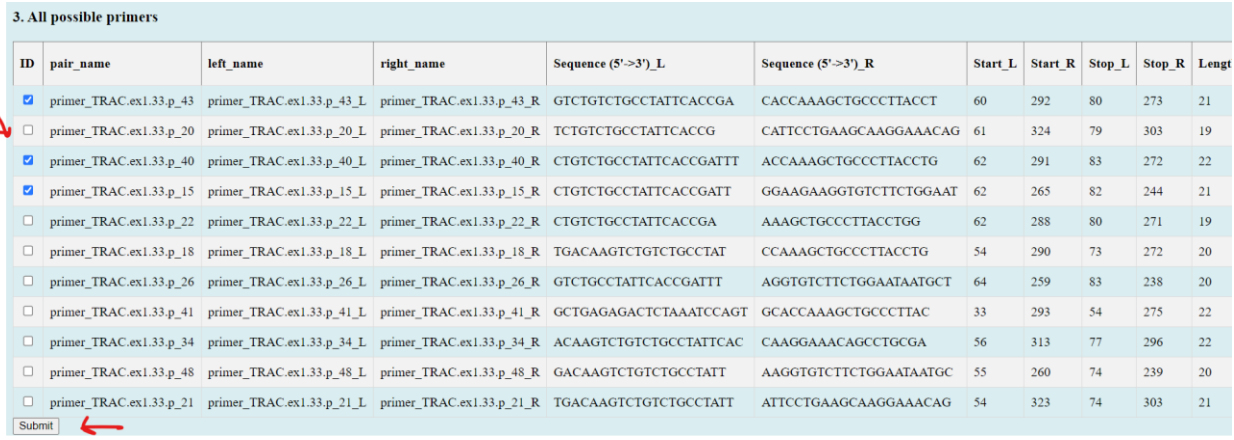
h. Input job_key to Blast results id and click Submit.



i. Below, you will see a table with good primer pairs. Scroll the page to the right to see all necessary parameters: Tm, GC%, cut site distances, amplicon sizes, thermodynamic parameters, and number of off-targets.

The table is sorted by probability of having an off-target effect. The first results are better.

Select the best primers and click submit.



3. All possible primers

ID	pair_name	left_name	right_name	Sequence (5'->3')_L	Sequence (5'->3')_R	Start_L	Start_R	Stop_L	Stop_R	Lengt
☑	primer_TRAC.ex1.33.p_43	primer_TRAC.ex1.33.p_43_L	primer_TRAC.ex1.33.p_43_R	GTCTGTCTGCCTATTCACCGA	CACCAAAGCTGCCCTTACCT	60	292	80	273	21
☐	primer_TRAC.ex1.33.p_20	primer_TRAC.ex1.33.p_20_L	primer_TRAC.ex1.33.p_20_R	TCTGTCTGCCTATTCACCG	CATTCCTGAAGCAAGGAAACAG	61	324	79	303	19
☑	primer_TRAC.ex1.33.p_40	primer_TRAC.ex1.33.p_40_L	primer_TRAC.ex1.33.p_40_R	CTGTCTGCCTATTCACCGATTT	ACCAAAGCTGCCCTTACCTG	62	291	83	272	22
☑	primer_TRAC.ex1.33.p_15	primer_TRAC.ex1.33.p_15_L	primer_TRAC.ex1.33.p_15_R	CTGTCTGCCTATTCACCGATT	GGAAGAAGGTGTCTTCTGGAAT	62	265	82	244	21
☐	primer_TRAC.ex1.33.p_22	primer_TRAC.ex1.33.p_22_L	primer_TRAC.ex1.33.p_22_R	CTGTCTGCCTATTCACCGA	AAAGCTGCCCTTACCTGG	62	288	80	271	19
☐	primer_TRAC.ex1.33.p_18	primer_TRAC.ex1.33.p_18_L	primer_TRAC.ex1.33.p_18_R	TGACAAGTCTGTCTGCCTAT	CCAAAGCTGCCCTTACCTG	54	290	73	272	20
☐	primer_TRAC.ex1.33.p_26	primer_TRAC.ex1.33.p_26_L	primer_TRAC.ex1.33.p_26_R	GTCTGCCTATTCACCGATTT	AGGTGTCTTCTGGAATAATGCT	64	259	83	238	20
☐	primer_TRAC.ex1.33.p_41	primer_TRAC.ex1.33.p_41_L	primer_TRAC.ex1.33.p_41_R	GCTGAGAGACTCTAAATCCAGT	GCACCAAAGCTGCCCTTAC	33	293	54	275	22
☐	primer_TRAC.ex1.33.p_34	primer_TRAC.ex1.33.p_34_L	primer_TRAC.ex1.33.p_34_R	ACAAGTCTGTCTGCCTATTCAC	CAAGGAAACAGCCTGCGA	56	313	77	296	22
☐	primer_TRAC.ex1.33.p_48	primer_TRAC.ex1.33.p_48_L	primer_TRAC.ex1.33.p_48_R	GACAAGTCTGTCTGCCTATT	AAGGTGTCTTCTGGAATAATGC	55	260	74	239	20
☐	primer_TRAC.ex1.33.p_21	primer_TRAC.ex1.33.p_21_L	primer_TRAC.ex1.33.p_21_R	TGACAAGTCTGTCTGCCTATT	ATTCCTGAAGCAAGGAAACAG	54	323	74	303	21

Submit

For each pair of primers, we count a number of off-targets:

- **score_1nt** - the proportion of complimentary nucleotides for the last one nucleotide.

bad_count_1nt - number of off-targets, which have complimentary last one nucleotide.

If **score_1nt** = 0 it means that all off-targets for this pair of primers have non-complimentary last nucleotides to off-target sequence.

If **score_1nt** = 1 it means that at least one off-target for this pair of primers has a complimentary last nucleotide to the off-target sequence.

- **score_2nt** - the proportion of complimentary nucleotides for last two nucleotides.

bad_count_2nt - number of off-targets, which have complimentary last two nucleotides.

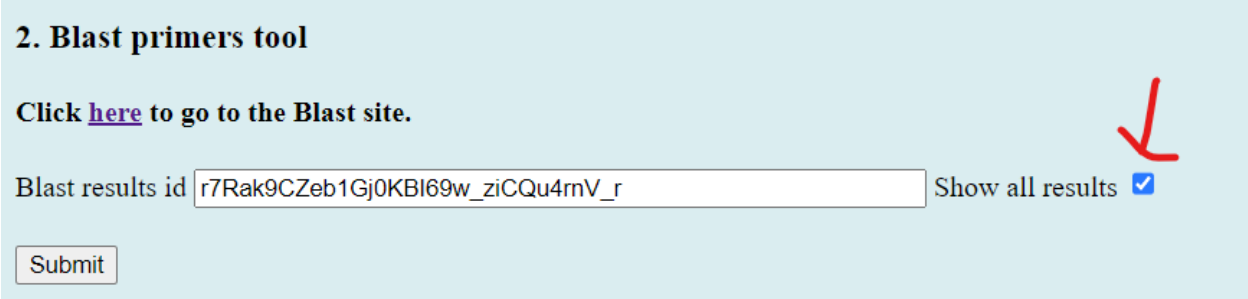
If **score_2nt** = 0 it means that all off-targets for this pair of primers have non-complimentary last two nucleotides to off-target sequence.

If **score_2nt** = 1, it means that at least one off-target for this pair of primers has complementary last two nucleotides to the off-target sequence.

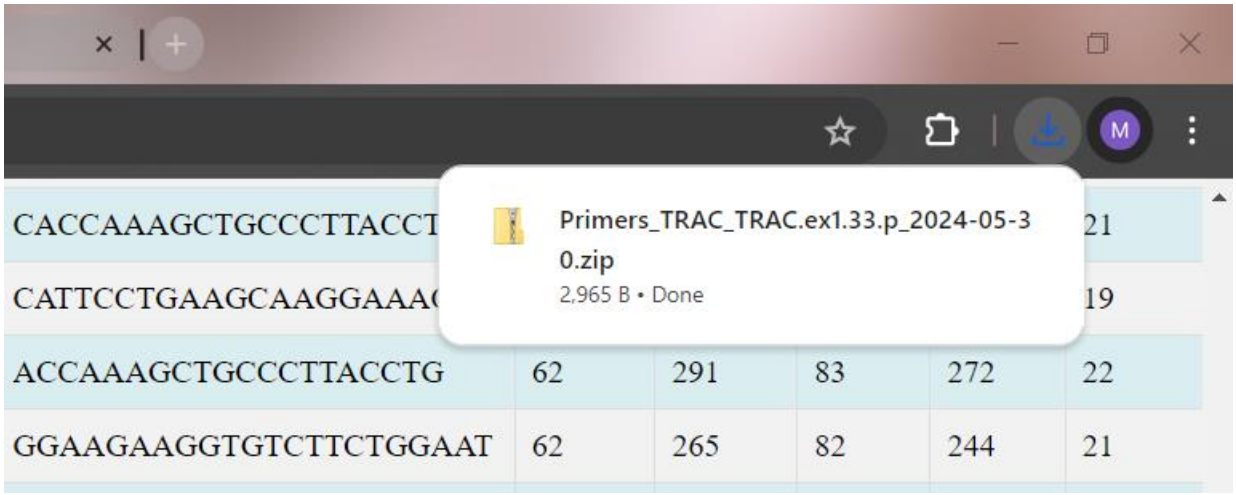
If **score_2nt** = 0.5 it means that at least one off-target for this pair of primers has complimentary one of two last nucleotides to off-target sequence.

- **score_3nt, score_4nt, score_5nt** - similar to score_2nt for the last three, four, and five nucleotides, respectively.

This table shows only primers with a lower probability of off-targets. If you want to see all Blast results, choose "Show all results" and click Submit. You will see the full table.

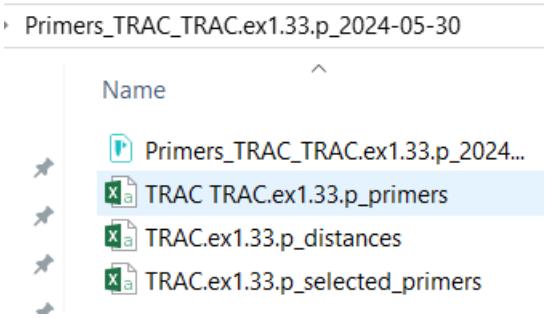


j. Your results will be downloaded as an archive.

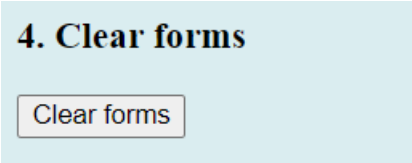


In archive, you will find:

- Snapgene file with all selected primers and guide. You can check it visually.
- Table with all primers.
- Table with selected primers.
- Table with amplicon size for all possible combinations between selected primers. If you want to check it.

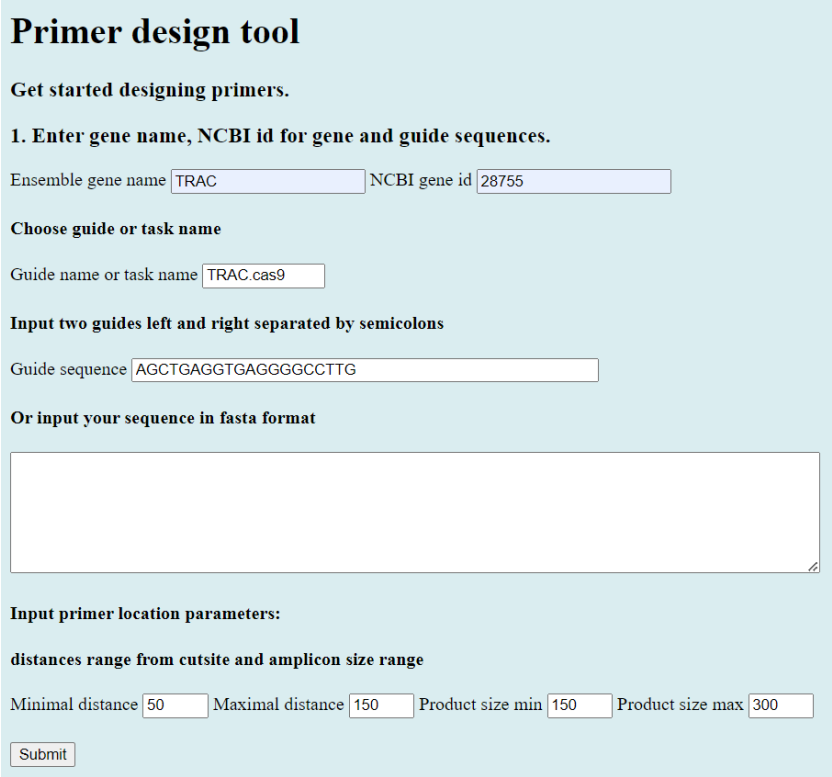


k. If you want to find new primers, clear form.



4. Search primers for guide Cas9

a. Input Ensemble gene name, NCBI gene ID, guide name, and guide sequence, and click Submit. Wait.

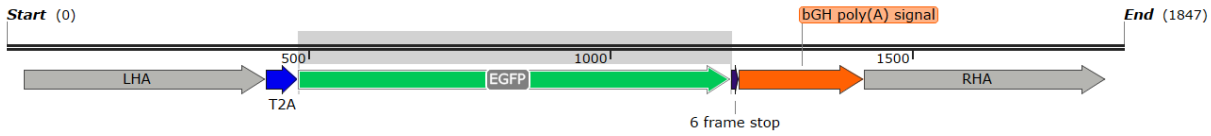


b. Repeat all other steps from section 3.

5. **Search primers for insert sequence.**

   For homology arm (PCR3) or GFP (PCR2), for example.

a. Copy the sequence for which you want to find primers. In my case, it's GFP.



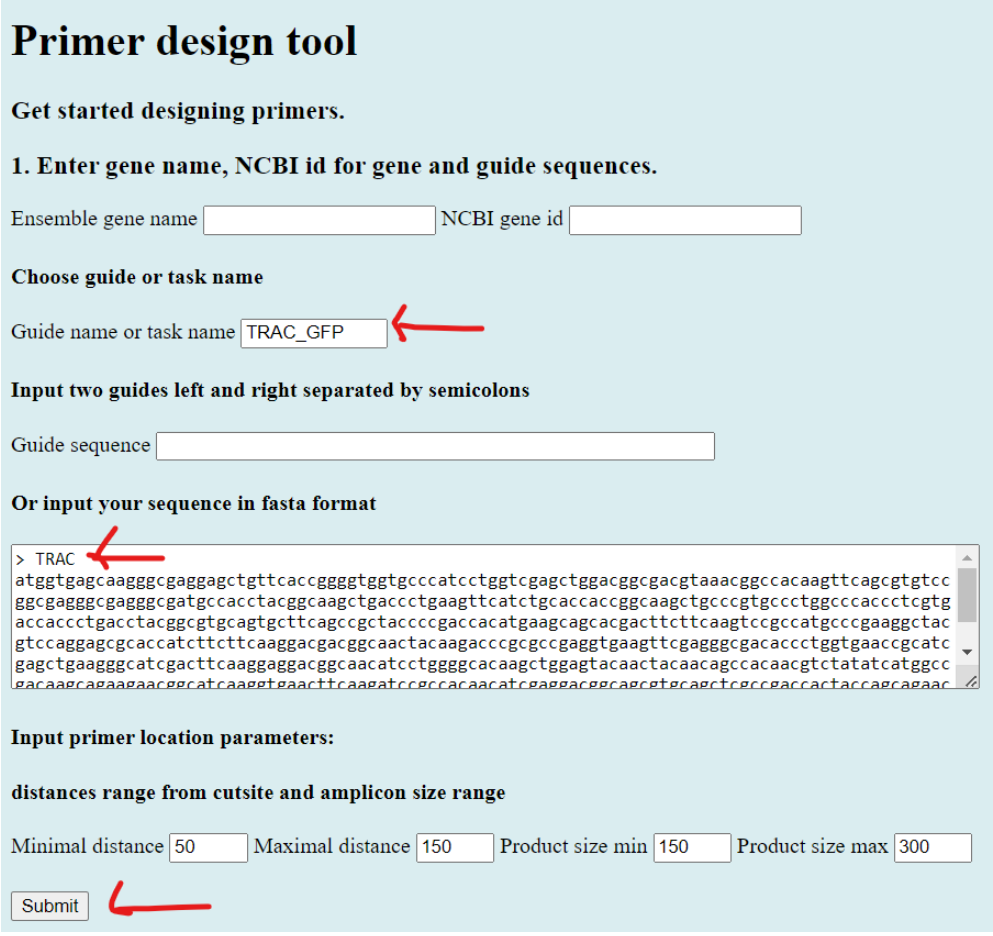
b. Input sequence in fasta format. Add ">" and sequence name.

   Input task name.

   Choose product size range. If you do not input a guide sequence, distances are not used.

   You can also input a 20nt sequence from your insert sequence. In this case, all primers will be found around this sequence (as a guide).

   Click submit. Wait.



c. Repeat all other steps from section 3.

d. In primer table, you will see all results from Blast Primers site.

6. Make table with distances and amplicon sizes for few guides.

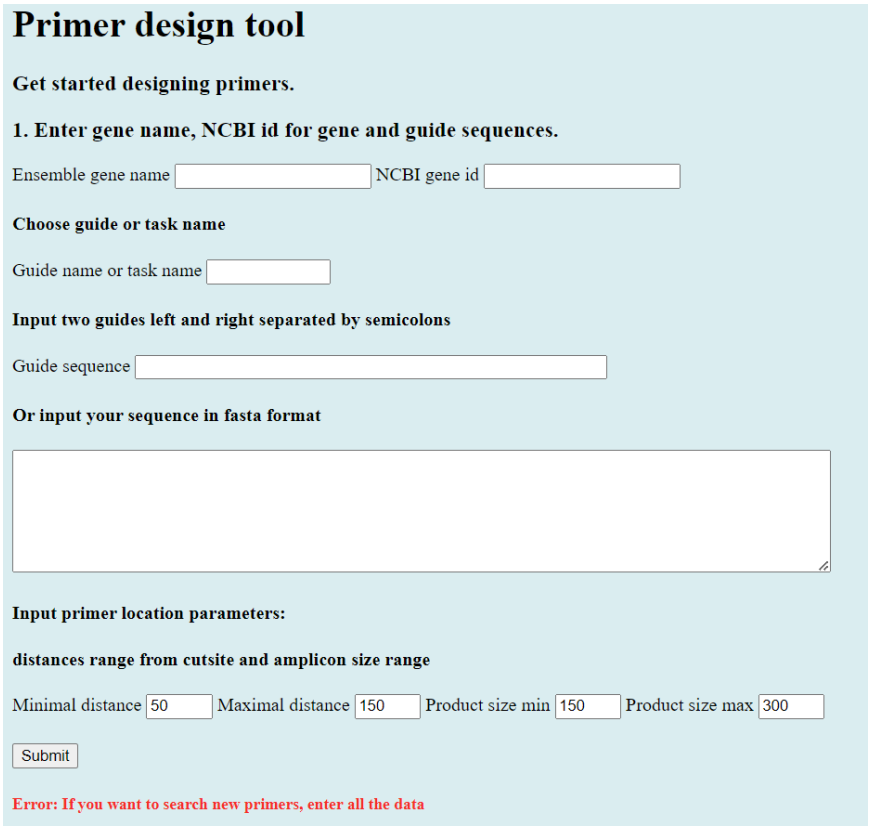
If you found primers for few guides (for example, TRAC exon 3) and want to check all possible primer pairs, you can make pivot table with this information.

a. Choose primer location parameters.

Keep other forms empty.

Click Submit.

Ignore the error message; it is not relevant for this case.



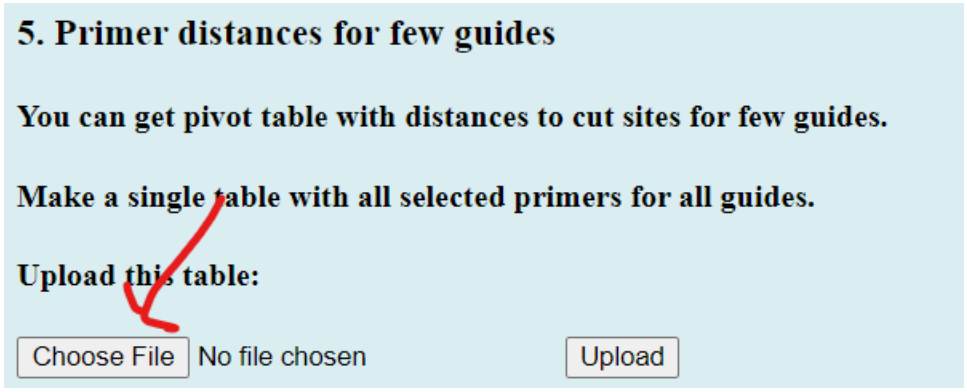
b. Copy all selected primers to one Excel table and save as .csv or .xlsx file.

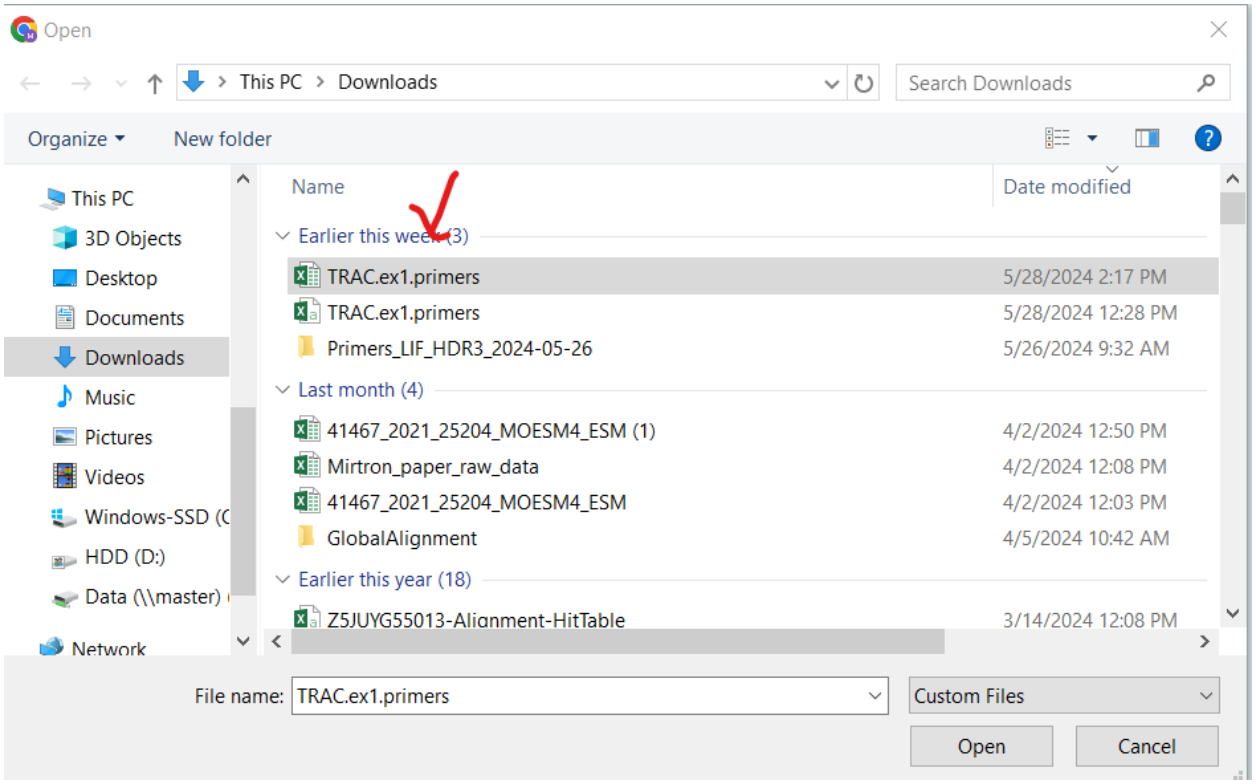
Copy all data from tables with selected primers. It is important.

Do not repeat column name.

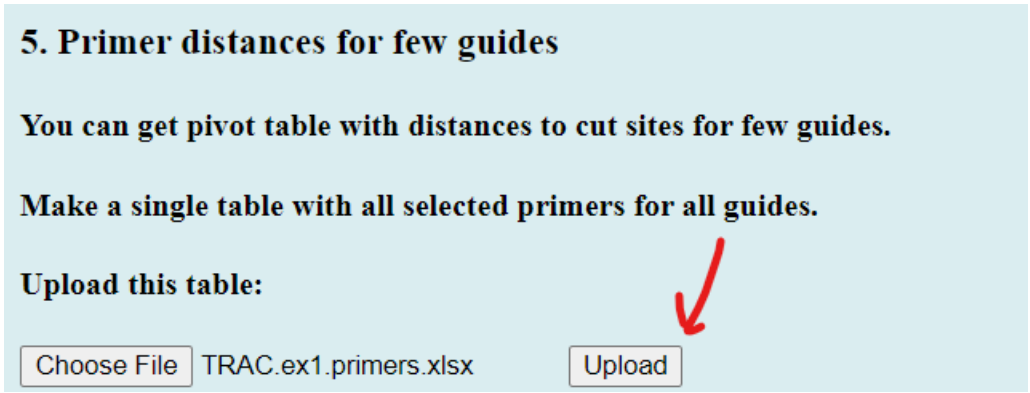
	A	B	C	D	E	F	G	H
1	pair_name	left_name	right_name	Sequence (5'->3')_L	Sequence (5'->3')_R	Start_L	Start_R	Stop_
2	primer_TRAC.100_1	primer_TRAC.100_1_L	primer_TRAC.100_1_R	ATGCAAGCCCATAACCGCTG	CTTAGGATGCACCCAGAGAC	46	253	
3	primer_TRAC.91_1	primer_TRAC.91_1_L	primer_TRAC.91_1_R	AAACCGTGGGTGTGTCCTG	GACCCGCGTCCCTAAAC	19	249	
4	primer_TRAC.74_1	primer_TRAC.74_1_L	primer_TRAC.74_1_R	CTGGGACATGCAAGCCCATA	GAGACCCGCGTCCCTAAAC	99	298	

c. Click “Choose file” and find the file that you made above. Select it and click ‘Open”.

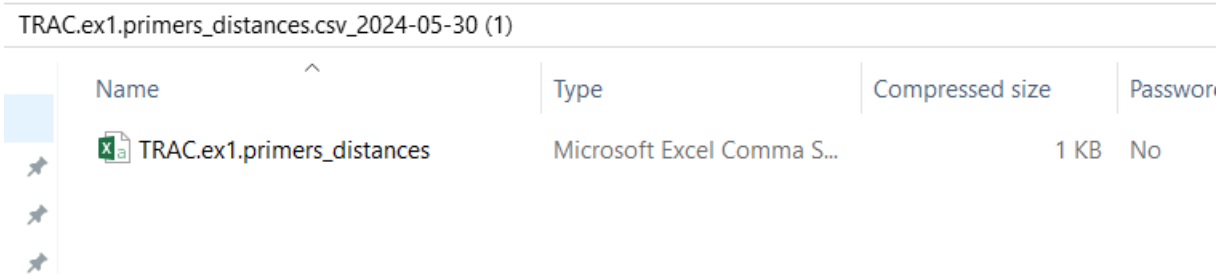




d. Click ‘Upload”.



e. Archive with results will be downloaded.



f. In the .csv table, you will find possible primer pairs that have correct distances and amplicon sizes.

	A	B	C	D	E	F
1	**primer_L**	**primer_R**	**TRAC.100**	**TRAC.74**	**TRAC.91**	**amplicon_size**
2	primer_TRAC.100_1_L	primer_TRAC.100_1_R	132/76	73/135	119/89	208
3	primer_TRAC.100_1_L	primer_TRAC.74_1_R	132/61	73/120	119/74	193
4	primer_TRAC.100_1_L	primer_TRAC.91_1_R	132/59	73/118	119/72	191
5	primer_TRAC.74_1_L	primer_TRAC.100_1_R	139/76	80/135	126/89	215
6	primer_TRAC.74_1_L	primer_TRAC.74_1_R	139/61	80/120	126/74	200
7	primer_TRAC.74_1_L	primer_TRAC.91_1_R	139/59	80/118	126/72	198
8	primer_TRAC.91_1_L	primer_TRAC.100_1_R		113/135		248
9	primer_TRAC.91_1_L	primer_TRAC.74_1_R		113/120		233
10	primer_TRAC.91_1_L	primer_TRAC.91_1_R		113/118		231

For all formatting of the Excel table, you need to do it manually.

Enjoy!